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CONTENTS

CUPRINS

REVIEW

NEW APPROACHES TO ADAPTATION

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ABSTRACT

This topic has been widely explored from the adaptation by natural selection (Darwin) to the general adaptation syndrome (Selye). This paper is focused on nowadays issues of the research on adaptation linked to stress. Allostasis is an oscillatory stabilization which allows the adjustment to various states. "Allostatic load" is the cumulative cost ongoing to repeated cycles of adaptation by physiological responses to stress and to possible adverse effects. "Psychological resilience", a component of positive adaptation during stress, represents the capacity to take on a traumatic event leading to disturbances and the subject's ability to offer protection against future challenges. Has been proved the importance of "posttraumatic growth and of thriving" – after psycho traumas; it explains way a large proportion of subjects submitted to adverse events recover fast. "Maladaptation" leads to PTSD and to other stress-syndromes which comprise psychoneurological, immunological, occupational and endocrine mechanisms. In contrast to the chronic or severe stress, a light or moderate stress named eustress has positive stimulating issues, related especially to the secretion of endorphins. Is in progress the foundation of adaptology which integrates the structural, functional and biopsychosocial sides of adaptation. In Romania, adaptology is promoted by several publications. We mention two books: "Human adaptation" (Badiu & Papari, 1999) and "Stressology, adaptology and mental health" (S. Riga & D. Riga, 2008).

Key words: allostasis, resilience, posttraumatic growth, stress-syndromes, eustress, adaptology

INTRODUCTION

The topic of adaptology is very large.

The first scientific account belongs to the Lamarckian concept of the inheritance of acquired traits. The main crucial advance has been performed by Darwin with his theory of evolution by natural selection, developed later by neodarwinism.

Selye's general adaptation syndrome is a seminal contribution to the links between stress and adaptation.

The above mentioned historical steps covered to explore adaptive processes can not be detailed in this paper; its aim is to discuss only the present-day directions dealing with the relationships between adaptation and various aspects of stress.

Further on will be underlined six main dimensions of this area of interest.

ALLOSTASIS

The term "allostasis" has been introduced by Sterling & Eyer (28). Its definition and peculiarities have been outlined by Mc Ewen (19-21).

Mc Ewen & Seeman (22) and Sterling (27) published comprehensive papers dealing with principles of allostasis, pathophysiology, therapeutic aspects and the links to stress.

Complementary information is given by Mc Ewen & Wingfield (23) and in Wikipedia (35). Are proposed two forms of allostatic load resulting in different responses: type 1 allostatic overload found especially among animals when energy demand exceeds supply, and type 2 when allostatic overload occurs when there is sufficient or even excess energy consumption accompanied by social conflicts or dysfunctions; this type present particularly in human society.

Allostasis means maintaining stability through change. This notion has been applied to the adjustment of the cardiovascular system, but can be used to explain other physiological processes of the body, such as secretion of cortisol or catecholamine (22).

Allostasis has a wider implication in the regulator mechanisms of the organism, such as homeostasis (3) and can be even used instead of the term "stress". Allostatic adaptive systems have much broader boundaries than homeostasis. They enable to respond to many physiological states: awake, asleep, standing, exercising, isolation, hunger, extreme temperatures, danger, microbial infections (19).

"Allostatic load" is the cumulative cost to cover repeated adaptive cycles of allostasis, experienced by the body as well as the turning on as shutting off of the neuroendocrine, systemic, and behavioral or by positive or sometimes negative events (22).

Examples of allostatic loads are the adverse effects of job strain on the cardiovascular system and the inhibition of cellular immunity resulting from chronic stress.

"Allostatic support" refers to mechanisms that confer resistance to individuals making them more robust to various kinds of stress and chronic illness.

The above described theoretical information is illustrated in Figure 1.

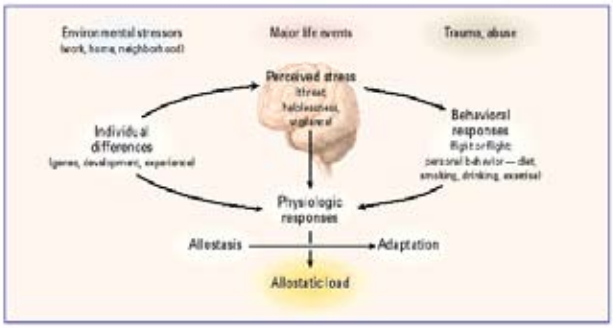

Fig. 1. The stress response and development of allostatic load (19)

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Mc Ewen (13) described four types of physiological response to an allostatic load: a. normal oscillatory responses over time; b. lack of adaptation with disturbed oscillations; c. prolonged response without recovery; d. inadequate low responses.

Physiological and pathophysiological effects of allostasis involve the action of glucocorticoids (GC) through intracellular, protoplasmatic steroid receptors and of catecholamines (CA) via membrane receptors and the second-messenger system. As well GC and CA act finally at the cell nucleus.

PSYCHOLOGICAL RESILIENCE

This psychological dimension represents the capacity to take on a traumatic event which could lead to functional disturbances and even to psychiatric conditions and to recover successfully.

Resilience implies good outcomes regardless of high-risks status, constant competition under stress, and efficient coping mechanisms. The negative effects of adverse life situations depend on the vulnerability of subjects which in stressful situations can be directed as well to pessimistic as to optimistic behaviors.

This dynamic process has been firstly described by Emmy Werner (34) on Taiwanese children, which grew up in very bad conditions. The results showed that one third of these youngsters did not exhibit destructive behaviors; this fact is explained by Werner by their resilience.

In 1980's this research topic emerged from observations on children of schizophrenic mothers.

Later, the resilience concept has been developed by Bonnano (2) in the USA and Cyrulnik (9) in France. Further details can be found in Wikipedia (36).

POSTTRAUMATIC GROWTH

The negative reactions to psycho traumas and extreme events (grief, torture, natural catastrophes, etc.) have been widely explored and categorized in DSM and ICD (see also the following section).

The possible/potential responses to trauma are outlined in Figure 2.

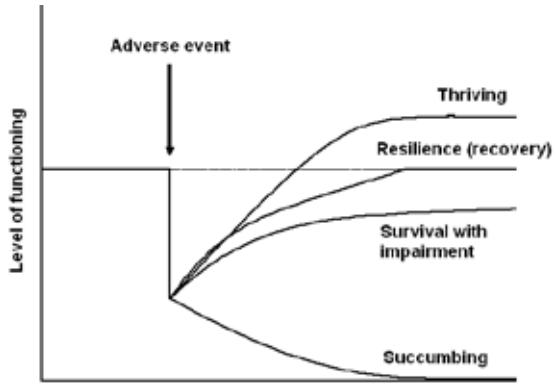

Fig. 2. Potential responses to trauma (O'Leary & Ickovics) (5)

An adverse event can induce four main consequences shown in Fig. 2.

The thriving reaction has been studied especially in USA by Carver (5), Calhoun & Tedescky (4), Tedescky & Calhoun (29, 30), who proposed a model of posttraumatic growth (PTG). Tedescky et al. (31) identified three main dimensions of PTG:

a. Friendly and altruistic interpersonal relationships;

b. Self-perception with better acceptance of limitations of the self;

c. Life-philosophy with shifts in an existential perspective.

Tedescky et al. (31) have designed a PTG inventory measuring the positive reactions of traumas. In Romania, Kallay (16, 17) has studied systematically this topic.

Posttraumatic growth explains why large proportion of subjects submitted to adverse events recovers successfully and fast returning to their pre-trauma functioning. Most important is the fact that sometimes the initial functional level is even raised. The psychological background of these positive reactions implies the intervention of meaning making. For details see Kallay & Miclea (18).

MALADAPTATION AND STRESS-SYNDROMES

Maladaptation leads to various stress-syndromes.

This topic has been developed in the last decades in several directions.

Horowitz (13, 15) has explored systematically the clinical significance of traumatic reactions, designed an event scale and elaborated a theory of traumatic reactions based on a social-cognitive approach.

A comprehensive book is devoted to the treatment of stress response syndromes (14). Other Horowitz's contributions are reported by Kallay (16).

The neuro-endocrine mechanisms of the stress-syndrome have been studied extensively by Chrousos (6, 7), the occupational outcomes by Theorell (33), and the endocrine syndromes by Coculescu (3).

The other directions of this topic deal with the cardiovascular stress-syndrome (for instance Tako-Tsubo syndrome) and with stress-syndromes described in exercise (overtraining, female athlete triad). The literature on the theories, models and peculiarities of posttraumatic stress reactions is very wide. For details see Kallay (16) and Derevenco (10-12).

EUSTRESS

In contrast to the familiar terms distress and strain, eustress is less used in the stress topic.

Eustress, aiming a light or moderate stress with positive stimulating issues, has been introduced by Selye and mentioned in his later writings (26). His expression "stress is the salt of life" belongs probably to eustress.

Eustress implies the secretion of endorphins and of other neuromediators including likely serotonin. The exercise-dependence of some athletes is probably related to the euphoric state produced by moderate exercise and social environmental factors.

Eustress represents a basis of the stress-inoculation method with preventive and curative effects.

ADAPTOLOGY

At present is in progress the foundation and promotion of adaptology, a new, distinct discipline which integrates the structural, functional and biopsychosocial components of adaptology.

This area of interest deserves to be explored in details elsewhere.

For the time being, we mention some contributions of the Romanian scientists. A pioneer of studies on adaptation is Schneider (25). He organized also a first meeting devoted to adaptology – topic of the 15th national Conference of Physiology, being author/coauthor of 16 works aiming adaptation. Recently, he has been published 2 comprehensive volumes.

The authors of the first book ("Human adaptation") are Badiu & Papari (1). This book comprises four parts including 21 chapters. Part I, general adaptology, Part

II, ontogenetic adaptology, Part III, environmental adaptology, Part IV, adaptology and physical exercise.

The second valuable volume signed by S. Riga & D. Riga is entitled "Stressology, adptology and mental health" (24). The section adaptology comprises five chapters: 1. adaptation, coping maladaptation, troubles of adaptation; 2. homeostasis, allostasis, vitality and vulnerability; 3. the stress binom: distress and eustress; 4. the general syndrome of adaptation; 5. capacity to cope with stress, antistress. S. Riga and D. Riga have also published other four papers on adaptation between 1988 and 2002.

CONCLUSION

Allostasis, resilience, posttraumatic growth, stress-syndrome, and eustress offer at present and in perspective a roadmap for the development of adaptology as a new integrative science and for surpassing over simplified and narrow-minded views on stress.

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NOI ABORDARI IN ADAPTOLOGIE

REZUMAT

Problematica adaptarii, de la teoria evolutionista (Darwin) la sindromul general de adaptare (Selye), a fost larg explorata. Lucrarea puncteaza unele directii actuale vizand studiul adaptarii si relatiile acestuia cu stresul. Allostaza este stabilirea oscilatorie ce asigura adaptarea la variate stari ale organismului. "Incarcarea alostatica" este costul cumulativ de a parcurge cicluri repetate de adaptare prin reactii la stres, cu posibile efecte adverse. Rezilienta psihologica, componenta a adaptarii pozitive la stres, consta in a lua act de un eveniment traumatizant care provoaca disfunctii fiziologice si abilitatea de a oferi protectie provocarilor viitoare. Intre raspunsurile la evenimente adverse, s-a dovedit importanta cresterii posttraumatice si a reusitei (thriving) care explica de ce o proportie insemnata de subiecti supusi psihotraumelor se reechilibreaza rapid. Maladaptarea se traduce prin sindromul dereglarilor posttraumatice de stres si prin alte sindroame care comporta mecanisme pe plan psihoneurologic, im unologic, ocupational, endocrin. Spre deosebre de stresul cronic sau prelungit (distres), eustresul, deci stresul usor/moderat, are valori pozitive, stimulatoare, legate in special de secretia endorfinelor. In prezent se contureaza adaptologia, disciplina de integrare a aspectelor structurale, functionale, biopsihosociale ale adaptarii. Adaptologia este promovata in Romania prin mai multe publicatii. Semnalam cartile "Adaptologia umana" (Badiu, Papari, 1999) si "Stresologie, adaptologie si sanatate mintala" (S. Riga, D. Riga, 2008).

Cuvinte cheie: adaptare, alostaza, rezilienta, crestere posttraumatica, sindroame de stres, eustres, adaptologie

MECHANISMS INVOLVED IN MESENCHYMAL STEM CELLS DIFFERENTIATION TOWARD EPITHELIAL LINEAGE. THE EFFECT OF SOME CHEMICAL INDUCTORS

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ABSTRACT

Adult mesenchymal stem cells (MSCs) are extremely attractive in order to study the regeneration and reparation features in various type of tissues. For maintaining the differentiation potential of MSCs there are used several strategies: the in vitro culture of mesenchymal cells in media supplemented with specific growth factors, the transfection of MSCs with the genes which are specific to the differentiated cells, co-culture of MSCs with differentiated cells from the target tissue. In this study we verify the effect of some chemical inductors to differentiate adult MSCs into epithelial-like cells. We used various cytokines and growth factors, added in the culture media alone or in combination. The results were analyzed using immunocytokemistry and molecular biology (PCR) methods. The experiments revealed that MSCs differentiation toward the cells expressing epithelial markers is relatively easily to obtain using a certain combination of inductors, without genetic manipulation of the cells

Key words: mesenchymal stem cells, epithelial differentiation, epithelial-like cells, chemical induction

INTRODUCTION

The mesenchymal stem cells (MSCs) role in the adult human body is the generation of mesenchymal cell lines and afterwards, through mesenchymal progenitors is involved in development, maintaining and restoration of connective and muscular tissue. Having these outstanding properties MSCs represents the best alternative for cell therapy applications; they have self renewal, great plasticity with differentiation potential in functional cell lines. Their efficiency was proven in numerous therapeutic protocols: bone marrow recovery, osteogenesis imperfecta, bone regeneration. Another advantage seems to be the fact that MSCs could be obtained from autologous sources (bone marrow) eliminated thus the complication of allogeneic transplantation. In past years were developed many researches in the field of MSC and now we have lot of information regarding plasticity, homing and differentiation potential in vivo, but less known are the intrinsic mechanisms which allow the self-renewal and differentiation. The understanding of these mechanisms at the molecular level is still a challenge for the medical community and the elucidation of mechanisms could offer a supplementary safety to cell therapies and make possible a large scale application of these methods (1,2). The MSCs capacity to differentiate in various cell lines was extensively studied beginning with the 1960. The results confirm the possibility to obtain specialized cells in vitro and in vivo. MSCs pluripotency is explained based on two theoretical models. The first one considering all of MSCs as pluripotent cells and differentiation could be induced by various environment factors. In the differentiation process MSCs loose their self-renewal capacity, passing through intermediary states (mesenchymal progenitors) and finally acquiring the characteristic phenotype of specialized cells. The alternative model, which is more confirmed by experimental studies sustained that the major determinant of pluripotency is the MSCs heterogeneity a property identified even in a single cell derived colonies. From the entire population of adherent cells only 30% are having a tri-differentiation potential (adipocytes/osteoblasts/chondrocytes) and the others having only a double osteo/chondrogenic or a unique osteogenic potential (3). A possible explanation is that in bone marrow reside primitive pluripotent MSC with limited capacity of self renewal which generates mesenchymal progenitors in various differentiation studies.

The concept of plasticity means the property of stem cells to differentiate in a distinct cell line apart from the originated tissue. The in vitro differentiation techniques are based on using a differentiation agent, co culture with specific cells or structures and modification in some gene expression. In vivo the differentiation process can be followed by marked SC transplantation in experimental animals or CS transplantation in genetic modified animals (with deficiency on some cellular lines). The differentiation methods used for the MSCs plasticity studies included MSCs transplant, chemical stimulation in culture and culture in special conditions. Till now there are cited successful differentiation processes through the cell lines belonging to all the embrionary layers. By MSC transplantation was obtained differentiation in: astrocytes (4); neurons; epithelial cells from skin, digestive and respiratory tract (5), cardiomyocites, (6), chondrocytes, adipocytes and marrow

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stromal cells (7). After chemical stimulation MSC was differentiate in: adipocytes (1-metil 3izobutilxantine, dexametasone, insulin and indhometacin), chondrocytes (without bovine serum and stimulation with TGF β), osteoblasts (dexametasone, β-glicerophosphate and ascorbate), neurons and astrocytes (EGF and BDNF or β-mercaptoetanol), skeletal muscle fibers and cardiomyocites (5-azacitidine). After prolonged cultures and co culture with another cell lines was obtained the MSC differentiation into neurons.

In our study we proposed to evaluate the differentiation of human adult bone marrow derived mesenchymal stem cells in to epithelial like cells by stimulation with some chemical inductors: growth factors and cytokines.

MATERIAL AND METHODS

The experiment was developed after obtaining the agreement of Ethics Committee of the University of Medicine and Pharmacy Victor Babes Timisoara. The bone marrow samples (2-4 ml) was harvest from 5 patients (4 females, 1 male; medium age 45+/-2) suffering by different forms of anemia. In accordance with the working protocol previously established, the written consent was obtained from patients. The sternal punction maneuvers were performed using specific instruments, in conditions of perfect safety and sterility, with minimum invasive procedure for the patients.

Isolation and culture of mesenchymal stem cells

The procedure used for mesenchymal stem cells isolation was based on plastic adherence, following a protocol described elsewhere. Briefly, the procedure steps are:

- The mononuclear cells (MNCs) were separated from the whole bone marrow cells by density gradient centrifugation using Ficoll-Paque plus (Amersham Biosciences Inc.)

- the MNCs were seeded on a cell concentration of 50,000 cells/cm^2 in 25 cm^2 plastic T flasks in on IMDM media (Invitrogene) supplemented with 10% fetal bovine serum (Sigma), 2 ng/ml Fibroblast Growth Factor (R&D Systems).

- Complete medium change was done at day 1 to 3 after the initiation of culture.

- The cultures were maintained at 37□in a humidified atmosphere with 5% CO2.

- The medium was replaced one to two times every week, every third to fourth day.

- When layers were subconfluent (70-75% confluence), cells were treated with 0.25% (v/v) trypsin/1 mM EDTA (Sigma) and replated at 1000 cells/cm^2.

- for determination of cell concentration the standard procedure of cell cont in Neubauer counting chamber (hemocytometer) was followed

The cells were examined daily using an inverted microscope (Olympus) in order to detect morphological changes and the initiation of confluence. The cell viability was determined both in the moment of isolation and placement in culture medium (day 0 of each passage), as well as at different time intervals, during their evolution, using small amounts of cellular suspension (approximately 200 μl). We used Trypan Blue microscopic method: dead cells, having lysis of cellular membrane, would be stained in blue, while viable cells, having intact membrane, would not be colored.

Confirmation of differentiation potential characteristic for mesenchymal stem cells

For this study MSCs at 3rd passage were used. The cultures subsequently were maintained in this media for 21 days, with media changes of 3 times a week.

1. Adipocytic differentiation

The induction medium was supplemented with 200 μ M indomethacin (Sigma), 1 μg/mL rosiglitasone, 1 mM dexamethasone, 0.5 mM isobutylmethylxanthine (IBMX). (8)

2. Osteogenic differentiation

The medium culture was replaced with osteoinductor medium, containing 50μg/ml ascorbic acid 2-phosphate (Sigma), 10nM dexamethasone (Sigma) and 10mM ß-glycerol phosphate (Sigma). (9)

3. Chondrogenic differentiation

The chondrogenic media consisted of 30 μg/ml ascorbate-2-phophate, ITS premix (BD Biosciences) and 10ng/ml TGF-β1 (R&D Systems) (10).

To evaluate the occurrence of differentiation, at the end of this period the cells were fixed with 10% formalin for 10 minutes and stained with specific antibodies. To monitor adipogenic differentiation, an antiFAB4 antibody was used (R&D Systems), as osteogenic marker was used antiosteocalcin antibody (DAKO) and for evidentiation of chondrogenic induction the choice was the antiaggrecan antibody (R&D Systems). For further confirmation of differentiation, RNA extraction and RT-PCR analysis of gene expression have also been performed. For RNA extraction the Gen Elute Mammalian Total RNA Miniprep Kit (Sigma) was used in accordance with the manufacturer instructions. The RNA quality and concentration was determined using a spectrophotometer (Nanodrop 2000C, ThermoScientific) and measuring the absorption at 260 and 280 nm. For reverse-transcription the One Step RT-PCR kit (Quiagene) was used and the thermal conditions were specific for each primer pair. The primer sequences used were found in literature (11) or were custom designed according to Gene Bank data (Table I).

Table I. Primers used for RT-PCR

Primer	sequence
PPARγ2	S: 5'GCTGTTATGGGTGAAACTCTG 3' AS: 5'ATAAGGTGGAGATGCAGGCTC 3'
Lipoprotein lipase	S: 5'GAGATTTCTCTGTATGGCACC 3' AS: 5'CTGCAAATGAGACACTTTCTC 3'
FAB 4 (AP2)	S: 5'-GTACCTGGAAACTTGTCTCC 3' AS: 5'-GTTCAATGCGAACTTCAGTCC 3'
GAPDH	S: 5'GGGCTGCTTTTAACTCTGGT 3' AS: 5'TGGCAGGTTTTTCTAGACGG 3'
Alkaline phosphatase	S: 5'TGGAGCTTCAGAAGCTCAACACCA 3' AS: 5'ATCTCGTTGTCTGAGTACCAGTCC 3'
Osteocalcin	5'ATGAGAGCCCTCACACTCCTC 3' 5' GCCGTAGAAGCGCCGATAGGC 3'
CBFA-1 (Runx2)	S : 5'CCACAGAACCACAAGTGCGG 3' AS : 5'ACGGAGCACAGGAAGTTGGG 3'
Osterix	S : 5'-CAGCTGCCATCTTAGATGTGC 3' AS : 5'-CCATTCCACAATGTTCTCTCC 3'
Agreccan	5'GCCTTGAGCAGTTCACCTTC 3' 5'CTCTTCTACGGGGACAGCAG 3'
Collagen type X	5'CCCTTTTTGCTGCTAGTATCC 3' 5'CTGTTGTCCAGGTTTTCCTGGCAC 3'
Collagen type II	5'GAACATCACCTACCACTGCAAG 3' 5'GCAGAGTCCTAGAGTGACTGAG 3'

Induction of epithelial-like cells

For this study MSCs at passage 3 were used. In a first set of experiments, the media was supplemented with one of the cytokines and growth factors: epidermal growth factor (EGF) 20 ng/ml, keratinocyte growth factor (KGF) 10 ng/ml, bone morphogenic protein 4 (BMP4) 10 ng/ml, tumor growth factor beta 1 (TGF beta1) 20 ng/ml, tumor growth factor beta 3 (TGF beta3) 25 ng/ml, hepatocyte growth factor (HGF) 10 ng/ml, beta cellulin 100 ng/ml, nicotinamide 25 ng/ml, fibroblast growth factor (FGF1) 10 ng/ml, insulin like growth factor II (IGF2) 50 ng/ml. All reagents were purchased from R@D Systems. Also, an experiment with a combination of fibroblast growth factor (FGF) 10 ng/ml, EGF 20 ng/ml and KGF 10 ng/ml

was carried out. .

In a second set of experiments, the effect of different combination of this inductors were evaluated. Four groups of experiments were used and the exact composition of the media is shown in figure 1.

GROUP 1	GROUP 2	GROUP 3	GROUP 4
•DMEM +	•DMEM +	•DMEM +	•DMEM +
•10% FCS	•10% FCS	•10% FCS	•10% FCS
•FGF 10 ng/ml	•IGF2 50 ng/ml	•IGF2 60 ng/ml	•HGF 10 ng/ml
•IGF2 50 ng/ml	•KGF 10 ng/ml	•KGF 10 ng/ml	•BMP4 10 ng/ml
•KGF 10 ng/ml	•EGF 30 ng/ml	•EGF 20 ng/ml	•KGF 10 ng/ml
•EGF 20 ng/ml	•BMP4 10 ng/ml	•HGF 10 ng/ml	•EGF 10 ng/ml

Fig.1. The composition of media for epithelial induction on each group

To evaluate the presence of some characteristic markers for undifferentiated MSCs respectively for epithelial lineage at the day 14 the cells were fixed with 10% formalin for 10 minutes and stained with specific antibodies: anti-vimentin (Beckton Dickinson) and anti-pancytokeratin (R&D Systems). For further confirmation of differentiation, RNA extraction and RT-PCR analysis of gene expression have also been performed. The primers used were for cytokeratin 19 (R&D Systems) and for E-cadherin (R&D Systems)

RESULTS

Isolation and culture of mesenchymal stem cells

From all the bone marrow samples were obtained primary cultures of adherent, spindle-shape cells (Figure 2). The cells had a good proliferation capacity and are able to survive in our lab for 5 to 6 passages. The cell viability on each passage was around 90-95%.

Fig. 2. MSCs, day 5, ob. 20x

Confirmation of differentiation potential characteristic for mesenchymal stem cells

The differentiation studies occurred in MSCs at passage 2. The cells were able to differentiate in all three lines (adipocytic, osteogenic and chondrogenic) which are characteristic for stromal/mesenchymal progenitors. The characteristic markers for those three lineages were expressed at the gene level and were evidenced by RT-PCR reaction (Figure 3). The presence of some markers at the protein expression level could be evidenced also, using immunocytochemistry staining (Figures 4, 5, 6).

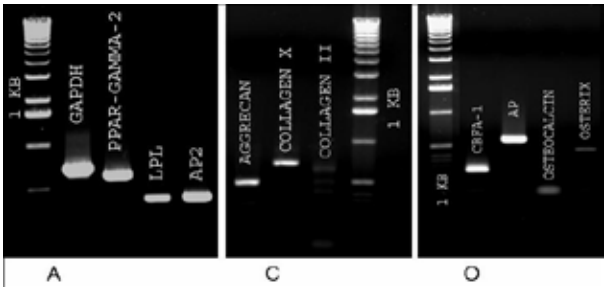

Fig.3. RT-PCR for MSCs after 2 weeks in adipocytic (A), osteogenic (O) and chondrogenic (C) media

Induction of epithelial-like cells

The results of RT-PCR expression of cytokeratin 19 is shown in figure 7. The cytokeratin expression was enhanced by media supplementation with EGF, KGF, BMP4, IGF2 and HGF. The addition of a mixture containing EGF, KGF and FGF seems to decrease the cytokeratin expression. The effect is possible to be generated by FGF addition because in experiments using only FGF as supplement the cytokeratin

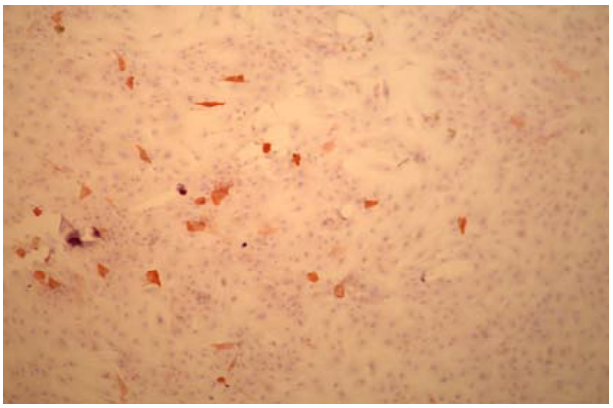

Fig.4. MSCs in adipocytic media. IHC for FAB4. ob. 10x

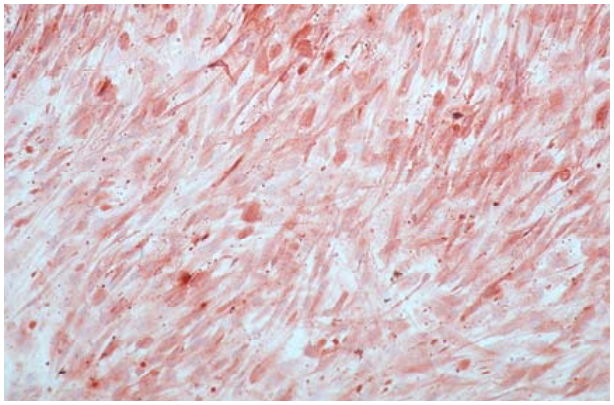

Fig.5. MSCs in osteogenic media. IHC for osteocalcin ob. 20x

expression was also very weak (Figure 7).

Regarding the second set of experiments, in all studied groups some modifications of cell morphology were noticed. The proliferation rate seems to be slower, cells became polygonal and, mainly for the cells from group 3 was noticed a tendency of multilayered culture (figure 8). A specific marker for MSCs, vimentin, had a weaker expression in the epithelial inducing media in comparison with undifferentiated

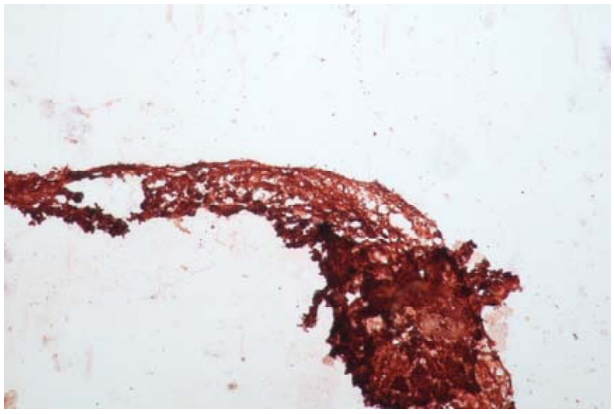

Fig.6. MSCs in chondrogenic media. IHC for aggrecan ob. 20x

MSCs (figure 9). In parallel, the expression of pancytokeratin is stronger in the cultures supplemented with epithelial-induction cocktail (figure 10).The best results were obtained in group 3. Regarding the detection of expression of cytokeratin 19

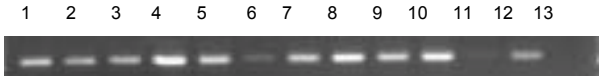

Fig.7. RT-PCR for cytokeratin 19 in MSCs cultured in media supplemented with TGF beta1(1), TGF beta3(2), beta-cellulin (3), BMP4(4), EGF(5), FGF(6), nicotinamide (7), HGF(8), KGF(9) IGF2(10), EGF+KGF+FGF(11), undifferentiated MSCs (12), negative control (13)

Fig.8. MSCs in epithelial inducing media (group 3), day 20, ob 20x

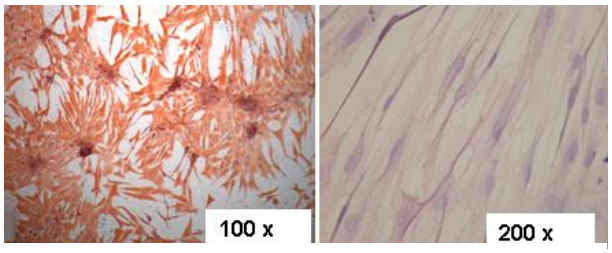

Fig.9. Vimentin expression in MSCs placed in undifferentiated media (left) and in epithelial inducing media (right)

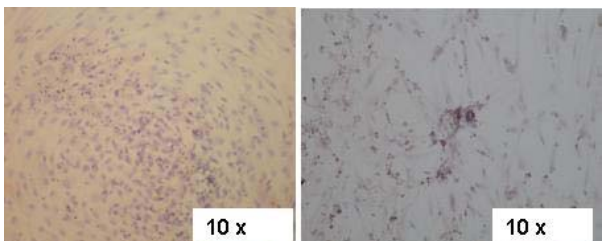

Fig.10. Pancitokeratin expression in MSCs placed in undifferentiated media (left) and in epithelial inducing media (right)

and E-cadherin by RT-PCR (figure 11); these markers are very well expressed in group 2, 3 and 4 without notable differences between groups. In cells from group

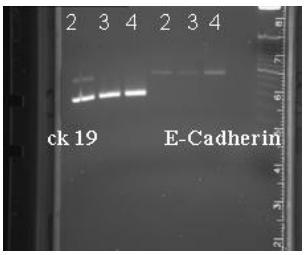

Fig.11. RT-PCR for Citokeratin 19and E-cadherin in experimental groups

1 the expression of E-cadherin and cytokeratin was not detectable, suggesting a possible role of FGF in inhibition of epithelial lineage.

DISSCUSION

The processes of MSCs differentiation in the cells belonging to all the embryonic layers are poorly understood. Although exist a theoretical model which try to explain the process sat the molecular and genetic level (12). According to this model the differentiation process have to distinct phases. In the first step, Go/G1 MSC suffer transcriptional modifications and generate mesenchymal progenitors but without any alteration of phenotype or self-renewal. The result is a cell identical with the mother cell and a mesenchymal pluripotent progenitor with restricted differentiation potential but phonotypical similar. After that the pluripotent progenitor cells generate by division tri and bipotent progenitors which differ only at the transcriptional level. The differentiation potential is restricted to osteogenic / chondrogenic / adypogenic for tripotent progenitors and to muscle/tendon for the bipotent progenitors. In the second step bi and tripotent progenitors generate unipotent specific progenitors for each line, with the phenotype modification and becoming finally adult cells with the specific specialized structures. The microarray analysis of global gene expression identified the genes which are involved in the MSCs differentiation process. The genes which trigger the progression toward a specialized cell line were evidenced by analyzing the transcriptional profiles of three cell lines: osteoblasts, chondrocytes, undifferentiated MSC. For analyses was used Affymetrix human genome U133 array set (13). The genes with strong expression in the differentiation process was identified and divided into three categories depending on the cell type. There was identified eight genes expressed in the differentiation process toward all three cell lines, which suggest that they are major determinants of the differentiation process: period homolog1 (PER1), nebulette (NEBL), neuronal cell adhesion molecule (NRCAM), FK506 binding protein 5 (FKBP5), interleukin 1 type II receptor (IL1R2), zinc finger protein 145 (ZNF145), tissue inhibitor of metalloproteinase 4 (TIMP4), serum amyloid A2. The functions of these gene address to the large spectrum of biological processes: cell adhesion, the tertiary structural protein organization, the citoskeletal organization and inflammatory response. The genes involved in final steps of differentiation process were not identified.

The cell plasticity is a hallmark of embrionary development. Although was demonstrated that MSCs can differentiate in vitro in a raw of cell types with the possibility to use them for therapeutic purposes in organ and tissues reconstruction. The molecular mechanisms involved in MSCs plasticity and in MSCs transdifferentiation are still unclear. The cells have a heterogeneous genetic pattern suggesting that they are in a permanent „standby stat" in which lot of genes is expressed at the minimal level making possible the changing of the cell fate. Some researchers showed that this remarkable plasticity appear mainly in the cells isolated from bone marrow which could under adequate circumstances differentiate into any cells belonging to the embryonic layers. (14) Is still controversially if in some cases

is involved a fusion process with the differentiated cells likes epithelial, Purkinje or muscular cells. The bone marrow derived stromal cells can differentiate in renal epithelial and non epithelial cells. The evidence of this fact appears when the female kidney is transplanted into the male and in the transplanted kidney could be identified the epithelial cells positive for Y chromosome (15). There are many experiments in animals which demonstrate that the cells from bone marrow can form bronchiolar epithelium and type 2 pneumocytes. Kotton et al. demonstrate in an experiment in which the MSCs were injected in mice with bleomycin induced pulmonary lesions that the injected cells differentiate in type 1 pneumocytes (16) MSCs can differentiate in vivo in gastrointestinal epithelial cells like esophageal and gut epithelium (17); it was noticed that the administration of the bone marrow derived mesenchymal progenitors leads to the differentiation in the crypt cells which represent the proliferative compartment from the tissue. Regarding the MSCs differentiation in epidermal cells, Herzog et al. showed that MSC can reach at the cutaneous lesion site and differentiate in proliferative keratinocytes which later are integrated in the scar tissue. (18) For maintaining of MSCs differentiation potential lot strategies are mentioned, like cell culture in the presence of specific growth factors, enrichment of the number of undifferentiated cells prior the addition of differentiation factors or even co culture of MSCs with the cells belonging to the specific tissue. On the other hand the analysis of the in vitro MSCs differentiation in the conditions mimicking the in vivo cell microenvironment could omit some essential factors for MSCs commitment toward a certain cell line. These factors are soluble molecules and their receptors (TGFbeta), molecules of the extracellular matrices (collagen, proteoglycans), cytoskeletal actine, transcription factors (Cbfa1/Runx2, PPARgamma, Sox9, MEF 2). Multiple protein interactions (focal adhesion, cytoskeleton) allow the cells to generate complex signals which lead to various in vitro cells behaviors. The initiation of cell adhesion integrin mediated has a great impact on cell proliferation. The integrins can regulate in a cooperative manner the members of cycline family and interfere in the cell cycle progression (19). Establishment of specific stimuli integrin-extracellular matrices can induce an increased expression of the genes involved in the differentiation processes.

CONCLUSIONS

From the anterior presentation we can conclude that adult MSC are extremely attractive for the researchers involved in the study of the various tissues regeneration-restoration processes. In the scientific literature can found references to the plastic potential of MSC to the mesodermal cell lines: bone, cartilage, adipocytes. There are data about the differentiation inducing agents and about the molecular events which can induce the cell switching from stem cell state able of self renewal in a commitment state toward a specific cell lineage. Concerning the capacity of MSC to differentiate in the cells belonging to the other embrionary layers there are lot of studies regarding in vivo and in vitro generation of the cell with neuronal characteristics; also a lot of interests was noticed regarding the possibility of obtaining the insulin secretory cells by manipulating the MSC. Concerning the differentiation in the epithelial lineage there are very few studies and the existing ones are about the murine MSC or referring to the in vivo experiments. Our study demonstrated that MSCs differentiation toward the cells expressing epithelial markers is relatively easily to obtain using a certain combination of inductors, without genic manipulation of the cells.

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MECANISME IMPLICATE ÎN DIFERENȚIEREA CELULELOR STEM MEZENCHIMALE SPRE LINIA EPITELIALĂ. EFECTUL UNOR INDUCTORI CHIMICI

REZUMAT

Celulele stem mezenchimale adulte (CSM) sunt extreme de attractive pentru studiul proceselor de reparatie si regenerare in diferite tipuri de tesuturi. Pentru mentinerea potentialului de diferentiere al CSM au fost utilizate o serie de strategii: cultivarea celulelor in vitro in mediu suplimentat cu factori de crestere specifici, transfectia MSC cu genele specifice celuelor differentiate, cu-cultivarea MSC cu cellule differentiate din tesutul tinta. In experimental nostrum am utilizat diferiti factori de crestere si cytokine adaugati in mediul de cultura singuri sau in combinatii. Rezultatele au fost evaluate prin imunocitochimie si metode de biologie moleculara (PCR). Studiul a demonstrat ca diferentierea CSM la celule ce exprima markeri de tip epitelial este relativ usor de realizat utilizand anumite combinatii de agenti inductori, fara a fi necesara manipularea genelor celulare.

Cuvinte cheie: celule stem mezenchimale, diferentiere epiteliala, celule epitelial-like, inductie chimica

ONTOGENETIC CHANGES OF BONE MINERAL COMPOSITION

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ABSTRACT

The goal of the study was to assess the dynamic of the bone mineral content at different ontogenetic stages. In the study were involved 80 rats that were divided in 4 groups by age – young, adult, old and senile. The dynamics of the calcium and phosphate quantities in the bone were similar – the maximal amounts were revealed in adult rats and their concentrations decreased gradually in old and senile animals. The highest mean concentrations of magnesium, chloride, potassium and copper were revealed in young animals (1.46 $\pm$ 0.21, 3.53 $\pm$ 0.28, 0.02 and 0129 $\pm$ 2.97 $\pm$ 0.18 mM/g tissue). The amounts of copper and sodium changed from one age group to another – moderate decreased in the adult compared with young (by 33% and 51%, $p < 0.0001$), increased in the old compared with those adults (by 13% and 62%, $p < 0.05$) and subsequently decreased again in the bone of senile rats (by 18% and 73%, $p < 0.005$). The amount of zinc was virtually identical in the bone of young, adult and old rats and varied between 0.017 and 0.0183 mM/g bone tissue while the sulphates concentration increased significantly in adult animals versus the young (14 times) and decreased gradually with age in the subsequent groups.
Our results revealed divers and significant quantitative changes of the mineral compound's content in the bone at different ontogenetic stages, their knowledge being important in the evaluation of bone status in different physiological and pathological conditions.
Keywords: bone, mineral composition, ontogenetic changes

INTRODUCTION

Along with increasing life expectancy and changing age structure of population, diseases of the osteo-articular system have grown in importance. Thus, investigations were directed to a greater extent to bone metabolism and the most common bone disease – osteoporosis. Particularly intense were studied bone composition and metabolism in persons during risk periods –postmenopause and senile. Strategies for prevention and treatment methods developed and implemented, also were targeted at older people, aimed at halting the decrease bone mineral density and recovery of lost bone.

However, it was acumulated a significant amount of information on the parentage in childhood and adolescence of many factors that determine the quality of bone tissue and predispose to osteoporosis (1) .Osteopenia and osteoporosis are not an exclusively adults and elderly issue, there are a lot of evidence of direct correlation between mineral density in elderly bone and bone mass accumulated in the first two decades of life and increased risk of osteoporosis if the accumulated peak bone mass was less than optimal (5).

Most studies have focused on the evaluation of specific markers of bone remodeling that can be assessed in blood or urine (2) in various primary and secondary osteo-articular diseases (6), on establishing correlations between bone mineral density and the dynamics of bone tissue markers in normal and pathological conditions (4), the analisys of drugs and osteotrope remedies influence on bone remodeling (3), etc.

However, there is no sufficient scientific data about the detailed bone composition and the dynamic of individual compounds in various diseases at different ages, necessary to build a scientific based diagnosis and differential, coherent prevention and therapy strategies.

It is obviously necessary to study the particularities of composition and metabolism of bone at different ontogenetic stages of development, to determine the parameters and characteristics of each age, to create database that would differentiate the age-related physiological changes from the pathological one determinated by various osteo-articular conditions or bone diseases.

Research goal was to determine the peculiarities of the bone mineral composition at different ontogenetic stages of development in experiment.

MATERIAL AND METHODS

The experiences were performed on 80 rats of different age:
- group I - young rats until sexual maturation (2 months);
- group II - adult rats in reproducible period (6 months);
- group III - old rats in postmenopause period (18 months);
- gloup IV - senile rats (24 months).

Rats were sacrificed under light narcosis with diethyl ether. Femoral bones were extracted, stripped of adjacent soft tissues and released of the bone marrow by repeated washing with glacial solution of 0.9% NaCl. Subsequently femoral bones were triturate in liquid nitrogen to the state of powder.

In the femoral bone powder was determined the amount of some bone mineral compounds. The calcium, phosphorus, magnesium, copper and chlorine contents were assessed with standard kits Elitech Diagnostic (France), according to the instructions attached. The amount of sodium, potassium and zinc was assessed with standard kits Centronic GmbH (Germany), according to the instructions attached. The amount of sulfates was determined turbidimetric with barium chloride.

The results were evaluated statistically according to Student t-criterion, neparametric Mann-Whitney criterion and the correlation coefficient r (Statistica 6.0, Stat Soft Inc.., 2002).

The research was approved by the Ethics of biomedical research Board of

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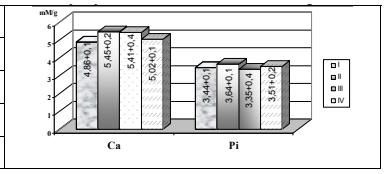
the State University of Medicine and Pharmacy "N. Testemitanu" from Republic of Moldova.

RESULTS AND DISCUSSION

It was established that the contents of calcium and phosphate in bone tissue of rats are subjected to similar changes, but of different magnitudes (Table I). The results show the highest average concentrations of calcium and phosphate in adult animals. The content of calcium increased significantly in adult rats compared with young (12%, p <0005) and decreases gradually thereafter, the differences being statistical conclusive between the concentrations detected in the adult animals and in the senile one (8%, p <0.05). The content of phosphates in the bone of adult rats is higher than in the young by 6% (p <0.05), in the old – by 8% (p <0.01) and the senile rats – by 5%. Thus, the total amount of phosphates in bone tissue of rats is changing less pronounced than that of calcium, the contents of these mineral elements are correlated between them. Strong positive correlation were revealed in young (r = 0.79, p <0.0001) and old rats (r = 0.91, p <0.0001).

Table I. The concentrations of calcium and phosphate in bone tissue of rats of different ages

Group	Calcium	Phosphate
I	4.86 ± 0.10	3,44 ± 0,09
II	5.45 ± 0.18^{***I}	3.64 ± 0.08^{*I}
III	5.41 ± 0.40	3.35 ± 0.42^{**II}
IV	5.02 ± 0.09^{*III,IV}	3.51 ± 0.17



Note a) table includes M±m; b) the concentrations of calcium and phosphate are expressed as mM/g bone tissue; c) the reliability of differences caused by age: *-p<0.05, **-p<0.01, ***-p<0.005, ****-p<0.001; the number indicates the group that the comparisont was carried out with

The highest average concentrations of magnesium, chlorine, potassium and copper in the bone tissue were attested in young animals, respectively, – 1.46 ± 0.21, 3.53 ± 0.28, 0.02 and 0129 ± 2.97 ± 0.18 mM/g tissue (Table II). In adult rats decreases the amount of magnesium compared with young animals (69%, p < 0001) and no significant changes were established in the following age groups. Same dynamic was characteristic for the potassium level – the highest concentration was detected in young animals, and then it decreased by about 50% (p < 0005) and remains virtually at the same level in the bone of adult, old and decrepit rats (0.068 – 0.080 mM/g tissue). The level of chlorine in bone was moderate decreased in the adult rats compartiv with young (27%, p < 0.0005) and more marked in old animals compared with adult (with 93%, p < 0.0001) and young one (95%, p < 0.0001). In group IV (senile rats) the chlorine content significantly increased comparative to the levels found in old animals (by 255%, p <0.0001), but not to the specific quantities of mature or young one were detected.

Table II. The contents of magnesium and chlorine in the bone tissue of rats of different ages

Group	Magnezium	Potasium	Chloride
I	1.460 ± 0.21	0.115 ± 0.02	3.53 ± 0.28
II	0.655 ± 0.03^{****I}	0.068 ± 0.002^{****I}	2.24 ± 0.09^{****I}
III	0.660 ± 0.06	0.073 ± 0.007^{****I}	0.162 ± 0.02^{****I,II}
IV	0.645 ± 0.03	0.080 ± 0.004$^{****I;\ ***II}$	0.58 ± 0.04^{****I,II,III}

Note. a) the table includes the M ± m; b) the contents of magnesium, chloride and potassium are expressed as mM/g bone tissue; c) the reliability of differences caused by age: * - p < 0.05, ** - p < 0.01, *** - p < 0.005; **** - p < 0.001; the number indicates the group that the comparison was carried out with

The amount of copper changed from one age group to another-moderate decreased in the adult compared with young (33%, p<0.0001), increased in the old compared with those adults (13%, p<0.05) and subsequently decreased again in the bone of senile rats (18%, p<0.005). Similar changes but less pronounced in magnitude of sodium content had been revealed in bone tissue of experimental rats (Figure 1).

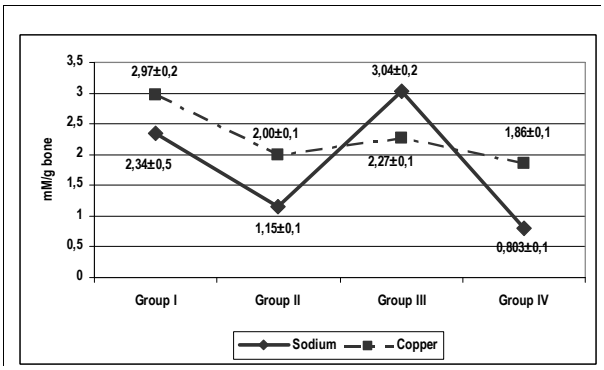
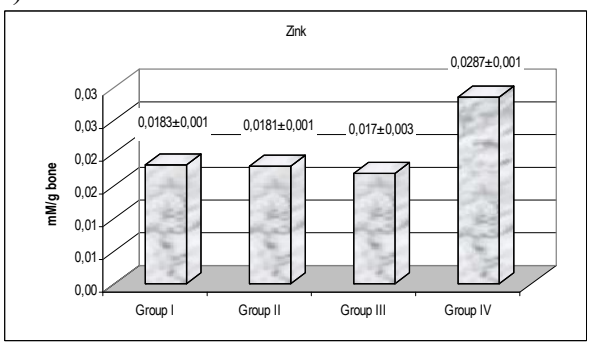


Fig. 1. Age depending dynamics of sodium and cooper contents in bone tissue of rats (mM/g bone tissue)

Note: a) the content of sodium and cooper is expressed as mM/g bone tissue;
b) figure includes M±m.

The amount of zinc was virtually identical in the bone of young, adult and old rats and varied between 0.017 and 0.0183 mM/g bone tissue (Figure 2). Only in senile animals was detected a significantly higher amount of zinc in bone tissue – 0.0287 mM/g bone tissue (p < 0.0001). The average content of sulphates in the bone tissue of young rats was 21 times less (p <0.0001) compared to adult and old animals. In senile rats were detected in comparisone with adult and old animals moderate decreased concentrations of sulfates (24%, p < 0.05 in both cases).

a)



b)

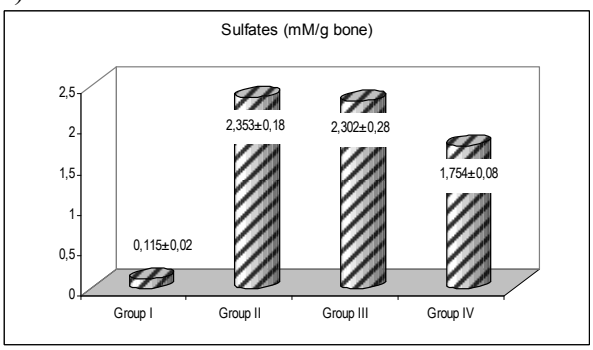


Fig. 2. Age depending dynamics of the zinc (a) and sulphates (b) contents in the bone tissue of rats at different ontogenetic stages

Note. a) the concentrations of zinc and sulphates are expressed in mM/g bone tissue;
b) figure includes M ± m.

Table III. Correlations between the contents of the mineral compounds of the bone tissue of rats of different ages

	Young					Adult					Old	Senile			
	Ca	Mg	Na	Pi	Cl	Ca	Mg	Na	Pi	Cl	Ca	Mg	Na	Pi	Cl
Ca		0.79	0.57	0.8	0.54					0.61					
Mg	0,79		0.75	0.68					0.52				1.0	0.8	0.68
Na	0,57	0.75		0.72	0.65				-051	053		1.0		0.8	0.68
Pi	0,8	0.68	0.72		0.6		0.52	-0.51		-0.58	0.88	0.8	0.8		0.77
Cl	0,54	0.61	0.65	0.6		0.6		0.53	-0.58			0.68	0.68	0.77	

Note a) table includes the values of correlation coefficient r,
b) in all cases $p<0.05$. 1.

Many correlations between the quantities of mineral compounds in bone tissue were found. These correlations vary in number, power and related substances in animalsof different age (Table III).

The greatest number of singifcant positive correlations of medium and strong intensity with r between 0.54 – 0.80 ($p < 0.05$) were attested in young and senile rats. In adult rats a moderate number of medium intensity correlations both positive and negative were found. The strongest positive (r between 0.71 and 0.89, $p <0.05$) correlations were established in old animals.

CONCLUSIONS

Research data attested statistically significant changes, but at moderate scale of the calcium and phosphate content – cardinal constituent elements of bone mineral phase, in animals of different age.

2. Quantities of magnesium, chlorine, copper, sodium, potassium, zinc and sulphates undergo significant age-dependent changes in the bone tissue of rats.

3. Correlations between the quantities of the mineral elements of the bone tissue of rats vary in number, intensity and type of the related substances in animals of different age.

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MODIFICARILE ONTOGENETICE ALE COMPOZITIEI MINERALE OSOASE

REZUMAT

Obiectivul cercetării a fost evaluarea compoziției minerale osoase la diferite etape ontogenetice de dezvoltare. Experiențele au fost efectuate pe 80 şobolani divizați în 4 loturi – tineri, adulți, bătrîni şi senili.

S-au depistatt modificări de acelaşi sens ale cantităților de calciu şi fosfați, care ating valori maxime la animalele adulte şi în următoarele grupe de vîrstă diminuează treptat. Concentrațiile medii maxime de magneziu, clor, potasiu şi cupru în țesutul osos se atestă la animalele tinere (respectiv, 1.46 ± 0.21, 3.53 ± 0.28, 0.02 and $0129 \pm 2.97 \pm 0.18$ mM/g țesut). Concentrațiile de cupru şi sodiu alternează de la o grupă de vîrstă la alta – scade la cei adulți comparativ cu cei tineri (cu 33% şi 51%, $p < 0,0001$), creşte la cei bătrîni comparativ cu cei adulți (cu 13% şi 62%, $p < 0,05$) şi ulterior iarăşi descreşte la şobolanii senili comparativ cu cei bătrîni (cu 18% şi 73%, $p < 0,005$). Conținutul de zinc este practic identic la şobolanii tineri, adulți şi bătrîni şi variază între 0,017 şi 0,0183 mM/g țesut osos, pe cînd concentrația sulfaților în os creşte semnificativ la animalele adulte comparativ cu cele tinere (de 14 ori), odată cu înaintarea în vîrstă înregistrîndu-se descreşterea treptată a cantității lor.

Studiul efectuat denotă modificări semnificative cantitative şi variate ca direcție ale concentrațiilor substanțelor minerale în țesutul osos al şobolanilor la diferite etape ontogenetice de dezvoltare, cunoaşterea căreea este necesară pentru evaluarea stării țesutului osos în diverse condiții fiziologice şi patologii

Cuvinte cheie: țesut osos, compoziție minerală, modificări ontogenetice

GLUTATHIONE PEROXIDASE ACTIVITY AFFECTS PROGNOSIS IN NON-ST SEGMENT ELEVATION ACUTE CORONARY SINDROM

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ABSTRACT

Introduction: Cellular antioxidant enzymes such as glutathione peroxidase play a central role in the control of reactive oxygen species. In vitro, data and studies on animal models suggest that these enzymes may protect against atherosclerosis, but few are known for their relevance to human disease.
Aim: We have examined the association between redox status and the prognosis of non ST elevated acute coronary syndrome.
Methods: We have evaluated 55 patients, age under 75, consequently hospitalized in the Cardiology Clinic of Emergency Clinic Hospital of Constanta, during May 2008 – May 2009, diagnosed with non ST elevated acute coronary syndrome and 19 healthy volunteers (without cardiovascular affections, hypercholesterolemia, diabetes and non-smoking). The patients were divided in two groups: the first group - patients with unstable angina (37 patients, out of which 10 were readmission in the following 6 months) and the second group – patients with non-Q wave myocardial infarction (18 patients, out of which 6 were readmission in the first 6 months after the heart attack). Glutathione peroxidase (GPx) activity was measured over a fixed time: T1 – the first 24 hours after hospital admission, T2 – at 48 hours and T3 – at discharge. After discharge, the patients were monitored and the following data was recorded: months of follow-up, death due cardiovascular cause and onset of major cardiovascular events.
Results: This prospective study of patients admitted with non-ST-segment elevation acute coronary syndrome (unstable angina and non-Q wave myocardial infection) showed a direct association between baseline GPx and the onset of major acute coronary events in the group with unstable angina and inversely associated with future fatal and non-fatal cardiovascular events in the group with non-Q wave myocardial infarction.
Conclusion: In the case of the patients with non-ST-segment elevation acute coronary syndrome, the antioxidative enzyme GPx seems to protect against adverse oxidative effects. The analysis of GPx activity provides superior information on cardiovascular risk assessment compared with the measurement of traditional risk factors alone.
Key words: acute coronary syndrome, biological markers/blood, glutathione peroxidase, oxidation-reduction, oxidative stress

INTRODUCTION

Controversial data existing concerning the relation between the activities of scavenger antioxidant enzymes and coronary heart disease (CHD) risk.

Oxidative stress may be defined as an imbalance between the production and degradation of reactive oxygen species such as super oxide anion, hydrogen peroxide, lipid peroxides and peroxynitrite. Aerobic organisms possess antioxidant defense systems that deal with reactive oxygen species (ROS) produced as consequence of aerobic respiration and substrate oxidation. Low levels of ROS are indispensable in many biochemical processes, including intracellular signaling, defense against microorganisms and cell function. In contrast, high dose and/or inadequate removal of ROS, results in "oxidative stress", which has been implicated in the pathogenesis of many cardiovascular diseases, including hypercholesterolemia, atherosclerosis, hypertension, diabetes, and heart failure. Enzymatic inactivation of reactive oxygen species is achieved mainly by glutathione peroxidase, superoxide dismutase, and catalase (7, 8). In mammalian cells, glutathione and the glutathione peroxidases are the principal antioxidant defense system (13, 20).

There are at least four different glutathione peroxidases, all of them containing selenocysteine at their active sites (2).

Glutathione peroxidase 1, the ubiquitous intracellular form and key antioxidant enzyme within most of the cells, including those of endothelium, uses glutathione to reduce hydrogen peroxide to water and lipid peroxides to their respective alcohols (6,9), and it also acts as a peroxynitrite reductase (17).

On the basis of the experimental evidence, we addressed the hypothesis that enhanced activity of cellular glutathione peroxidase 1 would be protective against cardiovascular events in patients with coronary artery disease.

METHODS

We have evaluated 55 patients, age under 75, consequently hospitalized in the Cardiology Clinic of Emergency Clinic Hospital of Constanta, during May 2008 – May 2009, diagnosed with non ST elevated acute coronary syndrome and 19 healthy volunteers (without cardiovascular affections, hypercholesterolemia, diabetes and non-smoking) – control group.

The patients were informed about the study protocol and a written consent was obtained from each patient. All the patients were evaluated within the same clinical and paraclinical protocol: medical history, physical examination, 12-lead electrocardiography, biochemical analysis.

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The patients were divided in two groups: group I - patients with unstable angina (37 patients, out of which 10 were readmission in the following 6 months – group IA and 27 without being re-hospitalized – group IB) and group II – patients with non-Q wave myocardial infarction (18 patients, out of which 6 were readmission in the first 6 months after the heart attack – group IIA and 12 with good evolution, without being re-hospitalized – group IIB).

Glutathione peroxidase activity was measured over a fixed time: T1 – in the first 24 hours after hospital admission, T2 – at 48 hours and T3 – at discharge. After discharge, the patients were monitored by means of a telephone survey, a personal interview, a chart consultation or any other combination of these methods. The following data was recorded: months of follow-up, death due cardiovascular cause and onset of major cardiovascular events (MACEs defined as cardiovascular death, readmission with acute coronary syndrome or the need for coronary revascularization).

Glutathione peroxidase (GPx) in whole blood quantitative determination is based on Paglia and Valentine method. GPx catalyse the oxidation of gluthatione (GSH) by Cumene Hydroperoxide. In the presence of gluthatione reductase (GR) and NADPH the oxidized glutathione (GSSG) is immediately converted to the reduced form with a simultaneous oxidation of NADPH to NADP+. The decreased in absorbance at 340nm is measured.

The main reaction is:

$$2GSH + ROOH \xrightarrow{GPX} ROH + GSSG + H_2O$$

$$GSSG + NADPH + H^+ \xrightarrow{GR} NADP^+ + 2GSH$$

Glutathione peroxidase concentration was calculated using the following formula: U/l whole blood = 8412 x ΔA 340 nm/min and expressed in U/l.

Through routine methods we have determined: the complete blood count (CBC) and the lipidic profile of the patients (high cholesterol, LDL - cholesterol, HDL – cholesterol and triglyceride)

The presence of diabetes at baseline was defined as fasting plasma glucose >110 mg/dl or use of oral hypoglycemia agents or insulin (19). A surrogate marker for obesity content is the body mass index (BMI), which is determined by weigh (kilos), divided by the square of the height in meters. In clinical terms, a BMI of 25-29 kg/m^2 is called overweight; higher BMI (30 kg/m^2) are called obesity. The waist circumference was measured on admission, midway between the last rib and iliac crest and the average of 2 measured was recorded (12).

All data is presented as the mean and standard deviation. Continuous data analyzed using Student test for independent samples. The hazard ration and their 95 percent confidence intervals are reported. The p-value is two-sides; a p-value of less was considered to indicate statistical significances.

RESULTS AND DISCUSSION

The study's results are presented as tables and graphs.

Table I offers the base-line characteristics of the 55 study participants. It can be noticed that there are no important differences between the two study groups as far as it concerns the clinic characteristics of the patients. Also, there are no significant variations of the incidence of cardiovascular risk factors (diabetes, obesity, dyslipidemia). The age of the patients hospitalized with non-Q wave infarction is significantly bigger than the age of the patients with unstable angina (65+/-9.57 vs 60.86+/-10.49, pT=0.0036). In table I it is also shown a significant grow of the fibrinogen (535±163.22 vs 646±193.23, pT=0.043), total creatine kinase (128±76.21 vs 639±680.34, pT=0.0055), troponin I (0.409±0.39 vs 35.2±40.84, pT=0.0219), and of the leucocytes number (7.96±2.16 vs 9.71±2.31, pT=0.0119) at the patients with non-Q wave myocardial infarction.

Table I. Base-line characteristics of the study patients

		patients with unstable angina 67.25 % (N = 37)	patients with non-Q infarction 32.73 % (N = 18)	p value NS
Characteristics				
mean age		60.86±10.49	65±9.57	0.0036
sex	women	19	9	NS
	male	18	9	NS
body-mass index		26.8±3.4	26.4±4.1	NS
diabetes (%)		18.92 (7)	27.78 (5)	NS
history of miocardial infarction (%)		16.21 (6)	33.33 (6)	NS
hypertension (%)		35.14 (13)	66.67 (12)	NS
lipid variables				
	cholesterol (mg/dl)	230±61.48	226± 74.28	NS
	LDL- cholesterol (mg/dl)	130±33.98	129 ± 31.46	NS
	HDL- cholesterol (mg/dl)	67±16.97	62±16.46	NS
	triglycerides (mg/dl)	156±64.55	180±60.71	NS
inflammatory variables				
	ESR (mm/h)	22±17.64	30±25.73	NS
	fibrinogen (mg/dl)	535±163.22	646±193.23	0.043
	Total CK	128±76.21	639±680.34	0.0055
	CK – MB	12±16.65	27.25±24.78	NS
	TnI	0.409±0.39	35.2±40.84	0.0219
CBC	RBC (10^6/mm3)	4.4±0.5	4.51±0.63	NS
	HGB (g%)	13.29±1.65	13.51±1.85	NS
	HCT (%)	40.39±4.62	41.18±5.37	NS
	WBC (10^3/mm3)	7.96±2.16	9.71±2.31	0.0119
	PLT (10^3/mm3)	215.49±62.24	263.22±102.57	NS

Continuous numerical data were expressed as mean ± std.dev.; NS, not significant; ESR, erythrocyte sedimentation rate; total-CK, total creatine kinase; TnI, troponin I; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; WBC, white blood cell; PLT, platelets

At the patients with unstable angina (UA) and good evolution (group IB) we can see a significant decrease GPx at the time of hospitalization toward the witness lot, but also toward the group with unstable angina and unfavorable evolution (group IA), followed by a significant grow within 48 hours, and at the discharge the GPx values returning to normal (Fig. 1). Unlike the patients with unstable angina and good evolution, at the patients with unstable angina who have been re-hospitalized in the following 6 months, it is noticeable a precocious grow of GPx, grow that is still available after 48 hours, the returning to normal values being slower that in the cases of patients with favorable evolution within 6 months of surveillance (Table II). No significant difference is noticeable of the GPx activity within 48 hours and at externalization between the two lots (IA and IB) of patients with unstable angina.

Table II. The variations of glutathione peroxidase at the patients with unstable angina (UA)

Pacients with unstable angina			**Admission (T1)**		**48 hours (T2)**		**Discharge (T3)**	
		age	u/l	u/gHb	u/l	u/gHb	u/l	u/gHb
Control group	average		**198.444**	**1.491**				
	std.dev.		16.964	0.303				
UA with cardiovascular event	average	**63.70**	**256.900**	**1.956**	**272.500**	**1.969**	**207.100**	**1.550**
Group I A	std.dev.	9.44	55.551	0.412	48.415	0.508	33.351	0.367
	pT (group IA vs control group)		***0.009***		***0.001***		***0.458***	
	pT (T1 vs T2 and T3)				***0.512***		***0.026***	
	pT (T2 vs T3)						***0.002***	
UA without cardiovascular event	average	**59.81**	**178.741**	**1.354**	**274.296**	**1.980**	**197.222**	**1.465**
Group I B	std.dev.	10.83	45.191	0.399	80.513	0.653	52.491	0.368
	pT (grup IB vs control group)		***0.047***		***< 0.001***		***0.911***	
	pT (T1 vs T2 and T3)				***< 0.001***		***0.172***	
	pT (T2 vs T3)						***0.000***	
	pT (IA vs IB)		**0.001**		**0.935**		**0.505**	

At the patients with myocardial infarction (MI) non-Q that were re-hospitalized in the first 6 months after the discharge it is noticeable o quick, significant grow of GPx toward the witness lot but also toward the patients with IM non-Q with good evolution after 6 months (317.667 vs 198.44 vs 142.917, pT<0.05) (Table III). Afterwards, the GPx values are significantly decreasing and remain decreased until the discharge, unlike the patients with good evolution, the significance of the

variations being statistical (T<0.05) (Fig.2)

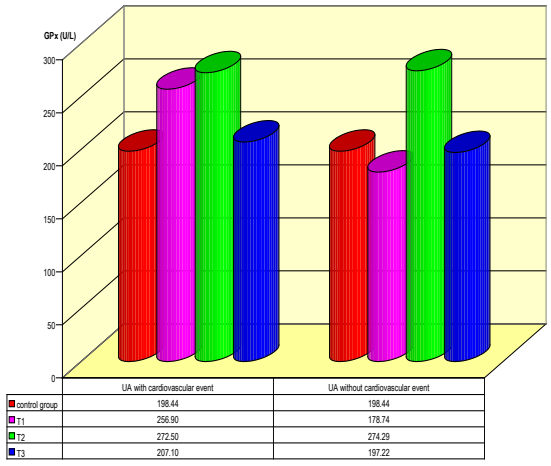

Fig. 1 The average variations of GPx at the patients with unstable angina (UA)

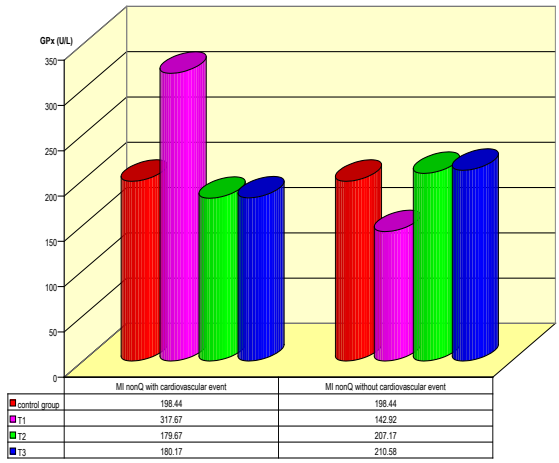

Fig. 2 The average variations of GPx at the patients with non-Q wave myocardial infarction

Table III. The variations of glutathione peroxidase at the patients with non-Q wave myocardial infarction

Pacients with MI nonQ			Admission (T1)		48 hours (T2)		Discharge (T3)	
		age	u/l	u/gHb	u/l	u/gHb	u/l	u/gHb
Control group	*average*		**198.444**	**1.491**				
	std.dev.		16.964	0.303				
MI nonQ with cardiovascular event	*average*	**67.00**	**317.667**	**2.398**	**179.667**	**1.539**	**180.167**	**1.430**
Group II A	std.dev.	7.18	89.415	0.595	11.776	0.438	35.114	0.449
	pT (grup IIA vs control group)		***0.023***	***0.062***			***0.322***	
	pT (T1 vs T2 and T3)			***0.009***			***0.006***	
	pT (T2 vs T3)						***0.937***	
MI nonQ without cardiovascular event	*average*	**64.00**	**142.917**	**1.066**	**207.167**	**1.484**	**210.583**	**1.571**
Group II B	std.dev.	10.73	37.049	0.296	33.701	0.233	26.294	0.347
	pT (grup IIB vs control group)		***0.000***		***0.736***		***0.666***	
	pT (T1 vs T2 and T3)				***0.003***		***0.030***	
	pT (T2 vs T3)						***0.824***	
	pT (IIA vs IIB)		**0.004**		**0.023**		**0.049**	

The enzymes in the GPx family scavenge ROS in the vascular and protect the bioavailability of NO, thereby maintaining normal endothelial function and antithrombotic vascular milieu. The cellular isoform of GPx (GPx-1) has impaired endothelium-dependent vasodilatator function (7), and decreased levels of GPx-1 in humans have been recently associated with coronary heart disease in a dose-dependent manner (11).

This prospective study of patients admitted with non-ST-segment elevation acute coronary syndrome (unstable angina and non-Q wave myocardial infarction) showed a direct association between baseline GPx and the onset of MACEs in the group with unstable angina and inversely associated with future fatal and non-fatal cardiovascular events in the group with non-Q wave myocardial infarction.

Although the association between oxidative stress and the development of both endothelial dysfunction and coronary arteriosclerosis has been studied before (18,21,22,23), the prognostic role of markers of oxidative damage had comparatively received less attention and the results so far are not clear. Indeed, in the particular case of GPx, only 3 reports (4,10,15) have examined its association with the onset cardiovascular events and the results (an inverse association between higher GPx and the rate of adverse event during follow-up) were partly in disagreement with those of our study. Admittedly, those study populations comprised mostly persons with stable ischemic heart disease, whereas in our study all patients were admitted with on-ST-segment elevation acute coronary syndrome. Another factor that must be taken into consideration is that the method by which we have determined the GPx was different from the one used in the earlier studies, which might also yield different results.

Most prognostic studies have analyzed the onset of cardiovascular events in relation to systemic oxidative status (1,3,5,13,15,23) whereas the role of antioxidant status has received less attention, perhaps because its action was assumed to be protective against cardiovascular events during follow-up. Our study show that, in the event of unstable angina, higher GPx probably reflects greater antioxidant response due to greater oxidative status, and that this response, at least during the acute phase, is not associated with a better prognosis but with a worse one. In opposition, at the patients with non-Q wave myocardial infarction, the decreased level of GPx at externalization is associated with the growth of the risk of future cardio-vascular complications (cardiovascular death, readmission with acute coronary syndrome or the need for coronary revascularization).

CONCLUSIONS

- The coronary acute syndromes are associated with the alternation of the balance between the oxidant and anti-oxidant systems.
- GPx is an essential component of the enzymatic system of anti-oxidant defense of the body.
- At the patients with non-Q wave myocardial infarction who have been readmission, the GPx values have precocious increased (at 24 hours from hospitalization). Afterwards, the values have decreased, remaining under the values of the control group also at externalization.
- At the patients with unstable angina with unfavorable evolution, after 24 hours from hospitalization, the GPx values were bigger that at the patients with unstable angina with good evolution.
- Taking into consideration the anti-oxidant mechanism's dynamic related to the evolution of the patients with non-ST-segment elevation acute coronary syndrome, the anti-oxidant therapy could be a good method to improve the prognostic (25)

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ACTIVITATEA GLUTATION PEROXIDAZEI AFECTEAZA PROGNOSTICUL IN SINDROMUL CORONARIAN ACUT FARA SUPRADENIVELARE DE SEGMENT ST

REZUMAT

Enzimele celulare antioxidante, ca glutation peroxidaza detin un rol central in controlul speciilor reactive de oxigen. In vitro, datele si studiile efectuate pe animale sugereaza ca aceste enzime au rol protector impotriva aterosclerozei, dar sunt putine date relevante la om. Scopul studiului a fost determinarea asocierii dintre statusul redox si prognosticul pacientilor cu sindrom coronarian acut fara supradenivelare de segment ST (angina instabila si infarct miocardic fara unda Q). Metoda: Au fost evaluati 55 de pacienti, cu varsta sub 75 ani internati consecutiv in Clinica de Cardiologie a Spitalului Clinic de Urgenta Constanta in perioada mai 2008 – mai 2009 cu diagnosticul de sindrom coronarian acut fara supradenivelare de segment ST si 19 persoane voluntare (fara afectiuni cardiovasculare, hipercolesterolemie, diabet zaharat si nefumatoare). Pacientii au fost impartiti in doua grupuri: grup I – pacientii cu angina instabila (37 pacienti, din care 10 au necesitat reinternare in urmatoarele 6 luni) si grup II – pacientii cu infarct miocardic acut fara unda Q (18 pacienti, din care 6 pacienti au suferit reinternare in primele 6 luni post-infract). Activitatea glutation peroxidazei (GPx) a fost determinata la perioade fixe: T1 – primele 24 de ore de la internare, T2 – la 48 ore de la internare si T3 – la externare. Dupa externare pacientii au fost monitorizati si s-au notat urmatoarele date: perioada de urmarire, decesul de cauza cardiovasculara si incidenta evenimentelor cardiovasculare majore. Rezultate: Studiul demonstreaza o asociere directa intre nivelul GPx si incidenta evenimentelor coronariene majore in grupul cu angina instabila si o asociere inversa cu evenimentele cardiovasculare fatale sau non-fatale in grupul pacientilor diagnosticati cu infarct miocardic fara unda Q. Concluzii: La pacientii cu sindrom coronarian acut fara supradenivelare de segment ST enzima antioxidanta GPx pare a avea un rol protector impotriva efectelor oxidative adverse. Analiza activitatii GPx poate oferi informatii superioare in evaluarea riscului cardiovascular comparativ cu masurarea doar a factorilor de risc traditionali.

Cuvinte cheie: sindrom coronarian acut, markeri biologici/sange, glutation peroxidaza, oxidare-reducere, stress oxidativ

THE INVOLVEMENT OF OXIDATIVE STRESS IN THE ULCEROUS PATHOLOGY INDUCED IN THE RATS BY ASPIRIN

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ABSTRACT

Various studies show that reactive oxygen species (ROS) are involved in ulcerous disease. This has multiple etiopathogenetic factors, among which the administration of NSAIDs plays an important role. The digestive lesions may be prevented by the association of proton pump inhibitors (PPI), and there are also data that indicate the advantages of the therapeutic supplementation of potentially antioxidant potions.The aim of the study was to evidence the growth of the oxidative stress level due to the administration of aspirin, and its decrease following the association of the antiulcerous protection, the antioxidant. There are considerable variations of the oxidant-antioxidant balance among the study groups. The aspirin treatment alters considerably the serum oxidative stress parameters and the gastric tissue in favor of prooxidants. The preventive administration of PPI considerably improves the status of serum antioxidants. PPI therapy decreases the level of tissue antioxidants (probably due to excessive consumption), but pathomorphologically improves the gastric mucous membrane. The association with antioxidants decreases the level of reactive oxygen species (ROS) and improves the anatomo-pathological aspect of the gastric mucous membrane.

Key words: oxidative stress, NSAIDs, peptic ulcer

INTRODUCTION

Peptic ulcer and gastritis have multiple etiopathogenetic factors, and one of the major factors of aggression is the formation of a reactive oxygen species (ROS). The involvement of free radicals derived from oxygen, such as the superoxide anion, hydrogen peroxide and hydroxyl radical, is demonstrated in the pathogenesis of the ischemic lesions of the gastro-intestinal mucous membrane, as well as in other types of its lesions that are induced by NSAIDs, ethanol, food diet and Helicobacter pylori (23). The reactive oxygen species generated by the metabolism of arachidonic acid, blood platelets, macrophages and cells of smooth brawn may contribute to the formation of gastric mucous membrane lesions. Therefore, through the scavenger action against free radicals, the reactive oxygen metabolites may be useful by protecting the gastric mucous membrane from oxidative lesions or by the acceleration of the healing of gastric ulcers (19).Gastric ulcerations associated with NSAIDs appear in 30% of the patients which require to be hospitalized and are associated with a high mortality rate (13). NSAIDs are frequently prescribed due to their efficiency in fighting pain, inflammation and fever. Their use is associated with the appearance of some digestive side effects, such as erosions of the gastric mucous membrane, ulcerations, bleeding, perforation, as well as the high risk of hemorrhage from the preexisting peptic ulcers. The pathogenesis of gastrointestinal lesions induced by NSAIDs may depend on the independent processes of prostaglandins, such as oxidative phosphorylation, the decrease of the proliferation of the mucous membrane cells and the activation of the neutrophils, followed by the increase of endothelial adhesion. Finally, they produce the decrease of microvascularization and the hyperproduction of reactive oxygen metabolites, able to induce tissue oxidative lesions which probably play an important role in the pathophysiology of digestive ulcerations induced by NSAIDs (5,10). NSAIDs inhibit the cyclooxigenase isoforms and decrease the level of prostaglandin E_2 in the gastric mucous membrane, causing ulceration, and it delays the cure of ulcer by the reduction of the level of prostaglandins and the prevention of angiogenesis mediated by these. In addition to this, NSAIDs decrease the gastric production of mucus, which may lead to hemorrhagic ulcer. So, the drugs that prevent the progression of ulcer by antioxidant action and also promote the secretion of PGE_2, gastric mucus and increase factors, may accelerate the cure of gastric ulcer (4). The proton pump inhibitors (PPI) are currently used in the treatment and prevention of gastroduodenal lesions induced by NSAIDs, their efficiency being related to the inhibition of gastric acid secretion. The gastric protection provided by pantoprazol is also given by its ability to interfere with NSAIDs secondary oxidative and inflammatory lesions. Pantoprazol acts with the help of direct and indirect antioxidant mechanisms, protects the gastric mucous membrane against the oxidative injuries caused by focal ischemia and the activation of neutrophils induced by NSAIDs (10). Vitamin C is a hydrosoluble antioxidant that eliminates the reactive oxygen species (ROS), among which: superoxide radical, hydroxyl radical, hydrogen peroxide, singlet oxygen and hypochlorous acid; it plays the role of antioxidant of vitamin E, by reducing the radical of vitamin E at the level of liquid / water. It prevents the burn of neutrophils in the endothelium by its scavenger action on the derived ROS at the level of the activated neutrophils. Vitamin E is a liposoluble antioxidant and plays the role of destroying the bonds in the peroxidation process of the cell membrane lipids. At the same time, it is a scavanger of ROS, such as superoxide radical, hydroxyl radical and singlet oxygen. Vitamin E exerts an anti-

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inflammatory action by inhibiting the O_2 production - in the activated neutrophils, the adhesion of neutrophils to endothelial cells and the transendothelial migration of neutrophils (17). The action of vitamin E is enhanced by selenium and protects other liposoluble antioxidants; it protects especially the lipids of the membranes (11). Selenium is part of the composition of a primordial enzyme that fights ROS (glutathione peroxidase- through selenomethionine and selenocysteine). Vitamin A presents precursors of vegetal origin (carotenoids) which may be used as very strong antiradicals; the most common are beta-carotene, lycopene and lutein (9). It is well known that in the case of chronic peptic ulcers and gastroduodenitis, the routine of applying the neutralizing or protective therapy, or agents that decrease gastric secretion, not only produces the reduction of the symptoms and the remission of the inflammatory changes, but also has an inhibitory effect on the engendering of free oxygen radicals. The results of the researches of other authors emphasize the favorable effect of vitamin C supplementation in patients with chronic recurrent gastroduodenitis, and a decrease in the risk of development of gastric cancer is also suggested for these patients. Even though the benefits of antioxidant vitamin supplementation are demonstrated in the treatment of some gastric diseases (for example for vitamin C), there are still not sufficient studies available to formulate specific therapeutic recommendations (3).

MATERIAL AND METHODS

The experimental study that we performed was one of prospective longitudinal type, and the collection of the data was the exposed – non-exposed type.

The major objective of this study was to monitor the variation of oxidative stress parameters as a result of aspirin administration; in parallel, the secondary objectives were to monitor the changes in the general state and the pathomorphological state at the level of gastric tissue.

The purpose of this study was to demonstrate the increase of oxidative stress (OS) after the administration of aspirin and the decrease of OS as a result of the anti-ulcer protection, antioxidant therapy, respectively.

CONDITIONS

The researches were conducted on white male Wistar rats, aged approximatively 10 weeks old, with a weight between 170 – 250 g. The animals were kept in the biobase of the Physiology Department of "Iuliu Haţieganu" UMPh Cluj-Napoca, under standard laboratory conditions, at a temperature of 23 +/- ^{0}C, with a duration of 12 hours of exposure to light and 12 hours exposure to darkness, with water and food ad libitum. The animals had a period of 1 week to adapt themselves before the experiment started.

GROUPS

4 groups of 10 male Wistar rats, aged approximatively 10 weeks old, with a weight between 170 – 250 g, were included in the study.

- group I (control) –received distilled water by gavage for 7 days;
- group II –received by daily gavage aspirin suspended in carboxymethylcellulose, in a dose of 200 mg/Kg body weight for 7 days;
- group III –received by daily gavage aspirin suspended in carboxymethylcellulose, in a body dose associated with PPI (pantoprazol in a dose of 60 µmol/Kg body weight) for the 7 days (4)
- group IV –received by daily gavage aspirin suspended in carboxymethylcellulose, in a dose of 200 mg/Kg body weight associated with PPI (pantoprazol in a dose of 60 µmol/Kg bodyweight) and a prepared antioxidant, each capsule of which contained: selenium 50 µgrams, provitamin A (beta-carotene) 10 mg, vitamin C (ascorbic acid) 100 mg and vitamin E (DL-tocopherol acetate) 40 mg (11).

After the last dose received by gavage, the animals were not given food for 24 hours before they were sacrificed. The animals included in the study were individually weighed both at the start and at the end of the experiment.

BIOLOGICAL SAMPLING

Blood samples were taken from the internal angle level of the eye – the venous orbital sinus – with the help of a capillary tube (approximately 1 ml/experimental animal). The blood was immediately centrifuged for 5 minutes at 3500 rotations/min. After the plasma was obtained, it was immediately frozen and kept at a temperature of -80^0 C until it was processed. After blood sampling, the animals were euthanized by cervical dislocation, the necropsy was performed. The abdomen was cut open along the white line, the peritoneal cavity was examined and the stomach was exposed. The stomach was harvested in isotonic solution of KCl, on ice, and subsequently it was cut along the great curvature so that the macroscopic examination could be done. During the evaluation, the samples were kept on ice. After the completion of the process of counting the lesions, parts of the gastric periulcerous zone were excised and frozen for the histopathological examination and in order to determine the oxidative stress markers. The samples were kept at a temperature of -80^0 C until they were processed.

The macroscopic examination of the gastric lesions

The lesions were categorized as follows:

- score 0 – no ulcerations or up to 3 punctiform ulcerations
- score 1 – more than 3 punctiform ulcerations
- score 2 – between 1 and 5 small-size ulcers (<2 mm)
- score 3 – more than 5 small ulcers with a diameter smaller than 2 mm
- score 4 – one or more gigantic ulcers, with a diameter of more than 2 mm (12,13).

The indicators which were determined from the blood and from the gastric tissue were:

- prooxidants: malondialdehyde (MDA) and the carbonilated proteins (CP)
- antioxidants: the hydrogen donor capacity (HD) and the thiol group (sulfhydryl) (SH)

METHODS TO DETERMINE THE OXIDATIVE STRESS INDICATORS

Malondialdehyde (MDA) was determined through the method of fluorescein dosage, according to Conti. The concentration values were expressed in nmol/ml, for the serum and in nmol/mg protein, for the gastric tissue (14). The carbonylated proteins (CP) were dosed using Rezsnick's method. The results were expressed in nmol/ml, for the serum and in nmol/mg protein, for the gastric tissue (15). The determination of the hydrogen donor capacity (HD) was made using a dosage method elaborated by Janaszewska. The results were expressed both in the serum and in the gastric tissue, as the inhibition percentage of the free radical (i%) (16). The determination of the content of the total thiol group (sulfhydryl) (SH) was made according to the dosage method of Hu. The values were expressed in µmol/ml in the serum and in nmol/mg protein, for the gastric tissue (17).

The statistical study of results

It was performed using the SPSS 13 program. For the detection of the significance of the statistical difference from the average, the „t" Student test (for two

groups) and ANOVA (for three or more groups) were used. Two aspects were studied in the „t" Student test: the descriptive analysis of the data and the analysis of the significance of the difference between the average values; a 95% confidence interval was used; the results in which $p < 0.05$ were considered to be eloquent.

RESULTS

Evolution of the oxidative stress markers

Serum MDA had the following variations: an insignificant increase of the values in group II compared to the control group; an insignificant decrease in group II compared to the first two groups; a significant decrease of the values of group IV compared to those of the control group and group II, with an insignificant variation compared to group III (Fig. 1).No significant variations in serum CP values were detected (Fig. 2).Serum HD presented the following alterations: they decreased significantly in group II compared to the control group; they increased significantly in group III compared to group II; otherwise, the variations of the values between the groups were insignificant (Fig. 3). For the serum thiol groups, the following were noticed: they decreased significantly in group IV compared to group II; they increased significantly compared to group III; they did not vary significantly compared to the control group (Fig. 4). Malondialdehyde at gastric tissue level presented: a significant decrease in group II compared to the control group; a significant decrease in group IV compared to the first two groups, with insignificant variations compared to the control group (Fig.5). The levels of carbonylated proteins (CP) in the gastric tissue did not undergo significant alterations between the rat groups under observation (Fig. 6). The level of hydrogen donors (HD) in the gastric tissue presented differences between the groups: they decreased significantly in group II compared to the control and in group IV compared to group II, the rest of the variations between the groups were insignificant (Fig. 7).Except one insignificant difference between groups II and III, the thiol groups (SH) varied as follows: they decreased significantly in the last three groups compared to the control group, meaning that they were situated at a lower level in group IV compared to the rest of the groups (Fig.8).

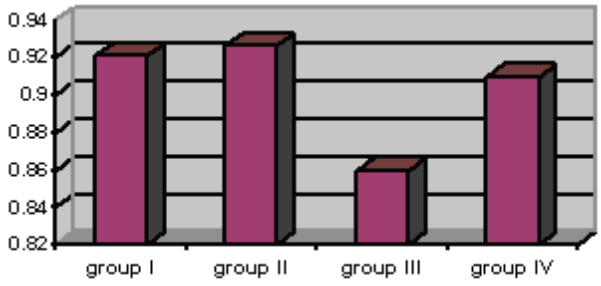

Fig. 1. Variation of serum malondialdehyde

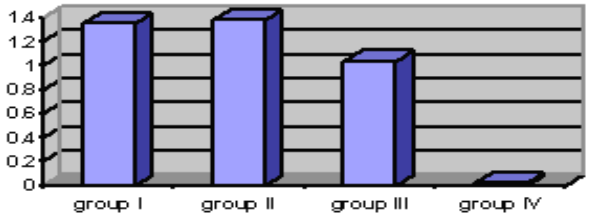

Fig. 2. Variation of serum carbonylated proteins

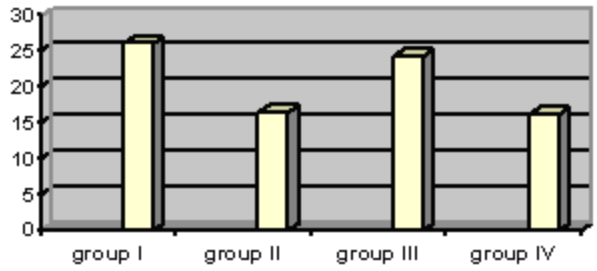

Fig. 3. Variation of serum hydrogen donor capacity

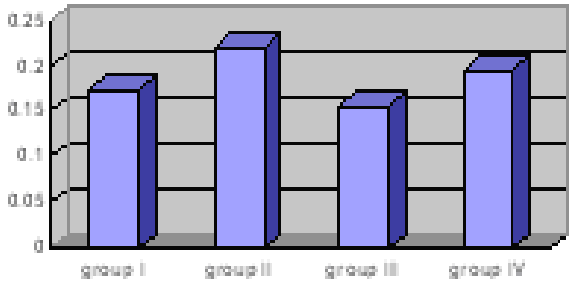

Fig. 4. Variation of serum sulfhydryl groups

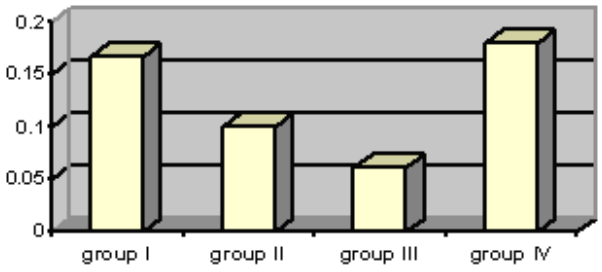

Fig. 5. Variation of malondialdehyde in the gastric tissue

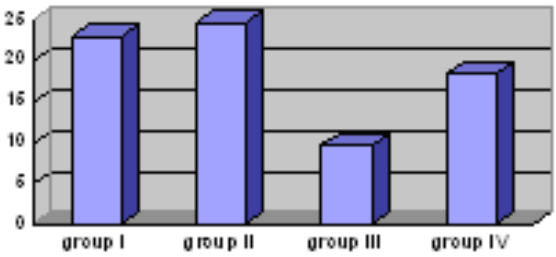

Fig. 6. Variation of carbonylated proteins in the gastric tissue

Evolution

No significant changes were identified in the general state or in the evolution of individual weight. Mortality was less than 5%, and was most probably caused by individual sensitivity.

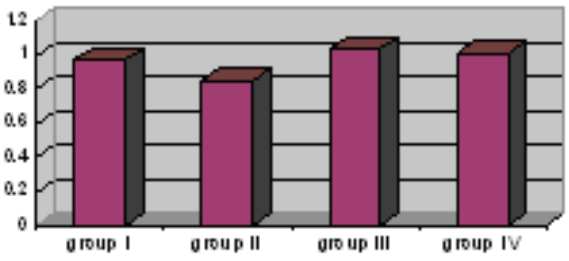

Fig. 7. Variation of the hydrogen donor capacity in the gastric tissue

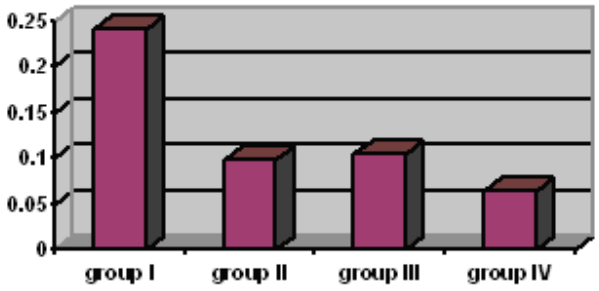

Fig. 8. Variation of sulfhydryl groups in the gastric tissue

The macroscopic examination of the gastric lesions

The macroscopic examination of the gastric mucous membrane, after the stomach was sectioned along the great curvature, showed that the animals in the control group did not present any pathological alterations.The second group showed lesions of the gastric mucous membrane categorized according to the score presented before: 40% score 1, 60% score 4.The third group presented lesions with a smaller degree of severity compared to group II, 40% presented score 1 lesions, and 60% presented score 0 lesions.The forth group presented score 0 lesions of the mucous membrane , meaning that the mucous membrane was affected comparably to the control group and less severely compared to the animals of group II, group III, respectively (Fig. 2).

Histopathological examination

No pathological changes were found at the level of the gastric mucous membrane of the control group. The gastric mucous membrane of the rats of group II presented: focal erosions and profound areas of ulcerations, superficial chorion with small hemorrhagic foci, an inflammatory neutrophilic infiltrate more abundant in the areas of discontinuity of the mucous membrane, deep chorion and submucosa (close to the muscularis of the mucous membrane). Group III presented an undamaged surface epithelium; the chorion contained a mixed inflammatory lymphoplasmacytic and neutrophilic infiltrate. The inflammatory cells were more abundant in the deep region of the mucous membrane and at the level of the muscularis of the mucous membrane.Group IV also presented an undamaged surface epithelium, with no erosions or ulcerations, but it had a reduced inflammatory lymphoplasmacytic infiltrate, located at the level of the deep chorion. Some lymphocytes in the muscularis of the mucous membrane could be observed, but with absent polymorphonuclear neutrophils (Fig.9).

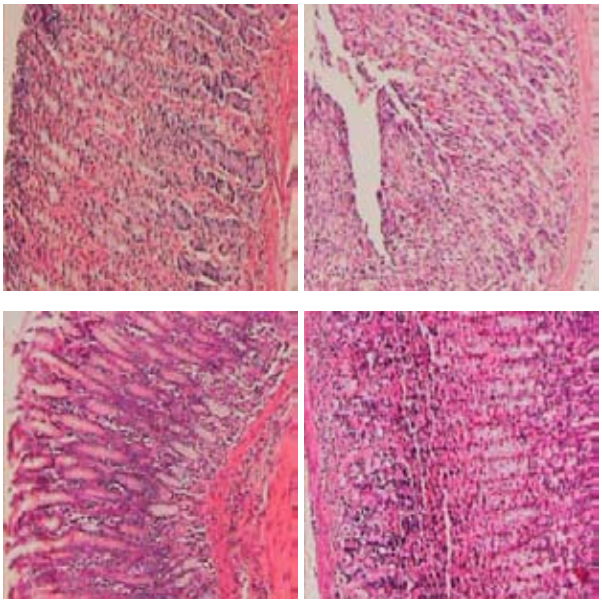

Fig. 9. Histopathological appearance of the gastric mucosa in the studied groups

Table I. The alterations of the indicators of the oxidant/antioxidant balance in the studied groups

		MDA		CP		HD		SH	
		serum	tissue	serum	tissue	serum	tissue	serum	tissue
Group I	Average	1.350	0.167	0.921	0.970	26.090	23.130	0.175	0.242
	Standard deviation	0.236	0.071	0.445	0.450	9.182	6.264	0.227	0.081
Group II	Average	1.380	0.101	0.926	0.850	16.410	24.830	0.221	0.099
	Standard deviation	0.352	0.037	0.372	0.392	7.114	4.523	0.172	0.218
Group III	Average	1.035	0.062	0.860	1.037	24.370	9.790	0.156	0.105
	Standard deviation	0.658	0.023	0.157	0.420	6.468	3.593	0.033	0.051
Group IV	Average	0.020	0.180	0.910	1.005	16.220	18.530	0.196	0.065
	Standard deviation	0.442	0.059	0.321	0.305	6.623	4.334	0.025	0.262

Table II. The statistical significance (p) of the difference in the indicators of the oxidant/antioxidant balance between different groups

Comparison between the groups:	Oxidative stress indicators							
	MDA		CP		DHD		SH	
	serum	tissue	serum	tissue	serum	tissue	serum	tissue
I-II	0.832	0.010	0.974	0.510	0.002	0.570	3.10	0.0007
I-III	0.080	0.0003	0.680	0.720	0.630	0.00002	0.120	0.0002
I-IV	0.020	0.660	0.950	0.830	0.320	0.07	0.065	0.000
II-III	0.088	0.040	0.572	0.350	0.009	3.140	4.35	0.777
II-IV	0.030	0.002	0.920	0.340	0.950	0.004	0.010	0.040
III-IV	0.960	0.0004	0.724	0.870	0.004	0.001	0.040	0.057

Table III. Distribution and categorization of the gastric lesions observed by macroscopic examination

	Group I	Group II	Group III	Group IV
Score 0	100%	-	60%	100%
Score 1	-	40%	40%	-
Score 2	-	-	-	-
Score 3	-	-	-	-
Score 4	-	60%	-	-

DISCUSSIONS

There were significant differences in the oxidant-antioxidant balance between the study groups.The results of the researches over the past years suggest that free oxygen radicals are co-responsible, in addition to the etiologic factor, for the lesions of the mucous membrane of the digestive tract and for the initiation of the inflammatory process. The inflammation, which is a defense reaction from the body, is at the same time a cause of tissue damage. During the respiratory explosion of the phagocytes of the inflammatory cells, almost 3.2 million superoxide anion radicals, and also 3.6 millionperoxide hydrogen molecules are generated in one second. The stimulated phagocytes represent one of the main sources of free oxygen radicals in the body (3).Our study has demonstrated that the administration of aspirin significantly alters serum and tissue oxidative stress parameters (meaning that it alters the oxidant-antioxidant balance in favor of the first). The rats that were given aspirin alone presented the appearance of necrotic areas of the mucous membrane, associated with extensive polymorphonuclear cell infiltrate. The results of our research are correlated with evidence showing that NSAIDs, acting through local and systemic mechanisms, lead to ischemic and inflammatory changes which result in gastric neutrophil infiltration, the release of oxygen metabolites and the peroxidation of the cellular mucous membranes. In particular, focal ischemia produced by the adhesion of neutrophils to the vascular endothelium, the capillary connection and the intravascular deposit of fibrin were recognized as an early event in the pathogenesis of gastric oxidative injuries produced by NSAIDs. These alterations are further amplified by subsequent tissue infiltrations of polymorphonuclear cells, activation due to which a massive release of oxygen radicals and other inflammatory mediators responsible for epithelial lesions is produced (10). The preventive administration of PPI has significantly improved the serum antioxidant status. There are studies that prove that they alone have antioxidant properties (2,7,10,21). Locally, the administration of PPI partially prevents gastric lesions or, at least, reduces their severity. In parallel, a reduction of tissue antioxidants was noticed, probably due to excessive consumption which contributes to local defense. The addition of antioxidants further decreases the oxidative excess and improves the macroscopic and anatomo-pathological aspect of the gastric mucous membrane. These aspects are to be found in other studies supporting that the addition of vitamin E to diet is able to protect the cellular mucous membrane against the alterations produced in the case of ischemia-reperfusion lesions induced in rats, by the inhibition of lipid peroxidation and the interference with neutrophilic infiltrates (16). Another experimental study of gastric lesions produced by ethanol or HCl showed that vitamin A and beta-carotene are able to significantly prevent the number and the severity of the lesions of the gastric mucous membrane, but these agents fail to compensate the decrease of the SOD activity of the mucous membrane diminished by the administration of HCl (15). We obtained the same findings regarding the local antioxidant capacity in the study above. Another experimental study demonstrated that selenium and vitamin E treatment in rats with stress induced ulcers significantly reduced the acid base gastric secretion, their combination producing a better inhibition of gastric acid secretion compared to their individual effects (1). In conclusion, we may state that the parallel administration of drugs that decrease acid gastric secretion (PPI) significantly reduces the risk and size of lesions produced by aspirin administration. At the same time, the association in treatment of products with an antioxidant potential, further improves the defense of the gastric mucous membrane through the growth of the antioxidant status, and results in an obvious decrease of tissue lesions.

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IMPLICAREA STRESULUI OXIDATIV IN PATOLOGIA ULCEROASA INDUSA DE ASPIRINA LA SOBOLANI

REZUMAT

Numeroase studii arata faptul ca speciile reactive ale oxigenului (SRO) sunt implicate in boala ulceroasa. Aceasta recunoaste factori etiopatogenetici multipli, intre care un loc important il ocupa administrarea de AINS. Leziunile digestive pot fi prevenite prin asocierea inhibitorilor pompei de protoni (IPP), existand si date care indica avantajele suplimentarii terapeutice aditionale cu preparate cu potential antioxidant. Scopul studiului a fost de a demonstra cresterea nivelului stresului oxidativ consecutiv administrarii de aspirina si, respectiv, scaderea acestuia secundar asocierii terapiei de protectie antiulceroasa, respectiv antioxidanta. Am constatat ca intre loturile din studiu exista variatii semnificative ale balantei oxidanti-antioxidanti. Tratamentul cu aspirina modifica semnificativ parametrii de stres oxidativ serici si din tesutul gastric in favoarea prooxidantilor. Administrarea preventiva de IPP imbunatateste semnificativ statusul seric antioxidant. Terapia cu IPP scade nivelul de antioxidanti tisulari (probabil datorita consumului in exces), insa imbunatateste morfopatologic tesutul gastric. Asocierea de antioxidanti scade nivelul speciilor reactive ale oxigenului (ROS) si amelioreaza aspectul anatomo-patologic al mucoasei gastrice.

Cuvinte cheie: stres oxidativ, AINS, ulcer peptic.

VARIATIONS OF SOME SALIVA MARKERS OF THE OXIDATIVE STRESS IN PATIENTS WITH ORTHODONTIC APPLIANCES

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ABSTRACT

The use of orthodontic appliances in the treatment of the various dento-maxillar anomalies most frequently presume the application of high intensity forces, non-physiological, which always will produce an inflammatory response localised around the tooth or the teeth subjected to displacement. The presence of an inflammatory process at this level will produce an increased synthesis of free radicals, secondary followed by the oxidative stress.

In order to asses the level of the oxidative stress, a series of its markers are used, including the reactive species of oxygen and nitrogen, and the oxidation products in particular, such as lipid peroxides and oxidized proteins respectively.

Thus, the objective of the present study is to determine and compare the levels of some oxidative stress markers (malondialdehyde, ceruloplasmine, hydrogen donors) in saliva of patients with orthodontic appliances, before and after the initiation of the treatment.

The patients included in this study were 7 girls and 4 boys, with the average age of 9.9 years, in which orthodontic biomechanical appliances were applied, in the purpose of treatment of some dento-maxillar anomalies. Saliva has been collected before the initiation of the orthodontic treatment, at 1 hour, at 24 hours, and at 7 days from from the orthodontic appliance application.

The variations in the concentrations of the saliva markers of the oxidative stress reached a maximum at 24 hours from the debut of the treatment for ceruloplasmine and malondialdehyde, and at one hour for the hydrogen donors respectively, while at 7 days from the device application the concentrations were close to the initial values.

The health condition at the level of the oral cavity in the patients taken into study did not change during the research. These results demonstrate that the utilization of an orthodontic appliance changes the levels of the saliva markers of oxidative stress, but these variations, even statistically significant, do not determine the appearance of certain pathological processes at the level of the oral cavity.

Key words: ceruloplasmine, malondialdehyde, hydrogen donors, saliva, orthodontic appliances

INTRODUCTION

Free radicals, such as the reactive species of oxygen, result in a high diversity of biochemical reactions produced during the progress of the cellular functions (e.g. the mitochondrial metabolism). The formation of these free radicals (pro-oxidants) in physiological conditions is balanced through their consumption by the antioxidants.

The oxidative stress is produced as a consequence of an unbalance between the synthesis and the neutralization of the pro-oxidants. A high number of pathological processes in the human organism can affect this balance by the increasing of the free radicals production related to the existing antioxidants.

Formation of pro-oxidants and their effect upon the cellular functions (which can finally lead to apoptosis) have the name of oxidative stress. Sies H., in his paper: „Oxidative stress: from basic research to clinical application", published in 1991 (1), stated the first definition of the oxidative stress – „an unbalance in the balance pro-oxidants / antioxidants behalf pro-oxidants, with possible repercussions on the organism".

The oxidative stress involves the adverse effects of oxygen and of other free radicals on the living tissues (2). The free radicals are unstable molecules, with increased reactivity, because they have a free electron, which combines with various cellular components, such as DNA, proteins, lipids and fatty acids. These reactions are finally followed by damages in the DNA molecules, mitochondrial malfunctions, alteration of plasma membrane and eventually the cell death.

A high variety of biological processes, such as the anti-microbial defence, inflammation, carcinogenesis, and the aging as well imply the involvement of the free radicals (3, 4), leading thus to oxidative stress. The DNA alteration, the cellular malfunctions, and other pathological processes mediated by these free radicals represented in time sources of interest for biochemists and physicians.

The main free radicals are represented by the reactive species of oxygen (ROS) and nitrogen (RNS).

The settlement of a certain causative relation between the oxidative stress and the physiological or pathological processes in which is involved, is achieved by the assessment of the oxidative stress markers in different fluids of the human body.

The biomarkers are defined as characteristics which can be objective measured, and evaluated as indicators of the normal biological processes, of the pathological processes, or of the pharmacological responses after a therapeutic intervention (5).

A series of markers of the oxidative stress are used, including the reactive species of oxygen and nitrogen, but most of them have a limited value because of a reduced

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sensibility or specificity, or because they need invasive methods of collection.

As ROS/RNS are unstable products, these reactive species were quantified by the evaluation of their stable metabolites, such as nitrates or nitrites, as well as by the measurement of the oxidized products' concentration: lipid peroxides and oxidized proteins respectively.

Malondialdehyde (MDA) is an aldehyde produced by the peroxidative degradation of the unsaturated lipids. The MDA excess secondary arisen after the lipid peroxidation is combined with the free amino groups in the protein structure, leading to the appearance of proteins with modified structure.

Ceruloplasmine, which is the major copper transporter within the organism, displays an increased level in the acute and chronic inflammations, thus representing an acute phase reactant.

The using of the orthodontic appliances in order to treat various dento-maxillar anomalies presumes most frequently the application of some forces of increased intensity, non-physiological, which always will generate an inflammatory answer localized around the tooth or teeth which need to be displaced. And, as was already mentioned, any inflammatory process stimulates a higher synthesis of free radicals, resulting in oxidative stress.

In these conditions, the objective of this study is represented by the quantification of the levels of some markers of the oxidative stress (malondialdehyde, ceruloplasmine, hydrogen donors) in the saliva of patients carrying orthodontic appliances, before, and after 1 hour, 24 hours, and 7 days from the beginning of the orthodontic treatment.

SUBJECTS AND METHOD

The written consent of the parents of the child patients taken into this study was obtained after a previous description of the study's protocol. In order to asses the saliva levels of the oxidative stress markers, 11 patients were taken into study, in which 142 measurements were made. The 11 patients, 7 girls and 4 boys (between 8 and 12 years, average age 9.9 years), displayed the following criteria for including in study:

- Well general status, without known affections;
- Lack of antibiotic therapy within the last 6 months;
- Absence of anti-inflammatory drugs administration in the month before the study;
- Healthy periodontal tissues and appropriate oral hygiene.

In each patient a brief instruction for teeth brushing and oral hygiene was made before the beginning of the orthodontic treatment. The orthodontic treatment for all 11 patients was performed using biomechanical orthodontic appliances.

The samples of saliva were collected as follows:

- Moment 0 – before the initiation of the orthodontic treatment;
- Moment 1 – at 1 hour from the device application;
- Moment 2 – at 24 hours from the device application;
- Moment 3 – at 7 days from the device application.

In order to perform the study, unstimulated saliva was collected, by the expectoration method, for 2 minutes. The test tubes with saliva were then kept at -80°C until the measurements were performed.

Malondialdehyde was dosed, after the previous protein precipitation, by the spectrophotometric method with 2-tiobarbituric acid, and reading the extinction at 530 nm.

The assessment of ability of hydrogen donor is based on the reduction of the stable radical 1, 1-diphenil-2-picrilhydrazil by a series of antioxidant compounds, pursued by the colour change from violet to wan yellow, monitorised by the modification of absorbance at 520 nm.

Ceruloplasmine is an alpha-2 globulin, which in vitro displays properties of phenoloxidase, and catalyzes the oxidation of p-phenylendiamine to a compound with a violet colour having a maximum absorbance at 530 nm.

Concerning the statistical analysis, in order to compare the mean values Student t-test was performed, because the values have displaied a normal distribution. The statistical analysis was accomplished with the Statistical Package for Social Sciences (SPSS), and with the statistical software of Microsoft Office.

RESULTS

The average level of the oxidative stress markers in the saliva from the patients taken into study, at the time intervals mentioned before is presented in Table I.

Table I. The mean concentration of the oxidative stress saliva markers in the patients taken into study

	MOMENT			
	zero	*1 hour*	*24 hours*	*7 days*
Ceruloplasmine (mg%)	2.277	3.627	4.881	2.768
Malondialdehyde (nm/ml)	0.263	0.309	0.536	0.309
H^+ donors (inhib. %)	20.127	14.581	16.109	19.472

Changes in the concentrations of ceruloplasmine (mg%), malondialdehyde (nm/ml), and hydrogen donors (inhib. %) in saliva are presented in Figures 1, 2 and 3.

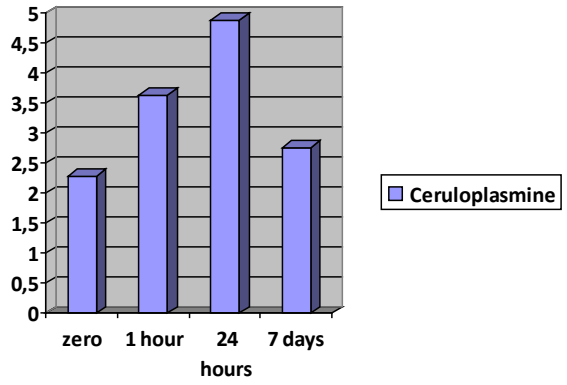

Fig.1. Changes in the ceruloplasmine concentration in saliva during the study (mg%)

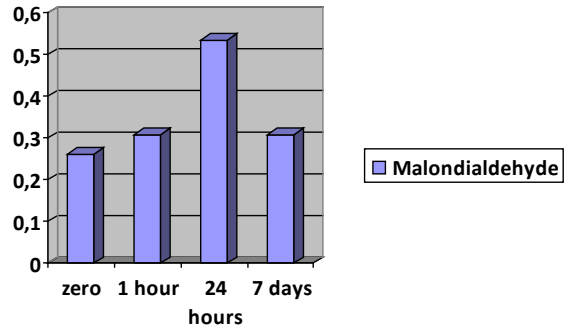

Fig. 2. Changes in the malondialdehyde concentration in saliva during the study (nm/ml)

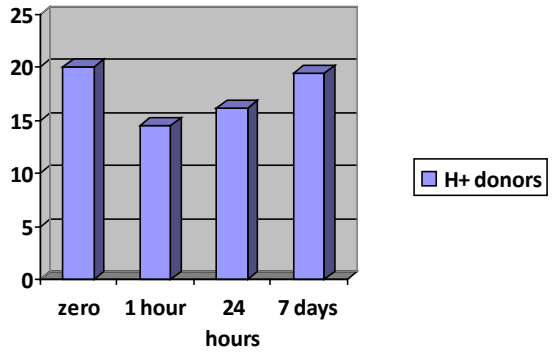

Fig. 3. Changes of the hydrogen donor ability in saliva during the study (inh.%)

DISCUSSIONS

At the moment 0, before the application of the orthodontic appliance, the saliva levels of ceruloplasmine and malondialdehyde were ranging in normal limits (average: 2.27 mg% for ceruloplasmine, and 0.26 nmols/ml for malondialdehyde respectively).

At 1 hour from the beginning of the force action, the saliva concentrations of ceruloplasmine and malondialdehyde display a slight increasing, statistically significant ($p<0.01$), but without implications on the pathology of the oral cavity (3.62 mg%, and 0.30 nmols/ml respectively).

At 24 hour from the device application, the levels of ceruloplasmine and malondialdehyde in saliva have a maximum of 4.88 mg%, and 0.54 nmols/ml respectively, values which are statistically significant increased ($p<0.01$), but again without the appearance of certain pathological changes at the level of the oral cavity.

At 7 days from the beginning of force action, the saliva levels of the two markers decrease, coming close to the values recorded before the application of the orthodontic appliance.

Before the beginning of the orthodontic treatment, the ability of hydrogen donor in saliva has the mean value of 20.13 inhibition %. At 1 hour from the force application, the minimum of level is reached, respectively a mean value of 14.58 inhib.% decrease which is statistically significant ($p<0.01$). At 24 hours from the force application, the value increases at an average of 16.11 inhib. %, while at 7 days from the debut of the orthodontic treatment, the level returns to values close to those evaluated at the moment 0, respectively an average of 19.48 inhib.%. All these variations in the ability of hydrogen donor are statistically significant, but without implications on the oral pathology.

A series of studies have proven increases of the plasma concentrations of malondialdehyde and ceruloplasmine in patients with rheumatoid arthritis (6,7), as well as of the saliva level of these markers of the oxidative stress in the same disease (8), in which one of the articulations of interest is the temporo-mandibular joint.

Because the use of the orthodontic appliances for the treatment of the various dental anomalies will produce an inflammatory response, localized around the tooth or the teeth to be displaced, the results of this study are in correspondence with the data reported in the literature.

Taking into account there is not known literature data to mention the variation of the hydrogen donors in the context of our study, a possible explanation for the reach of their minimal level at 1 hour from the beginning of the treatment could be the direct mechanism of annihilation of the oxygen free radicals by hydrogen, with water formation.

Therefore, the oxidative stress markers in saliva display a series of variations along the study, but which do not lead to the appearance of some pathological processes in the oral cavity of the patient with orthodontic appliance. The study proved that the highest variation in the levels of oxidative stress markers in the saliva of the patient with an orthodontic appliance is measured at 24 hours from its application for ceruloplasmine and malondialdehyde, and at 1 hour in the case of the hydrogen donors respectively, and at 7 days the values being close to the initial ones. These variations will be repeated after each activation of the device.
Variations of the oxidative stress markers in saliva are statistically significant, but they do not change the health status of the oral cavity, which denotes that even these markers undergo important variations, these do not prove the appearance of a pathologic process in the oral cavity in patients with orthodontic appliances.

CONCLUSIONS

• The application of an orthodontic appliance modifies the saliva markers of the oxidative stress, but these variations, even statistically significant, do not change the health status at the level of the oral cavity.

• Even if the application of an orthodontic appliance is followed by the appearance of an inflammatory process localized around the tooth which needs to be displaced (otherwise strictly necessary to achieve the dental displacements), and consecutively the appearance of the oxidative stress at this level, this does not manifest generally at the level of whole oral cavity.

• This is due to the fact that the inflammatory process is a localized one, and the markers of the oxidative stress are diluting in the oral cavity, thus their variations, even statistically significant in the saliva of the patient carrying an orthodontic appliance, do not produce the appearance of pathological changes at this level.

• The application of an orthodontic appliance does not produce pathological changes in the oral cavity of the patient to be treated, in the conditions in which the forces which act on the tooth to be displaced are close to the physiological forces.

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VARIAȚII ALE UNOR MARKERI SALIVARI AI STRESULUI OXIDATIV LA PACIENȚII PURTĂTORI DE APARATE ORTODONTICE

REZUMAT

Utilizarea aparatelor ortodontice pentru tratarea diverselor anomalii dento-maxilare presupune cel mai frecvent aplicarea unor forțe de intensitate crescută, nefiziologice care întotdeauna vor determina apariția unui răspuns inflamator localizat în jurul dintelui sau dinților care urmează a fi deplasați. Prezența unui proces inflamator la acest nivel va produce o sinteză crescută de radicali liberi, cu apariția secundară a stresului oxidativ.

Pentru determinarea nivelului stresului oxidativ, sunt utilizați o serie de markeri ai acestuia, incluzând aici speciile reactive ale oxigenului și azotului, dar mai ales produșii care sunt oxidați, respectiv peroxizii lipidici și proteinele oxidate.

Astfel, obiectivul lucrării îl constituie determinarea nivelului unor markeri ai stresului oxidativ (malondialdehida, ceruloplasmina, donorii de hidrogen) în saliva pacienților purtători de aparate ortodontice, înainte și după debutul tratamentului.

Pacienții cuprinși în studiu au fost 7 fete și 4 băieți, cu vârsta medie 9,9 ani, la care s-au aplicat aparate ortodontice mobilizabile, în scopul tratării unor anomalii dento-maxilare. Saliva a fost recoltată înainte de debutul tratamentului, la 1 oră, 24 ore și 7 zile de la aplicarea aparatului ortodontic.

Variațiile concentrațiilor markerilor salivari ai stresului oxidativ prezintă un maxim la 24 ore de la debutul tratamentului pentru ceruloplasmină și malondialdehidă, respectiv la o oră pentru donorii de hidrogen, la 7 zile de la aplicarea aparatului, concentrațiile apropiindu-se de valorile inițiale.

Pe tot parcursul desfășurării cercetării, starea de sănătate de la nivelul cavității orale a pacienților luați în studiu nu s-a modificat. Aceste rezultate demonstrează că aplicarea unui aparat ortodontic modifică markerii salivari ai stresului oxidativ, dar aceste variații deși sunt semnificative statistic, nu determină apariția unor procese patologice la nivelul cavității orale.

Cuvinte cheie: ceruloplasmina, malondialdehida, donori de hidrogen, saliva, aparate ortodontice

CONSUMPTION OF CALORIGENIC FOODS IN ADOLESCENCE

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ABSTRACT

Daily consumption of cereals and derivates contributes to covering ¾ of the glucide requirements, ½ of the energetic needs and up to ½ of the protein necessities of the organism. Fast-food products are rich in fats, sugar and salt, and they are included into the lifestyle of adolescents and they often replace traditional nutrient sources. The working method was a transversal populational study, by use of the CORT 2004 questionnaire for the investigation of some health risk behaviours in young subjects, on a representative population of adolescents in Timiș County, including 2908 pupils. We found that 96.4% of the adolescents consume bread daily, ¾ of the girls eat 2-7 slices of bread a day, while ½ of the boys consume at least 8 slices daily. The consumption of pasta, rice, cereals is present daily in 10.5% of the young subjects, with a 1.3/1 ratio between boys and girls. Chips and snacks were consumed by 20.5% of the adolescents, hamburgers and pizza by 10.2% and fried potatoes by 15.5%. The results may corelate mainly with an energetic intake replacing basic foods and with a deficit of high biological value proteins.

Key words: adolescents, alimentation, fast-food

INTRODUCTION

Cereals represent an important source of vegetal proteins, with a content of 7-16 g%, represented in proportion of 430-40% by gliadin and gluteins, and by low quantities of albumins, nucleoalbumins and globulins (1). Regarding the biological value, they do not signify a qualitative fulfilment of the food portion because proteins in cereals and in dry vegetables contain all essential aminoacids but not in optimal proportions for the organism (2).

Among glucids, starch has the highest proportion, at an average of 80-90% in cereals and 50-55% in dry vegetables, and this cathegory of foods is an important source of B group vitamins, vitamin E and minerals, especially phosphorus (200-400 mg%), potasium (100-350 mg%) and magnezium (50-150 mg%) and they lack vitamin C (3).

The requirement of cereals and cereal derivates in adolescence: 300-450 g bread/day (6-11 slices of 40 g); 60 g cereal derivates/zi (1 porion of 60 g). Reported to the caloric value of the portion, these foods will cover, on average, 50% of the needs, meaning 70-80% of the necessary glucides and 45-50% of the protein needs (4).

In the great fast-food era, good home-learned practices are replaced by some not very advisable feeding trends. There is a tendency to rapidly eating spicy foods, sandwiches, conserved food, steaks, pasta, avoiding vegetables, fruits, bread (5).

Consumption of fast-food products causes the dramatical increase in the number of obese individuals. Obesity is no longer an American problem and it is spreading throughout the world at an increasing rate (6).

Many snacks have a very high level of fats, sugar or salt, ingredients which should be restricted to a small part of foods (7). Eating a healthy diet does not mean complete elimination of favourite foods, but young people must be selective and limit the total of fats, saturated fats, cholesterol or sodium. Consumer organizations worldwide released the code which bans promotion of junk food among children. The code is meant as a strategy against obesity and diseases occuring as a consequence of uncontrolled diets, because statistics show that, worldwide, over 177 million children are threatened by obesity. The code addresses the marketing system for foods with high caloric value, rich in fats, sugar and salt.

METHODS

The study was performed on a representative population of adolescents in high-schools, postgraduate and apprentice schools in Timiș county, in urban areas, and it included a total of 2908 pupils aged between 14 and 25 years (99% for the group of adolescents aged between 15-19 years), 51.5% girls and 48.5% boys (9).

The work method was a transversal populational study, by use of the group and anonimous CORT 2004 questionnaire for investigating some health risk behaviours in young subjects, designed by a CNCSIS accredited research team, by adaptation of some international questionnaires (ESPAD, YRBSS) to the reality of Romanian life, in the period 2003-2005 (10).

RESULTS AND DISCUSSIONS

1. Bread consumption (Figure 1)

The cereal intake in the daily portion of most young people, 96.4%, was covered by consumption of bread, and only 3.6% of the adolescents did not consume this aliment. Among the young bread consumers, 36.0% reported a 2-4 slices a day intake (around 80-160 g), 27.8% of them consumed 5-7 slices daily (around 200-280 g), and 32.6% of them ate more than 8 slices of bread a day (over 320 g).

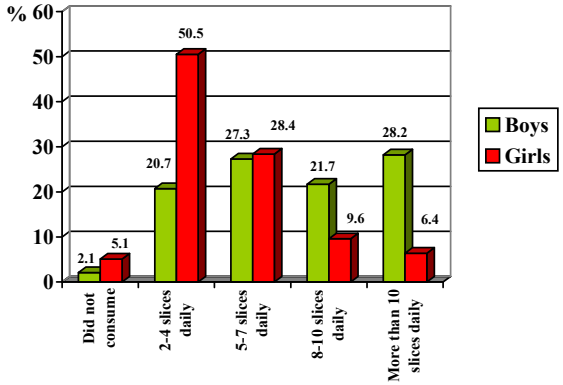

Fig.1. Percentual distribution of boys and girls according to the frequency of bread consumption during one week

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Between the frequencies of bread consumption depending on gender, there are statistically significant differences, with a χ^2 value of 459.7 and a probability cutoff $p<0.001$, differences in favour of boys who recorded a daily bread consumption of over 10 slices (around 400g or more). Half of the girls, 50.5%, and 20.7% of the boys consumed between 2-4 slices of bread a day, and the percent of those who do not eat bread is double for girls, 5.1% as compared to only 2.1% in boys.

2. Consumption of pasta, rice, cereals (Figure 2)

Per sample, most young subjects, 66.9%, consumed cereal derivates, others than bread, with a frequency of 1-3 times a week. The 4-6 times a week consumption was recorded in 12.0% of the adolescents, 10.5% of them reporting an intake of at least once a day, and 10.6% did not eat food from this cathegory.

Differences between percentual distribution in girls and boys, depending on the frequency of cereal derivates consumption are statistically significant, with a χ^2 value of 19.5 and a probability cutoff $p=0.001$, and they are in favour of boys. Thus, 12.0% of boys and 9.3% of girls consumed this type of food at least once a day. The percent of boys, 13.5%, who had an intake of cereal derivates 4-6 times a week is higher than in girls, 10.5%.

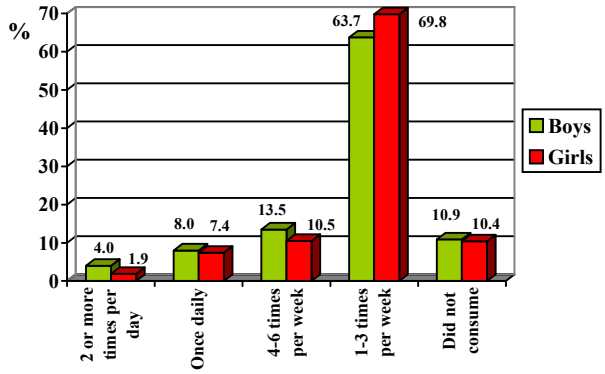

Fig.2. Percentual distribution of boys and girls according to the frequency of pasta, rice, cereals consumption during one week

3. Consumption of fast-food

3.1. Consumption of chips and snacks (Figure 3)

Regarding the consumption of chips and snacks, most young subjects, 47.0%, reported consumption 1-3 times a week, and the proportion of adolescents who consumed these "unhealthy" foods, at least once a day, is rather important, 20.5%.

Among the two genders, the hierarchy of consumption frequency of chips and snaks is identical and similar to consumption frequencies for the entire sample, and differences between genders are not statistically significant. Thus, 48.0% of boys and 46.2% of girls, consumed 1-3 times a week, and 19.3% of boys and 21.7% of girls, had a daily intake of chips and snaks. Only 16.9% of boys and 16.6% of girls did not consume these foods.

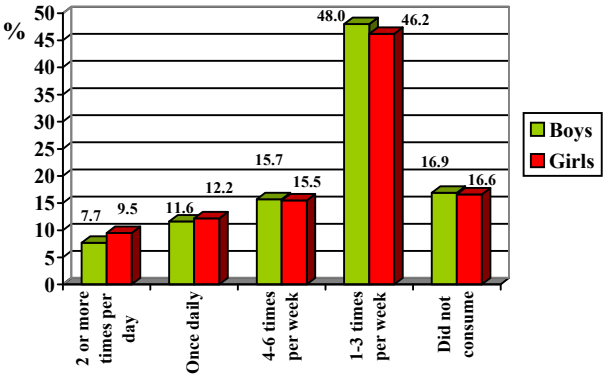

Fig.3. Percentual distribution of boys and girls according to the frequency of chips and snaks consumption during one week

3.2. Consumption of hamburgers and pizza (Figure 4)

An important percent of the sample, 57.5%, reported hamburger consumption with a frequency of 1-3 times a week, which represents a higher proportion as compared to chips and snaks consumers, 10.2% consuming at least once a day, and 24.8% of the young subjects never consuming these foods.

As for hamburgers and pizza consumption, there is a statistically significant difference between consumption frequencies in boys and girls, with χ^2 of 39.6 and a probability cutoff $p<0.001$. An increased proportion persists in boys, 59.2% and girls, 55.8%, who consumed hamburgers and pizza 1-3 times a week, and 12.3% of boys and 8.2% of girls consumed these foods at least once a day.

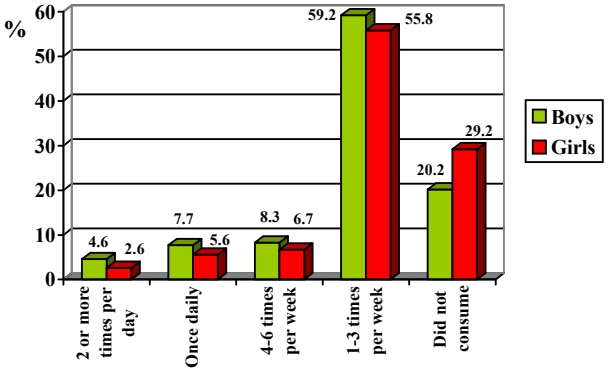

Fig.4. Percentual distribution of boys and girls according to the frequency of hamburgers and pizza consumption during one week

3.3. Fried potatoes consumption (Figure 5)

In the case of fried potatoes, 56.4% of the adolescents consumed them at least 1-3 times a week, and to these subjects a 21.1% is added represented by young subjects who consumed this food 4-6 times a week, and only 7.0% of them did not eat this aliment.

Like in the case of chips, snaks, hamburgers and pizza, fried potatoes were also more frequently eaten by boys. Gender differences, regarding fried potatoes

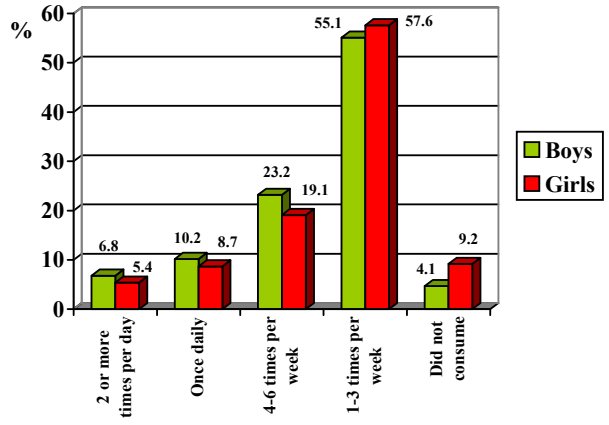

Fig.5. Percentual distribution of boys and girls according to the frequency of fried potatoes consumption during one week

consumption, is statistically significant, with χ^2 of 31.20 and a probability cutoff $p<0.001$ and it is in favour of boys. Thus, 78.3% of the boys and 76.7% of the girls consumed this type of food 1-6 times a week, and 17.0% of the boys and 14.1% of the girls consumed fried potatoes at least once a day. Only 4.7% of the boys and a double percent, 9.2% of the girls did not consume it.

CONCLUSIONS

Daily consumption of cereals and derivates contributes to covering ¾ of the glucidic needs, ½ of the energetical needs provided by glucides, and especially by starch, and up to ½ of the proteic needs.

Daily intake of bread is present in 96.4% of the teen-agers, 4/3 of the girls consume 2-7 slices of bread daily, whereas ½ of the boys eat at least 8 slices/day and 1/3 of the boys consume more than 10 slices/day. By age groups, consumption differences are not significant.

The consumption of cereal derivates, other than bread, (pasta, rice, cereals) is present daily in 10.5% of the adolescents, with a boys/girls consumer ratio of 1.3/1. The most frequent consumption is 1-3 times/week throughout the entire sample and in all age groups.

The obtained results may corelate with an energetic intake replacing deficiencies found in meat, milk and eggs intake, but also with a deficit in high biological value proteins obtained from animal foods.

Fast food aliments are rich in fats, sugar and salt and are included into the lifestyle of adolescents. They often replace traditional nutritional sources, such as cereal derivates, dry and fresh vegetables, fruits (11).

Daily consumption of chips and snaks has been reported by 20.5% of the adolescents, and the boys/girls consumer ratio was 1/1.2. Reported to age groups, the daily consumption was 25.3% in 15 year olds and 12.7% in 19 year olds.

Daily consumption of hamburgers and pizza is declared by 10.2% of the adolescents, and with a boys/girls consumer ratio of 1.5/1. In 15 year old subjects the consumer percent was 11.7% and in 19 year olds it was 9.3%.

Daily consumption of fried potatoes was reported by 15.5% of the adolescents, with a boys/girls consumer ratio of 1.1/1 and with 15.8% consumption in 15 year olds and 10.7% in 19 year old subjects.

High/excessive consumption of fast-food type aliments increases the risk of obesity, diabetes, depressive moods (7).

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CONSUMUL DE ALIMENTE CALORIGENE ÎN ADOLESCENȚĂ

REZUMAT

Consumul zilnic de cereale şi derivate de cereale contribuie la acoperirea a 3/4 din necesarul de glucide, 1/2 din necesarul energetic şi până la 1/2 din necesarul de proteine. Alimentele de tip fast-food sunt bogate în grăsimi, zahăr şi sare, sunt incluse în stilul de viață al adolescenților şi înlocuiesc frecvent sursele tradiționale de nutrienți. Metoda de lucru a fost studiul populațional transversal, prin aplicarea chestionarului CORT 2004 de investigare a unor comportamente cu risc pentru sănătate la tineri, pe o populație reprezentativă de adolescenți din județul Timiş, totalizând 2908 elevi. S-a constatat că, 96,4% dintre adolescenți consumă zilnic pâine, respectiv, 3/4 dintre fete consumă 2-7 felii de pâine/zi, în timp ce 1/2 dintre băieți consumă cel puțin 8 felii/zi. Consumul de paste făinoase, orez, cereale, este prezent zilnic la 10,5% dintre tineri, cu un raport al consumatorilor băieți/fete de 1,3/1. Chips-urile şi snacks-urile au fost consumate de 20,5% dintre adolescenți, hamburgerii şi pizza de 10,2% dintre ei, iar cartofii prăjiți de 15,5% dintre tineri. Rezultatele obținute se pot corela, în principal, cu un aport energetic care înlocuieşte alimentele de bază şi cu un deficit de proteine de valoare biologică superioară.

Cuvinte cheie: adolescenți, alimentație, fast-food

MOLECULAR CHANGES IN PRECANCEROUS LESIONS OF THE LARYNX

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ABSTRACT

Precancerous lesions of the larynx are imtermediary states between the hpysiological mucosa and cancer.
Recent molecular pathology studies in head and neck cancer support a carcinogenesis model in which the development of a field with genetically altered cells plays a central role. Information from the genetic analysis of laryngeal cancer has grown enormously in the last 20 years.
There are two classes of genes implicated in the progress of malignancy: the oncogenes and the tumor supressor genes.
The molecular biology techniques have identified mutations at the level of chromosomes 9p, 3p, 17p, 11q and also the accumulation of nuclear proteins as Ki-67 and PCNA. These genetic alterations are represented by the inactivation of tumor suppressor genes- p16, p53, FHIT- and by the activation of oncogenes- cyclin D1.
Advances in the understanding of the molecular basis of the laryngeal carcinogenesis will help in the identification of new molecular markers that could be used for a more accurate diagnosis and assessment of prognosis and may open the way for novel approaches to treatment and prevention.
Keywords: benign scuamous hyperplasia, dysplasia, carcinoma in situ, oncogenes, tumor supressor genes, nuclear proteins

INTRODUCTION

The same year Watson and Crick published their article on the tridimensional structure of the DNA 50 years ago, Slaughter et all, described for the first time the phenomenon of "field cancerization", hypothesizing that there are alterations at the level of the entire mucosa determined by carcinogens in patients with cancer of the upper aero-digestive tract. The authors examined pathology slides from 783 patients with head and neck cancers in an effort to identify alterations of the epithelium that surrounds tumors and to explain its clinical behaviour. 11% of the patients presented histologic alterations independent of the tumor area. At the time there was no molecular basis for their observations, but the term has been used to describe three phenomena: (1) wide field of aero-digestive mucosa tends to be affected by the premalignant disease; (2) frequent occurrence of multiple primary tumors in the epithelial areas affected by widespread premalignant disease and (3) possible distant related primary tumors in the upper aero-digestive tract.

In 1990, Fearon and Vogelstein proposed the first model for the molecular progression of the colorectal cancer stipulating that tumors progress by the activation of the oncogenes and the inactivation of the tumor suppressor genes, each producing growth advantage for a clonal population of cell. Generally there are specific genetic events in a distinct order, but the order in which they are produced isn't necessarily the same for a certain type of tumor. Genetic events summation determines the progression of the tumor (Ha and Califano, 2003)(13).

Laryngeal carcinogenesis is a multistage process, initiated by various carcinogens (tobacco, alcohol, gastro-esophageal reflux, the human papillomavirus, etc.) which induce genetic alterations and if they remain undetected by the DNA repairing mechanisms, they confer growing advantage with increased cellular proliferation (Monier R et al., 2008)(20).

The aim of this article is to review and highlight some of the recent advances in the molecular biology of the laryngeal carcinogenesis with particular clinical relevance for the diagnosis, management and prognosis of the precancerous lesions of the larynx and laryngeal carcinoma.

A GENETIC MODEL FOR THE LARYNGEAL CARCINOGENESIS

The transition from the normal epithelium to the scuamocellular carcinoma of the larynx is a long, complex and multistage process, with the accumulation of genetic alterations- between 6 and 10- that translate initially by the presence of precancerous conditions (benign squamous hyperplasia, dysplasia, carcinoma in situ) and which can suffer malignant transformation (Braakhuis B et al., 2004)(2). The number of genetic alterations grows with the malignity level established by the histologic examination (see Figure 1)(Califano et al., 1996)(5).

Fig. 1. Genetic model for the laryngeal carcinogenesis (Califano et al., 1996)(5)

There are two classes of genes involved in the progress of malignancy: the oncogenes and the tumor supressor genes. The activation of the oncogenes induces the initiation and progression of the tumor while the inactvation of the tumor suppressor genes contributes to the progression of cancer (Crissman J et al., 2004)(7).

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Normal cells present protooncogenes that turn into oncogenes when activated by different carcinogens, radiations or viruses. This activation is produced by chromosomal translocation, gene amplification, point mutations or deletions. Oncogenes activity is expressed by the level of the growing factors and of their receptors, the signal transducer systems and the nuclear proteins.

The tumor suppressor genes, active in normal cells, suffer mutations or deletions under the action of the same agents, which leads to their inactivation and consecutive disregulation of the pathways that control cellular growth and differentiation.

Studies have demonstrated that patterns of genetic alterations can be used to predict the behavior and tumorigenetic potential of pre-malignant laringeal lesions. In a retrospective study, it has been demonstrated that loss of chromosomes 9p21 and 3p14 were more frequent in patients whose premalignant disease eventually developed into scuamous cell carcinoma (Chang S et al., 2008)(6).

Cromosome 9p

The 9p21-22 deletion is the most frequent genetic alteration in laryngeal carcinogenesis, occuring in about 70% cases (Kim et al., 2004)(18). At this level p16/p14ARF gene, responsible of tumor supressor activity is located. This alteration is found in 20% of the benign squamous hyperplasias and in 57% of the dysplasias of the larynx (Califano et al., 1996)(5).

Gene p16 encodes a 16kDa protein, part of the cyclin dependent kinase inhibitors (CDKIs), among p15WAF/CIP1 and p27KIP1. The product of the p16 (CDKN2A/MTS1) gene binds to CDK4 and CDK6, inhibiting their association with the cyclinD1. The inhibition of cyclin D1/CDK4/6 complex activity prevents the Rb phosphorylation and the release of E2F, thus preventing the G1-S transition in the cell cycle. The genetic alterations that inactivate p16 gene offer growth advantage to the cell, contributing in the carcinogenesis process (Hardisson D, 2003)(15).

Gene p14ARF inhibits the association between p53 and its inhibitor, MDM2, resulting an antiproliferative effect (Ha P et al., 2008)(14).

The p16/p14ARF genes can be inactivated by an alternative mechanism by the promoters hypermethylation (Fan, 2004)(8). This is an epigenetic phenomenon consisting in the abolishment of the gene expression without mutations in the sequence of the genetic code. The methylation of the cytosine residues at the level of the CpG regions of the promoters is responsible of the reduced expression (Baylin and Herman, 2000)(1). Thus, in a significant number of cases the inactivation of the p16 gene can occur in the absence of homozygous deletions. Loss of gene function due to promoter hypermethylation has several characteristics that bear striking similarity to loss of the tumor suppressor gene function by somatic mutation. First, promoter methylation in one allele is frequently accompanied by deletion of the opposite allele, resulting in loss of heterozygosity of the gene. Second, gene inactivation in association with promoter hypermathylation is fully heritable. Finally, loss of gene function due to epigenetic alterations leads to selective growth advantage in a manner identical to loss of tumor suppressor gene function due to somatic mutation (Herman and Baylin, 2000)(16).

Chromosome 3p

Another genetic alteration that occurs early in the laryngeal carcinogenesis process is the deletion at 3p14.2, present in 16% of the laryngeal squamous benign hyperplasias, 52% of the dysplasias and 60% of the carcinoma in situ (Califano et al., 1996)(5).

At this level is located the FHIT (fragile histidine triad), member of the histidine triad superfamily. It seems to work as a preapoptotic tumor supressor gene, that, when affected by deletions, can induce multiple epithelial tumors. The low level of protein expression of the FHIT associates low rates of apoptosis and increased tumoral cell proliferation (Kim M. and Califano J, 2004)(18).

Chromosome 17p

At the level of the 17p13.1 chromosome there is located the p53 tumor supressor gene, one of the most involved genes in carcinogenesis. It was initially thought to be an oncogene, as there was aberrant overexpression of mutated forms of TP53, but it was subsequently shown that these forms were not functional (Oliver et al., 2002)(21). It is constituted of 11 exons that encode a nuclear phosphoprotein with functions in the regulation of gene transcription, DNA synthesis and reparation, cell cycle coordination and reparation, cell cycle coordination and apoptosis. Thus, the loss of function of the p53 gene determines genomic instability and accumulation of genetic alterations.

Most frequently, mutations involve guanine, including G:C to T:A transversions and G:C to A:T translocations at the level of non GpC sites. These changes are frequently associated with benzo(α)pyren, nitrosamines and oxygen reactive species from tobacco smoke (Fan CY, 2001)(8). These selective mutations seem to be caused by the formation of specific compounds between DNA and the carcinogens mentioned above and usually affect the codons 238-248 (Hardisson D, 2003)(15).

The product of the mutant p53 gene, characterized by conformational changes, has a prolonged half-life and stability, and can be detected at nuclear level by IHC staining. Molecular assessment of surgical margins for the TP53 mutations observed in primary laryngeal carcinoma have shown that if the margins are positive for the clonal genetic alteration, there is an increased risk of local recurrence (Ueno T. et al., 2003)(25).

Califano et al. Found p53 mutations in 11% of the benign squamous cell hyperplasia of the larynx, in 33% of the dysplastic lesions and 47% of the carcinoma in situ.

Chromosome 11q

On the 11q13 chromosome there are located the bcl-1, int-2, hst-1 and cyclin D1/ PRAD1 genes. The function of the cyclin D1 protooncogene, also known as PRAD1 and CCND1, is the Rb activation by phosphorilation, forming complexes with the cyclin dependent kinase cdk 4 and cdk 6, facilitating the progression from the G1 phase to the S phase of the cell cycle (Kim m. et al., 2004)(18).

The cyclin D1 supraexpression shortens the G1 interval and reduces the mitogen dependence for the cellular proliferation.

Alterations in the cyclin D1 expression are found according to Califano et al. in 6% of the benign squamous hyperplasia, 29% of the dysplasia lesions and 40% of the carcinoma in situ.

Amplification of the cyclin D1 gene may also have prognostic significance. In a homogenous cohort of 51 patients with laryngeal carcinoma treated surgically with or without postoperative radiotherapy, Belacosa et al. demonstrated independent prognostic values for both tumor stage and cyclin D1 gene amplification in predicting poor overall survival (Jeannon J.P. et al., 2004)(17).

Beside the activation of the oncogenes and the inactivation of the tumor suppressor genes, laryngeal carcinogenesis is also characterized by the supraexpression of proteins as result of gene amplification and the increased DNA transcription and translation.

The epidermal growth factor receptor (EGFR)

The EGFR gene is located on the chromosome 7p12 and it has 110kb. It is a transmembranar receptor, regulator of growth. Its structure consists of a glycoprotein of 170 kDa, that influences cell division, migration, adhesion, differentiation and apoptosis, acting on the tyrosine kinase pathway, activating STATs through

phosphorilation (Pomerantz R.G. and Grandis G.R., 2004)(23).

The EGFR overexpression is an early event in laryngeal carcinogenesis. Its level increases with the degree of the epithelial lesions (Gale N.et al., 2008)(11). The retinoic acid, used in the treatment of the laryngeal precancerous lesions, decreases the level of the EGFR mRNA by low gene transcription.

Ki-67

Ki-67 is a nuclear protein expressed only in cells actively going through cell cycle and it can be used to appreciate the proportion of proliferating cells in a population. It can be determined by IHC techniques and quantitated by determining the percent of nuclei that stain (Brown J et al., 2003)(4).

As a marker of cell activity, Ki-67 can be useful in the prognosis of laryngeal precancerous lesions. The researchers at Fox Chase Center in Philadelphia observed that Ki-67 is nine fold more increased in the basal layer of the dysplastic lesions compared to normal mucosa and more increased in the superficial layers in high grade dysplasia compared to low grade ones (Koch W., 1999)(19).

In another report, 9 of 10 low grade preneoplastic laryngeal lesions that eventually progressed to invasive scuamous cell carcinomas, had detectable Ki-67 staining. In contrast only 2 of 22 lesions that did not progress expressed Ki-67. Thus, as a marker of biologic agressiveness in premalignant lesions, the sensitivity and specificity of Ki-67 approached 90% (Pignataro L. et al., 1998)(22).

The proliferating cell nuclear antigen (PCNA)

PCNA is a nuclear protein as Ki-67 that appears late in the G1 phase, increases in S phase and decreases through G2 and M phases of the cell cycle. It is involved in the regulation of DNA synthesis and cell proliferation. It can be detected by IHC techniques with PC10 antibody (Sarac S. et al.)[24].

CONCLUSIONS

The term "field cancerization" reunites today more concepts that its initial use 50 years ago. What at the beginning was only the description of histologic changes includes today the molecular bases of the transformation of the normal epithelium in premalignancy and cancer.

Precancerous lesions of the larynx are intermediary states between the physiological mucosa and cancer. The development of molecular techniques promises to facilitate early diagnosis, the creation of screening protocols for population at risk and a better surveilance for cancer treated patients. The creation of a molecular profile of the lesions will facilitate the analysis of surgical margins, the prediction of the behavior of precancerous lesions and laryngeal malignancy and the therapy response.

The use of adenoviral vectors to restore p53 expression, demethylating agents to re-express p16, anti-EGFR immunotherapy and small molecule kinase inhibitors are in various stages of testing and clinical trials.

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ALTERARI MOLECULARE IN LEZIUNILE PRECANCEROASE LARINGIENE

REZUMAT

Leziunile precanceroase ale laringelui sunt stari intermediare intre mucoasa fiziologica si cancer.

Studiile recente de biologie moleculara in cancerele capului si gatului au scos in evidenta un model al carcinogenezei in care rolul central este jucat de campul de celule alterate genetic. In ultimii 20 de ani s-a obtinut o cantitate enorma de informatii din analiza gentica a cancerului laringian.

In progresia malignitatilor sunt implicate doua clase de gene: oncogenele si genele supresoare tumorale.

Tehnicile de biologie moleculara au identificat mutatii la nivelul cromozomilor 9p, 3p, 17p, 11q si acumularea de proteine nucleare precum Ki-67 si PCNA. Aceste alterari genetice sunt reprezentate de inactivarea unor gene supresoare- p16, p53, FHIT-si de activarea oncogenelor-ciclina D1.

Progresele in intelegerea bazei moleculare a carcinogenezei laringiene vor ajuta la identificarea de noi markeri moleculari care ar putea fi folositi pentru un diagnostic mai exact si pentru stabilirea prognosticului si ar putea deschide calea unor noi protocoale terapeutice si de preventie.

Keywords: hiperplazie scuamoasa benigna, displazie, carcinoma in situ, oncogene, gene supresoare tumorale, proteine nucleare

THE HYPOLIPEMIANT EFFECT OF HIGH DOSES ATORVASTATIN THERAPY IN DIABETIC PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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ABSTRACT

In cardiovascular patients, the presence of diabetes mellitus is a major cardiovascular risk, according to all the clinical evidences in the present. However, there is a lack of information on the acute coronary patients with diabetes mellitus witch arrived late at specific medical cardiovascular assistance, because of the distance.

This trial evaluates the long term hypolipemiant effect of the early therapy with high doses Atorvastatin 80 mg in diabetic coronary patients hospitalized in The Cardiology Department of The Cardiology Institute in Timisoara.

We studied 352 acute coronary patients, only 81 being with diabetes mellitus type 2. The period from the first symptomatic evidence to the hospitalisation was late, 8-12 hours. The present observational study is undertaken in accordance with all valid ethical principles, all the patients included benefited from pharmaceutical therapy conformable with the current therapeutic guides. Written informed conscience was obtained from all patients. We had two groups of patients: the control group (A) and the group of patients who received the early therapy with high doses of Atorvastatin 80 mg (B).

At the baseline, the level of seric lipids was similar for both lots of patients with an average level of the LDL cholesterol of 124 mg/dl, triglycerides 184 mg/dl and HDL 46 mg/dl. At 6 months the reduction of the level of total cholesterol, of the LDL and of the triglycerides was complete in the lot of patients with high dose of Atorvastatin; this fact maintained at 12 months and at 24 months. By the end of the study, after 24 months treatment, the diabetic coronary patients treated with 80 mg Atorvastatin had mean reduction of LDL cholesterol of 61%, approximately 90% of the maximum reduction in plasma LDL cholesterol levels was achieved by the end of the first 2 weeks.

Patients treated with 80 mg Atorvastatin had reductions in total cholesterol of 46% and reductions of Apo B of 50%, respectively. Atorvastatin 80 mg reduced plasma triglycerides concentrations in 24 months with 25%. There was no consistent pattern in the percent changes from baseline for HDL cholesterol, apo A-I and LP (a).

The present observational study is undertaken in accordance with all valid ethical principles, all the patients included benefited from pharmaceutical therapy conformable with the current therapeutic guides. Written informed consent was obtained from all patients.

The results of our trial confirm and sustain all the present medical evidences, that the early therapy with high doses of statin has a lot of benefits in short and long term evolution of coronary patients.

Key words: acute myocardial infarction, Atorvastatin, hypolipemiant, triglycerides, cardiovascular risk

INTRODUCTION

Diabetes has been shown to increase the cardiovascular risk in all populations studied. However, there is a lack of information on the acute coronary patients with diabetes mellitus witch arrived late at specific medical cardiovascular assistance, because of the distance. The objective of this analysis was to evaluate the long term evolution of these type of patients with high doses of Atorvastatin therapy 80 mg.

MATERIAL AND METHOD

The present observational study is undertaken on acute coronary patients with diabetes mellitus non insulin-dependents, adults (above 18 years), that hospitalized relatively tardy, 8-12 hours after the onset of the myocardial infarct, regardless of the presence or absence of dyslipidemia.

We excluded the coronary patients that were scheduled for revascularization interventions, the myocardial infarct patients with Q wave in the last 4 weeks, coronary by pass undertaken in the last 3 months, PTCA in the last 6 months, severe anaemia (HGB < 8mg%), dialysis patients (IRC stadium IV), insulin-dependent diabetes, pregnancy and nursing.

The present observational study is undertaken in accordance with all valid ethical principles, all the patients included benefited from pharmaceutical therapy conformable with the current therapeutic guides. Written informed consent was obtained from all patients. The hospitalized patients, with acute myocardial infarct (with or without pre-hospital thrombolysis) and severe unstable angina, were treated and monitored adequately during the hospitalization period and received high doses of Atorvastatin (80mg/day) after which they were attentively monitored both clinically and biologically at 6 months, 12 months and 24 months. All the patients received recommendations for hygiene-dietetic regime, conformable with the current therapeutic guides.

After 24-96 hours of hospitalization, the acute coronary patients received Atorvastatin in maximum dose of 80mg/day; the clinical and paraclincal monitoring of the patients has been duly undertaken, both during the hospitalization period

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and after the hospitalization at 6, 12 and 24 months. The clinical and paraclinical evolution of these patients was compared with hospitalized patients with similar characteristics who received usual pathology specific treatment, except the maximum Atorvastatin dose administered just after admission in the study. Preliminary analyses of the results at 2 weeks, 3 months, 6 months, 12 months and 24 months from the hospitalization of acute myocardial infarct patients were effectuated.

Upon entering the baseline phase and continuing throughout the therapy phase of the study, patients were counselled on to use of the National Institutes of Health (NIH) NCEP Diet. This diet limits dietary cholesterol to <300 mg/d and total fats to <30% of total calories, with <10% of total calories from saturated fats, 10% from polysaturated fats and 10-15% from monosaturated fats.

STATISTICAL ANALYSIS

The sample size of the study was chosen to detect a significant linear dose effect for a 25% difference between the mean percent changes of control and the highest dose of Atorvastatin. Statistical analyses were performed with SAS statistical package. Analyses included data from all patients with at least one baseline and one double blind measurement of the parameter of interest regardless of the patient compliance protocol. ANOVA was used to evaluate the effect of Atorvastastin on the percent change from the baseline in LDL cholesterol, the primary efficacy parameter. Baseline was define as the mean of each patient's LDL cholesterol values at start, with the analysis of percent change from the baseline being performed at the last visit of the study. On the basis of this model, a sequential, "step down" trend test was performed to determine the significance of the duff effect. Dunnett's test was used to compare each group when the percent change was not monotonic across the dose levels. All analyses were done using a two-sided significance level of 5%. The same analysis was performed for secondary efficacy parameters except that the baseline value for Apo-I, apo-B and Lp (a) was the mean of the measurements during the study.

RESULTS

From a total of 584 acute coronary patients, we included 352 patients with acute myocardial infarction and severe unstable angina in the study, and only 81 patients with diabetes mellitus, in accordance with the preset inclusion criteria, the period of the inclusion of the patients being February 2004-December 2005. The moment of the admission of the patients to the study overlapped with the moment of their hospitalization. The maximum daily dose of Atorvastatin (80mg/day) was administered to a lot of 179 patients, the rest of the patients received the usual treatment according to the valid therapeutic guides (other statins in usual doses), meaning the control lot was composed of 173 patients. The clinical and paraclinical monitoring of all the patients was undertaken at 6, 12 and 24 months from the inclusion to the study. The clinical and demographical data of the patients from the two groups were similar at the moment of the inclusion to the study (Table I).

Table I. Adjusted mean percent changes from baseline in lipid and apolipoprotein values at 24 months therapy

Percent (%) change	Control	Atorvastatin 80 mg
LDL Cholesterol	7.6	-61.0*
Total cholesterol	4.8	-45.7*
HDL cholesterol	-2.5	3.5*
Triglycerides	-0.7	-27.2
Apo A-I	-3.5	0.8
Apo B	5.8	-50.3*
Lp (a)	7.1	-14.2*

* Significant different than control by sequential, step-down, trend test, $p<0.05$

At the baseline, the level of seric lipids was similar for both lots of patients with an average level of the LDL cholesterol of 124 mg/dl, triglycerides 184 mg/dl and HDL 46 mg/dl. At 6 months the reduction of the level of total cholesterol, of the LDL and of the triglycerides was complete in the lot of patients with high dose of Atorvastatin; this fact maintained at 12 months and at 24 months (Table I).

By the end of the study, after 24 months treatment, the diabetic coronary patients treated with 80 mg atorvastatin had mean reduction of LDL cholesterol of 61%, approximately 90% of the maximum reduction in plasma LDL cholesterol levels was achieved by the end of the first 2 weeks.

Atorvastatin treated patients had dose-related reductions from baseline in total plasma cholesterol and Apo B (Table I). Patients treated with 80 mg Atorvastatin had reductions in total cholesterol of 46% and reductions of Apo B of 50%, respectively. Atorvastatin 80 mg reduced plasma triglycerides concentrations in 24 months with 25%. There was no consistent pattern in the percent changes from baseline for HDL cholesterol, apo A-I and LP (a).

SAFETY

No serious adverse effects appeared in more than 1% of the patients. Increased hepatic transaminases (three times the normal value) appeared in 8 patients (4.5%), for whom the treatment with Atorvastatin was stopped and who where excluded from the study. No case of documented myositis was registered.

DISCUSSIONS

Atorvastatin has a rapid onset of action; approximately 90% of LDL-cholesterol reduction from baseline occurred within the first 2 weeks of treatment. Reduction of LDL cholesterol was 61% in Atorvastatin 80 mg group of patients, after 24 months. In addition to LDL cholesterol, apo B, the major protein component of Ldl cholesterol, was reduced from baseline with 51% after 24 months. There were no clinically important changes in HDL cholesterol, Apo A-I or LP (a). Triglycerides were reduced from baseline with 32% after 24 months of treatment with 80 mg Atorvastatin.

Atorvastatin was well tolerated in this study. No serious adverse effects appeared in more than 1% of the patients. Increased hepatic transaminases (three times the normal value) appeared in 8 patients (4,5%), for whom the treatment with Atorvastatin was stopped and who where excluded from the study. No case of documented myositis was registered.

This study suggests that 80 mg Atorvastatin is very effective in precocious treatment of diabetic patients with acute myocardial infarction.

CONCLUSIONS

The present study proves that the early administration of 80mg/day Atorvastatin in diabetic patients with acute myocardial infarction late hospitalised, with or without ST segment depression, is necessary immediately after the ischemic event and in the long run, because it reduces the recurrence of the ischemic events in long term evolution and especially the ischemic cardiovascular recurrences that require hospitalization.

The aggressive decrease of the values of the serum lipids at the administration of Atorvastatin in maximum dose of 80mg/day is very effective in short time, approximately 90% of LDL-cholesterol reduction from baseline occurred within the first 2 weeks of treatment.

Atorvastatin was generally well tolerated in this patient population. There were no documented cases of myositis, witch is the most serious adverse effect of statins. Levels of serum transaminases exceeding 3 times the ULN were detected in 8 patients, but these patients were excluded.

In conclusion, the results of this trial indicate that the treatment with high doses

Atorvastatin 80 mg may provide adequate therapy for diabetic patients with acute myocardial infarction late hospitalised.

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EFECTUL HIPOLIPEMIANT AL DOZELOR CRESCUTE DE ATORVASTATIN IN TERAPIA PACIENTILOR DIABETICI CU INFARCT MIOCARDIC ACUT

REZUMAT

Diabetul zaharat este un factor de risc major suplimentar la patientii cardiaci, cu atat mai mult la pacientii coronarieni acuti, conform tuturos evidentelor clinice actuale. Totusi, exista o lipsa de evidente clinice effectuate la pacienti diabetici coronarieni acuti care sunt spitalizati tardiv pentru asistenta medicala de specialitate, datorita distantei. Prezentul studiu evalueaza efectul hipolipemiant al terapiei precoce cu atorvastatin in doza maxima, la pacientii diabetici coronarieni acuti, spitalizati tardiv pentru acordarea asistentei medicale de specialitate in Clinica de Cardiologie a Institutului de Boli Cardiovasculare Timisoara.

Din totalul de 352 pacienti coronarieni acuti monitorizati, 81 pacienti au fost diabetici. La 8-12 ore de la debutul bolii. Terapia administrata a fost conform ghidurilor terapeutice in vigoare, in lotul A (lot control), iar in lotul B (lotul cu atorvastatin) s-a administrat tuturor pacientilor, imediat de la internare (precoce) doza maxima de atorvastatin 80 mg/zi, cu monitorizare la 6, 1 si 24 luni. La debut, valorile serice lipidice au fost similare in cele doua loturi de pacienti: LDL_colesterol 124 mg/dl, triglyceride 184 mg/dl, HDL 46 mg/dl. Dupa 24 luni de tratament, s-au inregistrat diferente semnificative din punct de vedere statistic intre cele doua loturi: LDL-colesterol a scazut cu 61%, colesterolul total a inregistrat o scadere cu 45,7%, cresterea HDL-colesterolului cu 3,5%, iar Apo B a inregistrat o scadere cu 50,3%, comparative cu lotul control, in care s-a inregistrat o crestere cu 7,6% a LDL-colestrol, cu 4,8% a valorilor colesterolului total, scaderea HDL-colesterol cu 2,5% si cresterea Apo B cu 5,8%. De mentionat ca complianta pacientilor la trtament a fost similara in cele doua loturi, inclusive aparitia efectelor adverse secundare.

Rezultatele acestui studiu, confirma si sutin evidentele clinice effectuate pana in present, terapia precoce cu doze maxime de statine aducand numeroase beneficii pe termen mediu si lung in evolutia pacientilor coronarieni.

Cuvinte cheie: infarct miocardic acut, Atorvastatin, hipolipemiant, triglyceride, risc cardiovascular

URBAN NOISE ASSESSMENT IN TIMISOARA

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ABSTRACT

Noise is a modern plague of the urban society. In an effort made to asses the noise level of the urban area of Timisoara, a number of 103 noise level measurements, each 8 hours long were performed in the daytime. The measurements show that the urban noise in generated mainly by road traffic, affecting significantly residential, recreational and medical treatment areas.
Keywords: urban noise, daytime noise measurements, road traffic

INTRODUCTION

Noise consists of all unwanted sound - sound that is loud, unpleasant or unexpected. It has been increasing in urban areas to the point where it has become a matter of public concern.

The effects of noise can vary from one individual to another. However, a WHO report entitled "Community Noise - Environmental Health Criteria", published in 1996, and highlights such effects as disturbance of sleep, auditory or physiological effects (basically cardio-vascular) and interference with communication.

Initially, action to reduce noise was not considered an environmental priority - unlike action to reduce air pollution, for example. The effects of noise are unspectacular, and the decline in quality of life was accepted by the general public as being an inevitable consequence of technological progress and urbanization (1).

However it has been estimated that around 20 percent of the European Union's population or close to 80 million people suffer from noise levels that scientists and health experts consider to be unacceptable, where most people become annoyed, where sleep is disturbed and where adverse health effects are to be feared. An additional 170 million citizens are living in so-called 'grey areas' where the noise levels are such to cause serious annoyance during the daytime (2).

MATERIALS AND METHODS

In 2008, a number of 103 8h-daytime measurements were conducted in randomly selected measurement points in the urban area of Timisoara. The measurement points were later classified by the function of the selected urban area:

- Traffic area (roads, public transportation paths & railways)
- Industrial area
- Residential area
- Recreational area (playgrounds & parks)
- Medical treatment & care area

Traffic area measurements were made at the edge of the pathway (25m from the axis in the railways case), industrial and medical treatment area measurements were made along the specific perimeter, recreational area measurements were made inside the area, as central as possible and in the residential area measurement were conducted at a distance of 3m form the façade of the buildings, facing the noise sources, according to Romanian measurement standards (6).

The measuring instrument chosen was a Bruel&Kjaer 2238 Mediator (Class 1 Integrating Sound Level Meter with logging software) fitted with an outdoor kit (free-field microphone with windscreen, cable preamplifier extension and tripod)

The microphone was set at a height of 1.3 m form the ground level, according to Romanian noise measurement standards (6).

A 100 ms sampling rate was selected in order to maximize the amount of recorded data (288,000 samples/measurement) for statistical purposes and for further analysis.

Before each measurement the SLM was recalibrated using a class 1 acoustic calibrator.

Measurement data included LAeq (A-weighted equivalent noise level) L90 (background noise) and L10 (maximum noise). Lowest sound pressure levels and peak levels were also recorded. The measurement unit was the dBA (A-weighted decibel) (5).

RESULTS

Road traffic measurement conducted on the main roads at the city limits area indicated higher noise levels due to higher vehicle speed and increased traffic density. It is known that at speeds above 50 km/h tire noise becomes significant in the overall noise emission of a vehicle. The equivalent noise level was in all cases above 60 dBA, although the background noise was in some 3rd category roads under 45 dBA indicating that the flux of vehicles was not constant (7). However, in 2nd category roads, the background noise was permanently over 55 dBA (Table I).

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Table I. Road Area Measurements

	Road Area	LA_{eq} (dBA)
1	Giroc – Centru	62.6
2	Halta Semenic	60.2
3	Calea Girocului 113 (UM)	70.8
4	Calea Girocului – Maldini	70.9
5	Calea Girocului – Vile Braytim	64.5
6	Calea Girocului -Albac	67.6
7	DN6 - Vama Timisoara	71.4
8	Versului	66.0
9	Uranus	68.4
10	Padurice	67.5
11	Sirius	65.9
12	Brancoveanu - Snagov	69.5
13	Calea Aradului	72.1
14	Amurgului	73.5
15	Calea Sagului	72.4

Table II. Public Transportation – Trams Measurements

	Public Transportation - Trams	LA_{eq} (dBA)
1	Rebreanu	62.6
2	Drubeta - Eneas	85.9
3	Gheorghe Doja	75.0
4	Piata Resita	59.8
5	Iosif Nemoianu	68.2
6	Piata Maria	67.5
7	Gara Nord	69.8

The public transportation, in the case of trams, generates a high level of noise. At an average 5 minutes per car passage, with a peak of 85-97 dBA, on canyon-like streets (Gheorghe Doja), tram generated noise could be a serious issue, especially at high speeds and poorly maintained rails and cars. A particular case was at the Drubeta – Eneas junction where the improper maintenance of the curved rails led to a LAeq of 85.9 dBA, the distance between the rails and the façade of the residential buildings in the area being less than 3 meters (Table II).

The railways crossing the town have an impact on a small percentage of the population. Train passages generate high peak levels, but the relatively long interval of time between the passages permits the 8 hours value of LAeq to settle between 58 dBA and 68 dBA. In some sectors railway noise is combined with road traffic noise leading to increased noise levels. Studies have shown that, psychologically, people tend to support easier railway noise than road traffic noise even when railway noise levels are higher than road traffic noise levels (3) (Table III).

Table III. Railway Measurements

	Railway Area	LA_{eq} (dBA)
1	Spitalul Nou	67.7
2	DAB stația Meteo	63.7
3	Gheorghe Lazăr	67.0
4	Halta Semenic	58.2
5	Gara Sud	68.4

Table IV. Industrial Area Measurements

Nr. crt.	Industrial Area	LA_{eq} (dBA)
1	Prelucrare lemn, Dragalina	54.8
2	Spalatorie auto, Eroilor	55.7
3	Centrala, Piata Victoriei	66.9
4	Prelucrari metalice, Dragalina	59.7
5	Tinichigerie, Titulescu	50.0
6	Depozit,Take Ionescu	67.1
7	Fabrica mobilier, Brediceanu	65.0
8	Chiller, Aries	58.8
9	Service auto, Eroii de la Paulis	59.9
10	Brutarie, Caprioarei	51.8
11	Benzinarie, Lidia	65.0
12	Fabrica boltari, Buziasului	52.4
13	Fabrica electrice, Orion	50.8

Noise in the industrial area, LAeq ranging between 50 dBA and 67 dBA, was mainly generated by the use of electric motors, compressors, ventilators, chillers, sawing mills, dryers and other stationary equipment generating an almost constant noise. These areas were characterized by a high background noise, above 50 dBA, and occasionally with high peak levels during loading/unloading, maintenance and construction operations. In all cases measurements results were influenced by the nearby road traffic generated noise (Table IV).

Residential areas had a wide range of noise levels recorded, noise generated almost entirely by road traffic, mainly because of the size and paving of the streets and because of the continuity and amount of vehicle traffic (4). There are areas with low background noise and a passage of few cars/hour, like Codrului Street, LAeq 47.1 dBA, and areas with almost continuous noise levels of 72 dBA, like Calea Aradului. Romanian standards recommend a maximum accepted outdoor level of 50 dBA daytime equivalent noise level for residential areas. This limit is surpassed in the majority of cases (Table V).

Noise levels in the recreational areas (parks and playgrounds) are relatively high due to the vicinity of the city's main roads. Especially for playgrounds that were built beside busy roads, for easy access, this positioning leads to an exposure of children to high levels of noise and air pollutants. Nevertheless there are still parks relatively calm during daytime like the Roses Park with a LAeq of only 49.7 dBA and a background noise of 43.3 dBA (Table VI).

Unfortunately a large number of medical treatment units and care facilities are built near major roads, traffic noise having a significant impact upon this kind of area. From 14 hospitals and other units investigated none was measured with a daytime outdoor LAeq smaller than 55 dBA which is with 10 dBA more than the maximum limit set for medical areas (Table VII and VIII).

Table V. Residential Area Measurements

	Residential Area	LA_{eq} (dB A)
1	Barbu Iscovescu	55.9
2	Aleea Ripensia 26	60.3
3	Piata Mărăşti	71.0
4	Calea Martirilor – Lidia	66.5
5	Calea Martirilor 37	67.7
6	Codrului	47.1
7	Aurelianus	56.6
8	Mihai Viteazul	68.5
9	Calea Martirilor – Siemens	66.6
10	Brediceanu 14	70.7
11	Eroilor	60.4
12	Nicolae Titulescu - Dragalina	68.6
13	Piaţa Traian	55.2
14	Piaţa Romanilor	58.2
15	Calea Aradului 18	72.8
16	Take Ionescu	69.0
17	Mures 33	67.2
18	Piata Unirii	58.6
19	Piata Concordia	70.5
20	Piata Dr Russel 5	72.4
21	Tudor Vladimirescu – Mangalia	65.6
22	Calea Sagului 37	69.7
23	Victor Babes - Cluj	77.0
24	Iepurelui – Piata Varful cu Dor	71.1
25	Simion Barnutiu 4	63.0
26	Mures 33	69.6
27	Iancu Vacarescu 8	59.5
28	Lidia 100	64.2
29	Brancoveanu 52A	69.2
30	Piata Aurel Vlaicu – str. Anton Pann	63.7
31	Liviu Rebreanu	70.5
32	Splaiul Galati 6	67.8
33	Roma	54.5

Table VI. Recreational Area – Park Measurements

	Recreational Area - Parks	LA_{eq} (dBA)
1	Parc Pârvan	60.6
2	Parc Corneliu Coposu	56.6
3	Parcul Universităţii	54.7
4	Parc Doina	62.4
5	Parcul Rozelor	49.7
6	Parc Padurice	48.6
7	Parcul Copiilor - Beethoven	53.2
8	Parcul Constructorilor – Eroilor	59.0
9	Parcul Zona Soarelui	58.6

Table VII. Recreational Area – Playgrounds Measurements

	Recrational Area - Playgrounds	LA_{eq} (dBA)
1	Piaţa Eforie	53.5
2	Calea Aradului – Moţul	67.1
3	Calea Aradului – Sever Bocu	65.6
4	Bucovinei	49.4
5	Piaţa Dacia 1	53.6
6	Piaţa Dacia 2	57.6
7	Eroilor – Parcul Constructorilor	58.8

Table VIII. Medical Treatment and Care Area Measurements

	Medical Treatment & Care Area	LA_{eq} (dBA)
1	Maternitatea Bega - Victor Babeş 12	67.9
2	Clinicile Noi - Piaţa Mărăşti	71.8
3	Spitalul Militar - Gheorghe Dima	69.1
4	Clinica Oftalmologie - 1 Mai 3	70.4
5	Spitalul Copii 3 Hematologie - Republicii	68.0
6	Spitalul Copii 3 Ortopedie - Iosif Nemoianu	65.8
7	Spitalul 5 Maternitate - 16 Decembrie 24	71.5
8	Spitalul Psihiatrie - Iancu Văcărescu 23	55.9
9	Spitalul CFR - Tudor Vladimirescu	62.7
10	Spitalul Clinic 1 Judeţean – Dr. Stanca	59.7
11	Spitalul 1 Ortopedie - Aleea Sănătăţii	55.2
12	Clinica 1 Pediatrie - Păltiniş	61.1
13	Clinica de Cardiologie - Spitalul Nou 13	70.1
14	Casa Austria – Iosif Bulbuca	69.2

CONCLUSIONS

By the Observer's point of view, the crushing majority of the urban noise sources in Timisoara during daytime are represented by the road traffic. In 96% of the cases, noise was generated by the traffic of cars, vans, trams, motorcycles and lorries. This type of noise affects all studied areas in a significant degree, leading to the proposal that a new sort of noise source should be introduced in the regulations, to be considered as a separate category from fixed and mobile sources: the traffic noise as a collective phenomenon.

Road noise increases proportionally with speed for all categories of vehicles. Road material, pavement design, road maintenance and timing of the traffic lights contribute as well to the noise level generated by the road traffic.

Although high values of noise were measured, railway noise in Timisoara is limited to a narrow path and affects a small percentage of the population.

Stationary noise sources in the industrial area represent only 4% of the total cases. With the enlargement of the city limits, industrial areas tend to move outside the town, therefore the pressure on the residential and protected area diminishes significantly with the passage of time.

Noise levels in protected areas (recreational and medical treatment) is high in the daytime, in the majority of cases being generated by the road traffic due to

the fact that Timisoara's main recreational areas and medical facilities are located near very busy roads.

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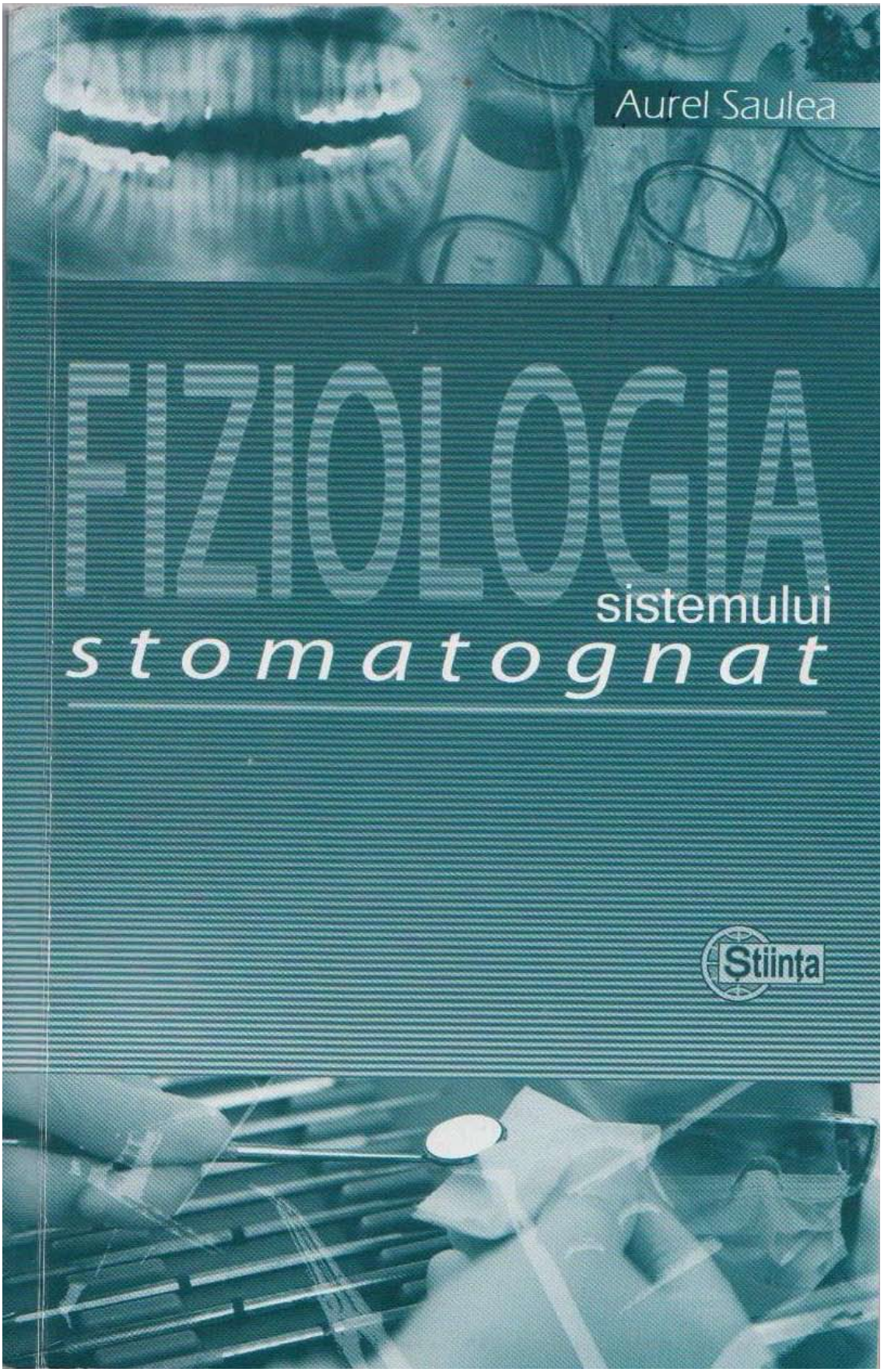
EVALUAREA ZGOMOTULUI URBAN IN MUNICIPIUL TIMISOARA

REZUMAT

Zgomotul este o problema a societatii urbane moderne. Intr-o incercare de a evalua nivelul de zgomot urban in municipiul Timisoara, au fost efectuate 103 masuratori ale nivelului de zgomot, pe timp de zi, cu o durata de 8 ore pentru fiecare masuratoare. Masuratorile au indicat faptul ca zgomotul urban este generat in mare masura de catre traficul rutier si afecteaza semnificativ zonele rezidentiale, recreationale si de tratament medical din oras.
Cuvinte cheie: zgomot urban, masuratori pe timp de zi ale nivelului de zgomot, trafic rutier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