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CUPRINS

ETHANOL SUPPRESSES SEIZURE INCIDENCE IN BOTH METAPHIT AND LINDANE MODELS OF GENERALIZED EPILEPSY IN RATS

DRAGAN HRNČIĆ[1], ALEKSANDRA RAŠIĆ- MARKOVIĆ[1], DRAGAN DJURIC[1], VESELINKA ŠUŠIĆ [2], OLIVERA STANOJLOVIĆ[1] , ADELA JOANTA[3], MANOLE COJOCARU[4]

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ABSTRACT

In different experimental epilepsy models, ethanol may have either proconvulsive or anticonvulsive effects on epileptic activity. The aim of the present study was to determine and compare the influence of ethanol on seizure incidence, main parameter of convulsive behavior, in models of generalized epilepsy induced by lindane and methaphit. Adult male Wistar albino rats were randomly divided into following groups: control, saline – injected, dimetilsulfoxide – treated, ethanol (2 g/kg, i.p.), lindane (8 mg/kg, i.p.), ethanol (2g/kg, i.p.) + lindane (8mg/kg, i.p) in experiment I and metaphit (10 mg/kg, i.p.) and ethanol (2 g/kg, i.p.) + metaphit (10 mg/kg, i.p.) groups in experiment II. Seizure incidence was defined as percentage of animals with seizure out of total number of animals in group. No signs of seizure behavior were observed in control and ethanol groups. Seizure incidence was significantly lower in groups that received ethanol in comparison with those received convulsive drug (either lindane or metaphit). These results indicate prominent anticonvulsive activity of acute ethanol in high dose in models of generalized seizures induced by lindane and metaphit.

Key words: ethanol, seizure incidence, metaphit, lindane, generalized epilepsy, rats

INTRODUCTION

In different experimental epilepsy models, ethanol may have either proconvulsive or anticonvulsive effects on epileptic activity (15). Chronic ethanol consumption has been considered as major risk factor for epilepsy (4). It is also known that even seizure series may occur during the withdrawal period (8). On the other hand, acute administration of high doses of ethanol exerts an inhibitory effect on central nervous system and increases the threshold of seizure activity (5, 19, 8).

The molecular mechanisms involving these interactions are still not well known since an ideal model for their study is currently unavailable. Ethanol exerts its behavioral effects largely by interacting with receptors of brain neurotransmitters (31). In particular, it has been shown that ethanol directly modulate the gamma-aminobutyric acid type A (GABAA), N-methyl-D-aspartate (NMDA), glycine (32), neuronal nicotinic (3) and 5-hydroxytryptamine type 3 (5-HT3) receptors (20). Namely, acute ethanol exposure potentiates ion currents at GABAA and glycine receptors (21) but inhibits them at NMDA receptors (9).

Metaphit (1-[1-(3-isothiocyanatophenyl)-cyclohexyl]-piperidine), a phencyclidine (PCP) analog (23) is known to induce audiogenic seizures in small rodents (29). Metaphit model of generalized, reflex audiogenic seizures in rats has been shown valuable for studying anticonvulsive profile of numerous drugs (10, 26, 27).

Lindane (γ-1,2,3,4,5,6-hexachlorocyclohexane), an organochloride pesticide, is extensively used in agriculture and in human and veterinary medicines. Lindane is postulated to interact with the picrotoxin site within the GABAA receptor chloride channel and to suppress the GABA-induced chloride flux into cells (1, 28). Model of generalized epilepsy induced by lindane may be used as a suitable model for testing new antiepileptic drugs (33). The aim of the present study was to determine and compare the influence of ethanol on seizure incidence, main parameter of convulsive behavior, in models of generalized epilepsy induced by lindane and methaphit.

MATERIALS AND METHODS

Animals

Adult 2-month-old Wistar rat males (180 - 220 g), raised at Military Medical Academy Breeding Laboratories, Belgrade (Serbia) were used. They were kept under controlled environmental conditions (22±1 °C, 50% relative humidity and 12/12 h light/dark cycle with light switched on at 9 AM) and housed individually with free access to standard laboratory animal chow and tap water. Each animal was used only once.

All experimental procedures were in full compliance with The European Council Directive (86/609/EEC) and approved by The Ethical Committee of the University of Belgrade (Permission No 298/5-2).

Experiment I

A total of 48 animals were divided into five groups: 1. control, saline-injected (n=8); 2. dimethylsulfoxide (DMSO)-treated (n=8); 3. Lindane-administered (L, 8 mg/kg; n=11), 4. Ethanol-treated group (E, 2 g/kg; n=8); 5. group received ethanol (2g/kg) 30 min prior to lindane (8 mg/kg), EL (n=16). For intraperitoneal (i.p.) administration lindane was dissolved in DMSO.

Behavioral changes were observed during 30 minutes after lindane administration. Seizure incidence was defined as percentage of animals with seizure out of total number of animals in group.

Experiment II

A total of 19 animals was divided into following groups: metaphit administered (M, 10 mg/kg; n = 12) and metaphit + ethanol administered (EM, 10 mg/kg metaphit + 2g/kg ethanol; n = 7). Ethanol was injected 8h after metaphit

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administration, when metaphit audiogenic seizures were fully developed.

None of the untreated animals screened for audiogenic susceptibility expressed seizure activity. Audiogenic stimulation (AGS) was applied for 60 s using an electric bell (on the top of the cage) generating 100 ± 3 dB and frequency of 5-8 kHz. The first stimulation was applied 60 min after metaphit administration and thereafter at hourly intervals during the experiment. Seizure incidence was defined as in previous experiment.

Drugs

Injected solutions given i.p. in a total volume of 0.1 ml were freshly prepared in sterile physiological saline. Metaphit methanesulphonate, lindane and DMSO were purchased from Sigma-Aldrich Chemical (St Louis, MO, USA). Absolute ethanol was a product of Merck KGaA, Germany.

Data analyses

Significance of the differences in the incidence of seizures between groups was evaluated by Fisher's exact probability test.

RESULTS

No signs of seizure behavior were observed in control and DMSO-treated groups, as well as in group of ethanol-treated animals (E). Seizure incidence in EL group was significantly lower in comparison with L group ($p<0.05$, Fig.1)

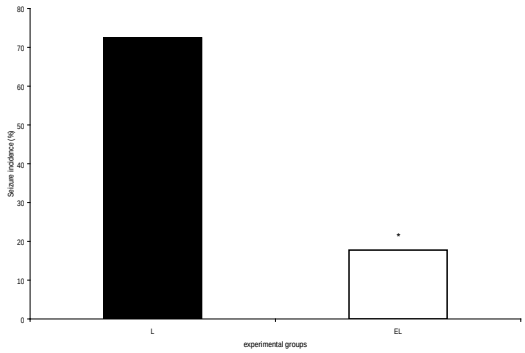
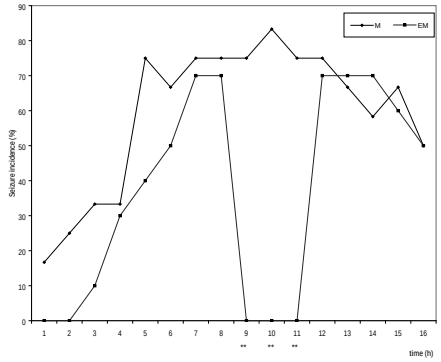


Fig. 1. Seizure incidence in lindane group (L, 8 mg/kg, i.p.) and group received ethanol (2g/kg, i.p.) 30 min prior to lindane (8 mg/kg, i.p.) (EL). Significance of the differences in the incidence of seizures between groups was evaluated by Fisher's exact probability test (*$p<0.05$)

Behavioral observation showed that the incidence of seizure responses on AGS were the highest 7-12 h after metaphit injection. About 25% of metaphit-treated animals never responded to AGS and behaved normally during this critical time period.



Time course study revealed high incidence reduction of all convulsive components of metaphit seizures occurring for 3 h after ethanol injection. Incidence of seizures in EM group was significantly reduced in comparison with group M in the three time points 9h ($p < 0.01$), 10h ($p < 0.01$) and 11h ($p < 0.01$) (Fig. 2).

Figure 2: Time course of seizure incidence of metaphit-induced seizure followed by ethanol injection after 8 h. All animals were exposed to an intense AGS (100 ± 3dB, 60 s) at hourly intervals after metaphit (10 mg/kg, i.p) injection during the experiment. Incidence of seizures in metaphit + ethanol-treated group (EM) was significantly reduced in comparison with only-metaphit-treated group (M) as indicated. Y-axis: seizure incidence (%) X-axis: time (h) after metaphit administration. Significance of the differences in the incidence of seizures between groups was evaluated by Fisher's exact probability test (**$p<0.01$).

DISCUSSIONS

In the present experiments, we showed that ethanol (2 g/kg, i.p.) significantly suppressed seizure incidence in models of epilepsy induced by lindane (8 mg/kg, i.p.) and metaphit (10 mg/kg, i.p +AGS).

Molecular mechanisms underlying the metaphit seizure are still unclear and there are several hypotheses on its convulsive activity. Metaphit irreversibly binds to the ligand-gated ion channels of the NMDA receptor complex, opening it for Na+ and Ca2+ influx and/or upregulating NMDA receptors and increasing receptor affinity for the binding of natural ligands, such as glutamate and aspartate (29). It is possible that metaphit inhibits the back-influx mechanism transport system for glutamate at the luminal side of the brain capillaries, resulting in an over-accumulation of glutamate in the brain, as suggested by Lipovac et al. (18), who hypothesized a metaphit related inhibition of the glial uptake of glutamate resulting in an increase in extracellular glutamate levels in the brain. Similarly, metaphit affects numerous neurotransmitter systems and receptors, such as serotonergic, dopaminergic, voltage-dependent sodium channels (24) and sigma receptors (34). Ishida et al. (12) demonstrated that audiogenic seizures induced by high-intensity sound stimulation in genetically suspectible mice and rats expressed typical and characteristic signs of epilepsy (running, clonus and tonus). In this model, convulsions were triggered by creating an imbalance between excitatory and inhibitory brain activities, mainly by increasing excitatory influences related to glutamate activity.

Lindane induces convulsions through different neuronal mechanisms. Some of them include modifications in neurotransmitter levels in different brain structures. Pro-convulsive effect of lindane may be explained by direct influence on GABA-ergic system. Namely, lindane was postulated to interact with picrotoxin binding site within the GABAA receptor chloride channel as well as to suppress GABA-induced chloride flux into cells (28), leading subsequently to seizure induction. Furthermore, excitatory amino acid antagonists reduced pro-convulsive properties and toxicity of lindane (2). Calcium mobilization may be an additional mechanism of lindane-induced convulsions (25).

Other authors reported a similar data that provide evidence of anticonvulsive effect of ethanol (15). Namely, ethanol was found to act as lindane antagonist and its central depressive effects were predominant (14, 15, 36). The ethanol doses used in the present experiment were similar to those applied in experiments of anticonvulsant effects of ethanol against NMDA-, kainic acid- and picrotoxin-induced convulsions in rats reported by other authors (16). Ethanol (2 g/kg, i.p.) offered protection against these agents, and it was most effective against picrotoxin and least effective against kainic acid. In co-medication with valproate and carbamazepine, ethanol significantly increased the anticonvulsant effectiveness of both antiepileptic drugs (6). Furthermore, a number of previous in vivo studies in the pentylenetetrazol

(PTZ)-kindling and ferric chloride models in rats also suggest anticonvulsant and antiepileptic effects of ethanol after acute application (7, 11).

The results of Rabe CS et al. (22) demonstrate that in vivo actions of ethanol on the NMDA systems may be dependent on glycine concentrations at these receptor sites. Hoffman PL et al.(9) suggested in different studies using various concentrations of NMDA, as well as phencyclidine (PCP) and glycine, that ethanol affected the co-agonist binding site of the NMDA receptor-channel complex, rather than the PCP recognition site. Yaka et al. (35) results suggest that the interaction between tyrosine kinase Fyn and the NR2B subunit of the NMDA receptor mediates the acute sedative effects of ethanol.

GABA systems have been also implicated as targets for ethanol at the cellular, molecular and behavioural level (13). Ethanol acutely enhances Cl- transport through the GABAA receptor channel (30). While much effort concentrated on the effects of ethanol on GABAA receptor-mediated synaptic transmission, GABAB receptors are acknowledged as targets for ethanol (17).

These findings suggested that the anticonvulsant actions of ethanol may be attributed to its ability to antagonize NMDA-mediated excitatory responses and facilitate the GABAergic transmission.

Based on our results it could be concluded that ethanol has significant and comparable anticonvulsant activity in metaphit model of seizures and model of seizure induced by lindane.

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ETANOLUL REDUCE INCIDENTA CRIZELOR IN MODELELE EXPERIMENTALE DE EPILEPSIE GENERALIZATA LA SOBOLAN CU METAFITATI SI LINDAN ETHANOL

REZUMAT

In diferite modele experimentale de epilepsie, etanolul are fie efecte proconvulsivante, fie anticonvolsivante asupra activitatii epileptice. Scopul acestui studiu a fost sa determine sis a compare influenta etanolului in incidenta convulsiilor, cel mai important parametru al comportamentului convulsivant, la modelele de epilepsie generalizata indusa de lindan si metafitati. Sobolani adulti, masculi rasa Wistar albino au fost impartiti in urmatoarele grupuri: control, solutie salina – injectare, dimetilsulfoxid – tratati, etanol (2 g/kg, i.p), etanol (2 g/kg, i.p.), lindane (8 mg/kg, i.p.), etanol (2g/kg, i.p.) + lindane (8mg/kg, i.p) in experimentul I si grupurile cu metafitati (10 mg/kg, i.p.) si etanol (2 g/kg, i.p.) + metafiti (10 mg/kg, i.p.) in experimentul II. Incidenta crizelor epileptice a fost definita procentual in functie de numarul de animale cu crize, raportat la numarul total de animale din grupul respectiv. Nu au fost observate comportamente convulsive la grupurile de control si la cele in care s-a folosit etanolul. Incidenta crizelor a fost semnificativ mai scazuta in grupurile tratate cu etanol, comparativ cu cele in care a fost administrate medicamente convulsive (fie lindan, fie metafitati). Aceste rezultate indica activitatea anticonvulsivanta proeminenta a etanolului administrat in doze crescute la modelele animale de crize epileptice generalizate induse de lindan si metafiti.

Cuvinte cheie: etanol, incidenta crizelor, metafitati, lindan, epilepsie generalizata, sobolani

SENSIBILITY OF FUNGI ISOLATED FROM HIGH RISK WARDS

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ABSTRACT

Background: The aim of this study was to establish the antifungal sensitivity of possible nosocomial fungi isolated from high-risk wards of County Emergency Hospital Timisoara.

Material and method: The study took place during January 8, 2007 – June 15, 2008. During this period we have collected 564 biological samples from the patients hospitalized in County Emergency Hospital Timisoara wards. Isolated pathological samples were striked on chloramphenicol and gentamicin supplemented Sabouraud agar. API 20 C AUX kits were utilized for fungal identification. Antifungal sensitivity was performed both by disk diffusion classical method and ATB fungus.

Results: Mycology analysis of the pathological samples lead up to 314 Candida sp. strains, 28 Cryptococcus neoformans strains, 19 Aspergillus sp. strains. Antifungal tests revealed a high resistance to amphotericin B for a number of isolates: C.albicans-27, C.non-albicans-13, Cryptococcus neoformans-8. Fluconazole resistance was present for: 51 C. albicans strains, 27 C.non-albicans strains and 4 Cryptococcus neoformans strains. High resistance to clotrimazole had: 61 C. albicans strains, 37 C. non-albicans strains, and 3 Cryptococcus neoformans strains. 8 from Aspergillus fumigatus strains showed resistance to fluconazole and miconazole.

Conclusions: The number of resistant fungi is continuously increasing in hospitals around the world and nocomial infections treatment for these microorganisms requires high costs, it's implied to introduce some guides and protocols for prevention and control of resistant fungal strains spreading.

Key words: antifungal sensitivity, nosocomial fungi, fungal diseases, biological samples, intensive care unit

INTRODUCTION

Fungal infections represent an important acute problem especially in hospital's high-risk wards. In the USA, fungi are thought to be the fourth in place as nosocomial infectious agent and these germs are responsible of more than 5% of all sepsis cases (5).

Fungi are causing nosocomial infections, especially in Intensive Care Wards where the number of immunocompromised patients is high. The importance of fungi in nosocomial infections etiology has increased during the last two decades and there have been reported worldwide nosocomial outbreaks. Candida species have become dominant germs for many immunosupressed patients. Localized or systemic, Candida infections can be found in neutropenic patients, in patients with immunosuppressive therapy, in patients of Intensive Care Units. Fungi are more frequently isolated in Intensive Care Wards, due mainly to the increased number of therapeutic and diagnostic invasive procedures used in Intensive Care during the last years and to the microorganisms' ability to survive long periods in the environment and on the skin of medical staff (13). The risk factors for fungal nosocomial infection are represented by previous hospitalization, immunosupressed patient status, mechanic ventilation, respiratory or cardiac failure, previous infections and anti infectious therapy and by the presence of central or urethral catheters.

From all human pathogen fungi, Candida albicans, Candida non albicans species and Cryptococcus neoformans are producing most of the infections, often very severe, which require prolonged treatment and secondary prophylaxis (3).

For many decades, only the polyenes, represented by amphotericin B, were used for systemic infection treatment. Amphotericin B has preserved its efficiency for a long time, together with its adverse effects, especially nephrotoxicity (4). The progress in antifungal therapy led to the rise of new and more effective derivates, less nephrotoxic, more expensive.

Dermato-therapy was marked by the presence of azole antifungals. Introducerea antifungicelor azolice a marcat dezvoltarea dermatoterapiei. The azoles are distinctive by their wide spectrum, being active on dermatophytes, yeasts and a large number of human pathogenic molds.

In 1969 under the name of imidazole derivatives clotrimazole and miconazole appeared, followed by econazole and ketoconazole used for topic treatment of skin mycoses. Ketoconazole was the first wide spectrum systemic antifungal from this group.

Systemic treatment of mycoses has been improved by the discovery of fluconazole and itraconazole, antifungal drugs from the class of imidazole derivatives (5, 9).

Amorolfine belongs to a new class (morolphine derivatives) – topic antifungal. It's fungistatic or fungicide effect is based on fungal membrane allteration, primary target being sterols biosynthesis. Terbinafine, an oral systemic antifungal belongs to the group of allylamines.

Voriconazole is a new wide spectrum triazole, available as an oral or intravenous drug. It is used in treatment of Fusarium spp infections of patients who do not tolerate other antifungal agents or of patients with resistant infections to other antifungals. It has been proved that voriconazole is more effective than amphotericin B (partial or complet response 53% versus 32% in treatment week 12, survival rate 71% versus 58%) in primary treatment of invasive aspergillosis. Like other triazoles, voriconazole is generally well tolerated (2, 7).

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The newest antifungal agents are from Echinocandins class – a new semi synthetic lipopeptides with a selective antifungal activity. Capsofungin is used in primary therapy of invasive candidosis, including candidemia, therapy of patients with resistant aspergillosis (or intolerant ones) to other antifungals. It has been proved that capsofungin has similar activity with amphotericin B in treatment of patients with candidemia. A good therapeutic response was obtained on 73.4% of the patients treated with capsofungin and on 61.7% of the patients treated with amphotericin B. Capsofungin was well tolerated besides amphotericin B (1).

Now there are different antifungal agents to study: a liposomal form of nystatin, new triazoles (posaconazole and ravoconazole), echinocandins (anidulafungin and micafungin) etc. So far it is known that: new agents of the respective class have a lower toxicity, drug interactions are less, pharmacokinetics and pharmacodynamic properties are more in favor, and possible a better activity on resistant pathogens (7).

MATHERIAL AND METHODS

This study took place in Clinical Emergency County Hospital Timisoara (SCJUT) and isolation and antifungal activity tests were performed at the Bacteriology Department of the Central Analysis Laboratory of SCJUT. Between January 2007- June 15th 2008, 564 biological samples were collected from the patients admitted in different wards of Clinical Emergency County Hospital Timisoara. Patients included in this study belong to both genders and are over 15 years old. Before including them in this study, all the patients were hospitalized at least 48 hours, so the nosocomial infection criterion was fulfilled.

Collected pathological samples were represented by: bronchial aspirates, sputum, wound secretions, blood for blood culture, urine, peritoneal fluid etc. Pathological samples were striked on Sabouraud with chloramphenicol and gentamicin media. The plates were incubated in aerobiosis environment, on 35-37° C, 24-48 h (up to 4 days).Gram stained smears were done from the pathological samples. Mycology diagnosis was based on colony appearance on culture media: Sabouraud and Chromagar (agar, peptone, special chromogenic mixture, chloramphenicol) (6, 8). For fungal identification we have used API 20 C AUX kits. Api Candida kits (sugar fermentation) allow quick identification (18-24 hours) of 14 yeasts species: C. albicans, C. famata, C. glabrata, C. guilliermondii, C. kefyr, C. krussei, C. lusitaniae, C. parapsilosis, Candida tropicalis, Cryptococcus neoformans, Geotrichum (candidum şi capitatum), Saccharomyces cerevisiae and Trichosporon sp.

Microscopy of culture smears was a method to confirm mycology diagnosis. Microscopicallly examined, A. fumigatus presents sporulare ends with vesicles and a chain of philides on the external two thirds of the surface. Conidia are disposed in parallel chains. A. niger has sporulare ends with conidiophores, globular vesicles and philides disposed in one or two rows all over the surface (10, 12).

Also, the antifungal sensitivity was determined, both by classical method of disk diffusion and by ATB fungus.

RESULTS AND DISCUSSIONS

During January 8, 2007 – June 15, 2008, 361 fungi strains were isolated from 191 patients. Fungal infections were proved on 71 women (37.17%) and 120 men (62.82%) with ages between 15 and 81 years old. (Figure 1) Pathological samples most frequent isolated were pharyngeal swabs (23.82%), blood (17.45%), bronchial aspirates (14.95%), urines (8.86%), wound secretions (8.58%) and lingual secretions (7.20%) (Table I).

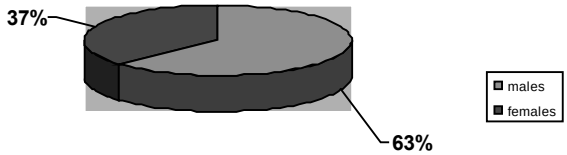

Fig. 1. Gender distribution of patients

Table I. Fungal strains distribution according to the pathological samples from where it have been isolated

Pathological sample	Number of strains	Percent
Pharyngeal swab	86	23,82
Blood	63	17,45
Bronchial aspirate	54	14,95
Urine	32	8,86
Wound secretion	31	8,58
Lingual secretion	26	7,20
Sputum	25	6,92
Catheter tip	15	4,15
Vaginal discharge	12	3,32
Peritoneal fluid	8	2,21
Ear discharge	6	1,66
Vomit liquid	3	0,83
Total	361	100%

Mycology analysis of the pathological samples allowed us the isolation of 361 fungi strains: 314 Candida sp. strains, 28 Cryptococcus neoformans strains, 19 strains of Aspergillus sp.

From 314 Candida sp. strains, 213 were Candida albicans and 101 Candida non albicans (C. tropicalis-46 strains, C. krussei-27 strains, C. glabrata-19 strains, and C. parapsilosis-9 strains). From 19 Aspergillus sp. strains, 12 were Aspergillus fumigatus, 4 Aspergillus flavus and 3 Aspergillus niger strains (Table II, III and IV).

Table II. *Candida* sp. strain distribution according to isolated pathological samples

Pathological sample	C. albicans	C. tropicalis	C. krussei	C. glabrata	C. parapsilosis
Pharyngeal swab	49	15	9	5	4
Blood	35	7	4	3	2
Bronchial aspirate	29	6	5	4	
Urine	13	3	5	1	2
Wound secretion	22	5		2	
Lingual secretion	20	2		1	
Sputum	19	4	1	1	
Catheter tip	10	1	1	1	
Vaginal discharge	9	2	1		
Peritoneal fluid	2	1	1	1	
Ear discharge	2				1
Vomit liquid	3				
Total	213	46	27	19	9

Table III. *Cryptococcus neoformans* strain distribution according to isolated pathological samples

Pathological sample	Cryptococcus neoformans
Pharyngeal swab	4
Blood	6
Bronchial aspirate	6
Urine	4
Wound secretion	2
Lingual secretion	2
Sputum	
Catheter tip	1
Vaginal discharge	
Peritoneal fluid	2
Ear discharge	1
Vomit liquid	
Total	28

Table IV. *Aspergillus* sp. strains distribution according to isolated pathological sample

Pathological sample	*Aspergillus fumigatus*	*Aspergillus flavus*	*Aspergillus niger*
Pharyngeal swab			
Blood	4	1	1
Bronchial aspirate	2	2	
Urine	3	1	
Wound secretion			
Lingual secretion	1		
Sputum			
Catheter tip	1		
Vaginal discharge			
Peritoneal fluid	1		
Ear discharge			2
Vomit liquid			
Total	12	4	3

During this study we have isolated 85 strains from the ICU ward of Clinical Emergency County Hospital Timisoara, 124 strains from the surgical wards of SCJUT (Reconstructive surgery – 31 strains, Surgical wards I, II and III – 17 strains, Orthopedics – 22 strains, Urology – 19 strains, Brain surgery – 18 strains, Vascular surgery – 17 strains), 33 strains in Nephrology department, Internal Medicine – 27 strains, Neurology – 10 strains, Gastroentrology - 18 strains, Cardiology – 18 strains, Endocrinology – 21 strains, Traumatology – 12 strains, Hemodialysis – 13 strains. These aspects are revealed in Figure 2

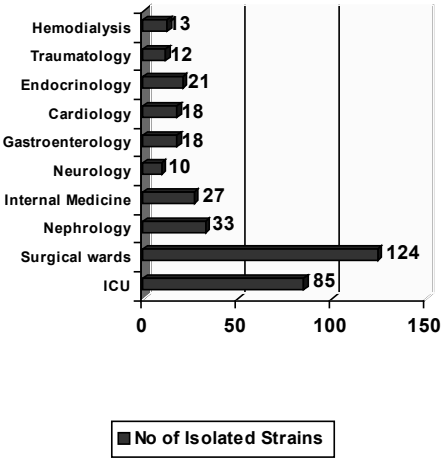

Fig. 2. Comparison between isolated strain numbers in SCJUT wards

In some pathological samples we have identified fungal and microbial association, especially Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter calcoaceticus baumannii complex. The most frequent associations were represented by Candida albicans + Staphylococcus aureus - 25 cases, Candida tropicalis + S. aureus- 11 cases, Candida albicans + Acinetobacter calcoaceticus baumannii complex – 7 cases, Aspergillus fumigatus + Pseudomonas aeruginosa – 6 cases, Cryptococcus neoformans + S. aureus – 5 cases, Aspergillus flavus + Escherichia coli – 2 cases, Aspergillus niger + Pseudomonas aeroginosa – 2 cases, Aspergillus niger + Klebsiella pneumoniae – 1 case (Figure 3).

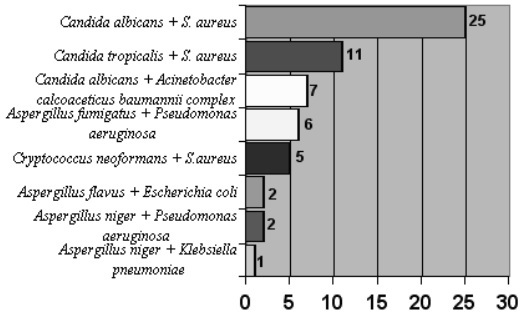

Fig. 3. Fungal and microbial association

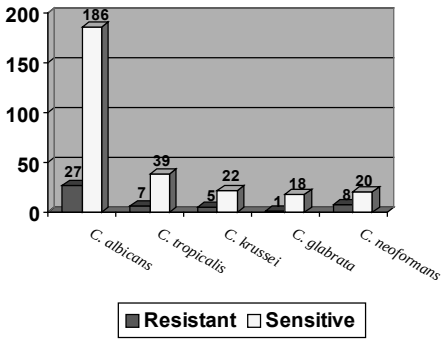

Fig. 4. Isolated fungal strains resistance to amphotericin B

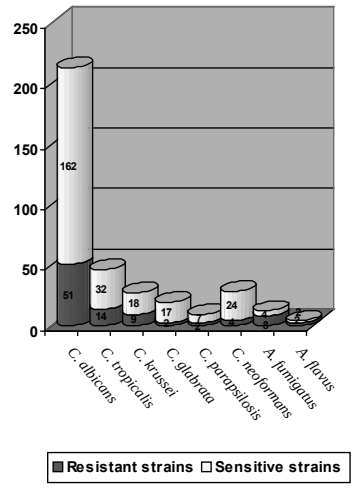

Fig. 5. Isolated fungal strains resistance to fluconazole

An important objective of this study was the antifungal sensitivity testing. This was performed for all 361 isolated fungal strains.

Disk diffusion test revealed a high resistance to amphotericin B for a number of isolates: C.albicans-27, C.non-albicans-13 (7 C. tropicalis strains, 5 C. krussei strains and 1 C. glabrata strain), Cryptococcus neoformans-8. Fluconazole resistance was present for: 51 strains of C. albicans, 27 C. non-albicans (14 C. tropicalis strains, 9 C. krussei strains, 2 C. glabrata strains and 2 strains of C. parapsilosis) and 4 Cryptococcus neoformans. High resistance to clotrimazole had: 61 strains of C. albicans, 37 strains of C. non-albicans (8 strains of C. tropicalis, 13 strains of C. krussei, 14 strains of C. glabrata, 2 strains of C. parapsilosis) and 3 Cryptococcus neoformans. 8 from Aspergillus fumigatus strains showed resistance to fluconazole and miconazole and 2 strains of A. flavus were resistant to fluconazole.

C. albicans strains were also tested to voriconazole. Form 213 C. albicans strains, 58 were resistant to voriconazole.

In this study were include patients hospitalized in different wards of Clinical Emergency County Hospital Timisoara, some of them being the second or the third time hospitalized. Blood cultures were performed only for febrile patients. 63 blood cultures were positive (51 Candida sp. strains, 6 Cryptococcus neoformans strains and 6 strains of Aspergillus sp.). In our study the oral candidosis was the most frequent infection, which led to isolation of 82 Candida sp. strains from pharyngeal swabs and 23 Candida sp. strains from lingual secretions. So it can be explained why repeated and often long treatment led to antifungal resistance. There are a lot of studies which proved that long time usage of fluconazole for oro-pharyngeal candidosis can led to fluconazole resistance by selective pressure, phenomenon which appears after using this drug for prophylaxis (5).

Like in other studies, Aspergillus sp. strains remained sensitive to amphotericin B, 10 Aspergillus sp. strains being resistant to fluconazole. Bronchial colonization with Aspergillus sp. was less frequent – 4 isolated strains from bronchial aspirates (2, 11).

Literature data show a marked and alarmed increasing of fungal strains number with resistance to antifungals. Thus, in Europe, similar studies have showed this, although resistance percent vary from country to country, partial because sensitivity data are obtained through different methods. Therefore it's implied to establish a standard method for antifungal sensitivity testing and a breakpoint for MIC, for a better observation of resistance tendencies. Antifungal resistance surveillance, study of resistance mechanisms, using in medical practice of some other antifungal classes to which the fungi are still sensitive, introducing in therapy of new antifungals (new triazoles: posaconazole, ravuconazole, echinocandins: anidaulafungin and micafungin) and preventing the spread of resistant strains are important measures required for impact control of these multiresistant microorganisms (11).

CONCLUSIONS

1. 361 fungal strains have been isolated from the hospitalized patients of SCJUT wards, during January 8 2007 – June 15 2008. Out of these, Candida sp. strains were 314, most of the strains being isolated from pharyngeal swab, blood and bronchial aspirates.

2. We have isolated 85 fungal strains in ICU ward of SCJUT, 124 strains from Surgical wards, 33 strains in Nephrology, Internal Medicine – 27 strains, Neurology – 10 strains, Gastroenterology – 18 strains, Cardiology – 18 strains, Endocrinology – 21 strains, Traumatology – 12 strains, Hemodialysis – 13 strains.

3. The most frequent fungal and microbial associations were represented by Candida albicans + Staphylococcus aureus - 25 cases, Candida tropicalis + S. aureus- 11 cases, Candida albicans + Acinetobacter calcoaceticus baumannii complex – 7 cases, Aspergillus fumigatus + Pseudomonas aeruginosa – 6 cases.

4. For some fungi strains we have pointed out an increased resistance to fluconazole, clotrimazole, miconazole and a good sensitivity to amphotericin B.

5. An alternative to the actual treatment of fungal nosocomial infections is the use in medical practice of some antifungals to which the fungal strains are sensitive (voriconazole, capsofungin etc.).

6. The number of resistant fungi is continuously increasing in hospitals around the world and nocomial infections treatment for these microorganisms requires high costs, it's implied to introduce some guides and protocols for prevention and control of resistant fungal strains spreading.

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SENSIBILITATEA FUNGILOR IZOLATI DIN SECTIILE SPITALICESTI CU RISC CRESCUT

REZUMAT

Scop: Scopul urmărit în acest studiu a fost determinarea sensibilității la antifungice a speciilor de fungi cu potențial nosocomial izolate în secțiile cu risc crescut ale Spitalului Clinic Județean de Urgență din Timişoara.

Material şi metoda: Studiul a fost efectuat în perioada 8 ianuarie 2007-15 iunie 2008. În acestă perioadă am recoltat un număr de 564 de probe biologice de la pacienții internați în secțiile Spitalului Clinic Județean de Urgență Timişoara. Produsele patologice izolate de la pacienții internați au fost însămânțate pe mediul Sabouraud cu adaus de cloramfenicol şi gentamicină. Am utilizat pentru identificarea fungilor galerii API 20 C AUX. S-a determinat de asemenea şi sensibilitatea la antifungice atât prin metoda clasică a antifungigramei difuzimetrice cât şi prin utilizarea ATB fungus.

Rezultate: Analiza micologică a produselor patologice ne-a permis izolarea unui număr de 314 tulpini de de Candida sp., 28 tulpini de Cryptococcus neoformans, 19 tulpini de Aspergillus sp. Antifungigrama a arătat rezistență crescută la amfotericină B pentru un număr de izolate: C.albicans-27, C.non-albicans-13, Cryptococcus neoformans-8. Rezistență la fluconazol au avut: 51 tulpini de C. albicans, 27 C.non-albicans şi 4 Cryptococcus neoformans.

Concluzii: Numărul tulpinilor de fungi cu rezistență la antifungice fiind în continuă creştere în instituțiile spitalicești din întreaga lume, iar tratamentul infecțiilor nosocomiale cu aceste microorganisme implicând costuri mari, se impune implementarea unor ghiduri şi protocoale de prevenire şi control a răspândirii tulpinilor de fungi rezistente.

Cuvinte cheie: sensibilitate la antifungice, fungi cu potențial nosocomial, infecții fungice, probe biologice, secția de terapie intensivă

REVIEW NEW LOOKS ON THE BIOLOGICAL MARKERS FOR ATHEROSCLEROSIS IN CORONARY PATIENTS

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ABSTRACT

At this moment, there are several models for the evaluation of the cardiovascular risk in the general population. These models include the classical cardiovascular risk factors: dislipidemy, high arterial essential blood pressure, diabetes, smoking, etc. Such models are not perfect, that is why, in the last years, a large number of biochemical parameters have been evaluated in several studies. The data existing today allows us to centralise these biological markers for atherosclerosis in five groups according to the pathogenetic mechanism: inflammation markers, lipoproteic markers, haemostasis markers, oxidation markers and other markers, all of these biological markers for atherosclerosis being presented in this paper.
Key words: risk factors, markeri, dyslipidemia, hypertension, atherosclerosis

INTRODUCTION

The classical factors of cardiovascular risk (dislipidemy, arterial blood pressure, diabetes and smoking, etc) have been integrated in multiple models for the evaluation of cardiovascular events risk in the general population (1). This concept of global estimation of the risk allows a more accurate prediction of the cardiovascular risk in coronary patients (9).

As it has been demonstrated in multicentric studies (7), the traditional risk factors explain over 90% of the myocardial infarct (7). The algorithms developed on the basis of these classical risk factors allow a classification of the individuals into three risk categories: low, medium and high (8). Nevertheless, one has to take into account the fact that these models are not perfect. It is demonstrated that one third of the patients initially classified as having a medium cardiovascular risk were the victims of a myocardial infarct. Therefore, during the last years a large number of biochemical parameters were evaluated as having potential cardiovascular risk (5). It were established the following biological parameter in order to determine its use in the clinical practice as a cardiovascular risk factor (Table I).

Table I. The Biological markers for atherosclerosis in coronary patients

Inflammation markers	Lipoproteic markers	Haemostasis markers	Oxidation markers	Other markers
Ultrasensitive PCR	Lp	fibrinogen	ADMA (asimetric dimethilarginina)	microalbuminuria
Seric Amiloid A (SAA)	LDL	plasma D-dimers	Lp-PLA2	natriuretic peptide
cytokine (IL6, IL8)	Residual lipoproteins	fibrinopeptides	homocystheina	adiponectina
Cell Adhesion molecules	oxidized particles of LDL	Plasminogen Activator Inhibitor-I (PAI-I)		
(VCAM-I, ICAM-I)	genotypes of Apolipoproteina E	Tissue Plasminogen Activator (TPA)		
LP-PLA2 (lipoprotein asociated to A2)		Coagulation Factors V, VII, VIII		
CD4 solubil ligand		activation and aggregation of thrombicytes		
number of leucocytes				

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The evaluation of these biological markers is precise and reproducible by means of standard international measures and they are better correlated with the evolution of patients in comparison with the old cardiovascular risk factors (3). The amelioration of the predictive value of the new cardiovascular risk factors has also greater importance, especially in subgroups of patients (e.g. women, kidney disease, cardiovascular disease, etc) as well as for therapy and finally for the knowledge of the psychopathological mechanism witch explains its role in the process of atherogenesis.

INFLAMMATION MARKERS

Ultrasensitive PCR and lipoprotein associated to phospholipase A (Lp-PLA2) are the two markers of inflammation with prognostic value in ATS (atherosclerosis) (3). There is a major interest in the ultrasensitive PCR, because its major role in the pathogeny of ATS is already known, representing an independent RF (risk factor). PCR with values over 1mg/l are considered to be associated with the moderate increase of risk, which becomes high with values over 3 mg/l. Numerous studies show that the decrease of ultrasensitive PCR induced by therapy with satins, besides the reduction of cholesterol LDL, determines additional clinical benefits(1). The patients with the most reduced levels of LDL cholesterol and of the ultrasensitive PCR benefit most of the treatment with satins (PROVEIT TIMI 22). The completion of the JUPITER study (for primary prevention) will bring decisive information to the patients with normal cholesterol, but with increased values of the ultrasensitive PCR(7).

Lp-PLA2, secreted by the inflammatory cells, monocytes, T cells and mastocytes is associated with high risk for cardiovascular events, independent of traditional RF and other inflammatory RFs. In addition, Lp-PLA2 is a RF for the relapse of the coronary accident.

LIPOPROTEIC MARKERS

The lipoproteic markers are numerous and many of them are known, but a special importance in the ATS process have the lipoprotein A (Lp A), small dense particles of LDL cholesterol and the apoliprotein E(2).

Lp A is the form under which the total cholesterol is transported in the blood, only 6% is transported in the form of Lp B. There are already many studies which have proved the connection between the blood level of Lp A and the coronary risk. Most laboratories have fixed the level of 300 mg/l as the threshold for cardiovascular risk. The fact that Lp A is a risk factor for stroke with elderly and young persons is already known. Unfortunately, medication does not influence the blood level of Lp A, with the exception of niacin (it reduces by 25% the Lp A level in the blood) and of anabolisant steroids(4).

The small and dense particles of LDL cholesterol are always present in trigliceridemy and type 2 diabetes. The macrophages in the arterioles phagocyte these particles and become yeasty cells, characteristic for the incipient phase of ATS(7). The biological effect of these small and dense particles of LDL cholesterol are the endothelial dysfunction, the recruitment of monocytes, the molecular expression of adhesion molecules at the surface of endothelial cells and the formation of yeasty cells.

The apolipoprotein E has a multifunctional role: it is involved in the lipidic metabolism and it has also a neurobiological function. In the plasma, it is present in three isoform: apo E2, E and E4. The most frequent isoform of the apolipoprotein E is apoE3, with an important role in the lipidic homeostasis. It seems that apo E4 has an important role in the pathogeny of Alzheimer disease, and apo E2 is involved in the pathogeny of the Fredrichson disease (8).

THE HAEMOSTASIS MARKERS

The final event in the pathogeny of ATS is thrombosis, which lies at the basis of myocardial infarct and of the ischemic stroke (4). This is the reason why great accent was put on the study of the coagulation factors, out of which the fibrinogen has an important role.

In many studies the fibrinogen was identified as an independent cardiovascular RF. Just like PCR, the fibrinogen is an acute phase reactive (2). Therefore, the fibrinogen is not useful in the estimation of the cardiovascular risk in the case of acute phase diseases. Currently, the serum value of the fibrinogen is not established consensually, but most clinical studies established the value of 3.5 g/l as the threshold from which the cardiovascular risk increases.

D-dimer is a marker of the activation of coagulation and the formation of the fibrin, as opposed to fibrinogen. With coronary patients, the increase of D-dimerics is associated with the increase of the cardiovascular risk (3).

THE OXIDATION MARKERS

An important process in the ATS pathogeny is the oxidative lipidic stress. The best studied markers are the already discussed Lp A, ADMA (asymmetric dimethyl arginine) or homocysteine (5). ADMA is an endogen inhibitor of all the major isoform of the endothelial synthesis of ON, thus contributing to the endothelial dysfunction. The ADMA values are higher in the case of IR, hypercholesteromy, hypertrigliceridemy, insulin resistance, diabetes, hyperhomocysteinemy, and high blood pressure. ADMA was identified as an independent cardiovascular RF of the ATS progression and of the cardiovascular mortality, of the total mortality in coronary patients (6).

Homocystein is an independent cardiovascular RF with a lower predictive value than the traditional RF, or than PCR and fibrinogen. Homocystein increases the cardiovascular risk of the subjects with higher global risk. The limit values of the homocystein vary between 10-16 ÷mol/l. The folic acid or its association with vitamin B6 and B12 reduce the value of the homocystein, but the studies concerning the decreasing of the level of homocystein are not conclusive yet (8).

OTHER MARKERS

Microalbuminuria (30-300 mg/24 hours) is considered a micro or macrovascular lesion marker in diabetes patients. Because of this reason and of the therapeutic benefit proved by IECA and the antagonists of the angiotensine II reception in the presence of albuminuria, certain recommendations suggest the identification of these urinary parameters for the prediction of the cardiovascular risk with diabetes and high blood pressure patients(8). It has been demonstrated in the specialized literature that microalbuminuria is a cardiovascular risk marker, going beyond the traditional RFs, but the use of this fact is limited because there is no clear consensus concerning the values of this marker.

The albumen concentration in the spontaneous urine has a good correlation with the urinary secretion/24 hours, only if the urine is collected in a well defined way (the second urine in the morning). The value of this parameter is strengthened by the ratio between urinary albumen and creatinine, ratio which depends on the muscle mass and requires reference to age and sex (2).

The association between advanced chronic kidney failure and the cardiovascular risk has been known for years. In the Rotterdam study it was proved that the cardiovascular risk is already increased in the case of deteriorated kidney function with normal creatinine, but also with increased C cyctatine (kidney function marker more sensitive than creatinine). The increase of the C cyctatine value is associated in many studies with the cardiovascular risk (11).

The adiponectine is a new marker which has a antiaterogenetic activity and is produced solely by the white adipose tissue. The adiponectine is reversely correlated with the resistance to insulin and the endothelial dysfunction (10). Low values of the adiponectine are associated with NO in low concentrations and with angiotensine II with high values. Knowing this, we can say that the adiponectine has an important role in the prediction of the cardiovascular risk. Currently, there is only one prospective study performed in the USA, with evidence concerning the significant reduction of the myocardial infarct risk associated with high levels of adiponectine(11).

BNP and pro-BNP is a peptide secreted by the cardiac muscle cells, as a response to the increase of the pressure consecutive to the ventricular volumetric increase and secondary to a pressure overcharge(9). Active BNP is secreted in the circulation in association with an inactive amono-terminal peptide (NT pro-BNP).

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NOI PERSPECTIVE ASUPRA MARKERILOR BIOLOGICI AI ATEROSCLEROZEI LA PACIENTII CORONARIENI

REZUMAT

In prezent, se cunosc cateva modele de evaluare a factorilor de risc cardiovasculari in populatia generala. Aceste modele include factorii clasici de risc cardiovascular: dislipidemia, hipertensiunea arteriala, diabetul zaharat, fumatul, etc. Insa aceste modele nu sunt perfecte si de aceea, in ultimii ani, s-a studiat un numar foarte mare de parametric biologici. Datelor existente astazi ne permit centralizarea markerilor biologici aterosclerotici in cinci grupe, dupa mecanismul pathogenic presupus: markerii aterosclerotici inflamatori, markerii aterosclerotici lipoproteici, markeri aterosclerotici ai hemostazei, markeri biologici si alti markeri aterosclerotici importnanit, toti acesti markeri fiind prezentati in lucrarea de fata.

Cuvinte cheie: factori de risc, markeri, dislipidemie, hipertensiune, ateroscleroza

USE OF EMG BIOFEEDBACK AS A METHOD OF MUSCULAR TRAINING DURING PHYSICAL EXERCISE IN ELDERS

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ABSTRACT

Objectives: Starting from the premise that physical exercise plays an important role in the maintenance of the clinical and functional status at elderly persons, we have proposed in the present study to follow the way in which associating the physical exercise program to the EMG biofeedback (EMGBF) as a muscle training method, can qualitatively and quantitatively influence its efficiency.

Methods: We performed a study on 30 patients aged > 65, divided in a randomized way into two groups. They were submitted to an identical force and resistance training program during 10 weeks. The EMGBF training was added to the experimental group. The patients were functionally (The Fullerton fitness test for seniors, global functional level through the ADL scale and the HAQ – DI scale, the Geriatric Depression Scale - GDS) and electrophysiologically (through surface EMG measuring the amplitude of contraction, of relaxation, the rise time, the drop time) evaluated. Such evaluations were performed at the beginning and at the end of the training period.

Results: There were no significant differences of the functional and electrophysiological parameters between the studied groups, before the beginning of the exercise program. After 10 weeks, all the functional and electrophysiological parameters increased on both groups, significant differences being recorded with the muscle force, balance, coordination, walking speed, in favor of the group which had BFEMG training.

Conclusions: Physical exercise has benefic effects on the fitness level, on the global functional status and on the depression condition, at elderly persons. Associating EMGBF to the training program provides several important advantages: objective diagnosis, targeted training, good control over the muscle activity phases, customized protocols, close communication patient-therapist, inciting, ludic therapy modality.

Key-words: elders, physical exercise, EMG biofeedback

INTRODUCTION

Physical activity is the factor with a determining role in the preservation as long as possible of the best functional status (fitness), and also in preventing/improving most of the chronic diseases on elderly persons (1). The regular practice of physical exercise ensures the increase of functional capacity, prevents osteoporosis, cardiovascular diseases, diabetes, obesity and depression, improves neuromuscular balance and coordination, and reduces mortality (2).

In general, exercise is practiced at a moderate intensity level. This can be monitored through a heart frequency level of 60-79% of the maximum frequency and a blood pressure under the risk values, or 11-13 quotation on the physical effort perception Borg scale. The exercise rhythm varies with each patient, according to the pursued objective and to the exercise type. The average rhythm is 3 times/week.

Unfortunately, elderly persons are rather reticent when it comes to regular practice of physical exercise, both as a therapeutic method, but mostly as a means of maintaining the good health. That is why we have proposed to introduce in the therapy "unit" an unconventional method – therapy through EMG biofeedback.

Biofeedback is a technique using an electronic equipment, in order to reveal to a certain person a part of his own physiological events, normal or pathological, as audio and/or visual signals, with a view to teach the person to manipulate such events, otherwise involuntary, by manipulating the displayed signals (3).

EMG biofeedback (EMGBF) is based on collecting the muscular bioelectric potentials through global electromiography, through the means of surface electrodes, their magnification and integration. This surface EMG, provides us an indirect measure of the subjacent striated muscles activity. The number of activated motor units increases gradually, with the increase of the developed force, achieving the spatial sumation or recruitment. Reversely, muscular relaxation is characterized by a reduction of motor units' electric discharges. EMG signals have three-phase morphology, its constituents being situated on both sides of the zero line. In order to benefit from all such constituents, all interchanges situated on one side of the zero line are redressed, resulting in the redressed EMG curve (full - wave rectification). The global surface of the redressed potentials is then automatically calculated and produces a curve with amplitude quantifying the muscular activity degree (signal integration). The muscular contraction is then acknowledged by the patient, through audio or visual signals, with an intensity which is directly proportional with the completed activity. The parameters of such signals can be introduced into the computer, thus facilitating the pursuit in time of the patients' evolution (4).

In the present paper we have approached the EMGBF use as a means of muscular training on elderly persons, within a complex program of physical exercise, conceived according to the needs established through a clinical and functional evaluation. We have followed the way in which associating EMGBF as a method of physical and behavior therapy to the exercise program can influence its efficiency from a qualitative and quantitative point of view (better results in a shorter period of time), and also the influence of such method on some psychical parameters like depression, attention, participation.

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MATERIAL AND METHOD

The study was performed on thirty patients, who showed themselves at the specialty medical office in Baile Calacea Resort during June 2008 - January 2009, for balneal-physiotherapy treatment. The criteria according to which the patients were included in the study were represented by the age of > 60 years old, the absence of cognitive troubles and of some absolute counter indications for physical exercise performance. Such counter indications are: unstable pectoral angina, uncompensated cardiac insufficiency, symptomatic severe aortic stenosis, hypertrophic cardiomyopathy, severe pulmonary hypertension, rest systolic tension > 200 mm Hg, rest diastolic tension > 110 mm Hg, pericarditis or acute myocarditis, dissecting aneurysm, thrombophlebitis, recent systemic or pulmonary emboli.

The patients' written approval was obtained, after they had been explained the study purpose, the performed measurements, the used means of treatment, the benefic effects and the existent risks. Anonymity was guaranteed.

The 30 patients were divided in a randomized way into 2 groups, made of 15 subjects: group A, as a control group and group B, for study. The allocation of patients in the 2 groups according to demographic parameters (age, sex, provenance environment and degree of education), is the following (Table I):

Table I. Structure of study groups based on demographyc parameters

Parameter		Group A (control)	Lot B (study)	Total
Age	60 - 75 years (youg old)	13 (86.66%)	14 (93.33%)	27 (90%)
	76 - 85 ani (middle old)	2 (13.33%)	1 (6.66%)	3 (10%)
Gender	Male	7 (46.66%)	6 (40%)	13 (43.33%)
	Female	8 (53.33%)	9 (60%)	17 (56.66%)
Residence	Urban	5 (33.33%)	4 (26.66%)	9 (30%)
	Rural	10 (66.66%)	11 (73.33%)	21 (70%)
Education	Primary school	5 (33.33%)	6 (40%)	11 (36.66%)
	High school	8 (53.33%)	7 (46.66%)	15 (50%)
	Faculty	2 (13.33%)	2 (13.33%)	4 (13.33%)

The main affection that the patients came at the medical office for was represented by osteoarthritis with different locations (vertebral - spondylosis and peripheral - coxarthrosis, gonarthrosis), light and medium forms (the advanced ones being recommended for surgery treatment). The patients also presented other associated diseases like: osteoporosis, cardiovascular diseases (ischemic cardiopathy, AH, varicose disease), pulmonary diseases (chronic bronchitis), metabolic diseases (hyperglycemia, dislipidemies, and obesity), and light visual deficiencies. Such associated diseases were evenly allocated within both groups, being in compensated states, under treatment. Their existence does not influence the study results.

The patients' evaluation was performed from a functional and electrophysiological point of view in two moments: before the beginning of the treatment and at the end of the proposed exercise program. The obtained values were compared one to the other, both within the same group (initial score – final score), and between groups. The initial scores were compared among them in order to avoid too big differences between the groups, while the final scores were compared in order to find out whether there was a statistically significant difference between the efficiency of the two practice methods.

The initial clinical evaluation comprised a general clinical exam, the measurement of the heart rate (HR) and of the rest TA, of the body weight (W) and the calculation of the body mass index (BMI). This evaluation was performed in order to identify the patients with possible counter indications for physical exercise and to evenly allocate the subjects in the two groups according to the associated pathology.

The functional evaluation comprised:

1. The global evaluation of the fitness level with the Fullerton fitness Test for seniors (5), which consists of 6 tests evaluating:

- the flexibility (expressed in centimeters) of the body upper extremity: "back scratch" and lower extremity respectively: "sit and reach"
- the muscular force (expressed in number of repetitions) of the body upper extremity: "arm curl" and lower extremity respectively: "30 second chair stand"
- the moving abilities and the dynamic balance: the "up and go" test (expressed in seconds)
- the aerobic resistance (expressed in number of repetitions): "2 minute marching step".

2. The estimation of the general functional status through a general score, with the ADL sheet: activities of daily living, of the Illinois University, short form (ADL score) (6), and also through the Health Assesment Questionnaire Disability Index : HAQ - DI (HAQ – DI score) (7) .

3. Depression was evaluated using the Yesavage's Geriatric Depression Scale, short form (with 15 items) (8), the evaluation result being under the form of a global score (GDS score).

Before beginning the study, the tolerance of patients to effort was also tested, in order to establish the initial intensity level of physical exercise. The six-minute walk test (6MWT) was used, well tolerated and easy to perform by elders, which measures the distance that the subject can cover walking on a flat, hard surface, during 6 minutes. This test is performed at a submaximal level of the functional capacity, in accordance with the level at which most of the daily activities are performed (ADLs) (9).

The electrophysiological evaluation consisted of measuring some parameters obtained as a result of the surface EMG (EMGs) recording. In the present study we used the Myomed 134 appliance manufactured by the company Enraf-Nonius. This appliance recorded the EMGs and it facilitated the practice through EMG biofeedback. The appliance was connected to a computer, thus the records were graphically presented and kept in the PC memory.

The EMG signal detection was accomplished through some self-adhesive single-use electrodes (Blue sensor Ambu - Denmark). The detection electrodes were placed in parallel with the muscular fibers, on both sides of the motor points, compared to the position that the muscle has during the contraction phase. The distance between the electrodes was 10 mm (10). Recordings were performed on two groups of muscles: for the upper member on the brachial biceps (the dominant arm) and for the lower member on the quadriceps muscle (the dominant member).

The evaluation protocol consisted of the following stages, each of them estimating a certain phase of the muscular activity:

1.) 60 sec. of pause – evaluation of the muscular relaxation (normal values = 1 - 2 μV or < 10 % of the maximum developed force);

2.) 5 contractions of 1 sec. with 10 sec pauses between contractions – evaluation of the phasic contraction;

3.) 5 contractions of 1 sec (during 10 sec.) = quick flicks – evaluation of the coordination;

4.) 5 contractions of 10 sec. with 10 sec. pause between contractions – evaluation of the tonic contractions;

5.) 3 submaximal contractions of 20 sec. with 20 sec. pause between contractions – evaluation of the muscular resistance (in case of muscular fatigue, or of decrease of muscular resistance, a great variability is recorded in the maximum amplitude) ;

6.) 60 sec. of pause.

After the EMGs recording the following were determined: the amplitude of the integrated signal (expressed in μV) and the standard deviation for each contraction and relaxation period (the appliance directly providing an average of the EMG signal amplitude for such phases), "rise time" (the rise time at contraction beginning), "drop time" (the return time at contraction end), expressed in seconds (normal values ≤ 2 sec.) and the variability coefficient (the ratio standard deviation/ amplitude). Electrophysiological recordings were also performed in two moments – at the beginning and at the end of the study, the data being compared among them. It was considered that a good evolution of the muscular function is given both by the increase of the contraction amplitude and the decrease of the relaxation amplitude, but mostly by the decrease of the contraction variability coefficient and by the value decrease of the "rise time" and "drop time", respectively (11).

After the completion of individual evaluations, the patients have started the exercise program. It was performed during 10 weeks, with a rhythm of 3 times/ week, at a moderate intensity level, relevant for a HR representing 75% of the max. HR (calculated according to the formula 220 - age) or for a 12 - 13 level on the physical effort perception Borg scale (RPE).

For group A (control), each exercise session lasted for approximately 55 minutes and consisted of the following protocol: 3 - 5 min. of warming up (light pedaling on the ergometric bicycle), 20 min. of exercise for muscular force strengthening, 5 min. of pause, 20 min. of endurance exercise, 5 min. of cooling (free walking or stretching exercise performed in a very slow rhythm). The force exercise was customized according to the evaluations, but they especially followed to train the hip flexors, extensors and abductors, the knee flexors and extensors and the plantar flexors, such muscles also being involved in the accomplishment of a good balance. The MS flexors were also trained. Resistance was set at 50% of the maximum level. 3 sets of 10 repetitions were performed for each muscle group. Progressivity was accomplished by increasing the lifted weight (evaluation at every 2 weeks of practice). Endurance exercise was performed on the ergometric bicycle, starting with a load of 25 W (according to the effort capacity), the intensity level being controlled by permanently monitoring the target HR (electronically displayed on the bicycle LCD screen). The BP was measured at the beginning an dat the end of each practice session.

Group B (study) was submitted to the same therapy protocol, to the exercise for muscular force strengthening being associated the EMG biofeedback practice.

For the biofeedback practice there have been selected the same muscle groups like in the force practice of group A: the quadriceps muscle, the middle buttock, the femoral biceps, the femoral triceps, the brachial biceps. The electrodes for the EMG potentials recording were located according to Zipp's recommendations, related to the indicated anatomic marks (12).

The specific EMG biofeedback practice was initially performed on an EMG channel for each muscle group, then, as the patient became more acquainted with the therapy, two EMG channels were used for the concurrent practice of two symmetric (right – left) muscle groups. The following protocols were used:

- Protocol 1: 5 sec. of contraction, 10 sec. of relaxation (protocol work - rest); repeated for 10 times for each muscle group (isotonic contraction);
- Protocol 2: progressive contraction, 20 sec. of maintenance, progressive relaxation (isometric contraction)
- Protocol 3: 5 rapid contractions in 10 seconds (quick fliks) – coordination practice
- Protocol 4: pattern type: graphics of various shapes appear on the screen, the patient has to maintain his level of muscle activity so that a slider present on the screen should follow the profile of such graphics.

Protocos 1 and 2 were used at the beginning of the practice program, then protocols 3 and 4 will be introduced after 2 weeks of practice.

These protocols were introduced into a program customized for each patient and stored in the appliance memory.

In addition to the visual biofeedback, an audio one was also used, the one generally used being a continuous sound appearing at a contraction intensity over the limit value. This value was determined by making the patient perform a maximal contraction. During the sessions, the limit value changed (increased).

The total duration of the EMGBF practice was 20 minutes.

RESULTS

The following tables present the final values (the arithmetic average) of the parameters obtained as a result of the functional and electrophysiological evaluations on the two groups under study. These values, compared with the unpaired t-test are presented bellow

(tab. 2, 3, 4).

Table II. Comparison between functional parameters at the two groups

EVALUATION		GROUP A	GROUP B	P
FITNESS	back scratch (cm)	8.4 ± 2.3	8 ± 1.3	0.88 (NS)
	sit and reach (cm)	1.4 ± 0.9	1.2 ± 0.9	0.88 (NS)
	30 sec. chair stand (no of repetition)	14.8 ± 2.3	18 ± 2	0.046 (< 0.05)
	arm curl (no of repetition)	13.8 ± 1.3	17.6 ± 0.8	0.049 (< 0.05)
	up and go (seconds)	9.8 ± 3.1	6.2 ± 1	0.049 (< 0.05)
	2 minute marching step (no of repetition)	93 ± 9.7	107.8 ± 9.7	0.042 (< 0.05)
FUCTIONAL STATUS	ADL score	168.6 ± 6.6	171.4 ± 3.5	0.47 (NS)
	HAQ - DI score	23.2 ± 1	23.8 ± 0.4	0.38 (NS)
DEPRESSION	GDS score	5.2 ± 0.8	4.6 ± 1.1	0.37 (NS)

Table III. Comparison between electrophysiological parameters of quadriceps muscle at two groups

PARAMETER	GROUP A	GROUP B	P
Contraction amplitude - mean (μV)	34.8 ± 14.1	49.4 ± 11.9	0.11 (NS)
Relaxation amplitude - mean (μV)	4.2 ± 0.8	2.4 ± 0.4	0.005 (< 0.01)
Rise time (sec)	3.2 ± 0.7	1.8 ± 0.2	0.01 (< 0.05)
Drop time (sec)	2.76 ± 0.4	1.82 ± 0.1	0.005 (< 0.01)

Table IV. Comparison between electrophysiological parameters of brachial biceps at two groups

PARAMETER	GROUP A	GROUP B	P
Contraction amplitude - mean (μV)	46.35 ± 15.3	55.7 ± 12.4	0.06 (NS)
Relaxation amplitude - mean (μV)	4.3 ± 0.5	2.15 ± 0.3	0.03 (< 0.05)
Rise time (sec)	3.35 ± 0.8	1.82 ± 0.2	0.03 (< 0.05)
Drop time (sec)	2.7 ± 0.5	2 ± 0.2	0.049 (< 0.05)

DISCUSSIONS

Comparing the initial values of the functional and electrophysiological parameters on the two groups, no significant difference was identified among them.

Comparing the evolution of the functional parameters, we observed the following:

- the evolution of parameters within the same group shows us a significant increase, both for the witness lot and for the experimental one, on all the followed parameters ($p < 0.05$).

- there is a statistically significant difference (a better evolution on the EMGBF group) in the evolution of fitness parameters: 30" chair stand, arm curl, up and go and 2 minute marching step. This shows a better increase of the muscle force (especially on the lower body), of the balance, coordination and walking speed.

- among the final values of the parameters estimating flexibility, the global functional status and depression, no statistically significant differences were observed between the two groupss ($p > 0.05$).

Comparing the evolution of electrophysiological parameters both in the recording performed on the brachial biceps muscle and on the lower right muscle, we observed:

- there are significant differences between the initial and final values within both lots, on all the followed parameters ($p < 0.05$).

- no significant differences were recorded between the lots regarding the increase of the muscle contraction amplitude ($p > 0.05$).

- there are, though, statistically significant differences in the evolution of the other electrophysiological parameters (relaxation, rise time, drop time) in favor of group B, such parameters showing an improvement of the contraction quality (increase of the muscle fibers recruitment, increase of the reaction speed, coordination improvement, muscle resistance improvement).

CONCLUSIONS

After the study it was proved the benefic effect of physical exercise on

the fitness level, on the global functional status and on the depression condition, at elderly persons.

Associating EMGBF to the training program provides several important advantages:

- it allows an objective diagnosis of the muscle function, determining certain well established recovery objectives and objectively estimating the final results (3).

- it permanently ensures the control of the muscle activity, providing us the possibility to follow the performance correctness, the exact moment of settlement of muscle fatigue (13).

- it allows the accomplishment of progress not only due to the quantitative aspect, but also to the qualitative one (which can be more rapidly observed), by acknowledging the action that must be performed and by differentiating the relaxation – contraction phases (12).

- it provides the possibility to establish some customized protocols, to memorize them and to record them for comparison.

- it can be even used at lower values of the muscle functional capacity, by establishing lower levels of the limit activity value and by gradually increasing it (better control over exercise progress).

- a closer communication is accomplished between the patient and his therapist.

- it is a modern method, the patient being involved in an inciting, even ludic way.

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UTILIZAREA BIOFEEDBACK-ULUI EMG CA METODĂ DE ANTRENAMENT MUSCULAR ÎN CADRUL EXERCIȚIILOR FIZICE LA VÂRSTNICI

REZUMAT

Obiective: Pornind de la premiza că exercițiul fizic joacă un rol important în menținerea statusului clinico-funcțional la persoanele în vârstă, în studiul de față ne-am propus să urmărim modul în care asocierea la programul de exerciții fizice a biofeedback-ului EMG (BFEMG) ca metodă de antrenament muscular, poate să influențeze eficiența acestuia din punct de vedere calitativ și cantitativ.

Metode: Am efectuat un studiu pe 30 de pacienți cu vârste > 65 ani, împărțiți în mod randomizat în două loturi. Aceștia au fost supuși unui program identic de antrenament de forță și rezistență pe o perioadă de 10 săptămâni. La lotul experimental s-a adăugat antrenamentul prin BFEMG. Pacienții au fost evaluați din punct de vedere funcțional (Testul de fitness Fullerton pentru vârstnici, nivel funcțional global prin scala ADL și scala HAQ - DI, Scala Geriatrică pentru Depresie - GDS) și electrofiziologic (prin EMG de suprafață măsurând amplitudinea contracției, a relaxării, rise time, drop time, coeficientul de variabilitate a contracției). Aceste evaluări s-au făcut inițial și la sfârșitul perioadei de antrenament.

Rezultate: Nu au existat diferențe semnificative ale parametrilor funcționali și electrofiziologici între grupele studiate, înainte de începerea programului de exerciții. După 10 săptămâni, toți parametrii funcționali și electrofiziologici au crescut la ambele grupuri, diferențe semnificative înregistrându-se în ceea ce privește forța musculară, echilibrul, coordonarea, viteza de mers, în favoarea grupului ce a făcut antrenament prin BFEMG.

Concluzii: Exercițiului fizic are efecte benefice asupra nivelului de fitness, statusului funcțional global și a stării de depresie, la persoanele în vârstă. Asocierea BFEMG la programul de antrenament oferă câteva avantaje importante: diagnostic obiectiv, tratament țintit, bun control asupra fazelor activității musculare, protocoale individualizate, comunicare strânsă pacient-terapeut, modalitate incitantă, ludică de terapie.

Cuvinte cheie: vârstnici, exerciții fizice, biofeedback EMG

SMOKING AND SALIVARY ANTIOXIDANT CAPACITY

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ABSTRACT

Cigarette smoke contains a large amount of oxidative species, and therefore smoking represents a significant source of oxidative stress. Saliva possesses protection mechanisms from oxidative attack. Smoking is one of the main and most prevalent, risk factor for chronic periodontitis and oral tumors.
The purpose of the study was to investigate the smoking effect on salivary antioxidant capacity, appreciated by glutathione level (GSH).
Material: There were recruited 10 persons (non-smoker group) and into the smoker group 14 healthy subjects who reported smoking more than 10 cigarette/day, for more than 2 years. Un-stimulated whole saliva samples were obtained in the morning, after two hours of tooth brushing and smoking. Salivary flow was noted. Salivary glutathione was performed by dithio-bisnitro-benzoic acid method (DTNB), in 1-2 hours after saliva collection, in order to avoid the release of GSH from destroyed cells.
Results: GSH level into the non-smoker group was higher (63.43 ± 32.64 microM/L), than into the smoker group (60.44 ± 28.83 microM/L). Salivary flow was 0.414 ± 0.171 ml/min. to non-smoker compared with 0.55 ± 0.14 ml/min. in smoker group. Also, in smoker group was observed high levels of desquamated epithelial cells and the decrease of cell viability under 90%. Early after one cigarette smoking the salivary flow and GSH increased.
Conclusion: Smoking modified the GSH levels and can produced noxious effects on oral cavity.
Key words: smoking, salivary GSH, antioxidant capacity

INTRODUCTION

Smoking is undoubtedly one of the main, most prevalent, risk factor for tumors. Tobacco smoking certainly modifies oral response to any injuries, such as microbial challenge or malignant transformation of cells. Smoking influences angiogenesis, adhesion molecule profiles, leucocytes recruitment, and multiple aspects of leucocytes development and function (1, 2).

Reactive oxygen species (ROS) play an important role in cell signaling and metabolic processes, but also contribute to pathogenic processes in a variety of inflammatory disorders and cancers by DNA base alterations, strand breaks, and an enhanced expression of protooncogenes (3). Oxidative stress is defined as the disturbance of the pro-oxidant-antioxidant balance in favor of former (4). It is well known that cigarette smoke contains a large amount of oxidative species, and therefore smoking represents a significant source of oxidative stress. In addition to this pro-oxidant burden, tobacco smoke can contribute to reactive oxygen species-mediated tissue damage through depletion of systemic endogenous antioxidant capacity (5).

Large amounts of pro-oxidants are produced by activation of leucocytes in prolonged inflammatory responses. Therefore, both tobacco smoke and inflammation are sources of reactive oxygen species and can compromise the antioxidant capacity of serum and tissues (6). Saliva possesses elaborate antioxidant defense systems (including superoxide dismutase, catalase, uric acid, vitamin C, glutathione, and others) to prevent oxidative damage in oral cavity (7, 8).

Thus, the aim of our study was to investigate the smoking effect on salivary antioxidant capacity, appreciated by glutathione level (GSH), correlated with ROS formation in salivary leucocytes.

MATHERIAL AND METHOD

In order to investigate smoking effects on oral environment there were studied physical, chemical and biological salivary parameters, in comparison smokers and non- smokers. 24 patients, 14 smokers and 10 non-smokers, aged between 30 and 60, who don't work in a polluted environment, were investigated.

The patients were questioned about the last oral hygiene, drugs used at the time of the study, eating after tooth brush, type of alimentation, and dental status. The smokers group was also questioned about the number of cigarettes smoked per day, and how long they smoked.

Lot distribution:

- Non-smokers: 10 healthy subjects, aged between 30-60, 7 females and 3 males,
- Smokers: 14 healthy subjects, aged 30-45, 12 females and 2 males, who smokes over 10 cigarettes daily, over 2 years.

Sampling

Sampling was performed at approximately 9.00 a.m. after a 10 hours fasting and avoidance of physical stress and cigarette smoking. Sampling also took place 2 hours after teeth brushing, food and liquids intake, chewing gum.

Salivary samples were taken from the subjects as follows: resting saliva samples were collected for almost 5 minutes in plain containers. Salivary gland flow rate are expressed as volume of saliva (ml) secreted per minute. In smokers saliva was obtained before and 30 min after one cigarette smoking.

Saliva samples were performed in 15-30 minutes after collection, in order to minimize the enzymatic degradation of the saliva. Saliva was centrifuged for 5 min at 2000-2500 rpm. It was separated salivary sediment and supernatant. Samples were stored at +4o C untill tests were performed.

GSH: from the supernatant it was determined salivary GSH by

spectrophotometric dithiobis- nitrobenzoic acid method. GSH microMoles/L = Absorption x 2020.

Salivary cells study

From salivary sediment were determined leucocytes and epithelial cells number with Burker camera. Results were noted as number of cells/µl. Cellular viability was determined with tripan blue vital dye, at the microscope, and were quantified the cells which exclude the dye (uncolored, live cells). Phagocytic capacity was ap-

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preciated by latex particle incorporation (IFL%), and by basal nitrobluetetrazolium test (NBT%) and stimulated (NBTs%) (9, 10).

Statistical analysis

The results were statistical analysed and were determined mean and standard deviation. In order to compare the results was used t Student test. Were compared the values of the investigted parameters before and after smoking, also, between smoking and non-smoking groups.

RESULTS

The results of investigated salivary parameters are showed in the tables. The variation of salivary rate at smoker's group, of salivary GSH, salivary cells parameters before and after smoking is extremely significant, compared with unsmoker's group (Table I).

Tabel I. Age, salivary flow/min, and GSH in non-smokers and smokers, before and 30 min. after smoking (M ± DS)

GROUP	Nr. cases	Age (years)	Salivary flow ml/ min.	GSH microM/L
Non-smokers (1)	10	54.4 ± 17,22	0.414 ± 0,171	63.43 ± 32.64
Smokers (2)	14	37.14 ± 5,89	0.55 ± 0.14	60.44 ± 28.83
Smokers 30 min after smoking (3)	14		0.71 ± 0.1	104.86 ± 22.70
Lot 1- Lot 2 compared		p = 0.002 VS	p = 0.0436 S	p = 0.808 NS
Lot 2- Lot 3 compared			p = 0.0018 VS	p < 0.0001 ES
Lot 1- Lot 3 compared		p = 0.002 VS	p < 0.0001 ES	p = 0.001 VS

NS=Not significant; S=Significant; VS=Very significant; ES=Extremely significant

Salivary leucocytes and epithelial cells were significant increased, but cells' viability was decreased in smokers compared with non-smokers (Table II).

Table II. Salivary cells parameters: salivary leucocytes, epithelial cells, cellular viability, in smoking and non-smoking group (M ± DS)

GROUP	Nr. cases	Salivary leucocytes /µl	Epithelial cells /µl	Cells viability %
Non-smokers (1)	10	1380 ± 657.26	4000 ± 3674.23	92.4 ± 2.509
Smokers (2)	14	7000 ± 4082.48	10742. 86 ±4375.63	88.14 ± 2.54
Smokers 30 min after smoking (3)	14	8357.14 ± 3902.07	12257.14 ± 4492.16	83.28 ± 2.42
Lot 1- Lot 2 compared		p = 0.0003 ES	p = 0.0007 ES	p = 0.0005 ES
Lot 2- Lot 3 compared		p = 0.378 NS	p = 0.374 NS	p < 0.0001 ES
Lot 1- Lot 3 compared		p < 0.0001 ES	p < 0.0001 ES	p < 0.0001 ES

NS=Not significant; S=Significant; VS=Very significant; ES=Extremely significant

The variation of salivary leucocytes phagocytosis tests before and after smoking is significant, in comparison with unsmoker's group (table III).

Tabel III. Mean values of salivary leucocytes phagocytosis tests (M ± DS).

GROUP	Nr. cases	IFL test %	NBT test %	NBTs test %
Non-smokers (1)	10	19.4 ± 0.894	2.2 ± 0.83	3.4 ± 1.14
Smokers (2)	14	19.14 ± 1.67	2.14 ± 0.69	3.71 ± 0.95
Smokers 30 min after smoking (3)	14	16.57 ± 1.71	1.57 ± 0,53	3.28 ± 0.48
Lot 1- Lot 2 compared		p = 0.659 NS	p = 0.818 NS	p = 0.475 NS
Lot 2- Lot 3 compared		p = 0.0003 ES	p = 0.0213 S	p = 0.142 NS
Lot 1- Lot 3 compared		p < 0.0001 ES	p = 0.0331 S	p = 0.726 NS

NS=Not significant; S=Significant; VS=Very significant; ES=Extremely significant

Increased salivary GSH after one cigarette smoking (Figure 1) was correlated with transitory high levels of salivary flow.

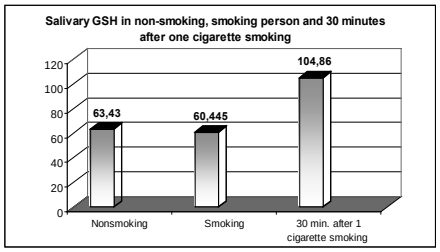

Fig. 1. Salivary GSH levels in non-smokers and smokers

DISCUSSIONS

According to Chiappin study we used un-stimulated whole saliva specimen for investigation of GSH and leucocytes function (11). Several methods of saliva collection are available, including the collection of un-stimulated whole saliva; whole saliva stimulated with, typically, paraffin wax, gum base or citric acid; or the collection of saliva from specific salivary glands.

For the purpose of analyzing salivary antioxidant status, whole saliva is the most relevant, as it contains gingival crevicular fluid, immune cells, and tissue metabolites (12) and reflects most closely the predominant intra-oral condition. Stimulation, on the other hand, may increase the flow of gingival crevicular fluid and this may result in false increases in the concentration of antioxidants in the saliva.

In our study salivary flow was significantly increased ($p < 0.05$) in smokers group (0.55 ± 0.14 ml/min) compared with non-smokers, and the elevated values (0.71 ± 0.1ml/min) was mentained after one cigarette smoking ($p < 0.001$). The variation of salivary flow was correlated with increased values of salivary GSH.

Pre smoking salivary GSH level was decreased. This may indicate that excesive ROS production in smokers individuals is leading to the situation of excessive oxidative stress which may be an important factor contributing to the destruction of oral tissues. After smoking GSH value was increased that can indicate mobilization of salivary antioxidant capacity.

Antioxidants, by counteracting the harmful effects of free radicals, protect structural and tissue integrity (13). Individuals with already low pre smoking glutathione concentrations are most prone to the noxious effects of cigarette smoking. The decreased antioxidant capacity may indicate either inherently low bazal antioxidant defence status or may result from an increase in oxidative stress (14).

Salivary cells were significantly increased in smokers and after one cigarette smoking ($p < 0.001$) associated with decreased cells viability, observed in present study.

It seems that tobacco use amplified expholiation of oral mucosa cells. The glutathione can be released from dead cells and for this reason the samples were performed in short time after salivary samples collection.

The resting saliva flow rate in young healthy smokers was 0.31 ± 0.18 ml/min and increased to 0.34 ± 0.22 ml/min after smoking associated with increased GSH concentration in a Charalabopoulos study (15).

Phagocytosis assays of salivary leucocytes performed in the present study showed the reduced values of NBT test in smokers. NBT reduction dye indirectly revealed ROS generation during phagocytosis. ROS are generated in vivo by multiple mechanisms including the respiratory redox chain in mitochondria, the respiratory burst in phagocytes, and the activity of various oxidase.

Low levels of salivary leucocytes phagocytosis in smokers group, reduced cells viability associated with high values of epithelial cells desquamation found in our study can decrease the oral mucosa barrier and can amplify local lesions induced by tobacco use. One potential mechanism is through tissue damage mediated by oxidative species originating from tobacco smoke and tobacco induced inflammation, in addition to the direct cigarette smoke-mediated depletion of antioxidants.

Abundant cross-sectional data support the adverse relationship between smoking and inflammatory modifications (1, 16, 17). A strong dose–response relationship between the amount smoked and the severity of inflammatory modifications has also been shown, further supporting the role of smoking as a risk factor for periodontitis (18).

CONCLUSIONS

Saliva samples may be used for smoking effects investigation on oral cavity. The measurement of salivary GSH in human saliva might be useful for estimating the level of oxidative stress on smoking habits.

Smoking modified the GSH levels and can produced noxious effects on oral cavity. GSH increased levels can be compensating defense mechanisms in saliva in order to withstand the oxidative stress imposed by smoking.

Low levels of salivary leucocytes phagocytosis in smokers group, reduced cells viability associated with high values of epithelial cells desquamation found in our study can decrease the oral mucosa barrier and can amplify local lesions induced by tobacco use.

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FUMATUL ŞI CAPACITATEA ANTIOXIDANTĂ SALIVARĂ

REZUMAT

Fumul de ţigară conţine o cantitate mare de specii oxidative, de aceea fumatul reprezintă o sursă semnificativă de stres oxidativ. Saliva posedă mecanisme de protecţie împotriva stresului oxidativ. Fumatul este factorul de risc cel mai întâlnit pentru parodontită cronică şi tumori maligne orale.
Scopul studiului a fost să investigăm efectele fumatului asupra capacităţii antioxidante salivare, apreciată prin nivelul de glutation (GSH).
Material: au fost selectate două loturi 10 nefumători, 14 fumători a peste 10 ţigări/zi de peste 2 ani.
S-au recoltat probe de salivă nestimulată, dimineaţa, la 2 ore de la periajul dinţilor şi fumat.
S-a notat debitul salivar. S-a determinat glutationul salivar prin metoda cu acid dithio-bisnitro-benzoic (DTNB), la 1-2 ore de la recoltare, pentru a evita eliberarea de GSH din celulele distruse.
Rezultate: Nivelul de GSH a fost mai crescut la grupul de nefumători (63,43 $\pm$ 32,64 microM/L), decât la grupul de fumători (60,44 $\pm$ 32,64 microM/L). Debitul salivar a fost de 0,414 $\pm$ 0,171 ml/min la grupul de nefumători comparativ cu 0,55 $\pm$ 0,41 ml/min la grupul de fumători. De asemenea, la grupul de fumători s-a observat creşterea celulelor epiteliale descuamate şi o scădere a viabilităţii celulare sub 90%. Imediat după fumatul unei ţigări debitul salivar şi GSH-ul au crescut.
Concluzie: fumatul a modificat nivelul de GSH salivar şi prin acesta poate avea efect nociv asupra cavităţii orale.
Cuvinte cheie: fumat, GSH salivar, capacitate antioxidanta

Minireview From autologous bone transplantation to tissue engineering in bone grafting orthopedic surgery

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ABSTRACT

Despite the many discoveries in bone grafting surgery, new materials and approaches to bone healing in bone defect continue to be investigated. One exciting area is tissue engineering, which can be defined as the application of biologic, chemical and engineering principles to the repair, restoration or regeneration of living tissues by using biomaterials, cells and growth factors, alone or in combination. In this review article is presenting the evolution of bone grafting used in orthopedic surgery, kinds, some remarks presented at the last congresses in this speciality and some aspects for future.
Key words: bone grafting, scaffold, mesenchymal stem cells, tissue engineering

INTRODUCTION

Large traumatic bone defects can be covered by soft tissues but reconstruction of the bone itself may be difficult. The use of autogenous bone has remained the golden standard in restoring bone defects, but it is not always possible to obtain enough bone.

Despite the evolution of surgical techniques, the reconstruction of large bone defects remains a major challenge for which a tissue engineering approach can provide a solution.

Bone defects and bone loss are a relatively frequent occurence in orthopedic and trauma surgery as in bone fractures, mal unions, non-unions, tumors, infections, primary or revision joint replacement procedures, spinal surgery.

The use of bone grafts is essential for bone reconstruction and restauration of bone functional integrity in a variety of clinical situations.

Bone grafts are second only to blood transfusions on the list of transplanted materials worldwide. The total USA bone graft market revenues were at 1.3 billions $ in 2006. The revenue for this market are expected to reach 3.3 billion $ in 2013. The total European bone graft substitute market alone is expected to reach 120 million $ by 2010(11).

In literature are discribed many methods for bone grafting. The conventional methods are autologous bone transplantation, allotransplant of bone and artificial bone graft materials(12). Even if autogenous bone grafts had been used before, Leopold Ollier was the first to study bone transplantation systemically (14). He also pointed out the difference between auto-, allo- and xenografts.

THE AUTOLOGOUS BONE TRANSPLANTATION

The autologous bone transplantation (patient's own tissue: cancellous bone, cortical -compact bone, microvascular grafts, the periosteum) has potential for bone regeneration, easy to model, bone harvesting damages healthy body parts, a secondary operation, the limited availability of bone. The autograft tissue bone grafts is harvested from the patient, usually from the iliac crest, but possibly from the distal femur or the proximal tibia. This tissue is ideal as a bone graft because it possesses all of the characteristics necessary for new bone growth- osteoconductivity, osteogenicity and osteoinductivity.

Osteoconductivity refers to the situation in which the graft supports the attachment of new osteoblasts and osteoprogenitor cells, providing an interconnected structure through which new cells can migrate and new vessels can form. Osteogenicity means the situation when the osteoblasts, that are at the site of new bone formation, are able to produce minerals to calcify the collagen matrix that forms the substrate for new bone. Osteoinductivity refers to the ability of a graft to induce nondifferentiated stem cells or osteoprogenitor cells to differentiate into osteoblasts.

In 1914 Phemister described clinically the healing of autogenous bone grafts and stressed the importance of vascularization, the various tissue components involved in bone healing and creeping substitution (16). In particular after the Second World War, when there were a great number of victims with severe bone defects, the research into bone grafting increased.

THE allotransplant oF bone

Comparative, the allotransplant of bone (donor tissue) is characterised through large quantities available, easy to model, nonuniformity of products (sex, age) and issues of antigenicity, infection through transmitting diseases and a lower osteogenic capacity. (12)

The xenogenic bone graft (from another species) presents similar problems to the allograft: acute antigenic response, a high failure rate and from this reasons indeed rarely used (7).

BIOMATERIALS

The artificial bone graft materials are uniform in quality, have no limit to amount of use and no capability of new bone formation. Approximately 60% of the bone graft substitutes currently available involve ceramics, either alone or in combination with another material. Ceramics together metal (titanium, vitallium, aluminium and stainless steel) and polymer (methyl methacrylate, polyhydroxyethylmethacrylate with calcium hydroxide coating) are biomaterials. A biomaterial can be defined as "a material intended to interface with biological systems to evaluate, treat, augment, or replace any tissue, organ, or function of the body" (18).

Ceramics family include calcium sulfate (the first material used in bone defect), bioactive glass and calcium phosphate. Nowadays, the last class-the calcium

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phosphate ceramics (hydroxiapatite: TRANS-OSSATITE, TECMA-FIXR, tricalcium phosphate: BIO-TECMAR, CALCIRESORB R , CEROSRTCP, CyclOS TM, biphasic calcium phosphate: BONE'X, EUROS SSBS, EUROCERR 400, ATLANTIKR , ATLANTIKR Genta) are frequent used in orthopedic surgery(10). They are bioactive, biocompatible, bioresorbable, osteoconductive and occasionally osteoinductive. The use of calcium phosphates as biomaterials is based upon their similarity with the mineral phase of bone and teeth. Biomaterials of calcium phosphates are available in various physical forms: particles, blocks (dense or porous), injectable compositions, coatings on metal implants, self-setting cements, composites with polymers(10).

TISSUE ENGINEERING

Since 1970 , the combination of cells and scaffolds has been investigated as an alternative for the autologous bone graft. This technology is now referred to as tissue engineering, popularized and defined in 1993 as "the application of principles of engineering and life sciences toward the development of biological substitutes that restore, maintain or improve tissue function"(8).

In 2002 tissue engineering has been defined as "the application of scientific principles to the design, construction, modification and growth of living tissues using biomaterials, cells and factors, alone or in combination"(6).

The main aim of tissue engineering is to fabricate artificial tissues or organs that can replace damaged or missing tissue in patients(9). One of the biggest advantages of tissue engineering is that new tissue, which is fabricated in vitro, is based on cells that originate from the patient that will receive the implant. This approach reduces the probability of rejection and bypasses the lack of organ and tissue donors.

The main components required for tissue engineering are cells, scaffolds, growth factors and proteins.

Stem cells are the cells which can be used in bone tissue engineering. They have extensive proliferation as well as differentiation capability. There are two types of stem cells, one is embryonic and the other is adult stem cells. The embryonic cells can be isolated from inner cell mass of blastocyst during the stage of embryonic development. But due to the cell source, are many ethical issues, possible transplantation rejection and teratoma formation for their use in clinical cases. In contrast to the embryonic cells, certain kinds of adult stem cells have the capability to differentiate into a number of different cell types. Also, there are the multi-differentiation capability of mesenchymal stem cells (MSCs) found in adult bone marrow and other tissues (12).

MSCs are bone marrow populating cells, different from hematopoietic stem cells, which possess an extensive proliferative potential and ability to differentiate into various cell types, including: osteocytes, adipocytes, chondrocytes, cardiomyocytes, myocytes and neurons. MSCs play a key role in the maintenance of bone marrow homeostasis and regulate the maturation of both hematopoietic and non-hematopoietic cells. The cells are characterized by the expression of numerous surface antigens, but none of them appears to be exclusively expressed on MSCs. Apart from bone marrow, MSCs are located in other tissues, like: adipose tissue, peripheral blood, cord blood, liver and fetal tissues. MSCs have been shown to be powerful tools in gene therapies and can be effectively transduced with viral vectors containing a therapeutic gene, as well as with DNA for specific proteins, expression of which is desired in a patient. Due to such characteristics, the number of clinical trials based on the use of MSCs increase. These cells have been successfully employed in graft versus host disease treatment, heart regeneration after infarct, cartilage and bone repair, skin wounds healing, neuronal regeneration and many others(3).

Presently, one basic research in tissue engineering is the development of "smart" multifunctional scaffolds for supporting and guiding the growth of cells.

Ideally, a scalfold should have the following characteristics (5) three-dimensional and highly porous with an interconnected pore network for cell growth and flow transport of nutrients and metabolic waste; biocompatible and bioresorbable with a controllable degradation and resorption rate to match cell/tissue growth in vitro and/or in vivo; suitable surface chemistry for cell attachment, proliferation, and differentation and mechanical properties to match those of the tissues at the site of implantation.

In a scaffold based tissue engineering approach, the following aspects must be considered: architecture-the scaffold must be porous with pore sizes sufficient for the ingrowth and vascularization of new tissue; structural mechanics, strength and stiffness of the scaffold must be as close as possible to the host tissue and the scaffold must resist traction and contraction forces; degradation- when cancellous bone is considered, scaffolds must retain their integrity for one until three months for cell seeding and culturing plus one -three months once they are placed in the location to repair. Then, the scaffold must be reabsorbed without foreign body reaction for another 12-14 months. Cortical bone regeneration requires that the scaffold maintain its integrity for 6-12 months. Materials surface should be favourable to protein adhesion and promote cellular proliferation and differentiation(17). No single material appears to be with the complex requirements needed for bone regeneration, thus the necessity of developing hybrid scaffolds.

The factors and proteins that exist in bone are responsible for regulating cellular activity. Growth factors bind to receptors on cell surfaces, stimulating the intracellular environment to act. This activity translates to a protein kinase that induces a series of events resulting in the transcription of messenger ribonucleic acid and then into the formation of a protein to be used intracellularly or extracellularly. The combination and simultaneous activity of many factors result in the controlled production and resorption of bone. These factors, residing in the extracellular matrix of bone, include TGF-beta, insulinlike growth factors I and II, PDGF, FGF, and BMPs. Researchers have been able to isolate and, in some cases, synthesize these factors. Much work continues in the research setting, and some products for clinical use have appeared on the market (4)

Transforming growth factor beta (TGF-ß) is an important regulator of bone development, induction, repair and remodelling. Beck showed in rabbits that a single application of TGF-ß1 leads to new bone formation (2).

Bone morphogenetic proteins (BMPs) play a key role in bone formation and repair. They have been demonstrated to elicit new bone formation both at orthotopic and ectopic sites in experimental animal models. Because of their osteogenic potentials, recombinant BMPs hold great promise for healing bone fractures (15). The improvement of bone healing properties of calcium phospates ceramics can be obtained through their association with osteoinductive growth factors such as BMP-2 or BMP -7 and/or bone progenitor cells.The adsorption of rhBMP-2 on sintered calcium phosphate ceramics can be significantly improved by coating the ceramic surface with apatite nanocrystals. It should certainly contribute to improve the osteoinductive properties of these materials. (1)

CONCLUSIONS

In long term the goal of the tissue engineering is the development of new clinical therapies and treatments in orthopedics surgery and not only(13).

At the beginning of the 21st century it is clear that the medical paradigm of tissue substitution must be replaced with tissue regeneration. Tissue engineering bone may represent a suitable alternative to the scarcely available autologous bone tissue and to allografts.

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DE LA TRANSPLANTUL OSOS AUTOLOG LA INGINERIA TISULARĂ ÎN CHIRURGIA ORTOPEDICĂ DE GRAFARE OSOASĂ

REZUMAT

In ciuda numeroaselor descoperiri din chirurgia grefării osoase, noile materiale şi tehnici în tratamentul defectelor osoase continuă să fie cercetate. Unul din domeniile ce suscita interes în prezent este ingineria tisulară, care poate fi definită ca o aplicaţie biologică, chimică şi principii de inginerie pentru repararea, restaurarea sau regenerarea ţesuturilor vii ce folosesc biomateriale, celule şi factori de creştere, utilizate separat sau în combinaţie. În acest articol review este prezentată evoluţia transplantului de grefă osoasă din chirurgia ortopedică, modalităţile de grefare, câteva aspecte punctate la ultimile congrese internaţionale ale acestei specialităţi, precum şi unele aspecte ce se prefigurează în viitor.
Cuvinte cheie: grefare osoasă, matrice, celule stem mezenchimale, inginerie tisulară

MILK AND ACID DAIRY PRODUCTS CONSUMPTION IN TIMIS COUNTY ADOLESCENTS. EVALUATION OF SOME RISK FACTORS

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ABSTRACT

Milk and acid dairy products are foods with high nutritional value especially due to the phospho-calcium complex and proteins. Reported to the caloric value of the food intake during adolescence, this animal origin nutrient cathegory will represent 15-20%.

The study was performed on a representative population of adolescents in high schools, postagraduate and vocational schools in Timiș County, in urban areas, including 2908 pupils.

We investigated the frequency of milk and acid dairy products consumption in adolescents, and the results showed that 31.7% of the young subjects had consumed milk and acid dairy products on a daily basis during the previous week, the boys/girls ratio being 1.3/1, and the daily milk intake decreased with age. Acid dairy products were consumed daily by 24.1% of the adolescents, the report between boys and girls being 1.1/1 and the proportion of daily consumers decreased with age.

We found that reported to the hygiene recommendation of daily intake of milk and acid dairy products, Timiș county adolescents may present a susceptibility towards retarded development and maturation, to harmful environmental agents and to osteomalacia.

Key words: adolescents, milk and acid dairy products, risks

INTRODUCTION

Milk is a complete aliment, with a high nutritional value, given by the presence of trophines which are indispensable for human nutrition at all ages. The most valuable trophines are the phospho-calcium complex and the proteins, involved in growth and maturation, in bone and teeth mineralization – antirachitic/antidecalcifiant (4).

The main calcium source is represented by milk and its derivates. The calcium concentration in milk is 120mg/100ml. The digestive use of milk calcium is also supported by some factors: Ca/P ratio over 1, 1.4 similar to the ratio in the human organism; the presence of natural D3 vitamin, lactose and lactic acid, citric acid and citrates, first class proteins. At the same time, some calcium inhibiting factors, such as the phitic and oxalic acids, are absent.

Milk proteins, 3.5g % on the average, are mainly represented by casein, 3 g% (2). Having a high biological value, milk proteins are proteinogenetic, they determine growth and a positive azotate balance, increase the resistence of the organism to biotic and abiotic aggressions.

Acid dairy products, obtained by milk fermentative processes (lactic, alcoholic fermentation) are recommended for daily alimentation, being characterized by: digestibility of proteins and lipids, 20-25% higher as compared to milk; high content of B complex vitamins (1).

The need of milk and acid dairy products in adolescence: 500 ml milk/day (2 cups of 250 ml or 3-4 glasses of 150 ml); 100 ml acid dairy products/day (1/2 cup or one glass). Reported to the caloric value of the portion, this animal origin food cathegory will represent 15-20% (7).

METHODS

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

The work method was the transversal populational study, by group and anonimous use of the CORT 2004 questionnaire for investigating some health risk behaviours in young subjects, as concieved by a CNCSIS accredited research, based upon the adaptation of some international questionnaires (ESPAD, YRBSS) to Romanian realities, during the period 2003 - 2005 (8).

RESULTS AND DISCUSSIONS

1. Milk consumption (Figures 1 and 2)

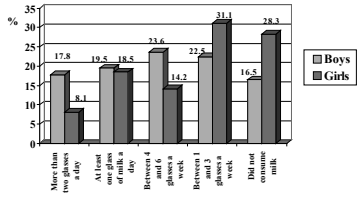

Fig. 1. Percent distribution of boys and girls depending on the frequency of a single milk glass consumption during one week

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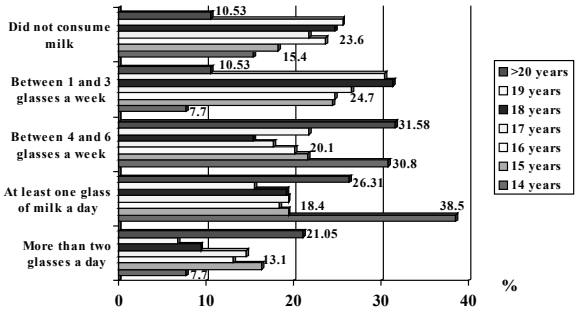

Fig. 2. Percentual distribution of adolescents by age groups depending on the frequency of one glass of milk during one week

Out of the questioned adolescents, only 12.8% consumed more than two glasses of milk a day, and 31.7% consumed at least one glass of milk a day, 18.7% of the young subjects consumed between 4 and 6 glasses a week, and 27% consumed between 1 and 3 glasses a week. The percent of those who did not consume milk is 22.6%.

We observed that boys had a better milk intake than girls, 17.8% consumed more than two glasses a day, as compared to 8.1%, and 22.5% of the boys and 31.1% of the girls consumed between one and three glasses of milk during one week. The percent of young subjects who consumed at least one glass of milk a day was low, 37.3% of the boys and 26.6% of the girls, given the importance of this aliment for adolescent nutrition (Figure 1).

The diference is statistically significant ($\chi 2$) as reported to gender, being 152.4, with a probability cutoff $p<0.001$, in favour of the boys who consume milk more frequently than girls.

Analyzing milk consumption in adolescents, by age groups, we found that almost half of the the 14 years olds, 46.2%, and 35.8% of the 15 year olds, consumed at least one glass of milk a day, and that the frequency of daily milk consumption decreases with age, namely only 22.3% of the 19 year old adolescents have a daily milk intake (Figure 2).

The age difference regarding milk consumption is statistically significant ($\chi 2$), 89.63%, with a probability $p<0.001$, the highest milk consumption being found in 16-17 year old adolescents.

2. Acid dairy product consumption (Figures 3 and 4)

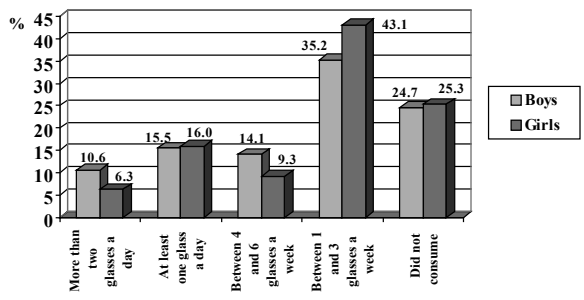

Fig. 3. Percentual distribution of boys and girls depending on the frequency of one glass of acid dairy products during one week

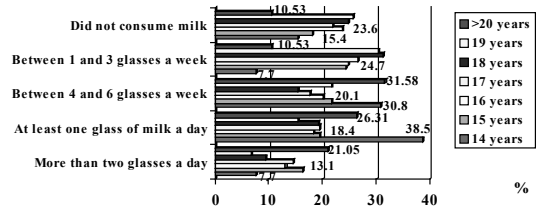

Fig. 4. Percentual distribution of adolescents by age groups depending on the frequency of consumption of one glass of acid dairy products (sana, kefir, yoghurt, sour milk) during one week

Considering the extremely valuable qualities of milk derivates, regarding their content of high quality nutrients, in an optimal combination for the develpoment and support of body functions, these have a great importance for adolescent nutrition as an alternative to milk consumption (5). Their nutritional value is similar to that of milk, but they have the advantage of presenting nutritional factors in an easier assimilable form (3).

The studied group had an acid dairy product consumption lower than milk consumption. Only 8.4% of adolescents consumed 2 or more glasses a day, 15.7% consumed one glass a day, and for 11.6% of the young subjects, the frequency of consumption was between 4 and 6 glasses a week. Most of the questioned adolescents, 39.2%, very rarely consumed milk derivates, namely, between 1 and 3 glasses a week.

Of the adolescents consuming at least one glass of acid dairy products a day, the highest proportion was represented by boys, 26.1%, as compared to girls, 22.3%. Only 6.3% of girls, as compared to 10.6% of boys, had a daily intake over two glasses. About one quarter of the young subjects, 24.7%, boys and 25.3% girls, do not consume milk derivates (Figure 3).

By gender, the diference is statistically significant ($\chi 2$), 32.6, in favour of boys, with a probability $p<0.01$, boys having a higher intake of acid dairy products because they are more preoccupied of increasing their muscular mass. By frequency hierarchy in consumption of acid dairy products, there is an identity between boys and girls.

We observed that the frequency of young subjects who rarely consumed acid dairy products is increasing from the age of 14, 30.8%, to the age of 19, 44.9%, as compared to those who had a daily intake of at least one glass, for whom the consumption frequency decreases from 14 years of age, 46.2% to the age of 19, 14.5% (Figure 4).

The difference between the frequency of consumption of acid dairy products, by age, is statistically significant, ($\chi 2$), 26.14, with a probability $p<0.01$.

3. Evaluation of risk factors for milk and acid dairy products (Table I)

We selected individual and family factors, possible predictors for insufficient milk and dairy products consumption in adolescents, by multivariated logistic regression.

Table I. Risk factors for daily consumption of milk and acid dairy products. Multivariated logistic regression analysis

Variable	Odds Ratio OR	Statistical significance cutoff p	95% C.I. for OR	
			Minim	Maxim
Individual Factors				
Masculine Gender	0.80	0.007	0.67	0.94
Residence				
Town	1(ref)			
Village	0.93	0.756	0.59	1.47
Municipium	1.32	0.016	1.05	1.65
Perceived Weight				
Under normal value	1(ref)			
Around the normal value	1.07	0.494	0.88	1.30
A little over the normal value	1.38	0.012	1.07	1.76
Much over the normal value	1.80	0.012	1.14	2.86
School situation (average grade in the last semester)				
Very good (average 9-10)	1(ref)			
Good (average 8-8.99)	0.87	0.184	0.70	1.07
Medium (average 6-7.99)	0.65	<0.001	0.52	0.81
Low (average 5-5.99)	0.91	0.693	0.58	1.43
Class				
The IX-th class	1(ref)			
The X-th Class	1.03	0.781	0.84	1.26
The XI-th Class	1.14	0.231	0.92	1.40
The XII-th Class	1.62	<0.001	1.27	2.06
Education level of the mother				
Faculty	1(ref)			
Postgraduate school	1.03	0.865	0.76	1.39
High school	1.34	0.009	1.08	1.66
Apprentice school	1.48	0.005	1.12	1.95
Gymnasium or less	1.30	0.108	0.94	1.80

By multivariated regression analysis, weak predictors (OR between 1-2, $p<0.005$) were revealed for daily lack of consumption of milk and acid dairy products in adolescents:

-the situation of XII-th class high school pupil (OR=1.62, $p<0.001$)

-the mother's average level of education (graduate of apprentice school) (OR=1.48, $p<0.005$).

The statistical behavioural model for daily lack of consumption of milk and acid dairy products in adolescents reunites the two significant predictors mentioned above.

CONCLUSIONS

Milk and acid dairy products are foods with high nutritional value especially given by the phospho-calcium complex and proteins.

Daily milk consumption during the previous week was declared in 31.7% of adolescents, boys more frequently being daily consumers, with a boys/girls ratio of 1.3/1. The daily milk consumption decreases with age from 35.8% at the age of 15, to 22.3% at the age of 19. Acid dairy products, defined by a higher digestability of proteins and lipids and a higher content of B complex vitamins, are consumed daily by 24.1% of the adolescents aged between 15-19 years, the boys/girls consumer ratio being 1.1/1. The daily consumption decreases with age, from 30.1% at the age of 15, to 14.5% at 19.

The statistical behavioural model for daily lack of milk and acid diary products consumption in adolescents reunites the two significant predictors: the XII-th class high school pupil status, the educational level of the mother (graduate of apprentice school).

Reported to the hygiene recommendation for daily consumption of milk and

acid dairy products, adolescents present susceptibility to osteomalacia, to developmental and maturation retardation, and to environmental harmful agents (6).

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CONSUMUL DE LAPTE SI PRODUSE LACTATE ACIDE IN RANDUL ADOLESCENTILOR DI JUDETUL TIMIS. EVALUAREA UNOR FACTORI DE RISC

REZUMAT

Laptele şi produsele lactate acide sunt alimente cu valoare nutritivă superioară dată mai ales de complexul fosfo-calcic şi proteine. Raportat la valoarea calorică a raţiei în adolescenţă, această categorie de alimente de origine animală va reprezenta 15-20%. Studiul a fost efectuat pe o populaţie reprezentativă de adolescenţi liceeni din licee, şcoli postliceale şi profesionale din judeţul Timiş, mediul urban, care a totalizat 2908 elevi. S-a investigat frecvenţa cu care adolescenţii consumă lapte şi produse lactate acide, iar rezultatele au evidenţiat faptul că 31,7% dintre tineri au consumat zilnic lapte în ultima săptămână, raportul băieţi/fete fiind de 1,3/1, iar cu vârsta, consumul zilnic de lapte scade. Produsele lactate acide au fost consumate zilnic de 24,1% dintre adolescenţi, raportul consumatorilor băieţi/fete fiind de 1,1/1 şi proporţia consumatorilor zilnici scade cu vârsta. Se constată că faţă de recomandarea igienică de consum zilnic de lapte şi produse lactate acide, adolescenţii timişeni pot prezenta susceptibilitate pentru retard în dezvoltare şi maturizare, faţă de noxele din mediul înconjurător şi pentru osteomalacie.

Cuvinte cheie: adolescenţi, lapte şi produse lactate acide, riscuri

ASSOCIATED RISKS TO THE SEXUAL ACT IN ADOLESCENTS FROM TIMIS COUNTY

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ABSTRACT

Risks associated with sexual act, such as the debut of sexual life under 13 years old, the lack of using contraception, sex with one-night partners, alcohol and drug consumption associated with intercourse, group sex, homosexual experiences are realities in adolescence. The study was conducted on a representative population of highschool students in Timis county, 2908 students from urban area of 15-19 years. The used work method was a cross sectional study. Results show: 3.1% of adolescents have started their sex life under the age 13.41.2% of boys and 10.4% of girls listed 4 or more sexual partners; 43.5% of boys and 21% of girls never use or only rarely condom with casual partners, 98.8% of the adolescents associated intercourse with different experiences. Promoting healthy sexual behavior as concept along lifetime is fundamental.
Keywords: adolescents, sexuality, risks

INTRODUCTION

Sexual freedom after puberty seems to be natural. In reality in this period sexual life hides many risks: sexual transmitted diseases, AIDS, abortions, unwanted child or born outside marriage, family conflicts (1).

Approximately 25% of sexually active young people in the U.S. get a sexually transmitted disease each year and 80% of infected young people do not even know they are infected, transmitting the disease to the partners. Factors contributing to such situations are combination of the intercourse with the consumption of alcohol or drugs in 23% of highschool students, intercourse in wich at 34% of them is unprotected by contraception. Some 14% of adolescents have had four or more sexual partners from the onset of their sexual life (7).

In multiple studies, adolescents have reported an early debut of sexual activity, multiple sexual partners, use of psychoactive substances, and sexual abuse in childhood. A relatively low percentage stated a condom was used at last intercourse. Unprotected sex was associated with lack of behavioral intentions to use condoms, pregnancy, having occasional partner and persons who practiced anal sex (3). In a study on a randomized group of 3000 adolescents of 17-19 years in Norway, during the recent intercourse, every fifth adolescent did not use any contraception; one in three used a condom and approximately 40% reported having used the pill. Multiple logistic regression analysis showed that among both girls and boys non-use of contraception increased with alcohol consumption prior to intercourse. Use of a condom most frequently occurred in standing relationships (4). Sexual orientation plays an important part in building identity during adolescence.

For a realistic understanding of sexual orientation in adolescents, is necessary a differential view on the dimension of sexual orientation. Thus, it can describe patterns of sexual orientation, including sexual attraction, fantasies, affiliations and behavior (5).

Sexual violence is all over the world. Childhood sexual abuse may weaken self esteem, self awareness, interpersonal relations and trust. Impact of sexual abuse on cognitive and emotional development of children is very important because the child feels betrayed, without force, stigmatized. The emotional response includes the anxiety, fear, depression, anger, self blame, low self esteem, poor capacity to make intimate relationships.

Fear of reprisals, attitude against homosexuality, and loss of self esteem they make boys to be less willing to confess abuse than girls. Studies on adolescent boys reported an association between sexual abuse and drug abuse, violent behavior, school absenteeism and theft. Even girls are included as sexual aggressors: sexual violence includes blackmail, intimidation, threats as forms of coerrction to compel to intercourse.

It may be taken into account the fact that sexual violence can occur on an individual who is unable to express consent, for example, drunk or under the influence of drugs (8, 9).

METHODOLOGY

Material

The study was conducted on a representative population of adolescents from high schools, post secundary schools and profesional schools in Timis county, urban area, and totalized 2908 students of 14-25 years (99% for the group of adolescents of 15-19 years), 51.5% girls and 48.5% boys.

Method

The method of the study was a cross sectional study through application of anonymous group questionnaire CORT 2004 of investigation risk behaviors for health to young people, designed by a team of an research project accredited CNCSIS, through adjustment of international surveys (ESPAD, YRBSS) to the reality of life in Romania during the period 2003-2005 (2, 10).

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1. Beginning of sexual life under the age of 13 years (Figure 1).

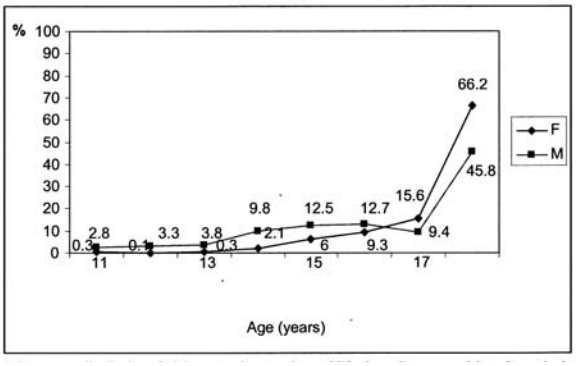

Fig. 1. Percentage distribution of adolescents who started sexual life, depending on age debut of sexual relations

A percentage of 43.8% adolescents between 15-19 years said they started sex life.

For both sexes, 54.2% of boys and 33.8% of girls have intercourse. On age groups at both sexes, the presence of sexual relations register increase percentage, from 11 years to 17 years or more. It is observed a beginning of sexual life at the age of 11 years or even earlier to a number of 1.5% subjects, 2.8% being boys and 0.3% girls.

Among boys, 3.3% began sex life from the age of 12 years, 3.8% from 13 years, 9.8% from 14 years, 12.5% from 15 years, 12.7% from 16 years and 9.4% from 17 years or more.

Girls at all age groups register lower percentages than boys in terms of debut of sexual life.

At the age of under 13 years, 3.1% of adolescents from Timis county began sex life, 2.8% boys and 0.3% girls.

2. The number of sexual partners, 4 or more (Figure 2).

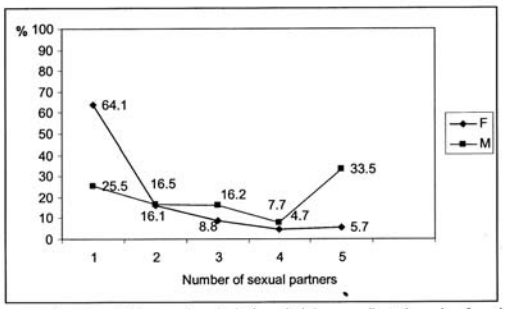

Fig. 2. Percentage distribution of adolescents who maintained sexual relations, according to the number of sexual partners

The most boys with sexual life, 33.5% indicated five or more partners before, 7.7% stated they had intercourse with four persons, 16.2% with three, 16.5% with two persons and 25.5% boys indicated one partner so far.

The most girls, 64.1% declare a single sexual partner, 16.1% stated they had intercourse with two persons, 8.8% with three, 4.7% with four and 5.7% with five or more partners until now.

A number of 4 or more sexual partners was mentioned by 41.2% of boys and 10.4% of girls.

3. Condom use in intercourse with casual partners (Figure 3).

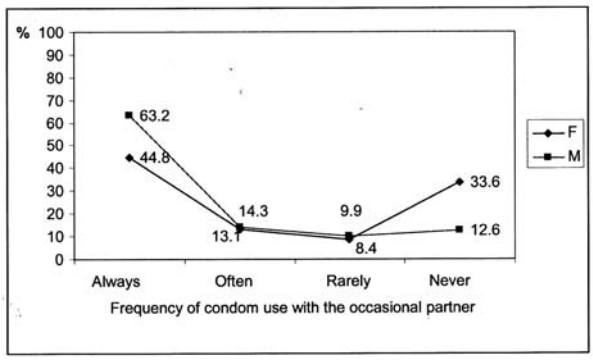

Fig. 3. Percentage distribution of adolescents who started sexual life, depending on the frequency of condom use with the occasional partner

The condom is used by a percentage of 87.4% boys and 66.4% girls who indicate casual sex partners. Among boys, 63.2% always use it, 14.3% often, 9.9% rarely and 12.6% never.

A percentage of 44.8% of young women who have answered the question, always indicate condom use, 13.1% often, 8.4% rarely and 33.6% never.

Never use a condom or only rarely in relations with casual partners, 43.5% of boys and 21% of girls.

4. Associated experiences to intercourse (Figure 4).

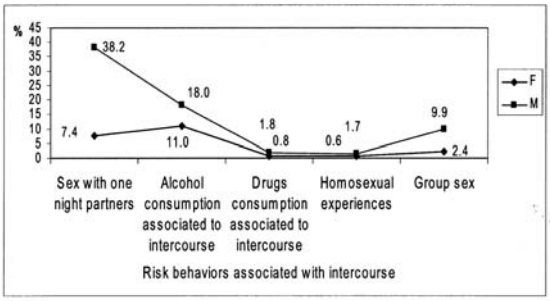

Fig. 4. Percentage distribution of adolescents who started sexual life, depending on the use of risk experiences associated with intercourse

A large proportion of adolescents with sexual life, 98.8%, of which 60.7% boys and 39.3% girls, answered the question concerning the association of sexual act with different experiences:

- 26.0% of them indicate sex with one night partners,
- 15.3% associate alcohol consumption and 1.4% drug consumption to intercourse,
- 6.9% of young people have practiced group sex,
- 1,1% had homosexual experiences.

The most boys, 38.2%, indicate sex with one night partners, 18.0% associate alcohol consumption and 1.8% drug consumption to intercourse, 9.9% practice group sex, and 0.6 % adolescents had homosexual experiences.

Among adolescent girls with sexual life, 11.0% associate alcohol consumption and 0.8% drug consumption to intercourse, 7.4% practice sex with one night partners and 2.4% group sex, while 1.7% of the young girls had homosexual experiences.

5. Sexual coercion of adolescents and coercion of other persons to sex relations by adolescents (Figure 5).

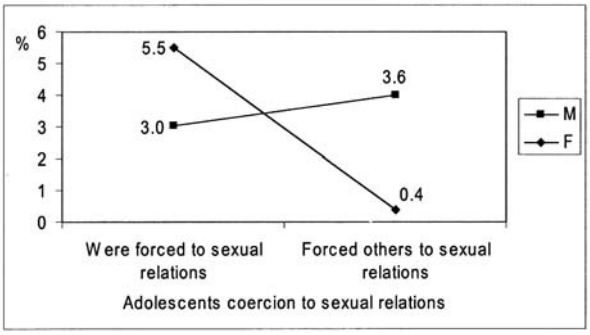

Fig. 5. Percentage distribution of girls and boys in terms of sexual coercion, supported or committed

In the CORT 2004 study, 5.5% of girls and 3% of boys reported that they were forced to sexual relations.

In turn, 3.6% of boys and 0.4% of girls admitted that they forced someone to maintain sexual relations with them.

CONCLUSIONS

A number of risks associated with sexual act, such as the debut of sexual life under 13 years, the lack of recourse to contraception, sex with one-night partners, alcohol and drugs consumption associated with sexual act, group sex, homosexual experiences, have been highlighted in the study of risk behaviors in adolescents from Timis county.

A substantial proportion of adolescents are at risk practices for unwanted pregnancies and sexually transmitted diseases. Despite the priority given to issues of sexuality in schools and health centers in recent decades, the percentage of those not using contraception is high. Development of health promoting activities capable to address those groups of adolescents not using contraception remains important (4).

Monitoring sexual behavior of the general population, focusing on risk behavior, rather than on the risk groups, is vital in promoting healthy sexual behavior as concept of lifetime (6).

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RISCURI ASOCIATE ACTULUI SEXUAL LA ADOLESCENȚI TIMIŞENI

REZUMAT

Riscuri asociate actului sexual, cum ar fi debutul vieții sexuale sub 13 ani, lipsa de recurgere la contracepție, sexul cu parteneri de o noapte, consumul de alcool şi droguri asociat actului sexual, sexul în grup, experiențele homosexuale sunt realități în adolescență. Studiul s-a efectuat pe o populație reprezentativă de liceeni din județul Timiş, mediul urban, 2908 elevi de 15-19 ani. Metoda de lucru a fost studiul populațional transversal. Rezultatele indică: 3,1% dintre adolescenți au început viața sexuală sub 13 ani; 4 parteneri sexuali sau mai mult, au fost menționați de 41,2% dintre băieți şi 10,4% dintre fete; nu folosesc niciodată sau doar rareori prezervativul cu parteneri ocazionali, 43,5% dintre băieți şi 21% dintre fete; 98,8%, dintre adolescenți asociază actul sexual cu diferite experiențe. Promovarea comportamentului sexual sănătos ca și concept de-a lungul vieții este fundamentală.

Cuvinte cheie: adolescenți, sexualitate, riscuri

THE EFFECT OF THE LONG TERM HIGH DOSES OF ATORVASTATIN THERAPY ON THE RECURRENT EVENTS IN ACUTE CORONARY PATIENTS

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INTRODUCTION

The statins has shown benefit in acute coronary patients. The long term high doses atorvastatin therapy has been very effective in improving outcomes of acute coronary patients with severe instable angina and acute myocardial infarction. There are only few studies of the effects of statins in acute coronary patients who hospitalised late in a specialised coronary unit. This study evaluates major and minor end points during a long period of time after acute coronary patients have been hospitalised.

MATERIAL AND METHOD

The present observational study is undertaken on acute coronary patients, 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 (Hb<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 consens 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 hygieno-dietethical 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 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 6 months, 12 months and 24 months from the hospitalization of acute myocardial infarct patients were effectuated.

END POINTS

All the patients were monitored for major and minor ischemic events (end-points), immediately after the onset of the acute coronary event: acute myocardial infarction or severe unstable angina.

The default primary end-points are the death, non-fatal acute myocardial infarct, cardiac arrest with resuscitation, or recurrent symptomatic myocardial ischemia with objective evidence requiring emergency re-hospitalization. Electrocardiographic entry criteria and the diagnosis of acute myocardial infarction included cardiac enzyme data criteria are for each patient.. Cardiac arrest with resuscitation and recurrent symptomatic myocardial infarction

The traced secondary end-points are the period of time until the occurrence of the primary end-points, the occurrence of fatal and non-fatal AVC, revascularizations (PTCA and by pass), re-hospitalization (aggravated angina, newly diagnosed or aggravated congestive cardiac insufficiency that needed hospitalization), the period of time until the occurrence of primary and secondary end-points and the seric level of the lipids in comparison with moment zero when the patients were admitted into the study.

All patients were monitored clinically and paraclincal: seric lipids (total cholesterol, triglycerides, LDL, HDL), PRC, fibrinogen, VSH, glycaemia, hemoleucograma, LDH, urea, creatinine, ionograma, ECG, echocardiography.

STATISTICAL ANALYSIS

Time to first occurrence or first primary or secondary cardiovascular event was analysed using stratified Cox proportional hazards models and were displayed using Kaplan-Meier curves. The Cox proportional hazards analyses were stratified by subgroups and index event (unstable severe angina or acute myocardial infarction). Stepwise regression analyses were performed to determine predictors of nonfatal myocardial infarction. The stratified Cox proportional hazards model that was used for the original analyses was used as the starting point for the stepwise analyses. Baselines variables (Table 1) were added one by one to the original Cox model in the stepwise analysis, beginning with the variable with the smallest probability value. The stepwise process ended when none of the variables outside the model had a probability value <0,05 and every variable in the model had a p<0,10.

RESULTS

From a total of 584 acute coronary patients, we included 352 patients with acute myocardial infarction and severe unstable angina in the study, in accordance with the preset inclusion criteria, the period of the inclusion of the patients being

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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/zi) 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 1).

Table 1. The baselines characteristics of the acute coronary patients

Age (years)	65	–
Men	224	64%
White raise	350	100%
Time from the onset (hours)	9	–
Cardiovascular history		
* Heart failure	30	8.50%
* Cerebrovascular disease	30	8.50%
* Periferic vascular disease	34	9.60%
* Miocardial Infarction	88	25.00%
* PTCA revascularisation	11	3.00%
* BY PASS revascularisation	25	7.30%
Cardiovascular Risk Factors		
* Smoking	98	28.00%
* HTA	193	55.00%
* diabetes melitus	81	23.00%
* dislipidemia	147	42.00%

Values are mean ±SD or number of patients (%).

We have split the acute coronary patients 3 groups corresponding to the two lots of patients (the control lot and the lot with acute myocardial infarct patients treated with atorvastatin 80mg/day) and in accordance with the ECG diagnosis. Group I includes 145 patients with severe unstable angina (Table 2). Group II includes 110 patients with acute myocardial infarction (AMI) without ST segment denivelation, group III includes 97 patients with AMI with ST segment denivelation (Table 2).

Table 2. Groups of acute coronary patients evaluated for 24 months

	Lot Atorvastatin 80mg/zi		**Lot Control**		**Total**
Patients	**nr patients**	**%**	**nr patients**	**%**	
Group I: Severe Unstable Angina	72	40%	73	42%	145
Group II: Non ST Denivelation IMA (NSTD)	57	32%	53	31%	110
Group III ST Denivelation IMA (STD)	50	28%	47	27%	97
Total	179		173		352

As a percentage, group I (patients with Severe Unstable Angina) comprises 41% of the patients, group II (Non ST Denivelation Myocardial Infarct) comprises 32% of the total patients and group III (ST Devinvelation Myocardial Infarct) comprises 27% of the total patients with IMA included in the study.

The average time between the hospitalization of the patients and the administration of the dose of 80mg/day of atorvastatin was 63 hours. The planning for the clinical monitoring of the patients was scheduled at 6, 12 and 24 months from the inception of the acute myocardial infarct. All the data about the patients was centralized in accordance to the preset planning, with the exception of 8 patients that got lost in the lot of patients that received atorvastatin 80mg/day (0,3%), respectively 3 patients that got lost in the control lot (0,1%). We considered as a "lost patient" the patient that could not be contacted at the domicile declared at the inclusion to the study and that could not be contacted using any of the data detained (administrative matters).

The compliance of the patients to the prescribed treatment, defined by the number of days in which the patient strictly followed the prescribed medication, was 86% in the atorvastatin lot and 87% in the control lot. The medication of the acute myocardial infarct patients was in accordance with the valid therapeutic guides, individualized according to the clinical and paraclinical diagnosis of each patient (table 3). Aspirin, heparin, nitrates and beta-blockers were administered to most of the patients included in the study.

Table 3. Medications During Hospitalization of the acute coronary patients

Aspirin	318	91.00%
Antiplatelet agents	42	12.00%
Heparin	263	75.00%
Oral anticoagulants	28	8.00%
Fibrinolitic Agents	24	7.00%
Nitrates	315	90.00%
Beta blockers	273	78.00%
Ca Chanel Blockers	168	48.00%
ACE Inhibitors	168	48.00%
Digoxin	42	12.00%
Statins (atorvastatin 80mg)	179	100.00%

Values are number of patients (%).

PARACLINICAL RESULTS

At the moment of the onset of the acute myocardial infarction, the level of seric lipids was similar for both lots of patients with an average level of the LDL cholesterol of 124mg/dl, triglycerides 184mg/dl and HDL 46mg/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 4).

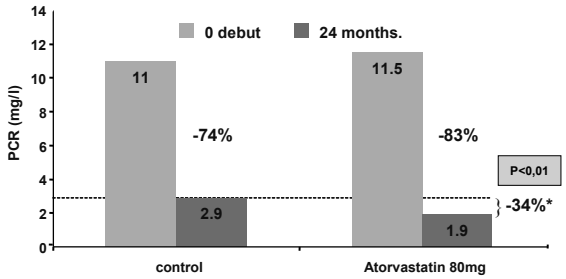
Table 4. The evolution of the lipidic markers of the acute coronary patients treated with 80mg atorvastatin comparative with the control group

	Lipid markers (mg/dl) :				
	LDL		TGL		HDL
	control	atorvastatin	control	atorvastatin	control
IMA debut	124		184		84
After 24	135	78	187	142	41
Results (%)	- 12%	- 40%*	+18%	- 16%*	- 6%

* P<0,001

By the end of the study, in the control group of patients, the seric level of the LDL cholesterol increased by 12% (average 135mg/dl), comparing to the atorvastatin lot where the seric level of the LDL cholesterol decreased by 40% (average 78mg/dl). Triglycerides increased by 8% (average 187mg/dl) in the control lot, compared to the decrease of 16% (average 142mg/dl) of the triglycerides level in the atorvastatin lot. In the control lot the seric level of HDL cholesterol decreased by 6% (average 41mg/dl) comparing to the atorvastatin lot where the seric level of HDL increased by 8% (average 50mg/dl).

Figure 1. The evolution of PCR inflamation marker of the acute coronary patients treated with 80 mg atorvastatin comparative with control group



In the patients with IMA treated with atorvastatin 80mg/day for 24 months, PCR decreased from an average value of 11,5mg/l to 1,9mg/l, representing a decrease of 83% compared to 79% in the control lot, at the debut PCR having an average value of 11mg/l and after 24 months 2,9g/l.

RESULTS OF THE END-POINTS

Over the six-months period of the study, in the lot of acute myocardial infarct patients treated with 80mg atorvastatin, major events (primary end-points) took place for 27 patients (14.8%) compared with 31 patients (18%) in the control lot (Figure 2). The administration of 80mg/day of atorvastatin diminished the risk for the combined primary end-point (Relative Risk RR) from 0.84 to 0.70-1.00, p=0.049, after 6 months of treatment from the debut. No statistical differences were registered between the control lot and the lot of patients treated with 80mg/day atorvastatin, regarding the death risk or the cardiovascular resuscitations with re-hospitalization after 6 months. However, in the patients from the lot with 80mg/day atorvastatin, a lower risk of the recurrent ischemic events with emergency re-hospitalization was registered at 6 months (RR, 0.74, 95% CI, 0.57-0.95, P=0.02).

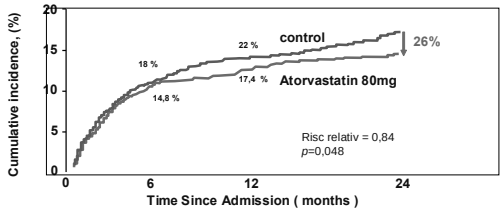
At 12 and 24 months the recorded differences between the two lots were significant from a statistical point of view, regardless of the ECG diagnosis of the IMA at the debut (group I, group II and group III).

Over the 12 months period of the study, in the lot of acute myocardial infarct patients treated with 80mg atorvastatin, major events (primary end points) took place for 31 patients (17.4%) compared to 38 patients (22%) in the control lot.

Over the 24 months period of the study, in the lot of acute myocardial infarct patients treated with 80mg atorvastatin major events (primary end points) were registered in 35 patients (19.6%) compared to 44 patients (25%) in the control lot.

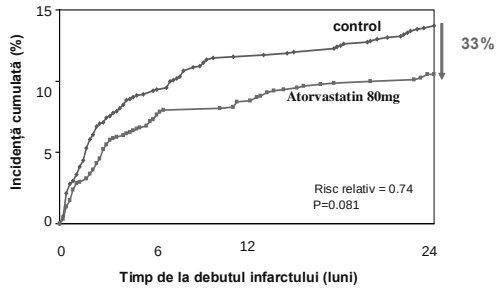
At 24 months the treatment with atorvastatin (80mg/day) significantly reduces the risk of the combined primary end points by 26%, p<0.5, Figure 2.

Figure 2. Results if the primary cumulative end points of the acute coronary patients treated with atorvastatin 80mg comparative with the control group



Significant statistical differences of the combined secondary end point were registered between the lot of patients treated with atorvastatin 80mg/day from the debut and the control lot at 24 months (Figure 3).

Figure 3. Results of secondary cumulative endpoints of acute coronary patients treated with atorvastatin 80mg comparative with the control group



treated with 80mg atorvastatin, the cumulative senondary endpoint was reduced with 33%, p=0.081. The administration of 80mg/day of atorvastatin diminished the risk for the combined primary end-point (Relative Risk RR) from 0.84 to 0.70-1.00, p=0.049, after 24 months of treatment from the debut.

In the lot of patients treated with atorvastatin 80mg/day, 13 fatal and non-fatal cerebrovascular events were registered (in 15 patients): 4 recurrent IMA, 3 hemorrhagic AVC, 1 embolic AVC, 4 of systemic tromboembolic nature, 1 case of unspecified etiology, compared with a total of 39 events the control lot.

Non-fatal recurrent AVC (stroke) was registered in 4 patients from the lot of patients treated with atorvastatin 80 mg (2.3%) and in 9 cases from the control lot (5.3%) with significant differences between the lots, $p<0.01$ (RR 0.41).

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

The control of the hypercholesterolemia in coronary patients is in general well documented, both in the primary prevention and the secondary prevention, especially in the acute coronary syndromes and the acute myocardial infarct. For the latter ones, the decrease of the cholesterol values is correlated with a favourable prognostic in the post-infarct evolution.

The prognostic of acute coronary syndrome patients in general, and the acute myocardial infarct in particular, with or without ST segment denivelation, proved to have the most beneficial effects if the total cholesterol level is significantly reduced below the normal values and the decrease of the LDL at values below 70mg%. These changes were demonstrated in the MIRACL study on a number of 3086 cases of acute myocardial infarct who received 80mg/day of atorvastatin, for 16 weeks: is has been demonstrated that the early administration of 80mg/day of atorvastatin reduces the risk for major cardiovascular events at 16 weeks by 16%, it also reduces the risk for re-hospitalization due to recurrent myocardial ischemia by 26%, as well as the LDL cholesterol level below 80mg%, with or without PTCA or other interventional procedures.

The trials existing up to date illustrate to a lesser extent the evolution of some groups of acute coronary patients, who arrive belated to specialized medical assistance specific for acute myocardial infarct for early coronary unobstruction. Such emergencies require emergency therapy insufficiently prompt, but in accordance with valid therapeutic guides. For this reason, we considered necessary such a lot of acute coronary patients, to whom a maximum daily dose of atorvastatin was administered, for a period of 24 months, with the monitoring of the clinical and paraclinical evolution.

In this trial, early treatment with 80mg atorvastatin of acute coronary patients with acute myocardial infarction and severe unstable angina, reduced major recurrent ischemic events with 26% after 24 motnhs, and with 33% minor recurrent ischemic events. The lipid markers evolution was very good: the seric level of the LDL cholesterol decreased with 40%, triglycerides decreased with 16% and the seric level of HDL cholesterol increased by 8%.

CONCLUSIONS

The present study proves that the early administration of 80mg/day atorvastatin in acute coronary patients late hospitalised, with or without ST segment denivelation, 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 seric lipids and of the PCR (atherosclerotic inflammation marker) at the administration of atorvastatin in maximum dose of 80mg/day, for 24 months, reduced the major cardiovascular events by 26% and the secondary cardiovascular events by 33%.

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 the late hospitalised patients with acute myocardial infarction and unstable angina reduces the early and long term risk of major and minor cardiovascular events.

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CAPSULE ENDOSCOPY: INDICATIONS AND FUTURE ADVANCES

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ABSTRACT

For many years the small bowel has been the least accessible portion of the gastrointestinal tract for endoscopic evaluation. Introduced for clinical use in 2001, capsule endoscopy has revolutionized the diagnosis and management of multiple small bowel lesions. The main indication of the wireless capsule endoscopy is the obscure gastrointestinal bleeding, where the upper digestive endoscopy and the colonoscopy did not find the source of blood loss. It can be useful tool in diagnosis and surveillance of intestinal tumors, Crohn's disease, celiac disease and polyposis, but the indications are continuously to be defined.
Key words: capsule endoscopy, gastrointestinal tract, evaluation, diagnostic

INDICATIONS

Capsule endoscopy (CE) represents a great advance of investigation methods for digestive tract, thus standing for revolutionary endoscopic modern techniques and providing the possibility to explore the digestive tract segments previously difficult to be visualized. Capsule endoscopy is presently unanimously accepted as diagnostic method, enterocapsule examination being considered the gold standard for exploring the small intestine, proving to be of superior or comparable value to the other methods presently available (barium tracking exam, push enteroscopy, entero-CT or entero-RMN, double balloon endoscopy). This new technique, easy to be performed and well accepted by the patients, is the only one that allows minimum invasive endoscopic evaluation of small intestine, without requiring sedation of patient and providing a great comfort to the patient.

Necessity to explore difficult accessible areas of digestive tract determined, even from the beginning of the nineties, initiation of studies regarding miniature video cameras, which could be swallowed and transmit images from these levels, using a wireless system. After a long period of research in this field, in 1999 administration of the first CE was decided to a human subject represented by the researcher C. Paul Swain (2). The success was extraordinary, and as a result of numerous clinical trials on patients developed during the following two years, in 2001 US Food and Drug Administration patented CE as investigation method for small intestine in adults, while the CE for pediatric use was released in 2003 (11).

INDICATIONS

1. Obscure gastrointestinal hemorrhages

According to the indited consensus (6), the main indication of enterocapsule is obscure gastrointestinal bleeding (imprecisely located) franc or occult, as well as iron deficiency anemia. Gastrointestinal hemorrhages are defined as persistent or recurrent gastrointestinal bleeding, whose source remains undetermined as a result of usual investigation techniques (gastroscopy and colonoscopy with ileoscopy). Under these circumstances, CE represents the third investigation method, following superior endoscopy and colonoscopy, except for massive hemorrhages, when an angiographic evaluation is required.

The first study performed on human subjects in 2001 showed that the bleeding sources were revealed in 100% as a result of standard investigation methods using CE. In addition to these, CE use helped in identifying other supplementary bleeding sources in 56% of the investigated patients (9).

Another study performed on 100 patients presenting obscure gastrointestinal bleeding showed that the CE accuracy is 91% in diagnosis of any hemorrhage, having 88.9% sensitivity and 95% specificity (7). According to this study, the most frequent revealed cause of hemorrhage seemed to be angiodisplasia in 29% of the cases, followed by inflammatory bowel disease in 6% of the cases.

2. Small intestine tumors

Before the CE entered in current clinical practice, the small intestine tumors were considered to have a low incidence, being incidentally diagnosed during laparotomies or some other investigations or therapeutically maneuvers. They were reported as 1%-2% from total tumors of digestive tract.

Recent studies showed that prevalence of small intestine tumors is much higher than thought before, clinical data from international studies showing an incidence between 5.4%-8.9%, while the malignity rate was estimated at 66%. The most frequent malignant tumor was adenocarcinoma, followed by carcinoma, lymphoma, and sarcoma (8).

3. Intestinal inflammatory bowel diseases

Numerous prospective studies referring to CE use in Crohn disease suspicion generally showed an increased rate of revealing the precocious inflammatory lesions in small intestine, comparative to other diagnostic methods (10). This investigation technique is much more sensitive than any other imaging technique, being useful in patients with Crohn disease suspicion and previous negative endoscopic evaluations.

CE is recommended both in Crohn disease suspicion as initial diagnostic method, as well as in monitoring of post-therapeutic tissue repair, and detection of extent and severity of disease at the level of small intestine for evaluation of disease activity and prognostic.

Nevertheless, there is a great problem represented by over-diagnosis of Crohn disease in cases of non-specific colitis or other lesions induced by AINS, due to

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impossibility to harvest mucosa biopsy for histological diagnostic. As consequence, AINS administration should be stopped 2 weeks before the capsule investigation is performed (8).

In a study performed on 30 patients having undetermined colitis symptoms and negative ASCA/p-ANCA antibodies, CE was able to diagnose presence of Crohn disease in 5 patients and had false negative results in other 5 patients, which were diagnosed consequently based on the biopsy performed during ileo-colonoscopy (5). This is one of the reasons why CE is not yet, unfortunately, an ideal diagnosis method for this pathology.

4. Celiac disease

Indication of CE use is made in cases of patients having positive serologic markers of celiac disease, but which do not want to have a superior endoscopy, or in which the endoscopic characteristic aspect is difficult to be visualized or irrelevant. Studies involving symptomatic patients, with positive serologic markers, showed a good sensitivity, as well as a good specificity for CE, with the only mention that the endoscopic signs are less specific comparative to the histological exam, considered as the gold standard in diagnosis of this disease (3).

CE is also indicated to patients known as having celiac disease which present alarm symptoms such as weight loss, abdominal pain, and fever.

5. Familial intestinal polyposis syndrome

According to a study in 2005, rate of polyps detection using CE is a lot more increased compared to the barium exam (4). Another study involving CE use in polyps detection ability in patients presenting familial polyposis compared to MRI, showed that polyps having more that 15 mm in size were detected with similar accuracy. In contrast, the detection rate of polyps with 5-15 mm in size was increased in CE compared to MRI, while the polyps less that 5 mm were better detected using MRI (1). As conclusion, this technique proved to be very useful in precocious evaluation of patients with family history of polyposis, which also present an increased risk of developing this disease.

6. Less frequent indications

Less used in current medical practice, CE can also be indicated in cases of monitoring the lesions of superior and inferior digestive tract (esophagitis, Brrett esophagus), polyps, malabsorbtion syndrome, and abdominal pains of undetermined origin, but these indications remain to be established in the future.

Table I. The main indications of wireless capsule endoscopy

I.	**Obscure gastrointestinal hemorrhages and Iron deficiency anemia of undetermined cause**
II.	**Small intestine tumor suspicion**
III.	**Familial intestinal polyposis syndrome** 1. Evaluation of patients with family history 2. Diagnosed patients monitoring
IV.	**Inflammatory bowel disease** 1. Diagnostic of patients with Chron disease suspicion 2. Chron disease extent monitoring
V.	**Celiac disease** 1. Diagnostic of patients with celiac disease suspicion 2. Monitoring of patients diagnosed with celiac disease

DISADVANTAGES (LIMITATIONS) OF CE

CE is an investigation technique very useful in diagnosis of different disorders of small intestine, which still has some disadvantages, thus limiting its use. The most important disadvantage is the impossibility to perform therapeutic maneuvers when the intestinal lesions are discovered, CE being only a mean of diagnosis, not a therapeutic one. Also, this capsule does not allow air inflating within the gastrointestinal tract for an increased visualization of the mucosa, thus the exact anatomic location of the revealed lesions cannot be determined.

The risk of capsule impastation at the level of diverticula's or narrowed areas must be also taken into consideration, due to the further possible consequences.

Sometimes, CE is not able to pass through the entire small intestine during the 8 hours of battery power, thus the investigation remaining incomplete. The relative cost of this investigation is increased (approximately 600 euros) and represents an important disadvantage, thus limiting its use in well selected cases.

CONTRAINDICATIONS

CE is generally very well tolerated by the patients, but its use is contraindicated in case of presence or even suspicion of obstruction/narrowing of intestine lumen, due to retention risk. This risk is defined by capsule stopping above the narrowed area, for over 2 week period, requiring removal of CE using medical, endoscopic or surgical procedures.

Presence of diverticulum's Zenker, or other sub-occlusive syndrome are circumstances which also contraindicate the use of CE because of the impastation risk of the capsule.

Passage disorders such as acute diarrhea in the moment of examination or in the last 30 days before the examination can also impair a good visualization of small intestine, due to an increase passage of the CE. Cardiac pacemaker implant, defibrillator or electro-mechanic devices also contraindicate administration of capsule due to interference risk in image transmission.

Table II. The contraindications of wireless capsule endoscopy

1.	Suspicion/presence of narrowed or partial/total obstructed intestine areas
2.	Deglutition disorders
3.	Severe motility disorders of digestive tract
4.	Sub-occlusive syndrome
5.	Zenker diverticulum
6.	Recent surgical procedure/resection of small intestine
7.	Cardiac pacemaker implant, defibrillator
8.	Pregnancy

CONCLUSIONS

CE represents a great advance of investigation techniques developed for digestive tract, developing into the standard evaluation procedure of small intestine. Similar to a large antibiotic capsule or a vitamin tablet, CE is small enough to be swallowed, is single use and do not require air inflation of the digestive tract. This capsule represents the non-invasive and non-harmful procedure for the digestive tract, providing the possibility of increased precision examination even in ambulatory conditions. Images obtained using this capsule have a high resolution, thus being possible to detect the lesions present on difficult access anatomic areas.

VIITOARE DIRECTII

The esophageal capsule is used in practice since 2004, indicated for patients with esophageal pathology, especially gastro-esophageal reflux and Barett esophagus. Due to a slower passage time, this capsule was coupled with two miniature sensors at both ends, so that the quality of the image obtained was improved.

In 2007 the colonic capsule was launched on the clinical market, having as indication the patients with incomplete colonoscopy and those having contraindications for colonoscopy. The advantage of this capsule is represented by the fact that does not require sedation or air inflating.

Possible future directions could be the necessity to implement a guiding system for the capsule and electric stimuli for movement control inside the intestine, as well as for improved visualization of intestine mucosa. In order to endow the CE with a therapeutic function, it can benefit of a delivery system for different drugs in certain anatomic areas.

Moreover, it becomes necessary that the CE would have a wireless power supplying system, due to the low power cycle of the batteries now in use of only 8 hours, which impairs visualization of the entire digestive tract, in cases of prolonged passage time. The technologic advance will improve in the future all present limitations of CE, the greatest challenge being the capsule having the possibility of sonography, thus enlarging even further the range of its use.

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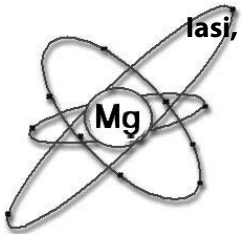
CAPSULA ENDOSCOPICA: INDICATII SI PERSPECTIVE

REZUMAT

De-a lungul timpului intestinul subtire a fost portiunea tractului gastrointestinal cea mai putin accesibila evaluarii endoscopice. Introdusa, in scopul utilizarii clinice, in anul 2001, capsula endoscopica a revolutionat metodele de explorare si diagnosticare a leziunilor prezente la acest nivel. Principala indicatie a capsulei endoscopice este hemoragia gastrointestinala obscura, atunci cand sursa de sangerare nu a fost decelata la endoscopia digestiva superioara si colonoscopie. Ea poate fi, de asemenea, utila in diagnosticarea si supravegherea tumorilor intestinale, boala Crohn, boala celiaca si polipoza intestinala, indicatiile sale urmand a fi definite in continuare.

Cuvinte cheie: capsula endoscopica, tract gastrointestinal, evaluare, diagnostic



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Magnesium in veterinary medicine
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Magnesium in pathology
Magnesium in neurology
Magnesium in psychiatry
Magnesium in immunity
Magnesium in reproductive system
Magnesium in gastrointestinal system
Magnesium in oro-maxilar area
Magnesium in nutrition
Magnesium in therapy
Magnesium's mechanisms of action at cellular level
Synthesis and biochemistry of new magnesium compounds
Pharmacology and toxicology of
Magnesium's sources
Other involvements of magnesium in biology