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Publication data: Fiziologia (Physiology) is issued quarterly

Subscription rates: Subscriptions run a full calendar year. Prices are give per volume, surface postage included.

Personal subscription: Romania - 100 RON, Outside Romania - 35$ (must be in the name of, billed to, and paid by an individual. Order must be marked "personal subscription")

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Bibliographic indices: We hope this journal will be regularly listed in bibliographic services, including "Current Contents".

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„FIZIOLOGIA – PHYSIOLOGY"

2. CORRESPONDENCE SHOULD BE ADDRESSED TO THE CHIEF EDITOR

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Editura **EUROSTAMPA**
Tel./fax: 0256-204816
ISSN 1223 – 2076

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Prof. dr. Francisc Schneider
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REVIEW

CANCER-ASSOCIATED FIBROBLASTS – INHABITANTS OF TUMOR, HOWEVER OBSCURE UNDERLYING THE HALLMARKS OF CANCER

LAURA CERNAT[2], FLORINA BOJIN[1], MEDA DEICA[1], GABRIELA TANASIE[1], VIRGIL PAUNESCU[1], CARMEN PANAITESCU[1]

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ABSTRACT

It becomes conspicuous the active role of stromal components in solid tumors proliferation, invasion and metastasis. Cancer-associated fibroblasts (CAFs) or tumor-associated fibroblasts (TAFs) are the main stromal cells which compete towards the malignant properties. It is undeniable that CAFs are distinguished from normal fibroblasts, epigenetically and perhaps genetically. Still, the origin and the molecular phenotype of CAFs are subjects of intensive debates. Any new findings regarding non-tumor elements and their interactions with the tumor cells could lead to new approaches to cancer therapy.

Key words: cancer-associated fibroblasts, tumor biology, stroma, cellular signaling, therapeutical targets

INTRODUCTION. HALLMARKS OF CANCER – INVETERATE AND NEW CONCEPTS

In a millenium issue article from January 2000, Hanahan and Weinberg have been proposed a suite of six characteristics of neoplastic disease, "hallmarks of cancer', features which consist of (1): self-sufficiency in growth signals; insensitivity to anti-growth signals; evasion of apoptosis; limitless replicative potential; sustained angiogenesis; tissue invasion and metastasis.

Same authors return upon this concept and consider two more hallmarks: reprogramming of energy metabolism and evade immune destruction (2).

Thinking about notable progress in cancer research, the two new traits are comprehensible and challenging for researchers. Past decades are extensive in understanding of molecular mechanisms, signaling pathways and recognition of active involvement of tumor microenvironment in neoplastic progression with its consequences.

Alteration of signals involved in cellular growth regulation, inactivation of tumor suppressors (such as RB protein (3) and TP53 protein), increase of antiapoptotic regulators expression (Bcl-2,Bcl-xL or of survival signals – Igf 1/2) (2), the significant expression of telomerase, upregulation of VEGF and FGF family gene expression (4), downregulation or even mutational inactivation of E-cadherin (5), are only a few examples of multiple mechanisms which are underlying the six hallmarks of cancer. Acquiring by tumor cells a multitude of characteristics through EMT (epithelial-mesenchymal transition) mechanism, leads to ability of tumor cells to disseminate and to be resistant to apoptosis (6) (Figure 1).

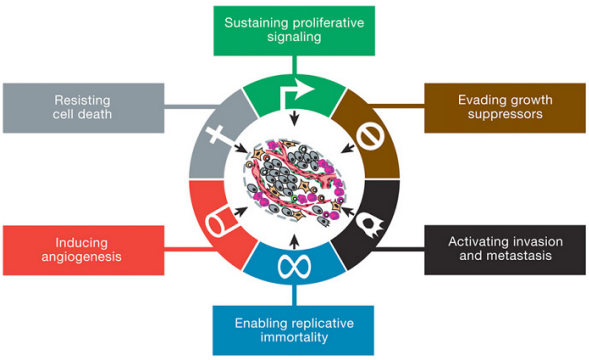

Fig.1. The hallmarks of cancer (1)

Emerging hallmarks proposed by Hanahan and Weinberg are the reprogramming of cellular metabolism (so that the nurture support will be assured) and the evasion of destructive activity of B cells, T cells, natural killer cells, macrophages that belong to host (2). From these points will derive two more features: genomic instability and inflammation which maintain all neoplastic characteristics (Figure 2).

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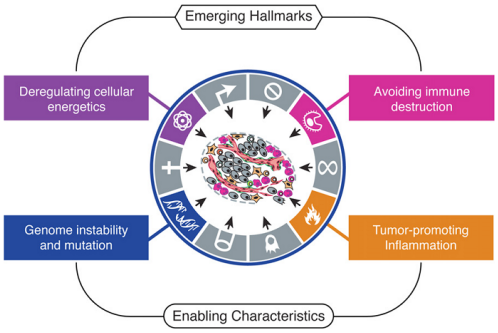


Fig.2. Emerging cancer hallmarks (2)

These hallmarks of cancer and not only them ought to permit exploring of new therapeutic horizonts, as a possible broad mapping could be assessed and the personalised medicine will be the first therapeutic choice.

FIBROBLASTS VERSUS CAFS – CHARACTERISTICS, ACTIVATION, PHENOTYPE

Beside alterations in cellular genome that develops the neoplastic potential of a tumor, recently data have revealed the outstanding involvement of microenvironmental component (tumor stroma) in neoplastic initiation, progression, invasion and metastasis (7,8).

Fibroblasts are elongated cells embedded in the connective tissue and are responsible for its synthesis and maintenance (9). They are non-vascular, non-epithelial and non-inflammatory cells and form the essential cellular component of connective tissue. Fibroblasts participate in the regulation of processes such as inflammation, wound healing and differentiation of their neighboring epithelial cells (10). They synthesize fibrillar Extra Cellular Matrix (ECM) proteins, such as type I, III and V collagens and fibronectin. At the same time, fibroblasts are also an important source of ECM degrading proteases such as matrix metalloproteinases (MMPs) which are involved in maintaining the ECM homeostasis. In healthy state, the rate of proliferation and the metabolic capacity of fibroblasts are low. During the wound healing, fibroblasts acquire secretory and contractile capacities and the index of proliferation increases (11). At the same time, activated fibroblasts secrete various chemokines, leading to recruitment of blood cells at the site of injury. After the reparation of damaged tissue, fibroblasts are returning to resting state (12). Main expression of fibroblast in resting state and upon activation can be observed in Figure 3.

Fibroblasts display different phenotypic characteristics, provided of anatomic region where they were isolated from (skin, articulation) (13). In addition they demonstrate diversity in topographic expression for genes involved in extracellular matrix synthesis, growth and differentiation, cell migration as well as genes involved in genetic syndromes. Chipev and Simon (14) indicated that fibroblasts from different body sites differ in size, with palmar fibroblasts being smaller than non-glabrous derived fibroblasts. In addition, growth kinetics and TGF (Transforming Growth Factor) -β1 Receptor II expression as well as the ability to contract collagen lattices were found to differ with palmoplantar skin derived fibroblasts having lower receptor levels and an increased mitotic rate. The authors speculated that this regional diversity may in part account for localized susceptibility to disease manifestation like scarring and keloid formation. Recent work on oral mucosal fibroblasts has demonstrated the differences in the capacity of these fibroblasts to reorganize collagen lattices by an increased matrix metalloproteinase (MMP)-2 expression as compared to skin derived fibroblasts (15).

Additional patterns of fibroblasts heterogeneity have been identified through the study of wounding and wound healing. Wound healing proceeds in three interrelated phases (16): inflammation phase, proliferation phase and maturation phase. Although fibroblasts have long been recognized as key cells in those process, it is only recently that we have begun to understand the origin and phenotypic differences of fibroblasts as related to the specific phases of wound healing. The initial inflammatory phase consists of an orderly extravasation of leucocytes like PMN and monocytes/macrophages into the wound. It has been shown that a population of fibroblasts which arise from blood-borne circulating progenitor cells migrate into the wound site and may account for as many as 10% of the cells that infiltrate sites of acute tissue injury (17). These cells were also termed fibrocytes and express markers for cells of both hematopoietic and mesenychmal origin. The collagen+/vimentin+/CD34+/CD13+/CD45+ positive fibrocyte cells may contribute to normal wound repair by antigen presentation but may also participate in pathologic fibrotic responses (18).

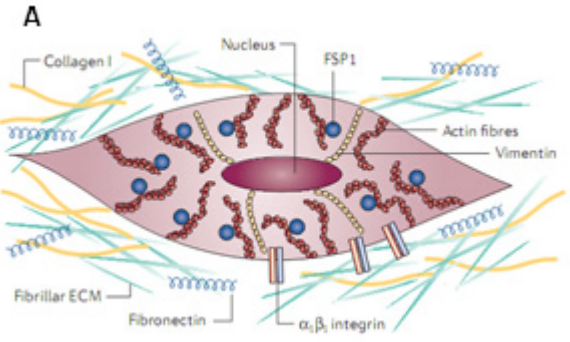
In the final phase of wound healing, a specialized population of differentiated fibroblasts occur (19). It has been demonstrated that fibroblasts under mechanical tension and under the influence of Platelet Derived Growth Factor (PDGF) express stress fibers. This cell type is termed proto-myofibroblast. Additional tension, TGF-β and a splice variant of fibronectin promote further differentiation into the myofibroblasts. These contractile myofibroblasts express *alpha* smooth muscle actin and are involved in the production of extracellular matrix and tissue contraction. Provided evidences showed that human wound fibroblasts represent a functionally different population than fibroblasts isolated from unwounded dermis (20). In their analysis, scientists concluded that 30–40% of wound-derived fibroblasts were myofibroblasts whereas only 1% of normal fibroblasts were alpha smooth muscle actin positive. Early studies demonstrated that fibroblasts of high population doubling which have left the cell cycle can carry out lattice contraction at least as effectively as cycling cells from early passages, leading the authors to conclude that the contraction of wounds is not due to myofibroblasts. *In vivo* data indicated that the number of myofibroblasts in the wound is dependent upon the amount of transplanted deeper dermal portion (21). Unfortunately it remains unclear whether this effect is due to the matrix or the resident cell population. Regardless of whether myofibroblasts are the true motile force for wound contraction, there is general agreement that wound contraction is an active cellular phenomenon depending on the activity of viable fibroblasts. Biological control of these cells either by the

matrix or by surrounding cells may minimize scar contraction (22).

During tumor progression, tumorigenesis and stromagenesis occur as parallel processes. The normal stroma serves as a natural tumorigenic barrier. During the initial stages of tumorigenesis, tumor cells induce normal stroma to become primed and engage in a feedback loop between stroma and tumor, which further promotes both processes (23). Additional alterations in the primed stroma will result in a series of gradual transitions, which will culminate in a phenotypically identifiable activated stroma (24) (25). Eventually, both stroma and tumor will become independent of one another irreversibly committed to a transformed phenotype (26). The 'point of no return' manifested either at the transition from primed to activated, or within the activated stromagenic stage remains to be identified.

One promising candidate for a stroma-specific marker is the fibroblast activation protein (FAP), a dipeptidyl peptidase of the serine protease family (27,28). FAP displays a restricted pattern of protein expression and enzymatic activity since FAP is expressed by stromal fibroblasts and fibroblasts in granulation tissue during wound healing, but not on fibroblasts in normal stroma or by epithelial cells regardless of their transformation stage (29). The selective expression of FAP postulates it as an attractive candidate for early therapeutic intervention. FAP has been shown to induce tumor growth in a murine animal model of tumorigenesis, and pretreatment with FAP antibodies attenuated FAP-dependent tumor growth (28). Another protein whose expression was originally thought to be restricted to the stroma is tenascin-C. Tenascin-C was initially considered to be a stromal marker for malignancy, since it was not detected in normal adult mammary tissue or its expression was limited to ductile tissue (27). However, recent studies have shown that tenascin-C is also expressed in breast hyperplasia, dysplasia and benign breast neoplasia (30). Thus, the relevance of tenascin-C as a diagnostic marker for tumors may be its enhanced levels of expression in primed and activated stroma.

On early stage of tumor, neoplastic cells form a lesion which is surrounded and separated by the rest of tissue by a basement membrane – carcinoma in situ (1). This membrane, along with capillary vessels, imune cells, fibroblasts and ECM, is forming the tumor reactive stroma (1,31). Due to an increased number of fibroblasts and capillary vessels, an increased amount of type I-collagen, it is assumed that this reactive stroma provides pro-tumorigenic signals. Some studies carried out on cancer-prone chickens are sustaining this assumption (32,33).



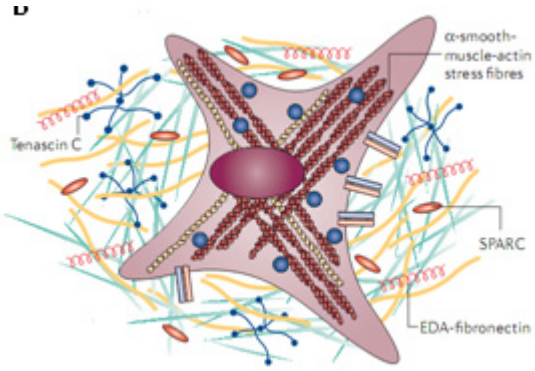


Fig.3. Fibroblast in resting state (A) and activated fibroblast (B) (10)

Fibroblasts are found in various proportions across the spectrum of carcinomas, constituting in many cases the preponderant cell population of the tumor stroma. The term "cancer-associated fibroblast" includes at least two distinct cell types: (1) cells with similarities to the fibroblasts that create the structural foundation supporting most normal epithelial tissues and (2) myofibroblasts, whose biological roles and properties differ markedly from those of tissue-derived fibroblasts. Myofibroblasts are identifiable by their expression of α-smooth muscle actin (α-SMA). They are rare in most healthy epithelial tissues, although certain tissues, such as the liver and pancreas, contain appreciable numbers of α-SMA-expressing cells. Myofibroblasts transiently increase in abundance in wounds and areal so found in sites of chronic inflammation. Although beneficial to tissue repair, myofibroblasts are problematic in chronic inflammation, contributing to the pathological fibrosis observed in tissues such as lung, kidney and liver. Recruited myofibroblasts and reprogrammed variants of normal tissue-derived fibroblastic cells have been demonstrated to enhance tumor phenotypes, notably cancer cell proliferation, angiogenesis, and invasion and metastasis; their tumor promoting activities have largely been defined by transplantation of cancer-associated fibroblasts ad mixed with cancer cells into mice, and more recently by genetic and pharmacologic perturbation of their functions in tumor-prone mice (34-36).

Because they secrete a variety of extracellular matrix components, cancer-associated fibroblasts are implicated in the formation of the desmoplastic stroma that characterizes many advanced carcinomas. The full spectrum of functions contributed by both subtypes of cancer-associated fibroblasts to tumor pathogenesis remains to be elucidated.

Fibroblasts communicate with cancer cells, resident epithelial cells, endothelial cells, pericytes and inflammatory cells through the secretion of growth factors and chemokines. Through the increased deposition of collagen types I and III and de novo expression of tenascin C they induce an altered extracellular-matrix microenvironment, which potentially provides additional oncogenic signals, probably leading to accelerated cancer progression. Fibroblasts mediate the inflammatory response by secreting chemokines such as monocyte chemotactic protein 1 (MCP1) and interleukins such as IL-1. Fibroblasts interact with the microvasculature by secreting matrix metalloprotein-

ases (MMPs) and vascular endothelial growth factor (VEGF). Fibroblasts also provide potentially oncogenic signals such as transforming growth factor-β (TGF-β) and hepatocyte growth factor (HGF) to resident epithelia, and directly stimulate cancer-cell proliferation and invasion by secreting growth factors such as TGF-β and stromal-cell-derived factor 1 (SDF1) (10).

Once activated to differentiate, myofibroblasts produce a myriad of mediators, including TGF-β, VEGF, bFGF, hepatocyte growth factor and the insulin-like growth factor – 1 (IGF-1) that contribute to the proliferation and expansion of the tumor cell clone. Additionally, TGF-β activation induces stromal myofibroblasts to overproduce various ECM components, including collagen I (27,37), and increased amounts of collagen types VI, XV, and XVIII, tenascin-C, plasminogen activator inhibitor-1 (PAI-1), and various growth factors are deposited in the ECM of human skin (38). During tumorigenesis, changes in the ECM microenvironment induce stromal fibroblasts and tumor cells to modify their complex network of signal transduction pathways (27,39). Although signaling pathways affected by TGF-β treatment comprise Smad pathways, as well as non-Smad signaling events including the mitogen activated protein kinase pathways (24,40), additional work remains to clarify signaling cascades downstream of TGF-β stimulation.

Next to these molecular markers of fibroblasts activation, some other proteins expressed by stromal fibroblasts are recognised to have a prognostic value for solid tumors. In particular, a poor prognosis has been associated with expression in CAFs of the hypoxia marker carbonic anhidrase IX in human lung adenocarcinoma (41), or periostin in cholangiocarcinoma (42), or p53 tumor suppressor in ductal carcinoma (43). On the other side, expression in CAFs of Caveolin-1, *PTEN* or podoplanin correlates with a favourable prognosis for several carcinomas (44-46).

In addition, genetic studies mainly carried out in breast cancers reported CAF somatic mutations in *TP53* and *PTEN*, as well as gene copy number alteration at other loci in tumor stroma (47). In keeping with this idea, p53 inactivation in stromal fibroblasts, as well as that genetic inactivation of *PTEN* in CAFs enhances tumor progression in breast carcinoma models (48) (44). These studies collectively evoke the idea that the tumor promoting activity of CAFs may be, above all, founded on these somatic mutations in key tumor suppressor genes. In addition, somatic alterations were consistently observed at a high frequency (>30%) in tumor adjacent fibroblasts (49).

Oppositely, more recent studies have alleged that genetic alterations were detected only in cancer epithelial cells and not in the stroma (50), and copy number and loss of heterozygosity analysis of CAFs derived from breast and ovarian carcinomas showed that somatic genetic alterations in CAFs are extremely rare and cannot be the basis of the carcinoma-promoting phenotypes of these cancers (51).

SIGNALING PATHWAYS BETWEEN CAFS AND TUMOR CELLS – A GENUINELY MAZE

Cancer cells have the ability to divide indefinitely and spread to different parts of the body during metastasis. In the past decade, many of the molecular mechanisms underlying tumorigenesis in cancer have been elucidated. JAK/STAT, Notch, MAPK/ERK, PI3K/AKT, NF-κB, Wnt and TGF-β pathways are involved in cancer progression. The genetic elements that are necessary for tumorigenesis have been well characterized in the past 20 years. Human somatic cells can become cancerous by activation of defined genetic elements such as introduction of oncogenic RAS, blockage of the p53/Rb pathway and activation of telomerase (52,53).

The JAK/STAT pathway is intimately linked to growth factor signaling, apoptosis and the cellular immune response. Deregulated JAK/STAT signaling can contribute directly and indirectly to tumorigenesis (54). Mutations, fusions, and/or amplification of JAK/STAT signaling components, such as the HER2/neu- in mammary and stomach carcinomas, or Epidermal Growth Factor-Receptor (EGF-R) in breast, brain and stomach tumors, can confer hypersensitivity to mitogenic signals and promote proliferation (55,56).

In some animal models, Notch plays a crucial role in the earliest stages of T-cell development and is dispensable for further differentiation to intrathymic T-cells. These results suggest that neoplasias could occur when Notch signaling remains active beyond normal development (57). Recent studies have implicated aberrant Notch signaling in human breast tumors, melanoma progression, medullablastoma and ovarian cancers. In the case of human breast tumors, amplification of Notch receptors and the presence of ligands, such as Jagged-1 correlate with a more aggressive disease phenotype. This phenotype can be relapsed by the expression of NUMB, a Notch antagonist (58).

RAS mutations are found in ~45% of colon cancers and ~90% of pancreatic cancers (59). RAF mutations, in turn, are found in just about two thirds of all melanoma (60). Disregulated PI3K/AKT signaling has been observed in various cancers. Mutations in the PI3K/AKT pathway inhibitor and tumor suppressor PTEN has been found in glioblastomas, lung carcinomas and melanomas whereas AKT overexpression or overactivation has been found in breast, ovarian, thyroid and other cancers (61). In conclusion, the PI3K/AKT pathway is constitutively active in numerous human cancers. Activation of this pathway can promote cell survival and proliferation.

Mutations and miss-regulation of NF-κB signaling has been involved in a variety of cancers (such as human B-cell malignancies).

Wnt-signaling is active in the cycling stem cells that reside in the bottom layer and turned off upon differentiation and migration to the top layer. In a majority of colon cancers (90%), APC protein, which under normal states targets β-catenin for degradation, is mutated condition producing β-catenin stabilization and initiation of the Tcf/Lef transcriptional program (62). Accordingly, APC deficient progenitor cells keep replicating, remain within the tissue, and increase the risk of gaining further mutations that alter them and become malignant (63).

Mutations or downregulation of TGF-β receptors, inactivation of SMAD4 or p15 INK4B can be found in a variety of cancers.

Primarily, SMAD4 inactivation arises in 53% of human pancreatic ductal adenocarcinomas (PDAC) (64) (65). Moreover, BMP2 is dramatically overexpressed in 98% of lung carcinomas (66) (67). TGF-*β* signaling can also amplify malignancy of epithelial tumors by stimulating metastasis: heterogeneous processes during which a cell disintegrates, develops increased motility and invasiveness to enter the circulatory system and settles a new niche within the body. In varoius aspects, metastasis resembles the highly coordinated cell movements that occur during embryonic development. EMT during metastasis causes cellular adhesion molecules, such as E-cadherins and cytokeratins, to be replaced by mesenchymal proteins N-cadherin and vimentin (68). Surprisingly, pathways and genes involved in EMT during development such as TGF-*β*, GSC or TWIST, are re-expressed during tumorigenesis and can initiate metastasis (69,70).

CAFs are actively implicated in communication with other cellular populations inside the tumor. In this condition, TGF-*β*, which is expressed by both cancer cells and CAFs, boosts tumorigenesis and causes the invasive phenotype and also avoiding the immune defense (71,72). Hepatocyte growth factor (HGF), also produced by CAFs, binds with high affinity to the receptor c-Met. The overexpression of c-Met signaling induces tumor proliferation, invasion and metastasis (73).

Figure 4 is illustrating the envolvement of CAFs in tumoral signaling mechanisms.

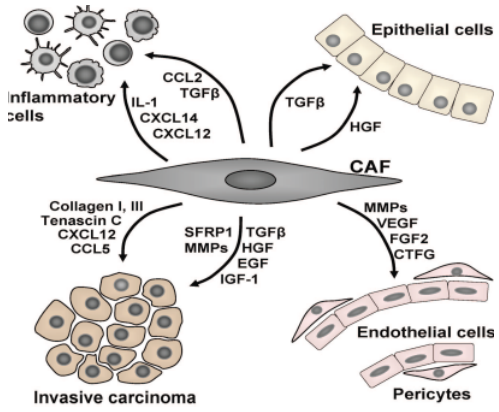


Fig.4. Signaling with envolvement of CAFs (10)

Many recent data reveal the essential role of WNT signaling in extracelullar, cytoplasmic and nuclear key regulation, in order to maintain tissues maintenance. WNT/PCP signaling pathway is implicated in embryogenesis as well as in carcinogenesis. Single nucleotide polymorphisms (SNPs) and copy number polymorphisms (CNPs) of cancer associated genes are utilized for the screening of cancer predisposed patients (74). Aberrant activation of the WNT/PCP signaling pathway leads to abnormal tissue polarity, which is one of characteristics of cancer cells. SNPs and CNPs for WNT/PCP signaling molecules are important candidates for use in screening of cancer predisposition, especially for gastric cancer. The deviant activation of the WNT/PCP signaling pathway also conducts to poor prognosis of human cancer patients via induction of invasion and metastasis. cDNA-PCR, microarray or ELISA reflecting the aberrant activation of WNT/PCP signaling pathway could be developed as novel cancer prognostics (75).

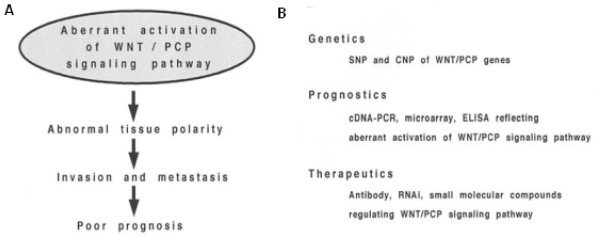


Fig.5. WNT/PCP signaling pathway and human cancer (75). (A) Aberrant activation of WNT/PCP signaling pathway leads to poor prognosis; (B) Clinical applications

EPITHELIAL - MESENCHYMAL TRANSITION (EMT) – ESSENTIAL IN BODY DEVELOPMENTAND POWERFUL "ALLY" OF NEOPLASTIC THREAT

EMT possess an essential role in formation and differentiation of tissues and organs during the body development. Throughout the embryonic ontogeny, EMT induces intense phenotypic changes that include the loss of cell-cell adhesion, the loss of cell polarity, and the acquisition of migratory and invasive properties. During gastrulation, the embryonic epithelium undergoes EMT to give rise to the mesoderm. During delamination of the neural crest, EMT is used to form a population of highly motile cells that ultimately incorporate into many different tissues (76). Induction of EMT alters cytoskeletal structure and leads to the breakdown of interactions between cells, their neighbors, and the underlying substratum. These phenotypic changes are driven by alterations in the expression of many genes including cytoskeletal components, transcription factors and enzymes. By any chance the most well-studied EMT stimulus is transforming growth factor (TGF)-β, which initiates and maintains EMT in a variety of biological systems (70).

Whereas embryonic EMT is normally a self-resolving process, EMT serving tissue repair or metastatic dissemination is often correlated with concomitant inflammation and continues until the provoking cause is eliminated (77). In this framework, CAFs are active in sustaining a chronic pro-inflammatory drive, eliciting multiple effects concurring to induce/select cancer cell phenotypes resistant to the hostile tumour microenvironment. In incipient tumors, CAFs have been shown to orchestrate macrophage recruitment and neovascularization in strict dependence on nuclear factor-κB (NF-κB) activation (78). Furthermore, CAFs exert their propelling role for EMT by eliciting similar pro-inflammatory pathways in metastatic cells, exploiting oxidative stress and involving the activation of COX-2, NF-kB and HIF-1 (79). The fact that cancer cells undergoing EMT in response to CAF contact share with inflammatory cells the same signals is in keeping with the idea that metastasis is a phenomenon reminiscent of the igratory/invasive behaviour of inflammatory cells. Besides epigenetic mechanisms influencing cross signalling between CAFs and cancer cells, malignancy may

also be regulated by stromal mutations affecting the motility and aggressiveness of cancer cells.

Epithelial to mesenchymal transition (EMT) is thought to be one of the sources of CAFs in tumors (77). Thus, epithelial cells, through an EMT process, achieve mesenchymal characteristic and become fibroblasts (80). This hypothesis arises from the evidence that epithelial cells exposed to MMP-driven oxidative stress undergo DNA oxidation and experience mutations, thereby undergoing to specialized EMT in which they transdifferentiate into activated myofibroblasts (81,10). Studies found that when normal fibroblasts were coinjected with cancer cells and then isolated by flow cytometry from the resultant orthotopic tumors, fibroblasts expressed the characteristic proinflammatory CAF signature. It was previously shown that coculturing of pancreatic or lung cancer cells with stromal fibroblasts induced the expression of COX-2 and IL-8 (82,83). The concept of *in vivo* education of normal cells into tumor-promoting stromal cells was previously described to the recruitment of monocyte progenitors and their differentiation into tumor-associated macrophages, which are functionally distinct from resident tissue macrophages (84).

EMT allows cancer cells to adopt a mesenchymal cell phenotype, characterized by an enhanced migratory capacity and invasiveness (77). EMT is induced by many factors (i.e. PDGF, TGFβ, EGF, etc) and is mediated by the activation of typical transcription factors like Snail, Slug, Twist and FOXC2 (85). Similarly, proliferating endothelial cells might contribute to CAF via endothelial to mesenchymal transition (EndMT). This process is characterized by the loss of endothelial markers like CD31, the expression of mesenchymal markers like FSP-1 and SMA under the stimulation of TGFβ , a growth factor abundantly present within tumor microenvironment (77). Conversely to embryonic EMT, EMT serving tissue repair or metastatic dissemination is often correlated with tissue inflammation and persists until the provoking stimulus is eliminated (77). In this context CAFs play a key role in sustaining a chronic pro-inflammatory stimulus, giving rise to multiple effects and culminating in prompting the escape from the hostile tumor microenvironment.

Inducing the EMT of tumor cells, CAFs contribute to the epigenetic programme leading to invasive process engaging a mesenchymal, motile and proteolytic phenotype (77) (6). In addition to the pro-migratory stimulus, EMT has also been correlated with the induction of a cancer stem cell phenotype. Indeed, in breast cancer cells, EMT, due to the overexpression of Snail or Twist transcription factors, is correlated with increased CD44/CD24 ratio as well as with the generation of tumor initiating cells which spread metastases (86,87). Congruent with this, Giannoni (79) reported that CAFs isolated from prostate carcinoma specimens, by means of activating the EMT epigenetic programme, promote/select the generation of cancer stem cells. Indeed, CAFs affect the self-renewal ability of carcinoma cells and enhance their expression of cancer stem cell markers (CD133+ and high CD44/CD24 ratio) and the formation of non-adherent prostaspheres, a property associated with prostate stem cells (86-88). Analysis of tumor-forming ability and spontaneous lung metastasis formation after contact with CAFs revealed that the activated stroma contributes to generate a population of prostate cancer stem cells with defined ability to form primary tumors and distant metastases (88). In keeping with this, in a onditional PTEN deletion mouse model of prostate adenocarcinoma, CAFs confirm their key role in the regulation of stemness properties as they enhance spheroid formation and prostate glandular structures with lesions, high proliferative index and tumor-like histopathology (89).

Mutations in stromal cells have been attributed to several reasons. First, EMT induced by CAFs in cancer cells may be directly responsible for the accumulation of mutations by tumor or stromal cells. Indeed, stromal-derived MMP-3, which is frequently upregulated in breast cancers, induces genomic instability through the upregulation of ROS (81). Alternatively, mutated stromal cells might directly derive from cancer cells that have undergone EMT and achieve the characteristics of CAFs (90). Finally, in mouse prostate carcinoma cells, a paracrine mechanism determines a selective pressure for the expansion of a p53-lacking subpopulation of CAFs. These CAFs lacking p53 contribute to cancer invasion and to the eventual loss of p53in the epithelium. Furthermore, p53 mutations in CAFs are also significantly associated with lymph node metastases in several sporadic breast cancers (91). Whatever their origin, these mutated CAFs show a profoundly altered behaviour and concur to tumor malignancy.

Because the EMT process generates cells of mesenchymal, migratory, contractile phenotype, these signals may cause both feed-back and feed-forward loops that dynamically impact the patterning of the tissues. Biological systems generate, transmit and concentrate mechanical stress into spatial patterns; these gradients in mechanical stress may serve to spatially pattern developmental and pathologic EMTs (92). Future studies investigating the signaling and mechanical regulatory networks within tissues undergoing EMT may reveal points and targets to augment the process (in the case of wound healing) or cut it short (in the case of metastasis).

CONCLUSIONS. FUTURE INSIGHTS

The stroma is generally made up of other cell types, extracellular matrix, and the vasculature and lymphatic systems feeding the tumors. Cell types in the tumor stroma include fibroblasts, myofibroblasts, granulocytes, macrophages, mesenchymal stem cells, and lymphocytes (93). In this context of the tumor microenvironment, some of these cell types additionally change phenotype and become accessories to tumorigenesis and metastasis. Hypoxic stress also promotes angiogenesis, lymphangiogenesis, and inflammation, leading to recruitment of new nutrient supplies and inflammatory cells that can then further potentiate the metastatic phenotype (94).

It is well known that origins of many human diseases, including cancer, diabetes, and neural disorders, are in the functioning (and malfunctioning) of signaling components. It is required to understand how individual components function within the context of the entire tumoral system. Tumoral microenvironment, through

its complicated and heterogenous mechanisms of maintaining the tumor homeostasis, could comprise a considerable variants of novel targets for anti-neoplastic therapeutics.

Nevertheless, there are enough obstacles which have to be regarded: the cellular components (CAFs, inflamatory cells, ECs) are not malignant cells, in consequence the therapeutic target must be as high as specific in order to avoid normal cells to be affected; there are a lot of mutual interactions between CAFs and tumor cells, multiple signaling interactions, such that it is highly difficult to establish a therapeutic target without the tumor to find an escape way or to develop resistance; there is strong evidence, *in vitro* and *in vivo*, demonstrating that EMT grants dedifferentiated malignant epithelial cells with mesenchymal, migratory and proteolytic properties that are required for local tumor invasiveness, a mandatory to metastasis formation.

Research on the role of EMT in cancer progression, is fully ongoing. It is plausible that specific targeting of EMT could potentially serve to decrease metastasis and overcome drug resistance. At the same time, depicting the molecular crosstalk between stromal cells and carcinoma cells may provide invaluable insights into the pathophysiological processes involved in cancer. In this respect, the occurence and improvement of varied methods of investigation based on high-quality, basic and translational research (eg. development of new mouse models, multiscale mathematical models, improved *in vivo* imaging techniques and the identification of new molecules important in regulating stroma-tumor interactions, etc.)

AKNOWLEDGMENTS

This work was supported by the Sectorial Operational Programme for Human Resources Development, financed from the European Social Fund, FSE POSDRU 107/1.5/S/78702.

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FIBROBLASTELE PERITUMORALE – COMPONENTE TUMORALE CU ROL DEFINITORIU IN CANCER

REZUMAT

Este evident rolul activ al componentei stromale in proliferarea, invazia si metastazarea tumorilor solide. Fibroblastele peri-tumorale sunt principalele elemente celulare stromale care contribuie la dobandirea caracterelor maligne. Se cunoaste faptul ca fibroblastele peri-tumorale difera de fibroblastele normale din punct de vedere epigenetic, posibil si genetic. Totusi, originea si caracterizarea fenotipica moleculara sunt aspecte intens dezbatute. Elucidarea caracteristicilor si comportamentului celulelor non-tumorale precum si interactiunea acestora cu celulele tumorale pot dezvalui noi tinte terapeutice in bolile neoplazice.
Cuvinte-cheie: fibroblaste peri-tumorale, biologia tumorii, stroma, semnalizare celulara, tinte terapeutice

THE EFFECTS OF RUTIN AND OMEGA-3 POLYUNSATURATED FATTY ACIDS ON THE OXIDANT-ANTIOXIDANT BALANCE IN A RAT MODEL OF ADJUVANT-INDUCED ARTHRITIS

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ABSTRACT

Oxidative stress plays an important role in the pathogenesis of rheumatoid arthritis (RA). Reactive oxygen species (ROS) produced during cellular oxidative phosphorylation and during the oxidative burst by activated phagocytes exceed the physiological buffering capacity and result in oxidative stress. The excessive production of ROS can damage proteins, lipids, nucleic acids and matrix components. Patients with RA have an altered antioxidant defense capacity. The purpose of this study was to observe and quantify the antioxidant effects of rutin and omega-3 polyunsaturated fatty acids (PUFA) in a rat model of adjuvant-induced arthritis (AIA). Five groups of rats were compared: a non-arthritic control group and four AIA groups treated via an intragastric tube for 21 days, 7 days after the induction of AIA, with: vehicle (saline solution), non-steroidal anti-inflammatory agents (indomethacin), rutin or omega-3 (PUFA) respectively. The arthritic score was determined in AIA rats as an indicator of destructive arthritis-associated clinical changes. After 28 days the serum levels of the malondialdehyde (MDA), carbonylated proteins (CP), sulfydryl groups (SH), hydrogen donor ability (HD) and glutathione (GSH) were measured. The results indicated an increase in the oxidative stress as seen by the increase in free radical production, MDA and CP levels, and impaired antioxidant defense systems shown by HD, SH and GSH levels in AIA rats. The MDA and CP levels were significantly decreased and HD, SH and GSH levels were significantly increased after the oral administration of rutin. Omega-3 (PUFA) increased the MDA, CP, HD, SH levels and decreased the GSH level. This study suggests that rutin and omega-3 (PUFA) treatment can be beneficial in attenuating the oxidative stress associated with RA.

Key words: oxidative stress, adjuvant-induced arthritis, rutin, rats.

INTRODUCTION

Rheumatoid arthritis (RA) is a frequent chronic disease characterized by persistent inflammation in multiple joints. Rheumatoid arthritis is associated with a high cardio-vascular mortality, which exceeds that of the general population. Pain and inflammation are the initial symptoms, followed by various degrees of joint destruction. Prostaglandin production (PG) by cyclooxygenase (COX) from the arachidonic acid's metabolism is one of the main pathways in the pathogenesis of acute inflammation. Non-steroidal anti-inflammatory drugs are used for the treatment of RA but have many side effects because of their COX inhibition (8). Therefore, the anti-inflammatory drugs available nowadays are not a very good therapy option.

Recent research shows that oxidative stress plays an important role in the pathogenesis of RA (7). Reactive oxygen species (ROS) produced during the cellular oxidative phosphorylation and during the oxidative burst by activated phagocytes exceed the physiological buffering capacity and result in oxidative stress. The excessive production of ROS can damage proteins, lipids, nucleic acids and matrix components. Epidemiological studies showed an inverse association between the antioxidant intake and the incidence of RA, and between the antioxidant level and inflammation (13, 14). During the lipid peroxidation, polyunsaturated fatty acids are oxidized, generating peroxide radicals, which oxidize other polyunsaturated fatty acids, in a chain reaction that affects cellular membranes. The degradation of extracellular matrix by cytokine-stimulated chondrocytes is mainly due to lipid peroxides and can be prevented by antioxidant intake (10).

Adjuvant-induced arthritis (AIA) is characterized by inflammation and aggressive pannus formation, which leads to cartilage and bone degradation. AIA in rats is a model of inflammatory joint disease associated with RA (8).

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Rutin is an important flavonoid due to its many biological properties, including anti-carcinogenic, cytoprotective, anti-platelet, antithrombotic, vasoprotective and cardioprotective effects, and, last but not least, it is a powerful anti-inflammatory and a potent antioxidant polyphenol because it scavenges ROS and free radicals directly (5, 21, 24, 25).

Alpha-linolenic acid (ALA) is an essential fatty acid from the human diet which can be metabolized into n-3 long-chain poly-unsaturated fatty acid (n-3 LC-PUFA) in the mammalian tissues (23). The major bioactive n-3 LC-PUFAs are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Recent research shows that omega-3 PUFA reduces the risk of cardiovascular disease (CVD), obesity, diabetes, inflammation, and several neurological diseases (17). The anti-inflammatory effects of omega-3 PUFA are due to its inhibiting the activation of the transcription factor NF-κB with consequent inhibition of pro-inflammatory cytokine production, such as IL-6 and TNFα (2, 9). Thus, new research shows that omega-3 PUFA modulates the oxidative stress (9).

The purpose of this study was to evaluate the role of rutin and omega-3 PUFA administration in attenuating the oxidative stress in RA.

MATERIALS AND METHODS

Based on the results of the preliminary test, we established the experimental design. The study was conducted on 35 adult male Wistar rats, weighing 225± 25 g, divided into 5 groups (n=7).

Two control groups: group I (healthy, non-arthritic control rats) and group II (arthritic rats) were treated with saline solution, administered daily via an intragastric tube (0.6 ml/rat/day) for a period of 21 days. Three arthritic groups (group III, IV and V) were treated with non-steroidal anti-inflammatory agents (IND, indomethacin 2 mg/kg/day, dissolved in saline solution), rutin (RUT, 80 mg/kg/day, dissolved in saline solution) or omega-3 PUFA (10 mg/kg/day, dissolved in saline solution) administered daily via an intragastric tube (0.6 ml/rat) for a period of 21 days.

Chronic AIA rats were used as a typical inflammatory arthritis pain model (16). A total of 35 animals were used in the present investigations, out of which 28 underwent chronic, unilateral inflammation, the remaining seven served as untreated (non-inflamed) control animals. Arthritis was induced by injection of 0.1 ml of Freund's adjuvant (FA; Sigma, UK) into the left hind footpad. AIA was confirmed 7 days after the injection by clinical examination. The rats were treated with indomethacin, rutin and omega-3 PUFA for 21 days after the arthritis confirmation.

At the onset of arthritis (day 7) and on days 14, 21 and 28, the severity of arthritis was assessed visually by two independent observers who were unaware of the treatment protocol the rats received. A modified macroscopic scoring system was used to monitor the severity of arthritis, as described previously (15). Briefly, the severity of inflammation in each paw graded on a scale from 0-4, where 0= no signs of arthritis, 1=detectable swelling and/or redness of the paw or one digit, 2= two joints involved with moderate swelling and redness, 3= more than two joints involved, 4= severe arthritis of the entire paw and all digits. The total score was the cumulative value in each of the 4 paws, with a maximum of 12 for each rat. Rats with a total score of >2 were considered to have arthritis.

After the last treatment (day 28), all the rats were fasted overnight and sacrificed by cervical decapitation with sodium pentobarbital (60 mg/rat ip). Venous blood samples were collected from the rats' retro-orbital sinuses. The serum was separated for the estimation of free radical production: malo-dialdehyde (MDA) (by fluorescein dosage, Conti method) (6) and carbonylated proteins (CP) levels (18). The results were expressed in mmol/mg protein. For the estimation of the defense systems: the assessment of hydrogen donor ability (HD) was measured by Janaszewska method and expressed in inhibition % (12); total SH groups of serum were assayed according to the Hu method (11), the concentration was expressed in micromol/ml and the serum gluthatione (GSH) level was measured using the Ellman method and expressed in nmol/ml.

The experimental protocol has been approved by the Ethics Committee of the University of Medicine and Pharmacy Cluj-Napoca and conforms to the Guide for the care and use of laboratory animals NIH publication No. 85-23, revised 1996.

Statistical analysis

The statistical analysis was carried out using the SPSS 17 - Statistical software package (SPSS Inc, Chicago, Illinois, USA). Data are expressed as the mean ± standard deviation. Statistical significance was tested at a confidence interval of 95% ($p < 0.05$).

RESULTS

Effects of indhomethacin, rutin and omega-3 PUFA on clinical signs of AIA

After 7 days, the rats began to show evidence of clinical inflammation in one or both hind paws. The first manifestation of disease was erythema of one or more ankle joints, followed by involvement of the metatarsal and interphalangeal joints. The typical time course for the development and progression of disease, as assessed by the mean arthritis severity score is shown in Figure 1. The disease was progressive, with joint recruitment following: tarsal, metatarsophalangeal and then interphalangeal articulations.

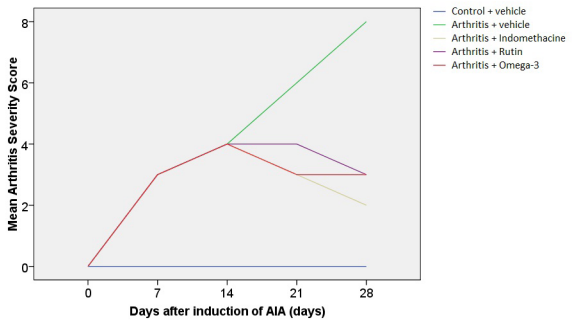

Fig.1. Effects of indhomethacin, rutin and omega-3 polyunsaturated fatty acids on clinical signs of adjuvant-induced arthritis

In the non-treated arthritic group (group II) the incidence of disease was 100% (all animals in the group were affected) at day 7, and remained as such throughout the duration of the experiment. In contrast, treatment with indomethacin, rutin and omega-3 PUFA exerted a significant attenuation in the incidence of AIA: 60% with IND treatment (P<0.05) and 50% with RUT and omega-3 PUFA treatment (P<0.05).

The serum oxidant-antioxidant balance

Median MDA levels were increased in the rats from group II (2.76±0.55) versus the rats from control group I (1.33±0.28). After the induction of AIA the MDA levels were very significantly (p<0.0001) increased compared to those of the control group (Figure 2). Median MDA levels were decreased after 21 days supplementation with IND (group III) (2.05±0.50) or RUT (group IV) (1.52±0.66) in the AIA rats. The difference was significant: p<0.05 and p<0.01 respectively versus the rats from group II. After 21 days of omega-3 supplementation (group V) the median MDA levels (3.28±0.57) were significantly increased (p<0.05) versus those of the rats from group II.

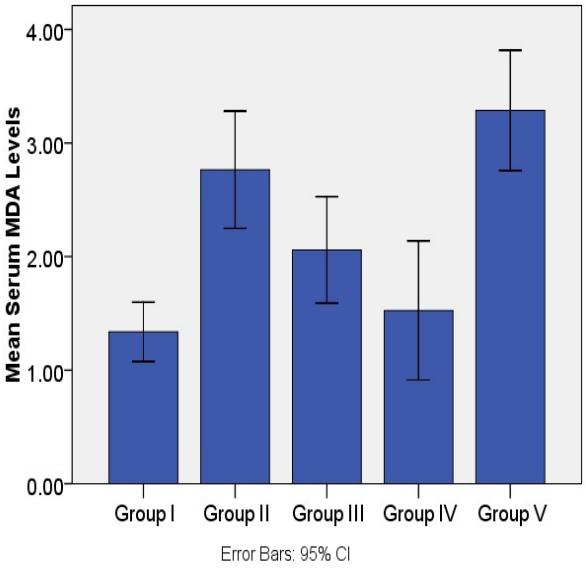

Fig.2. Serum MDA levels in tested groups and controls

Similar results were obtained for the CP determination. Statistical data showed a value of 2.62±0.16 in the rats from group II and 1.15±0.22 in the rats from the control group I. After the induction of AIA the CP levels were very significantly increased (p<0.0001) compared to the control group (Figure 3). After supplementation with IND (group III) (1.74±0.49) or RUT (group IV) (1.37±0.28) the median CP levels were very significantly decreased (p<0.002) versus those from group II. Supplementation with omega-3 (group V) (2.26±0.46) decreased significantly (p<0.05) the median CP levels versus those from group II, but significantly increased (p<0.05) versus those of the rats from group I.

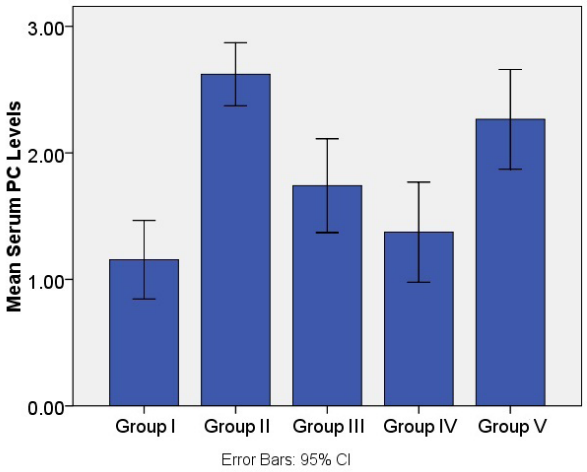

Fig.3. Serum CP levels in tested groups and controls

HD median levels were decreased after the induced of AIA in the rats from group II (26.10±2.75) versus the rats from the control group I (37.44±4.30). After the induction of AIA the HD levels were very significantly decreased (p<0.001) compared to the HD levels from the control group (Figure 4). After supplementation with IND (group III) (28.27±4.52) the median HD levels were not significantly increased versus those of the rats from group II. Supplementation with RUT (group IV) (36.48±3.00) in the AIA rats determined very significantly increased (p<0.0001) median HD levels compared to group II, and omega-3 supplementation (group V) (31.79±4.44) in the AIA rats determined significantly increased (p<0.0001) median HD levels.

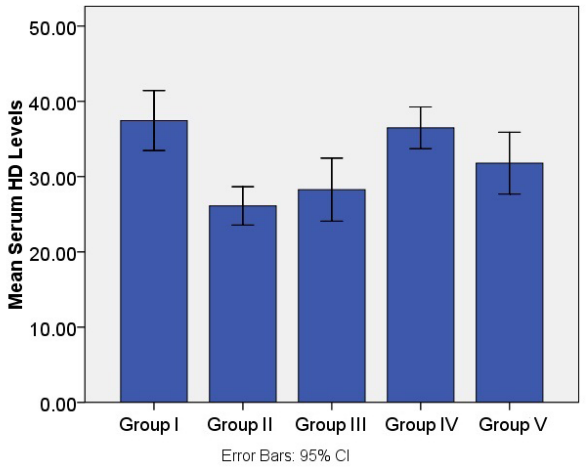

Fig.4. Serum HD levels in tested groups and controls

Median serum SH was lower after the induction of AIA in group II (0.09±0.01) versus the control group I (0.13±0.03). After the induction of AIA the SH levels were significantly decreased (p<0.01) compared to the SH levels in the control group (Figure 5). After supplementation with IND (group III) (0.16±0.06), RUT (group IV) (0.20±0.03) or omega-3 (group V), the median SH levels (0.15±0.02) in the AIA rats were significantly increased (p<0.01) or very significantly increased (p<0.0001) and (p<0.005)

respectively, versus those from group II.

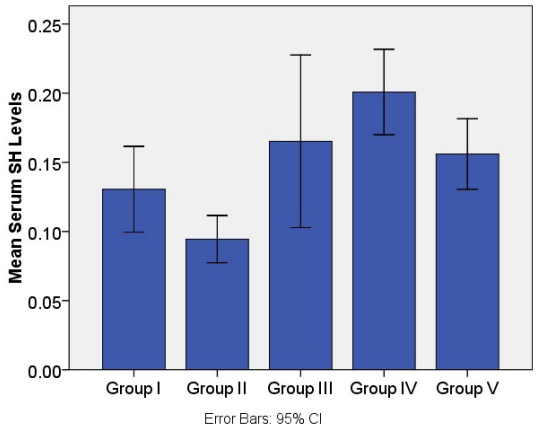

Fig.5. Serum SH levels in tested groups and controls

The median level of GSH was higher after the induced of AIA in group II (12.92±1.85) versus control group I (4.22±4.35). After the induction of AIA the GSH levels were very significantly increased ($p<0.005$) compared to the control group (Figure 6). After supplementation with IND (group III) (12.37±1.42) the median GSH levels were not significantly decreased versus those from group II. Supplementation with RUT (group IV) (16.66±3.58) in the AIA rats determined significantly increased ($p<0.05$) median GSH levels compared to group II, and omega-3 supplementation (group V) (6.77±0.92) in the AIA rats determined very significantly decreased ($p<0.0001$) median GSH levels compared to those from group II.

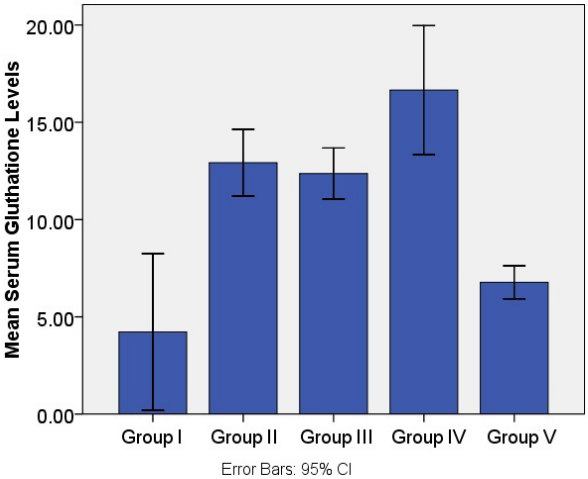

Fig.6. Serum GSH levels in tested groups and controls

DISCUSSIONS

Rheumatoid arthritis is characterized by persistent synovitis, systemic inflammation, and presence of auto-antibodies (7). The analgesic and anti-inflammatory effects of indhomethacin, a non-steroidal anti-inflammatory drug often used for the treatment of RA, partly produced by COX inhibition, are well known. The anti-inflammatory effect of indhomethacin is accompanied by a suppression of the oxidative burst of the stimulated blood phagocytes in animals treated with indhomethacin (4).

One of the mechanisms involved in the pathogenesis of RA is oxidative stress (10). A frequently used oxidative stress marker is determining the lipid and protein peroxidation by measuring MDA and CP levels. Clinical and experimental studies have shown increased plasmatic levels of MDA and CP in RA (3, 4, 20, 22).

The AIA model is robust, the incidence rate of the disease is 100%, and AIA rats share many features of RA with humans, such as in inflammation, marked bone resorption and periostal bone proliferation (19). AIA in rats has been adopted as an experimental paradigm for the pre-clinical screening of RA treatments. In this model we measured RA markers. Our data showed increased arthritis scores in AIA rats (Figure 1) and the fact that oral treatment with indhomethacin ameliorates the clinical severity of arthritis ($P>0.05$). The oral administration of rutin or omega-3 PUFA in AIA rats reduced the progression of arthritis by inhibiting the arthritis score.

Our present study provides clear evidence of increased oxidative stress status in an experimental rat model of RA. These observations are in agreement with previous reported data showing an increase of malondialdehyde and protein carbonyl in the serum of AIA rats. In the current study, we demonstrated that oral treatment with rutin reverses oxidative stress in AIA rats. This effect is accompanied by a decreased ROS production in serum, shown by the decrease of MDA and CP, and increased antioxidant status in serum as shown by HD levels, SH levels and GSH levels.

Recent studies clearly showed that high levels of unsaturated fatty acids induce an increase of n-3 PUFA levels in the tissues, which may lead to increased susceptibility to lipid peroxidation and in turn, oxidative stress. In our present study the oral treatment with omega-3 PUFA increased lipid and protein peroxidation (increased MDA and CP), increased the antioxidant status in serum: HD levels and SH levels and very significantly decreased the GSH levels.

CONCLUSION

Our results indicate that indhomethacin and rutin administration ameliorates the clinical severity of arthritis and decreases the oxidative stress. Thus we can conclude that oxidative stress plays an important role in AIA and could be controlled by a suitable combination therapy of indhomethacin and an antioxidant substance, as demonstrated for rutin. Omega-3 PUFA ameliorates the clinical severity of arthritis and moderately decreases the oxidative stress, and its administration can be beneficial in reducing the risk of CVD in RA.

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EFECTUL RUTINULUI ŞI AL ACIZILOR GRAŞI POLINESATURAȚI OMEGA-3 ASUPRA BALANȚEI OXIDANȚI-ANTIOXIDANȚI LA ŞOBOLANI CU ARTRITĂ INDUSĂ DE ADJUVANT

REZUMAT

Stresul oxidativ joacă un rol important în patogeneza artritei reumatoide (AR). Speciile reactive ale oxigenului (SRO) produse în cursul proceselor celulare de fosforilare oxidativă şi prin activarea celulelor fagocitare depăşesc capacitatea antioxidantă determinând apariţia stresului oxidativ. Producţia excesivă de SRO atacă proteinele, lipidele, acizii nucleici şi componentele matricei celulare. Pacienţii cu AR au alterată capacitatea antioxidantă a organismului. În acest studiu ne-am propus să evidenţiem şi să cuantificăm efectele antioxidante ale rutinului şi al acizilor polinesaturaţi (PUFA) omega-3 într-un model de şobolan cu artrită indusă cu adjuvant (AIA). Cinci grupuri de şobolani au fost comparate: grupul de control fără artrită şi patru grupuri cu AIA tratate prin gavaj timp de 21 de zile, după 7 zile de la inducerea AIA, cu vehicol (ser fiziologic), cu un agent antiinflamator non-steroidian (indometacin), cu rutin sau cu omega-3 (PUFA). Scorul artritic a fost determinat la şobolanii cu AIA ca un indicator clinic al afectării articulare. După 28 de zile a fost măsurat nivelul seric al malondialdehidei (MDA), proteinelor carbonilate (CP), grupărilor sulfhidril (SH), capacităţii de donor de hidrogen (HD) şi glutationului (GSH). Rezultatele obţinute ne arată o creştere a stresului oxidativ evidenţiat prin creşterea producţiei de radicali liberi, MDA şi PC, şi o scădere a capacităţii antioxidante prin măsurarea HD, SH şi GSH la şobolanii cu AIA. Nivelurile MDA şi al CP au scăzut semnificativ, iar nivelurile HD, SH şi GSH au crescut semnificativ după administrarea orală de rutin. Omega-3 (PUFA) a crescut nivelurile MDA, CP, HD, SH şi a scăzut nivelul GSH. Acest studiu sugerează că tratamentul cu rutin şi omega-3 (PUFA) poate fi benefic în atenuarea stresului oxidativ asociat AR.

Cuvinte cheie: stres oxidativ, artrită indusă de adjuvant, rutin, şobolani

QUERCETIN, LYCIUM BARBARUM AND CHITOSAN REVERSE THE EFFECTS OF HYPOBARIC HYPOXIA AND EXERT CARDIOPROTECTIVE EFFECTS IN RATS

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ABSTRACT

Chronic and acute exposure to hypobaric hypoxia induces changes of the pro-oxidant/antioxidant balance in the myocardium and throughout the body. It has been shown that natural antioxidants supplementation (Quercetin, Lycium barbarum and Chitosan) is effective in preventing the hypoxic stress. Therefore, the purpose of this study was to observe, highlight and quantify the cardio-protective effects of these natural antioxidants' administration. The study was conducted on male, Wistar rats, exposed to acute and chronic hypobaric hypoxia (HH) (5500 m, 23h/day) for different periods of time (1 or 14 days) or kept in normoxia for 14 days. Some of the rats were administered natural antioxidants one day before the first HH exposure and 14 days before and prior to every hypoxic exposure for the next 14 days. At the end of the study the animals were euthanized and the hearts were harvested and stored for further analysis. Myocardial sections were obtained and examined histopathologically to determine the cardiomyocyte viability and morphometry. The results show a negative effect of chronic hypobaric hypoxia on the cardiomyocyte viability and cardio-protective effects of natural antioxidants' administration. This study suggests that treatment with Quercetin, Lycium barbarum or Chitosan alone substantially restored the myocardial architecture and acted like cardioprotectants.

Key words: oxidative stress**,** hypobaric hypoxia**,** Lycium barbarum, Quercetin, Chitosan.

INTRODUCTION

Illness related to acute and chronic high-altitude exposure is primarily caused by hypobaric hypoxia (HH), which is considered a physiological oxidative stress that affects the myocardial tissue and determines several physiological alterations (13, 25). The severity and duration of the symptoms vary, depending on the altitude and rate of ascent, sometimes persisting after returning to lower altitudes (19). The development of these symptoms has been linked to hypoxia-induced oxidative and nitrosative stress (7). Exposure to high-altitude constitutes an oxidative stress, the reactive oxygen species (ROS) sources being alterations of: mitochondrial respiratory chain (11), cellular membrane and cytosol, endothelial cells and lipid and protein perturbations. ROS generation may be one of the mechanisms involved in the death of cardiomyocytes after hypobaric hypoxia exposure (21).

Quercetin is an important flavanone which is known to be a multi-functional agent, due to its anti-inflammatory and anti-cancerous activities, as well as a potent antioxidant because of its directly scavenging ROS and free radicals (9, 16, 17, 20).

The fruits of Lycium barbarum (LBG) have a wide anti-oxidant spectrum and have been used for a very long time in Easter-Asian medicine, especially in China (29). Recent research showed that LBG fruit extract has many beneficial effects, including anti-ageing, anti-cancerous, cytoprotective or immunostimulating properties. Eyesight improvement, blood pressure control, cholesterol level lowering have also been reported. Last but not least, this extract seems to have protective effects against the oxidative stress of different causes (18, 29).

Chitosan is a α-(1-4)-D-glucosamine polymer, a natural polysaccharide present in shellfish, clams, krill, oysters, fungi, etc (10). It has been shown to possess antilipidemic, anti-ulcerogenic, anti-ageing, membrane-stabilizing and antioxidant properties (3, 15, 26, 28).

Because hypoxic stress due to either acute or chronic hypobaric hypoxia is one of the mechanisms underlying the initiation of cardiac damage, the aim of this study was to evaluate whether Quercetin, LBG extract or Chitosan supplementation is effective

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in preventing cardiac damage.

MATERIALS AND METHODS

Male Wistar rats (180±10 g) were used for this experiment. Rats were fed standard laboratory diet and had free access to water. The experimental protocol has been approved by the Ethics Committee of the "Iuliu Hatieganu" University of Medicine and Pharmacy from Cluj-Napoca and conforms to the Guide for the care and use of laboratory animals NIH publication No. 85-23, revised 1996.

The animals were randomly subdivided into nine experimental groups (n=10): 1st Group- unprotected acute hypoxic hypobaric (HH) rats (control group); 2nd Group- treated acute HH rats, rats treated with quercetin; 3rd Group- treated acute HH rats, rats treated with LBG extract; 4th Group- treated acute HH rats, rats treated with Chitosan; 5th Group- unprotected normoxia rats, rats not exposed to HH (control group); 6th Group- treated chronic HH rats, rats treated with LBG extract; 7th Group- treated chronic HH rats, rats treated with Quercetin; 8th Group- treated chronic HH rats, rats treated with chitosan and 9th Group- unprotected chronic HH rats (control group).

The animals were exposed to a simulated altitude of 5500 m in a barochamber (in the Physiology Department of the UMF ″Iuliu Haţieganu″ Cluj-Napoca), where temperature and humidity were maintained at 28ºC and 55-60%, respectively, for one day (acute HH) and for 14 consecutive days (chronic HH). The rats were taken out of the hypoxic chamber once after every 24h exposure for 1 hour, for receiving food and water.

Some of the rats received Quercetin (30 mg/kg/day, dissolved in saline solution) or Lycium barbarum extract (LBG, 30 mg/kg/day, dissolved in saline solution) via an intragastric tube (0.6 ml/rat) for one day or 14 consecutive days prior to HH exposure and prior to every HH exposure respectively. Other groups were given intra-peritoneal injections of Chitosan (0,30-0,35 microg/animal/day, dissolved in saline solution) for a period of one day or 14 consecutive days prior to HH exposure and prior to every HH exposure respectively. The control groups were treated with saline solution (0.6 ml/rat) via an intragastric tube (ctrl groups).

All rats were employed the next day after the last hypoxic exposure and sacrificed by decapitation (with sodium pentobarbital, 60 mg/rat ip). Hearts were rapidly excised, washed in cold saline, than left and right ventricular walls and the septum were dissected, weighed and used for analysis.

For the histopathological examination the myocardial tissues were fixed in 10% neutral buffered formalin and, after proper fixation, were dehydrated in graded series of alcohol, cleared in Xilene and embedded in paraffin wax. Multiple longitudinal and transversal sections from each block were prepared at 5 µm, and stained with haematoxylin and eosin (H&E). The haematoxylin and eosin staining (H&E) was used, because it allows a good observation of different cell types. For measuring the cardiomyocyte diameter (morphological study) we used the technique of Aiello et al. (1). Tissue analysis was performed using an Olympus system for image acquisition and analysis, respectively an Olympus BX51 microscope equipped with Olympus Cell B software. 50 cardiomyocytes were measured from each section, from at least 10 different regions, with 200x or 400x magnification. Statistical analysis and all morphological data was performed using Shapiro-Wilk normality test, followed by the two-sample t-test, using R' software (R Development Core Team, 2010).

RESULTS

Histopathological exam

Cardiomyocytes' diameters in control rats, HH-exposed rats ant treated HH-exposed rats are illustrated in Figure 1. and Table I. Exposure to acute hypobaric hypoxia does not modify the cardiomyocytes size but exposure to chronic hypobaric hypoxia determines a statistically significant decrease in cardiomyocyte size after 14 days of exposure in all groups, compared to the control group (5th Group). However, the reduction of cardiomyocytes' size was less severe (cardioprotective effect) in the treated groups (6th, 7th and 8th group) compared to the 9th Group (unprotected ctrl group).

Table I. Cardiomyocyte diameter in the 9 experimental groups (Mean±SD, µm)

1st Group	2nd Group	3rd Group	4th Group	5th Group	6th Group	7th Group	8th Group	9th Group
15.7±0.9	15.9±0.8	16.6±0.7	15.8±0.4	15.5±0.4	15.5±0.5*	14.4±0.5*	14.4±0.6*	14.1±0.5**

*p<0.05; **p<0.01 as compared to the 5th Group (ctrl group)

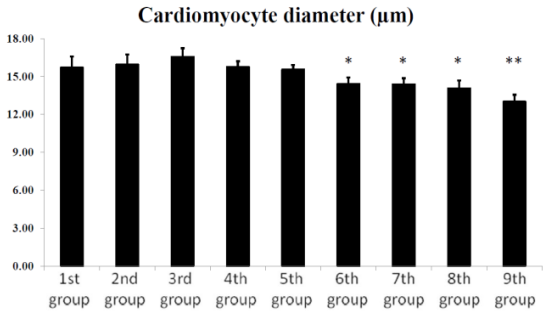

Fig.1. Quantification of cardiomyocytes diameters in the 9 experimental Wistar rats groups (Mean ± SD) *p<0.05; **p<0.01 compared to the 5th Group (ctrl group).

To further validate the effects of hypobaric hypoxia on myocardial tissue histopathologic examination was conducted. Figures 2-3 present the histopathological analysis of the sections of cardiac tissues stained H&E from the rats exposed to normoxia, acute hypobaric hypoxia, chronic hypobaric hypoxia and treated acute or chronic hypobaric hypoxia. Increased wall thickness, abnormal myocardial architecture and increased interstitial space were observed in rat hearts exposed to chronic hypobaric hypoxia (9th Group) compared to the 5th Group (ctrl group). The rats treated with Quercetin, LBG extract and Chitosan respectively (6th Group, 7th Group and 8th Group) and exposed to chronic HH presented a

restored towards normal myocardial architecture. No obvious differences of wall thickness, myocardial architecture and interstitial spaces were observed normoxia group (5th Group) and acute hypobaric hypoxia group (1st Group).

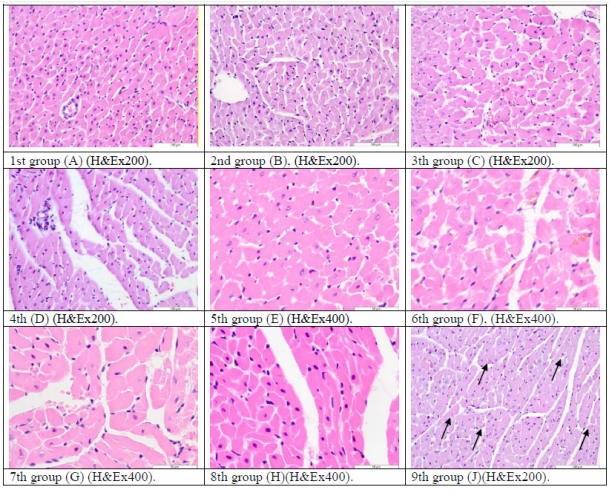

Fig.2. Cardiac changes of rats exposed to normoxia, acute hypobaric hypoxia, chronic hypobaric hypoxia and treated acute or chronic hypobaric hypoxia. (A-J). Representative examples of histopathological analysis in the transverse sections from the myocardium stained with haematoxylin and eosin (H&E). The images were magnified 200 or 400 times. 1st Group (A), 2nd Group (B), 3th Group (C) and 4th Group(D): no histological alterations were observed; 5th Group (E): no histological changes regarding cardiomyocytes or capillaries; 6th Group (F), 7th group (G) and 8th group (H): restored towards normal: mild decrease of cardiomyocytes diameter and increased number of capillaries; 9th Group (J): characteristic changes due to hypobaric hypoxia: moderately decreased diameters of cardiomyocytes and increased capillary density (arrows) compared to the 5th Group (ctrl group).

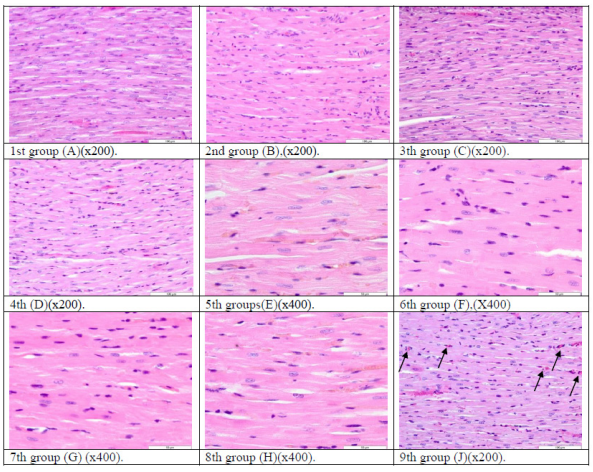

Fig.3. Cardiac changes of rats exposed to normoxia, acute hypobaric hypoxia, chronic hypobaric hypoxia and treated acute or chronic hypobaric hypoxia. (A-J). Representative examples of histopathological analysis in the longitudinal sections from the myocardium stained with haematoxylin and eosin (H&E). 1st Group (A), 2nd Group (B), 3th Group (C) and 4th Group (D): no histological alterations from normal cytoarchitecture were observed; 5th Group (E): no histological changes regarding cardiomyocytes or capillaries; 6th Group (F), 7th Group (G) and 8th Group (H): mild decrease of cardiomyocytes diameter and increased number of capillaries; 9th Group (J): characteristic changes due to hypobaric hypoxia: moderate decreases of cardiomyocytes' morphometric values (diameter, area and perimeter) and increased capillary density (arrows) compared to the 5th Group (ctrl group).

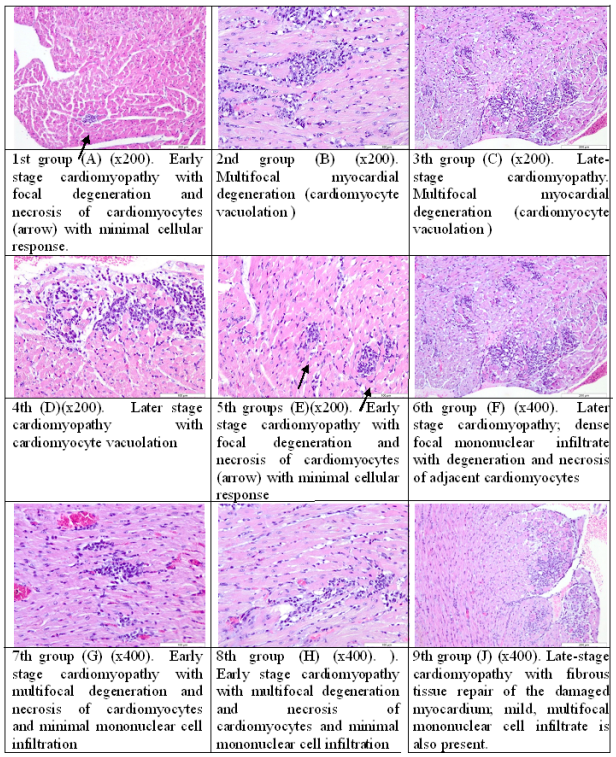

Fig.4. Cardiac and cardiomyopathie changes of rats exposed to normoxia, acute hypobaric hypoxia, chronic hypobaric hypoxia and treated acute or chronic hypobaric hypoxia. (A-J) Representative examples of histopathological analysis in the sections of cardiac tissue stained with haematoxylin and eosin (H&E). The images were magnified 200 or 400 times.

As for the non-specific myocardial lesions, we observed in this study that all the rats exposed to hypobaric hypoxia presented in the myocardium (Figure 4) small regions of degenerated cardyomyocytes surrounded by different degrees of more or less mononuclear inflammatory cells (lympho-histiocytes) - lesions of chronic progressive cardiomyopathy in different stages. Myocardial lesions were aggravated after chronic hypobaric hypoxia exposure. The rats treated with Quercetin, LBG extract and Chitosan respectively (6th Group, 7th Group and 8th Group) and exposed to chronic hypobaric hypoxia presented a reduction of the myocardial lesions and a minimal mononuclear cell infiltration.

DISCUSSIONS

Coronary artery disease (CAD) is the most common cardiovascular disease and a leading cause of cardiac death. Although numerous treatments are available, CAD treatment is still unsatisfying and limited because of its various side-effects and because of the lack of a preventive approach. This is why looking for alternative non-drug therapies for myocardial ischemia is essential. Long- term high altitude hypoxia has long been recognized to increase the resistance of the heart to ischemia-hypoxia injury. It has been shown that chronic intermittent hypoxia (CIH) is associated with increased production of reactive oxygen species that contributes to the adaptive mechanism underlying

the improved myocardial ischemic tolerance. Multiple mechanisms or pathways have been suggested to contribute to the cardioprotective effects of CIH, such as: increased coronary flow and myocardial capillary angiogenesis, activation of ATP-sensitive potassium channels, activation of protein kinase C, activation of MnSOD, etc (8).

Recent research suggests that ROS generated the adaptation period a CIH are important components of a signaling pathway leading to this form of cardioprotection (8). Starting from this hypothesis we tried to show in our study that antioxidant supplementation might have a beneficial effect in reducing the oxidative stress resulted from hypobaric hypoxia exposure, as well as a cardioprotective effect.

In our study we observed transverse and longitudinal myocardium stained sections, by histochemical analysis, which showed progressive increase of capillary densities associated with significant reductions in fiber area and diffusion distances in the rats exposed to chronic hypobaric hypoxia. Our results are in agreement with previous research (21, 24), and we interpreted the results as an adaptive response of rat myocardium to a more efficient O_2 delivery to the mitochondria of cardiac muscle cells. Higher capillarization parameters and fiber morphometry reductions indicate that there is a delay of about 14 days after the hypoxic stimulus ceases before complete angiogenesis is reached and fiber morphometry changes.

Quercetin is a flavonoid which is frequently used in altitude illness. Its cardioprotective effects on ischemic lesions in rats were shown by Annapurna et al. (4) as being due to a reduction of the oxidative stress and an increase of antioxidant enzymes. We observed in this study that rats exposed to chronic hypobaric hypoxia and treated with Quercetin presented a restored myocardial structure: cardiomyocytes' diameters did not decrease too much and there was a decrease in capillary densities compared to the control rats. We interpreted this as a result of Quercetin's antioxidant effect, which decreased the oxidative stress induced by chronic hypobaric hypoxia exposure, thus restoring the architecture of the myocardium.

Hypoxia and oxidative stress were shown to be potential inducers of cardiac hypertrophy (12). In the present study, abnormal myocardial architecture and increased interstitial space were observed after chronic hypobaric hypoxia exposure. Therefore, we speculate that a longer duration of chronic hypoxia could induce abnormal myocardial architecture and even lead to cardiac hypertrophy.

Different biological activities of L. barbarum have been demonstrated, including anti-aging, anti-cancerous, immunostimulatory or cytoprotective effects, and many studies suggested that the protective effect of L. barbarum mainly depends on its antioxidative action (14, 18, 23). In the present work we observed that rats exposed to chronic hypobaric hypoxia and treated with L. barbarum presented myocardial restoration in a way similar to Quercetin treated rats, clearly showing that myocardial restoration is due to its antioxidant effects.

Recent studies (2, 22) showed that Chitosan plays a role in the recovery of post-ischemic myocardial lesions by decreasing ROS, attracting chemokines and maintaining a normal level of antioxidant enzymes, thus proving its cardioprotective effects. Our study clearly shows that Chitosan administration in rats exposed to chronic hypobaric hypoxia restored the myocardial architecture.

In rats, the terms of progressive or degenerative cardiomyopathy are used to describe a series of degenerative myocardial lesions. Morphological modifications start around the age of 3-4 months, mainly in the apex, underneath the fibrous rings, papillary muscles and the free wall of the left ventricle. Sometimes, the modifications can be observed around the intramural coronary arteries. The initial lesions are focal accumulations of mononuclear inflammatory cells, oedema, and degenerating muscle fibers. In the more advanced stages, the lesions can either remain isolated or confluate to larger areas of fibrosis or cardiomyocyte necrosis (5, 27). The etiology of this cardiomyopathy is not very well known, but it has been suggested that the myocardial degeneration areas might be due to ischemia, as a result of a cardiovascular disease (6).

We observed that the rats exposed to acute and chronic hypobaric hypoxia presented progressive chronic cardiomyopathy lesions, in different evolving stages. Myocardial lesions were aggravated by the chronic hypobaric hypoxia exposure. The rats treated with Quercetin, LBG extract and Chitosan respectively, and exposed to hypobaric hypoxia presented a decrease of the myocardial lesions and minimal mononuclear cell infiltration.

CONCLUSION

These results demonstrate the beneficial effects of Quercetin, L. barbarum and Chitosan supplementation in natural and enriched foods. These substances presented a cardioprotective effect induced by hypobaric hypoxia. Our results demonstrate a cardioprotective role of Quercetin, Chitosan and L. barbarum in mediating the increased tolerance to ischemia observed in the chronically hypoxic heart.

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QUERCETINUL, LYCIUM BARBARUM ŞI CHITOSANUL CONTRACAREAZĂ EFECTELE HIPOXIEI HIPOBARE ŞI EXERCITĂ UN RĂSPUNS CARDIOPROTECTOR ÎNTR-UN MODEL DE ŞOBOLAN

REZUMAT

Expunerea la hipoxie hipobară acută şi cronică induce modificarea balanţei oxidanţi/antioxidanţi la nivelul miocardului şi a întregului organism. Cunoscând eficienţa suplimentării cu antioxidanţi naturali (Quercetin, Lycium barbarum şi Chitosan) în prevenirea mai multor tipuri de leziuni induse de stresul oxidativ în acest studiu ne-am propus să evidenţiem şi să cuantificăm efectele cardioprotectoare ale administrării unor antioxidanţi naturali. Şobolani masculi, rasa Wistar, adulţi au fost expuşi la hipoxie hipobară (HH) (5500 m, 23h/zi) pentru diferite perioade de timp (1 şi 14 zile) sau menţinute în normoxie (14 zile). O parte din şobolanii luaţi în studiu au fost trataţi cu antioxidanţi naturali înaintea expunerii la HH timp de o zi şi respectiv 14 zile şi apoi înaintea fiecărei expuneri la HH. La sfârşitul studiului toţi şobolanii au fost sacrificaţi şi inimile au fost prelevate şi conservate pentru analize. S-au efectuat secţiuni de miocard care au fost examinate histopatologic în vederea stabilirii morfologiei cardiomiocitelor (studiul morfometric). Rezultatele obţinute indică efectul negativ al hipoxiei hipobare cronice asupra arhitecturii miocardice şi efectele cardioprotectoare ale ale antioxidanţilor naturali administraţi. Rezultatele acestui studiu sugerează că tratamentul cu Quercetin, Lycium barbarum sau Chitosan restaurează substanţial arhitectura miocardului şi are efecte cardioprotectoare.
Cuvinte cheie: stres oxidativ, hipoxie hipobară, Lycium barbarum, Quercetin, Chitosan.

THE IMPORTANCE OF MYO-INOSITOL IN THE PHYSIOPATHOLOGY OF HEPATIC ENCEPHALOPATHY

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ABSTRACT

Hepatic encephalopathy is a clinical entity which is determined by the disturbance in the astrocitary-neuronal metabolic cycle, caused by excessive plasmatic ammonia in the context of liver failure to eliminate this by-product. Myo-inositol decreases were observed in this cases, as it seems to act as a buffer to compensate the osmotic imbalance. This paper seeks to identify correlations between myo-inositol levels and osmotic markers values in hepatic encephalopathy, with the intent of clarifying the physiopathological processes involved.

Keywords: Hepatic encephalopathy, physiology, physiopathology, myo-inositol, choline, glutamate, glutamine, MRI, spectroscopy]

INTRODUCTION

Hepatic insufficiency syndrome, indifferent of the determinating cause, has a suite of clinical and paraclinical manifestations, in which ammonia plays one of the leading roles (1), as it is being accumulated in the plasma due to the incapacity of the liver to metabolize it. Ammonia normally crosses the blood-brain barrier and enters the astrocitary-neuronal metabolic cycle, where it represents a substrate for glutamate to glutamine conversion. Its presence here, in amounts higher than usual, overloads this reaction and leads to increased amounts of glutamine, an osmotically active compound. High osmotic content of the cell produces swelling, and this is the moment when myo-inositol comes into play, as it is released from the cell to the interstitial space in the tendency to normalize cellular osmolarity (2).

Although these observations have been objectified by numerous studies dating since the early 2000s, the physiopathological processes are not fully comprehended, and also, the impact and efficiency of these mechanisms on the underlying disease and overall evolution of the hepatic encephalopathy are yet to be established.

Myo-inositol seems to be one of the main osmotically-active metabolites involved in the regulation of cellular volume in regard to the osmotic pressure, in the attempt to avoid swelling (3).

Magnetic resonance imaging (MRI) remains the spearhead in morphological central nervous system evaluation, and due to recent progress in the field, can now assess functional information about specific metabolite dynamics in the brain (4). Magnetic resonance spectroscopy (MRS) has been successfully used in creating a profile spectrum of hepatic encephalopathy, identifying the aforementioned metabolic imbalances as increased glutamate-glutamine peaks (Glx) and decreased choline (Cho) and myo-inositol (Ins) peaks, respectively. Choline seems to have an effect similar to myo-inositol in regulating osmotic disturbances (5).

Magnetic resonance imaging can also quantify the tissular water amount, defining the grade of edema and thus provide indirect information on the physiopathological processes involved.

The aim of this study is to evaluate the specificity of myo-inositol as a marker for minimal hepatic encephalopathy, describe its variation in relation to the other metabolites involved, and to discuss the dynamics of these metabolites in relation to the tissular water content in specific areas.

PATIENTS AND METHODS

Thirty-two patients were enrolled in our study (18 male and 14 female), aged 26 to 81, averaged 53.3 years. The patients underwent a neuro-psychological test, followed by an MRI examination with MRS profiling in the same day.

Twenty-one patients suffering from chronic liver insufficiency were divided into two groups concordant with the psychological test: one group consisted of patients with minimal hepatic encephalopathy (altered test): 6 male and 4 female; the other group gathered the patients without any kind of hepatic encephalopathy (normal test). The third lot of patients was healthy controls (6 male, 5 female).

The neuro-psychological testing consisted of the Porto-Systemic Encephalopathy (PSE) Syndrome Test, a standardized questionnaire with 5 tests (serial dotting test, line tracing test, two number connection tests, digit-symbol test) recommended in the evaluation of minimal hepatic encephalopathy (6).

The MRI examination followed the protocol: T2 weighed images (axial plane), diffusion-weighed images (par-axial plane), and fluid attenuated inversion-recovery images (coronal plane).

MRS imaging method protocol was the following: single voxel spectroscopy (SVS), short-TE (30 ms), 8 cm^3 voxel size (2 x 2 x 2 cm), and parietal white matter in the dominant hemisphere (Figure 1). Several calibration tests correlated with

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the literature studies confirmed that this particular location was optimal for detection of the metabolites of interest in this case (see discussions).

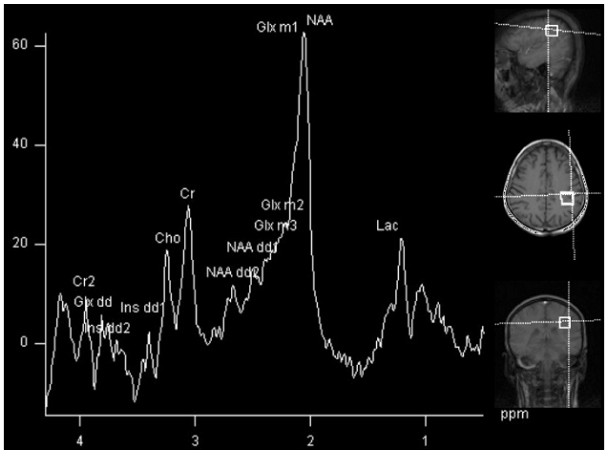


Fig.1. Normal MRS spectrum acquired in the parietal white matter in the dominant hemisphere. Three dimensional localization system on the right hand side. Assessed metabolites right to left: Lactate (Lac), N-Acetyl-Aspartate (NAA), Glutamate (Glx), Creatine (Cr), Choline (Cho), Myo-inositol (Ins); secondary and tertiary peaks displayed.

Statistical analysis tests were performed two-sided on the level alpha = 0.05; the dependencies between parameters were tested using Pearson correlation coefficient, and t (student) Test.

RESULTS

Patient distribution was represented in Figure 2. Male to female ratio was 1.2 in the control and no hepatic encephalopathy (No HE) lots, and 1.5 in the Minimal HE lot, respectively.

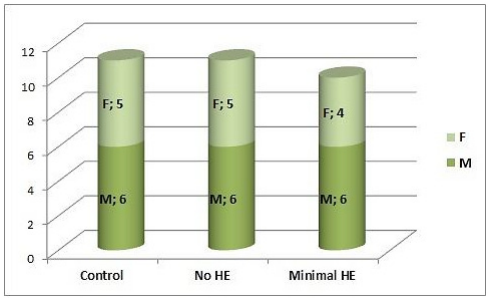


Fig.2. The 3 patients lots distribution by sex (M=male, F=female)

Various studies recommend different areas for MRS measurements in diffuse central nervous system metabolic disease. Our previous experience in the field of MRS demonstrated that for this particular study, the best location of the voxel would be in the parietal white matter. Tests on healthy controls and several patients suffering of HE showed that this region holds the most loyal ratio of metabolites measured by MRS compared to their actual levels in the intracellular and interstitial spaces (7).

The metabolites routinely measured through MRS were myo-inositol (Ins), glutamate-glutamine-gamma amino butyric acid complex (Glx), choline (Cho), N-acetyl aspartate (NAA), creatine (Cr), and lactate (Lac). Out of these, those relevant to our study and their inter-relation were summarized in Table I. Creatine is usually utilized as a reference molecule due to its high stability in various conditions, in different ages and metabolic states (8).

Table I. Metabolite average values in the study lots. Relevant ratios between Ins, Glx and Cho.

Lots\Parameters	M	F	Average Ins	Ins/Cr	Ins/Glx	Cho/Cr	Cho/Glx
Control	6	5	126.31±43	0.56	0.27	0.76	0.37
No HE	6	5	90.44±53.72	0.36	0.20	0.71	0.39
Minimal HE	6	4	63.78±58.95	0.29	0.11	0.66	0.26

The morphological assessment of the general tissular water content in the parieto-occipital matter and the cortico-spinal tracts bilaterally used T2 weighed images. Diffusion weighed imaging and fluid attenuated inversion recovery sequences were employed only to clarify the findings in cases of various artifacts. The results are shown in Table II.

Table II. Tissular water content evaluation through T2 weighed imaging

Lots\Location	Parieto-occipital white matter	Cortico-spinal tracts
Control	336.5 ± 52.15	358.75 ± 49.03
No HE	294 ± 75.81	323.6 ± 53.96
Minimal HE	336.25 ± 22.71	343 ± 15.29

DISCUSSION

Decreased myo-inositol values in the minimal hepatic encephalopathy lot seemed to correlate with the presence of this condition (p=0.00505). Also, lower values (though within the normal range) of myo-inositol were found in lot 2 (patients with chronic liver disease with no HE). This might suggest a tendency of these patients to evolve towards MHE (Figure 3).

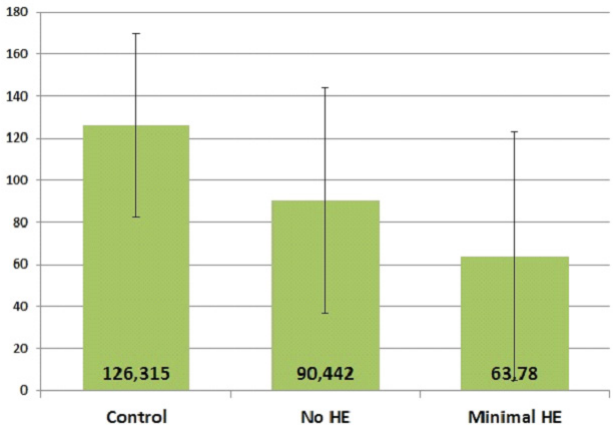


Fig. 3. Distribution of myo-inositol values in the 3 lots. Standard deviation overlay

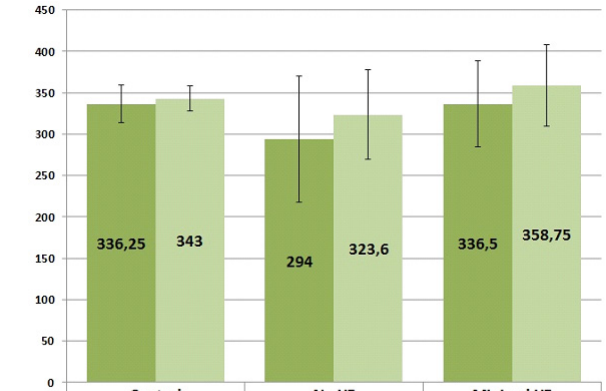
Nevertheless, we experienced high standard deviation values in the three lots, which might suggest the need for larger lots for a better statistical accuracy.

Tissular water content as measured by our method offered controversial data, as it seemed highest in the lot of MHE but nearly identical in the control group (Figure 4). Lowest values were found in the No HE lot. In all cases, we delineated higher values at the level of the corticospinal tracts than those in the parietal white matter. CST values followed the distribution of those in the parietal region.

In regard to the correlation between the myo-inositol values and the tissular water content, we failed to obtain statistical coherence. Though intuitive that lower values of myo-inositol measured by MRS suggest its release from the cell in the case of hyperosmolarity, increased water content in the location of the measurement was not demonstrated. This might be because the alteration in water content is very discrete, and though it has been identified in advanced stages of HE, in MHE the finding was not confirmed with the methods implied.

Choline variations were coherent with the myo-inositol trend in the study lots, supporting the idea of these two molecules having a corroborated effect in osmotic regulation ($p<0.001$).

Improvement of this study could be achieved by larger patient lots, and also by repeated MRS measurements to diminish the possibility of patient movement reading errors (image artifacts).



	Control	No HE	Minimal HE
P-VOI	336,25	294	336,5
CST	343	323,6	358,75

Fig.4. Tissular water content in the three lots measured at the level of the voxel placement (P-VOI) and at the level of the cortico-spinal tracts (CST)

CONCLUSION

Our findings confirm the fact that myo-inositol plays an important role in the cascade of events set in motion by the ammonia increase in hepatic encephalopathy. Its decrease correlates with the presence of minimal HE, validating the hypothesis that it acts as an osmotic buffer.

Choline variations line up with myo-inositol trends in the study groups proving a correlation of the two in the physiopathologic events of HE.

Higher tissular water content was expected in the case of MHE compared to the control lots, yet the findings were inconclusive, suggesting the need of a more sensitive method for detection.

ACKNOWLEDGEMENT: This paper is supported by the Sectoral Operational Programme Human Resources Development (SOP HRD) 2007-2013, financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/107/1.5/S/82839

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IMPORTANȚA MIO-INOZITOLULUI IN FIZIOPATOLOGIA ENCEFALOPATIEI HEPATICE

REZUMAT

Encefalopatia hepatică este o entitate clinică determinată de perturbarea ciclului metabolic astrocitaro-neuronal, produs de valori plasmatice crescute de amoniu în contextul eşecului ficatului de a elimina acest produs secundar. Scăderi ale valorilor mio-inozitolului au fost observate în aceste situaţii, întrucât acesta pare să funcţioneze ca un tampon în compensarea dezechilibrelor osmotice produse. Această lucrare caută să identifice corelaţii între nivelul mio-inozitolului şi valorile markerilor osmotici în encefalopatia hepatică, cu intenţia de a clarifica procesele fiziopatologice implicate.
Cuvinte cheie: encefalopatie hepatică, fiziologie, fiziopatologie, mio-inozitol, colină, glutamat, glutamină, IRM, spectroscopie

PULSE PRESSURE: PROGNOSTIC SIGNIFICANCE IN THE STRATIFICATION OF CARDIOVASCULAR RISK

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ABSTRACT

Aim: to evaluate the correlations between the pulse pressure and cardiovascular risk factor: hemodinamic parameters, metabolic factors and traditional risk factors.

Materials and methods: our survey included 398 consecutive patients with high cardiovascular risk, hospitalized in 2010 in the Preventive Cardiology Clinic of the Institute of Cardiovascular Diseases Timisoara. For every patient, demographic and anamnestic data as well as common cardiovascular risk factors, metabolic profile and hemodinamic profile (24h-BP Holter survey and 24h-PP) were registered.

Results: we have obtained highly significant statistical correlations between 24h pulse pressure and hemodinamic parameters: 24h-SBP ($p<0.001$, $r=0.752$) and 24h-MBP ($p<0.001$, $r=0.511$); significant statistical correlations were found between 24h-PP and metabolic parameters: LDLc ($p=0.038$, $r=0.156$) and glycemia ($p=0.001$, $r=0.162$). There were no significant statistical correlations found between 24h-PP and other common cardiovascular risk factors (smoking, history of coronary artery disease). 24h-PP variability was determined by linear regression equation by 2 factors: 24h-SBP and 24h-MBP.

Conclusions: the established cut-off value of the 24h-PP validates the fact that people with increased 24h-PP have elevated 24h-SBP, thus presenting high cardiovascular risk.

We proved the existence of a direct highly significant statistical correlation between 24h-PP and hemodinamic parameters, and between 24h-PP and some metabolic factors.

The 24h-PP showed its predicting significance in the assessment of the cardiovascular risk in direct correlation with the SBP and MBP.

Key words: pulse pressure, mean blood pressure , cardiovascular risk factors.

BACKGROUND

The pulse pressure is the pulsatile blood pressure component and is a marker of cardiovascular risk, reflecting large artery stiffness.

In recent years, seemingly simple direct relationship between cardiovascular risk and systolic and diastolic blood pressure was complicated by the results of observational studies showing that the risk is directly proportional to systolic and, for any given level of systolic blood pressure, the prognosis is inversely proportional to diastolic 1-2 with a strong predictive value of pulse pressure (1). The predictive value of pulse pressure may vary depending on the clinical characteristics of subjects.

In the biggest meta-analasys of the observational surveys that exists today (61 surveys including almost 1 million subjects without obvious cardiovascular disease-70% from Europe), both 24h-SBP and 24h-DBP presented independently similar predicting values concerning mortality due to stroke or coronary disease. In the case of hypertensive patients presenting cardiovascular risk or any other comorbidities, the 24h-PP was proved to have an important predicting significance for cardiovascular events (1,2)

Aim - to asses possible correlations between 24h-PP and the components of global cardiovascular risk: hemodinamic parameters (24h-SBP, 24h-DBP, 24h-MBP), smoking, metabolic factors, lipid profile.

MATERIALS AND METHODS

398 consecutive patients with cardiovascular risk, hospitalized in 2010 in the Preventive Cardiology Clinic of the Institute of Cardiovascular Diseases Timisoara were included in this study. We have used demographic and anamnestic data, full physical examination and laboratory examination of the patients. The inclusion and exclusion criteria are shown in Table I.

Table I. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
• Cardiovascular asymptomatic (Risk Score <5%) • Metabolic Syndrome • Dyslipidemia • Diabetus melittus • EF≥40	• Arrhytmias • Valvulopathies • Congenital cardiovascular disease • EF<40

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Clinical assessment:
For every patient, we took into consideration:

- Anamnestical data
- Family history of cardiovascular disease
- The evaluation or common risk factors:
  - changeable: hypertension, smoking, TC, LDL>115mg/dl, HDL<40mg/dl , diabetus mellitus, BMI
  - not changeable: age, male, genetic susceptibility
- For defining the asymptomatc subject, the ESC criteria were used (1)

Assessment of the cardiometabolic profile:

The assessment of the lipid profile and risk categorization were made according to ESC guidelines recomandations (1). Indirect measurement of LDL was performed by using the Friedewald equation:

LDLc = total cholesterol – HDL – (triglycerides/5)

NonHDLc was calculated by substracting HDLc from TC.The metabolic syndrome was defined according to IDF criteria, if at least 3 of 5 criteria were found (1).

Diabetus mellitus was defined by fasting plasma glucose >126 mg/dl or existing treatment.

Table II. Metabolic syndrome- defining criteria (IDF)

CRITERIA	NCEP-ATP III	IDF
Abdominal circumference(cm)	>88 (F),>102 (M)	>80 (F),>94 (M)*
24H-SBP (mmHg)	≥ 130/80	≥ 130/80
Glycemia (mg/dl)	> 110	> 100
Tryglerides (mg/dl)	>150	>150
HDL-c (mg/dl)	<50(F),<40(M)	<50(F),<40(M)

* If BMI ≥ 30 kg/m², the measuring of the abdominal circumference is no more required, according to the IDF

Assessment of the hemodinamic profile:

We assessed the profile of blood pressure according to te outcomes of 24 h recording of the BP, using BTL-08 ABPM. Measurements were made every 15 minutes during the day (7 am- 23 pm) and every 30 minutes during the night (23 pm-7 am), being repeated if more than 30% of them presented artifacts. During the monitoring period, medication was discontinued. From the protocol of 24 hour BP monitoring, we used the following: 24h-SBP, 24h-DBP, 24h-PP and 24h-MBP (1).

Automatic blood pressure monitoring allowed for defining hypertension category for the SBP>130mmHg. All the 24h-PPvalues above 60 mmHg were considered pathological.

Statistical analysis: All data was stored electronically and processed using S.P.S.S. 17.0. Data normality was assessed using the Shapiro-Wilk test. After that, the data was analysed using parametric tests like Independent Samples t test for coparing the mean values in two groups, and One Way ANOVA for comparing the mean values in more than three groups. The testing of the association between qualitative variables was made using Chi square test, and for testing the correlations between numeric variables, Pearson correlation coefficient was used. Using a model of variable logistic regression, the 24h binary dependency was analysed. We included the following independent variables: 24h-SBP, 24h-MBP, glycemia, LDL, HDL, total cholesterol and tryglicerides.

RESULTS

The prevalence of hypertension was 88,5% in the analysed group; 27.1% of patients were diagnosed with Diabetes mellitus type 2 and 10.5% of them, with metabolic syndrome.

Table III. General features

Cardiovascular risk factors*:	Primary hypertension	88.5
	Diabetes mellitus type 2	27.1
	Smokers (active smokers)	9.5
Metabolic profile **:	Metabolic syndrome	10.5
	Total cholesterol (mg/dl)	194 ± 69.32
	Tryglicerides (mg/dl)	151 ± 99.23
	LDLc (mg/dl)	100 ± 65.46
	HDLc (mg/dl)	43 ± 24.53
	Glycemia (mg/dl)	108±36.13

*-% of group ; **-mean +/- standard deviation

The correlation between 24h-PP and hemodynamic parameters: we have obtained a highly significant positive correlation between 24h-PP and 24h-SBP (p<0.001, r=0.752).

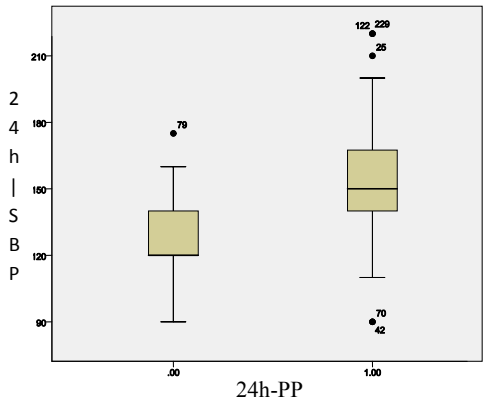

Fig.1. Mean 24h-SBP in 24h-PP categories

Mean 24h-SBP was significantly higher in the group of subjects with pathological values of 24h-PP. Those with 24h-PP ≥ 60mmHg showed a mean of 24h-SBP=154 ± 22 mmHg. Those with 24h-PP <60 mm Hg had a mean of 24h-SBP=126 ± 14 mmHg (p <0.001) (Figure 1).

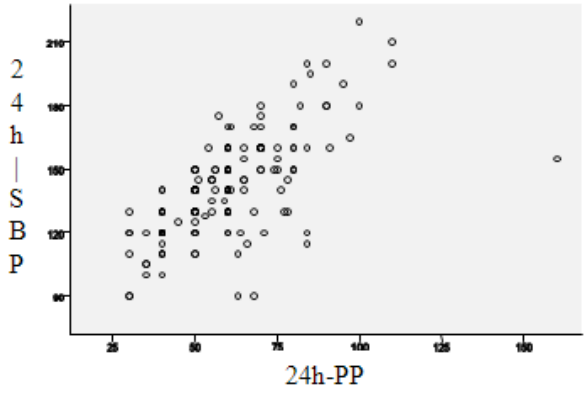

Fig.2. Correlations between 24h-PP and 24h-SBP

We also obtained a highly significant positive correlation between 24h-PP and 24h-DBP (p<0.001, r=0.246) as well as between 24h-PP and 24h-MBP (p<0.001, r=0.511) (Table IV).

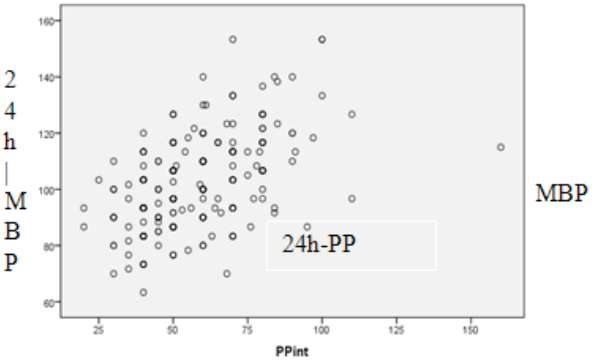

Fig.3. Correlations between 24h-PP and 24h-MBP

There is a statistically significant association between 24h-PP, 24h-SBP (fig.no.3) and 24h-DBP (p=0.003) categories. We have also obtained a statistically significant association between 24h-PP and 24h-SBP (p<0.001), respectively 24h-DBP (p=0.003) (Table IV)

Table IV. Correlations between 24h-PP and cardio-metabolic risk parameters

Dependent varianle	Independent variable	Pearson correlation coefficient	Statistical significance
24h-PP= pulse pressure	24h-SBP	r=0.752	p<0.001
	24h-DBP	r=0.246	p<0.001
	24h-MBP	r=0.511	p<0.001
	glycemia	r=0.162	p=0.001
	LDLc	r=0.156	p=0.038

Correlation between 24h-PP and metabolic parameters: we have obtained a highly significant statistical correlation between 24h-PP and glycemia (figure no. 3) (p=0.001, r=0.162). The correlation between 24h-PP and LDLc was also statistically significant (p=0.038, r=0.156) (Table IV).

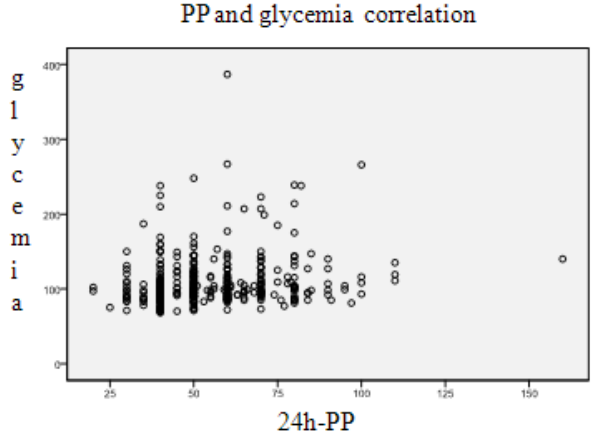

Fig.4. Correlation between 24h-PP and glycemia

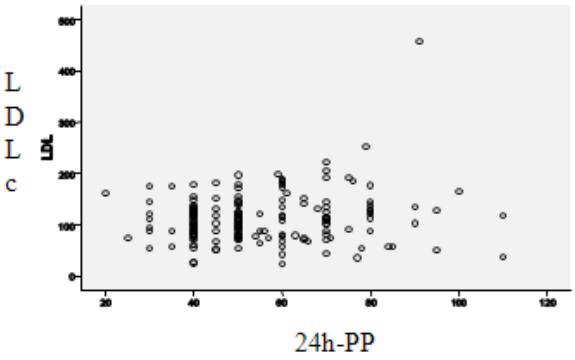

Fig.5. Correlation between 24h-PP and LDLc

Tabel V. Mean values of the metabolic parameters analyzed in the 2 groups of patients

Mean value *	24h-PP≥60mmHg(1)	24h-PP<60mmHg (0)
glycemia	112±44	105±28
LDLc	53±74	49±59
TC	187±69	175±69
TG	77±104	80±96
nonHDLc	166±65.4	153.5±65.4

*mg/dl

The patients with 24h-PP ≥ 60 mm Hg had mean values of the metabolic parameters higher compared to those with PPint < 60mmHg (Table V). Those with metabolic syndrome had mean 24h-PP of 56.12 ± 17.86 . Only 42.23% of those with metabolic syndrome had values of 24h-PP ≥ 60mmHg.

We didn't obtained statistically significant correlations between: 24h-PP and BMI (p = 0.708), 24h-PP and TC (p = 0.118), 24h-PP and TG (p = 0.374), 24h-PP and HDL (p = 0.457) and 24h-PP and nonHDL (p = 0.133).

The correlations between 24h-PP and BMI (p=0.708), 24h-

PP and total cholesterol (p=0.118), 24h-PP and tryglicerides (p=0.374), 24h-PP and HDL (p=0.457), 24h-PP and nonHDL (p=0.133) were not statistically significant.

The model of variable logistic regression used for analysing the 24h-PP binary dependency was significant for two predictors: 24h-MBP and 24h-SBP- p value<0.001.

We have obtained the following logistic regression model equation:

$$24h\text{-}PP=0.342 \times (24h\text{-}SBP) - 0.349 \times (24h\text{-}MBP) - 12.049$$

The variation of 24h-PP was explained at a rate of 71,9% by the predictor variables: 24h-SBP and 24h-MBP (Rsquare = 0.719).

DISCUSSION

The correlation between 24h-PP and the hemodinamic parameters (24h-SBP, 24h-MBP, 24h-DBP)

In our study, we have obtained statistical significant correlations between 24h-PP and 24h-SBP in the entire group of subjects. The 24h-PP reflects the tendency in the increasing of 24h-SBP and in the decreasing of 24h-DBP due to the increased arterial stiffness (3,4), thus reflecting the stiffness of large arteries. Thereby, when the 24h-SBP is pharmacologically controlled, the drecreasing of the 24h-DBP will expose the patient to a 24h-PP > 60 mmHg, thus to risk (5,6).

Therefore, in the assessment of the cardiovascular risk, it's wrong to focus only on one hemodinamic parameter, but we must take into account both the pulsating part (24h-PP) and 24h-MBP.

Dyer et all, in all four Chicago epidemiological prospective surveys which included 28360 subjects, men aged between 18-74 and women aged between 40-74, have obtained a statistically significant correlation between 24h-MBP, 24h-PP and cardiovascular risk (7,8).

There is clear evidence that, reflecting the arterial stiffness, the 24h-PP represents an independent and significant cardiovascular risk factor. Madhavan et all reported that pacients with 24H-PP > 63 mmHg present a high risk for cardiovascular complications (9) They have discovered that, in case of severe drop of 24h-DBP due to antihypertensive medication, there is an increased risk of myocardial infarction. In the asymptomatic cardiovascular patients, this shows the importance of 24h-PP and 24h-MBP assessment as variables which are to be considered in order to increase the accuracy of cardiovascular risk stratification.

The predictive significance of the 24h-PP may vary depending of the clinical features of the subjects. In the biggest meta-analasys of the observational surveys that exists today (61 surveys including almost 1 million subjects without obvious cardiovascular disease-70% from Europe (10), both 24h-SBP and 24h-DBP presented independently similar predicting values concerning mortality due to stroke or coronary disease, at hypertensive patients with cardiovascular risk factors or any other comorbidities. The 24h-PP was proved to have an important predicting significance for cardiovascular events (11,12).

Correlation between 24h-PP and metabolic parameters

In our study, we have obtained statistical significant correlations between the hemodinamic parameters (arterial stiffness-24h-PP) and LDLc.

The statistical significant correlations between 24h-PP and LDLc that we have found, suggest the fact that persistent hypertension combined with persistent lipoprotein molecules such as LDLc can lead to increased 24h-PP (13) Although Zanchetti et all have found a statistical significant correlation between 24h-PP and TC (14), at values of TC= 262,4 mg/dl, TG= 181.3 mg/dl, HDLc = 141mg/dl, LDLc=141mg/dl, this was not sustained by our study. This may be due to a mean of TC of only 194 mg/dl in our group of patients. Also, the mean of TG and HDLc was situated close to the cardiovascular protection cut-off limit in asymptomatic patients. We consider that this circumstance has led to lack of statistical significant correlations between 24h 24h-PP and lipid risk profile. Other studies emphasize the arterial stiffening condition, related with increased values of TC and LDLc, and analyzed in terms of 24h-PP behaviour (15,16).

In our group of patients, we have found statistical significant correlations between the hemodinamic parameters (arterial stiffness-24h-PP) and glycemia. Miyagi et all, in a multicenter cross-sectional study which included 939 hypertenive patients, obtained a statistical significant correlation between glycemic index, 24h-PP and the decreased value of HDL (17).

CONCLUSIONS

- The established cut-off value for 24h-PP confirm the fact that subjects with increased 24h-PP have increased 24h-SBP
- We have demonstrated the existence of a direct highly significant correlation between 24h-PP and hemodinamic parameters, and between 24h-PP and some of the cardio-metabolic parameters (LDLc, glycemia)
- In hypertensive patients, the 24h-PP cand be considered a factor with prognostic value concerning the cardiovascular risk stratification, due to its direct connection with BP

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PRESIUNEA PULSULUI: SEMNIFICATIA PROGNOSTICA PENTRU STRATIFICAREA RISCULUI CARDIOVASCULAR

REZUMAT

Scop: evaluarea corelatiilor dintre presiunea pulsului si factorii de risc cardiovascular: parametrii hemodinamici, factorii metabolici, factorii de risc traditionali. Materiale si metode: studiul nostru a inclus 398 pacienti cu risc cardiovascular crescut, spitalizati in anul 2010 in Clinica de Cardiologie Preventiva a Institutului de Boli Cardiovasculare Timisoara. Pentru fiecare pacient au fost evaluate datele anamnestice si demografice, factorii obisnuiti de risc cardiovascular, profilul metabolic si hemodinamic (24h-BP Holter si 24h-PP). Rezultate: am obtinut corelatii statistice inalt semnificative intre presiunea pulsului pe 24h si parametrii hemodinamici: 24h-SBP ($p < 0,001$, $r = 0,752$) and 24h-MBP ($p < 0,001$, $r = 0,511$); corelatii cu semnificatie statistica au fost evidentiate intre 24h-PP si parametrii metabolici: LDLc ($p = 0,038$, $r = 0,156$) si glicemie ($p = 0,001$, $r = 0,162$). Nu au fost gasite corelatii cu semnificatie statistica intre 24h-PP si alti factori comuni de risc cardiovascular (fumat, antecedente de boala coronariana). Variabilitatea 24h-PP a fost determinata prin ecuatia de regresie liniara cu 2 factori: 24h-SBP si 24h-MBP. Concluzii: valoarea cut-off a 24h-PP valideaza faptul ca pacientii cu 24h-PP crescut au valori crescute si ale 24h-SBP, astfel incat prezinta risc cardiovascular inalt. Am dovedit existenta unei corelatii inalt semnificative statistic intre 24h-PP si parametrii hemodinamici, precum si intre 24h-PP si anumiti factori metabolici. 24h-PP este un parametru cu semnificatie predictiva in evaluarea riscului cardiovascular in corelatie directacu SBP si MBP.
Cuvinte cheie: presiunea pulsului, presiune sanguina medie, factori de risc cardiovascular

JUVENILE NASOPHARYNGEAL ANGIOFIBROMA - HISTOLOGICAL AND IMMUNOHISTOCHEMICAL LANDMARKS

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ABSTRACT

Objectives: Juvenile nasopharyngeal angiofibroma is a rare, histologically benign tumor, very aggressive locally, affecting male adolescents or those at puberty (around age 15). In terms of histopathology, it is an angiofibroma, characterized by fibrous swirls and blood lakes, without walls. The fibrous elements initially have only a tumor supporting stroma role, then they become a predominant structural component, as the effect of estrogen decrease. Patients and methods: This study is a retrospective that includes assessment of tumor resection pieces of 10 patients diagnosed and treated in the ENT Clinic Timisoara between 2002-2012. It showsthe macroscopic tumoral aspects, the microscopic characteristics and Ac estrogen anti-receptor, Ac progesterone anti-receptor, Ac antivimentina-Ventana, Ac CD_{117}, Ac muscle specific actin, Ac ki_{67}, CD_{31} and Ac CD_{34}.Results: All patients were male. The most frequently encountered histological model contains in the stroma immature fusiform fibroblasts and thin-walled blood vessels in different proportions. The tumor does not have its own capsule. But, around these tumors, usually the non-ulcerative, one can occasionally observe a pseudocapsule derived from pharyngeal or nasal mucosa, of atrophic and stretched appearance. The dense fibrous stroma may show areas of hyaline altering or focal myxoid issues. Multinucleated stromal cells may be present, with "ganglion-like", intensely eosinophilic cytoplasm.Debate: The ratio between blood vessels and tumor stroma varies based on the tumor thickness. Ultrastructural studies have shown that stromal cells are myofibroblast, with variable expression of fibroblast and myofibroblast features. Conclusions: Although angiofibroma is a histologically benign tumor, it may have an aggressive behavior through its recurrence and ability to destroy adjacent bone structures.

Keywords: juvenile nasopharyngeal angiofibroma, benign, microscopical, macroscopical, histological.

INTRODUCTION

Juvenile nasopharyngeal angiofibroma is a rare, histologically benign tumor of the nasopharynx, affecting almost exclusively male teens or pubers, being diagnosed in boys between 14-25 years. It is a highly vascularized tumor with high tendency to local and loco-regional invasion, extending into the submucosa. For this reason it is a destructive tumor. From the histological point of view, it's a mesenchymal, vascular, and high cell tumor,made of fibrous connective tissue and an abundance of vascular spaces without endothelium (endothelium lined). There are various theories about the angiofibroma formation, but the most acknowledged theory is the theory of angiogenesis and histogenesis. This describes the angiofibroma as a pure vascular tumor that proliferates in ahemangiomatous manner and in which all other components, including fibrous connective tissue are derived from the undifferentiated vasoformative mesenchymal.

The macroscopic appearance indicates the juvenile nasopharyngeal angiofibroma to be a vascular, lobulated, firm, unencapsulated, reddish blue, sessile or pedunculatedtumor, with numerous crusts. The tumor surface, if it was not traumatized during surgery or during hemostasis, is covered by an intense red mucosa in the case of young subjects, and pale pink in older patients, or those in which the vascularization was reduced by radiation or hormone therapy (1). In patients who have had a history of bleeding or hemostatic tamponament, the tumor surface may showulceration or necrosis, with granular tissue appearing in such areas.

As texture, some tumors can be rubbery, otherscartilaginous and others soft, edematous, and occasionally brittle. When sectioned, the tumor may vary in color from pinkish-white to gray-yellow to red-brown (1). All tumors have a solid appearance, without cystic degeneration. Tumors that were previously radiated are pale and of fibrous consistency; tumors of young people and those not previously treated are soft and richly vascularized (2).

The microscopic appearance shows a tumor composed almost essentially of network-shaped blood vessels and con-

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nective tissue. Under the microscope, the appearance can vary from fully cavernous angioma, developed in a fibrous stroma, to occasionalmyxomatous fibroma, or dense cellularity.

The most frequently encountered histological pattern contains immature fusiform fibroblasts in the stroma and thin-walled blood vessels in different proportions. In fact, fusiform or stellate fibroblasts can be so numerous, that they may suggest a fibrosarcoma or angiosarcoma, tumors that are often confused with the juvenile nasopharyngeal angiofibroma (3).

In young subjects or untreated tumors, the angiomatous elements are more abundant, vessels become larger, irregular or sinusoidal. In old tumors or those who have already undergone radiotherapy or intensive androgen therapy,the vascular components are less represented or may disappear altogether, and thereby fibrous elements can predominate.

Lymphocyte holes or plasma cells may be present, especially in ulcerated or injured tumors. Myxomatous changes can be observed in various proportions, as well as areas of necrosis. Occasionally hyalinized thrombican be found, especially in old or previously treated tumors,as well ashyalinized stroma areas (3).

The tumor does not have its own capsule. But, around these usuallynon-ulcerated tumors, we can occasionally observe the pseudocapsule derived from pharyngeal or nasal mucosa, and it appears atrophic and stretched.

In this study we try to evaluate the histological and immunohistochemical aspects pieces found when examining the tumor resection.

PATIENTS AND METHOD

This is a retrospective study including the evaluation of tumor resection pieces of 10 patients diagnosed and treated in the ENT Clinic Timisoara between 2002-2012, and who received a histological confirmation of the preoperative diagnosis. The study includes a total of 10 registry blocks. Serial sections of 4-5 μ thick were performed according to the usual histological technique. For each case the sections made were stained with hematoxylin-eosin forthe histological diagnosis. The macroscopic aspects were studied retrospectively, as described when collecting the tumor resection pieces, from the pathological diagnosis report sheets, which confirmed the preoperative diagnosis. The macroscopic tumoral features are presented, the microscopic characteristics, as well as the Ac anti-estrogen receptor, progesterone receptor anti-Ac, Ac antivimentina-Ventana, CD_{117}, the muscle specific Ac actin, Ac $ki_{67,}$ Ac $CD_{31,}$ and Ac $CD_{34.}$

RESULTS

Macroscopically, it was found that the resected angiofibromas were well delimited, polypoid or lobulated tumors, covered by smooth and shinymucous membrane. They are like a firm and gummy mass. On the section surface, they have a fibrous or spongy appearance (due to the presence of numerous vascular spaces) and are gray-colored.

The **microscopic** aspect diagnosis is that of fibrous stroma, with few cells and partially containing collagen. The fibrous tissue cells are spindle or stellate, with rounded nucleus and small nucleon,inconsistently noticeable. The mitotic figures are few and typical in appearance.

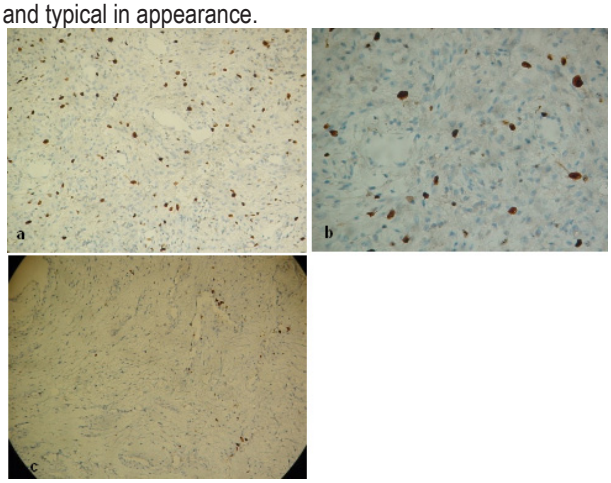

Fig.1. Ac Ki67 -Ventana / Roche, monoclonal, clone 30-9; a) lens 20x; b) 40x objective; c) overview, 20x objective.

Dense fibrous stroma may show areas of altered hyaline or focal myxoid issues. Collagen fibers are parallel. Vascular spaces have a dilated or slotappearance; the number, configuration and wall thickness are variable.

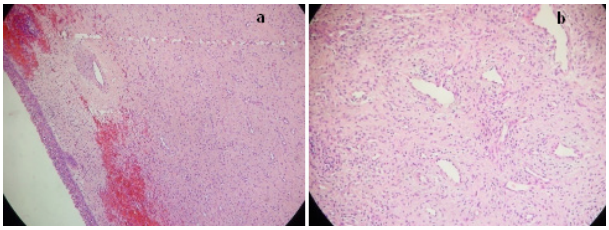

Fig.2. Nasopharyngeal angiofibroma - overview, H.E. stain; a, b) 20x objective.

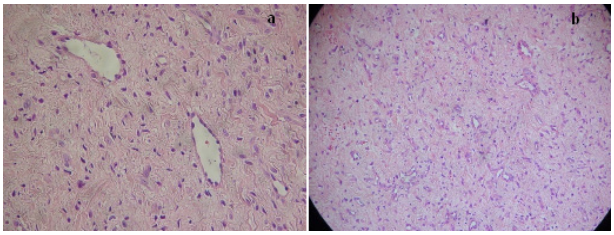

Fig.3. Nasopharyngeal angiofibroma - detail H.E. staining; a, b) 40x objective.

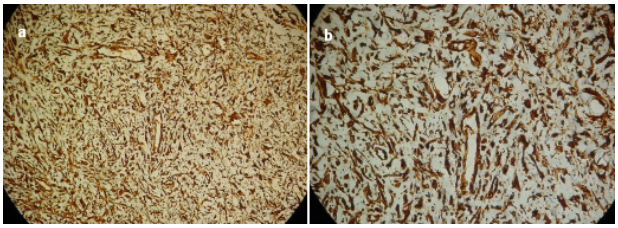

Fig.4. Ac antivimentin (Ventana / Roche monoclonal, Clone V9); a) lens20x; b) lens40x.

They are most often visible as being padded with a single row of endothelial cells.

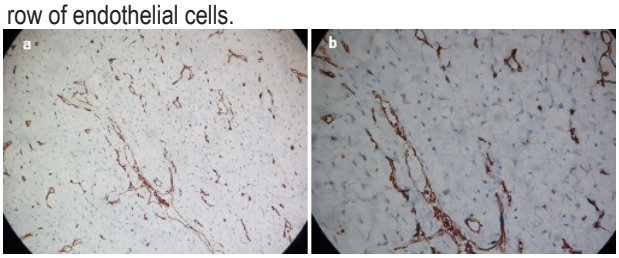

Fig.5. Ac CD31 - overview; a) lens 20x b) lens 40x.

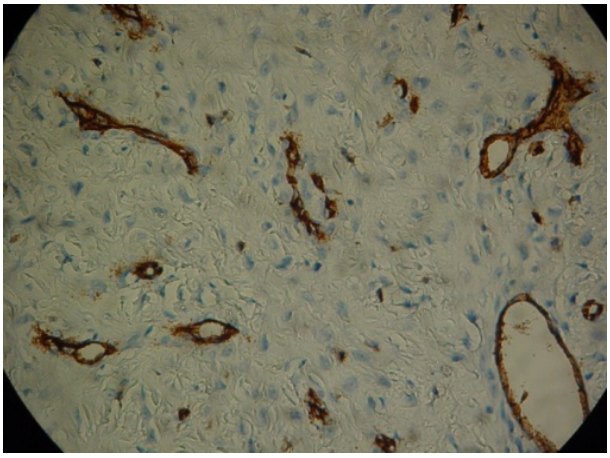

Fig.6. Ac CD34 – detail, lens 40x.

Vessels with thicker walls and lacking elastic fibers may be present. Few vessels can be surrounded by flat muscle cells.

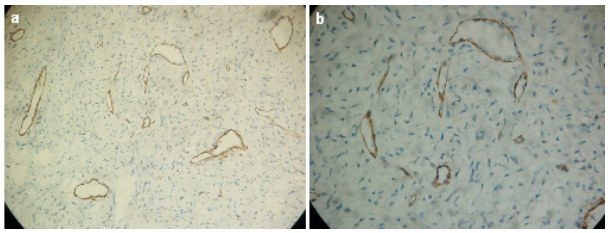

Fig.7. Muscle specific Ac actin, monoclonal, clone HHF 35; a) lens 20x; b) lens 40x.

The ratio between the blood vessels and tumor stroma varies depending on the tumor thickness. Thus, stromal elements are more numerous in the central area, while blood vessels are much less numerous. The blood vessels at the edges of the tumor, and are smaller, more uniform in size, thus the fibrosis is minimal.

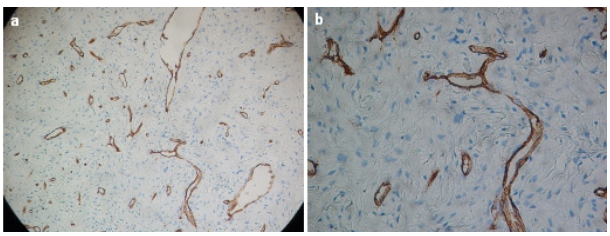

Fig.8. Ac CD34 - Cell Margue, monoclonal, clone QBE end/10; a) lens 20x; b) lens 40x.

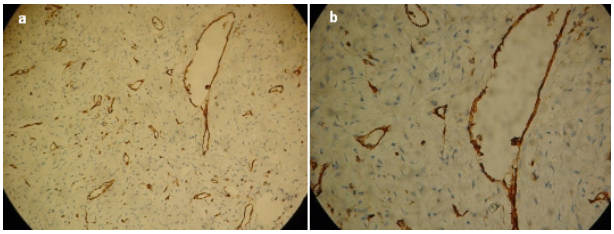

Fig.9. Ac CD31 Cell Margue, monoclonal, clone JC 70; a) lens 20x; b) lens 40x

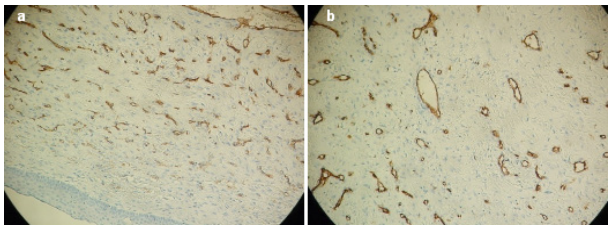

Fig.10. Ac CD34 -a) overview, lens 20x; b) detail, lens 20x.

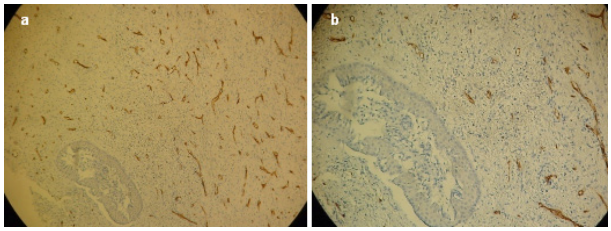

Fig.11. Ac anti CD34 ; overview, 20x lens b) detail, 40x objective.

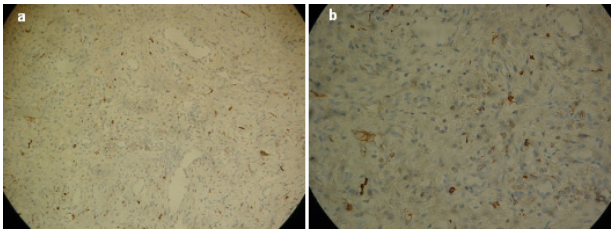

Fig.12. Ac CD117 (c-kit) - Ventana / Roche, monoclonal, Clone 9.7, a) 20x objective; b) 40x objective.

The arrangement of the collagen bands is random. Multinucleated stromal cells with intensely eosinophilic, "ganglion-like"cytoplasm may also be present.

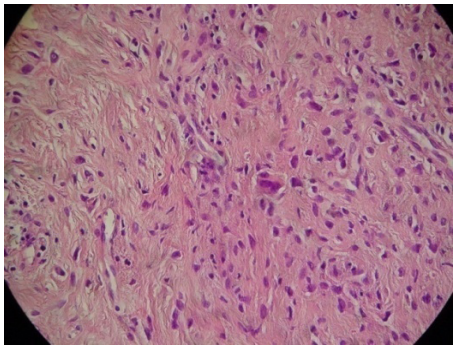

Fig.13. Nasopharyngeal angiofibroma -cell morphology; stromal cells with multiple nuclei: H.E. stain; 40x objective. Tumors showed no receptors for estrogen or progesterone (Ac anti estrogen receptor, 6F11/Novocastra clone, Ac anti progesterone clone and 1A6/Novocastra).

DISCUSSION

Ultrastructural studies have shown that stromal cells are myofibroblasts, with variable expression of fibroblast and myoblast features. These cells contain intranuclear dense granules, which apparently represent RNA-related protein complexes (4, 9, 10). The angiofibroma has been shown to have receptors for testosterone (6) but no receptors for estrogen (6, 7). In the study groups some tumors (little in number) showed positivity for progesterone. In our study, tumors did not show receptors for estrogen or progesterone (Ac estrogen anti-receptor, clone 6F11/Novocastna and Ac progesterone anti-receptor, clone 1A6/Novocastra). Preoperative administration of estrogen often leads to reduced tumor volume and number of blood vessels, assuming that the estrogen is inhibiting the release of gonadotropic hormones (7).

CONCLUSIONS

Although the angiofibroma is a histologically benign tumor, it may have an aggressive behavior by recurrence and through its ability to destroy adjacent bone structures. Macroscopically, the juvenile nasopharyngeal angiofibroma is a vascular, lobulated, firm, unencapsulated, reddish blue, sessile or pedunculated tumor. Microscopically, the tumor is composed almost entirely of network-shaped blood vessels and connective tissue. Appearance may vary from a complete cavernous angioma, developed in a fibrous stroma, to occasional or dense cellularity myxomatous fibroma.

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ANGIOFIBROMUL NASOFARINGIAN JUVENIL – CARACERISTICI HISTOLOGICE ŞI IMUNOHISTOCHIMICE

REZUMAT

Obiective: Angiofibromul nasofaringian juvenile este o tumoră rară, benign din punct de vedere histologic, dar foarte agresivă la nivel local, care afectează personale la vârsta pubertăţii sau adolescenţii de sex masculin (în jurul vârstei de 15 ani). Din punct de vedere histopatologic este un angiofibrom, caracterizat prin vârtejuri fibroase şi efuziuni sanguine, fără limitare clară. Elementele fibroase au iniţial doar rol de susţinere a stromei tumorale, mai târziu devenind elemente structurale predominante, ca efect al scăderii estrogenilor. Pacienţi şi metode: Acesta este un studiu retrospectiv care include evaluarea a 10 piese de rezecţie chirurgicală obţinute de la pacienţi diagnosticaţi şi trataţi în Clinica ORL Timişoara între 2002-2012. Evaluarea a inclus aspectul macroscopic tumoral, caracteristicile microscopice şi evidenţierea diferiţilor markeri – receptorul de estrogen, receptorul de progesteron, Vimentina, Ki67, CD31 şi CD34. Rezultate: Toţi pacienţii investigaţi au fost de sex masculin. Cel mai frecvent model histologic întâlnit a fost acela cu fibroblaste fusiforme imature prezente la nivel stromal şi vase sanguine cu pereţi subţiri în diferite proporţii. Tumora nu prezintă capsulă proprie, dar la nivel peri-tumoral, mai ales în cazul tumorile non-ulcerative, se observă ocazional o pseudocapsulă derivată de la nivelul mucoasei faringiene sau nazale, cu un aspect atrofic. Stroma fibroasă densă poate prezenta arii hialine care alterează aspectele mixoide focale. Celulele multinucleate stromale sunt prezente, cu citoplasmă intens eozinofilică, ganglion-like. Discuţii: Proporţia dintre vasele sanguine şi stroma tumorală variază în funcţie de dimensiunea tumorii. Studiile ultrastructurale au arătat că celulele stromale sunt miofibroblaste, cu expresie variabilă a caracteristicilor fibroblastice şi miofibroblastice. Concluzii: Cu toate că angiofibromul este o tumoră benignă din punct de vedere histologic, poate avea un comportament agresiv datorită recurenţelor şi capacităţii de a distruge structurile osoase adiacente.

Cuvinte cheie: angiofibrom nasofaringian juvenil, benign, microscopic, macroscopic, histologic

NEW INSIGHT INTO THE PULMONARY VASCULITIS

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ABSTRACT

Pulmonary vasculitides are non-infectious inflammatory disorders that mainly affect the blood vessels of the lung, from the main pulmonary artery to alveolar capillaries and are usually associated with systemic vasculitis. The presence of pulmonary vasculitis can be suggested by a clinical presentation that includes diffuse pulmonary hemorrhage, acute glomerulonephritis, chronic refractory sinusitis or rhinorrhea. Pulmonary vasculitis can occur in isolation without the involvement of other organs. Serologic tests, including the use of cytoplasmic antineutrophil antibody (ANCA) and perinuclear ANCA, are performed for the differential diagnosis.

Key words: pulmonary vasculitis, autoimmune diseases, pathophysiology, clinical and laboratory findings

INTRODUCTION

Pulmonary vasculitis refers to inflammation and damage to blood vessels in the lungs. Pulmonary vasculitis can be an acute reaction to a respiratory tract infection or a chronic condition related to an autoimmune disorder (1,2).

Several clinical symptoms and signs are suggestive of the presence of pulmonary vasculitis. Symptoms can vary, but many patients exterience shortness of breath, fatigue, and chest pains. Pulmonary vasculitis is associated with granulomatous, eozinophilic, lymphoplasmocytic, or neutrophilic inflammatory diseases and commonly is a manifestation of systemic illnesses such as primary systemic vasculitides, collagen vascular diseases, systemic diseases associated with the autoantibodies, and as a side effect of certain drugs. A positive cytoplasmic ANCA test result is specific enough to make a diagnosis of ANCA associated granulomatous vasculitis if the clinical features are typical. Perinuclear ANCA positivity raises the possibility of Churg-Strauss syndrome or microscopic polyangiitis. Treatment is generally geared toward eliminating the underlying cause (3).

VASCULITIS ASSOCIATED WITH COLLAGEN VASCULAR DISEASE

Pulmonary capillaritis can manifest as a complication of a disease course of various kinds of collagen vascular diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis, polymyositis, Sjögren syndrome, scleroderma, and primary antiphospholipid syndrome. The lungs and pleura are affected more frequently in SLE (50%–70% of cases) than in other collagen vascular diseases, and lung involvement manifests with diverse pathologic entities. SLE vasculitis is immune complex mediated and involves the arterioles and capillaries; necrotizing arteriolitis (much rarer than capillaritis) results in edema and fibrinoid necrosis in the arterioles within the secondary pulmonary lobule, and acute necrotizing capillaritis (more common than arteriolitis) chiefly causes diffuse alveolar hemorrhage (DAH). When DAH does occur, it is often seen concomitantly with other pulmonary manifestations of SLE such as acute lupus pneumonitis, pulmonary edema, or pleural effusion. DAH occurs in 4% of hospitalized patients with SLE, representing 22% of the pulmonary complications of SLE. Typically, patients with DAH present with rapid-onset tachypnea, cough, fever, hypoxia, and hemoptysis while displaying symptoms of generalized SLE vasculitis such as renal failure, arthritis, or rash. It is known that the presence of DAH should exclude scleroderma, rheumatoid arthritis, and dermatomyositis or polymyositis. However, DAH has been reported in a small number of patients with mixed connective tissue disease, and it has occurred predominantly in association with glomerulonephritis. Similarly, DAH has been reported in a small number of cases of rheumatoid arthritis. In SLE, pulmonary hypertension occurs in 0.5%–14% of patients. The above-mentioned necrotizing vasculitis may be a factor in pulmonary hypertension in SLE patients. Recurrent thromboembolic disease, bland vasculopathy as in primary pulmonary hypertension, and interstitial pulmonary fibrosis and hypoxia may also contribute to pulmonary arterial hypertension (4,5).

ETIOLOGY AND PATHOGENESIS

The exact mechanisms by which vasculitis occurs in the lungs is not always clear. Vasculitis can be pathologically defined by the presence of cellular inflammation, vessel destruction, and tissue necrosis. Although the inciting antigens or stimuli of vasculitis are not well understood, an immunologic mechanism is involved in the pathogenesis of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis. In ANCA-associated vasculitis, an intense infiltration of activated neutrophils results

Received February 2nd 2013. Accepted March 10th 2013. Address for correspondence: Dr. Manole Cojocaru, Titu Maiorescu" University, Faculty of Medicine, Str. Thomas Masaryk No. 5 Sector 2 Postal Code 020983, Bucharest, Romania, e-mail: mancojocaru@yahoo.com

in fibrinoid necrosis and dissolution of vessel walls, thus compromising the vascular lumen. ANCA may be involved in the pathogenesis not only by activation of neutrophils but also by enhancement of the adhesion of the activated neutrophils to cytokine-primed endothelial cells. The damage to the integrity of the vessels results in the leakage of blood into the alveolar space from the interstitial capillaries and causes stenoses, thrombotic obstruction, and aneurysms of the corresponding vessels. Cytoplasmic ANCA is mainly related to antibodies directed against proteinase 3 found in azurophilic granules of neutrophils and monocytes, whereas perinuclear ANCA is associated with antibodies directed against a variety of intracellular antigens (most commonly with myeloperoxidase, also found in azurophilic granules). The production of autoantibodies such as antiglomerular basement membrane antibody (type 2 hypersensitivity reaction), formation of immune complexes (type 3 hypersensitivity reaction), and T cell involvement (type 4 hypersensitivity reaction) are likely to link to the pathogenesis of other forms of small-vessel pulmonary vasculitis. This process may be collectively called immune complex–mediated vasculitis. Vasculitis in systemic lupus erythematosus (SLE) is typical of this immune complex–mediated vascular disease (1-3).

CLINICAL FINDINGS

Most commonly, patients with pulmonary vasculitis present with various multisystem manifestations. Although combined manifestations are more common, each of the following symptoms and signs can be seen as an isolated symptom or a sign: diffuse pulmonary hemorrhage, glomerulonephritis, upper respiratory tract lesions, imaging findings of nodules or cavitary lesions, mononeuritis multiplex, multisystem disease, and palpable purpura. In some instances, laboratory tests and physical exams may reveal an underlying problem (3).

LABORATORY TESTS

ANCA tests play an important role in the diagnosis of some pulmonary vasculitides, including ANCA-associated granulomatous vasculitis, CSS, and microscopic polyangiitis, and for idiopathic pauci-immune rapidly progressive glomerulonephritis. The use of cytoplasmic ANCA is highly sensitive (90%–95%) for the detection of active, systemic ANCA-associated granulomatous vasculitis and has a specificity of approximately 90%. In an adequate clinical setting, a positive cytoplasmic ANCA finding has sufficient positive predictive value (>90%) so that a biopsy may be deferred or exempted. Conversely, a positive perinuclear ANCA finding provides no more than suggestive evidence of CSS, microscopic polyangiitis, or idiopathic pauci-immune rapidly progressive glomerulonephritis. Perinuclear ANCA can be found in diseases such as rheumatoid arthritis, Goodpasture syndrome, infection (hepatitis C, fungal, and mycobacterial), and inflammatory bowel disease. When cytoplasmic or perinuclear ANCA finding is positive, the addition of proteinase 3 or myeloperoxidase ANCA enzyme-linked immunosorbent assay test can help increase the specificity in the diagnosis of ANCA-associated vasculitis. The importance of increasing ANCA titers to predict relapse in patients with vasculitides has also been evaluated. Current studies suggest that there is insufficient sensitivity and specificity of an isolated increase in the ANCA titer to accurately predict relapse in ANCA-associated granulomatous vasculitis and other vasculitides. Culture of blood and other affected organs (when applicable) must be obtained to exclude infection. Routine laboratories (complete blood count with differential, chemistries, liver tests, blood urea nitrogen, and creatinine) should be obtained; however, the findings are generally nonspecific. An elevated erythrocyte sedimentation rate and C-reactive protein are expected in active vasculitis but lack specificity. Urinalysis should be obtained in all patients, with the microscopic examination performed on a fresh sample (before red cell casts or other diagnostic features degenerate) by personnel skilled in its interpretation. Proteinuria and microscopic hematuria are common early findings in WG and MPA. Anti–glomerular basement membrane antibodies should be obtained in all patients with pulmonary hemorrhage or a pulmonary-renal syndrome and when present are indicative of Goodpasture's syndrome. Antinuclear antibodies and rheumatoid factor may be positive in primary vasculitis, although high titers and disease-specific antibodies, such as dsDNA, SS-A/SS-B, anti-RNP, and anti-Jo-1, favor a connective tissue disease. IgE should be obtained when CSS is being considered. Complement (C3 and C4) should be obtained whenever SLE is in the differential. Some fungal, bacterial, and parasitic agents are capable of causing blood vessel damage in the respiratory tract and lungs (6-10).

DIAGNOSTIC BIOPSY

Although a confident diagnosis may occasionally be made without tissue, diagnostic biopsy remains the bedrock of diagnosis. Diagnostic tissue may infrequently be obtained from easily accessible sites, such as skin or upper airway lesions. Percutaneous renal biopsy is commonly performed during the evaluation of an acute glomerulonephritis. Although features specific for vasculitis (e.g., granulomatous inflammation or necrotic vessels) are rarely found, the finding of a focal, segmental necrotizing glomerulonephritis without immune deposits (pauci-immune) generally reflects a systemic vasculitis. It is important that in addition to conventional histopathology, immunofluorescence (and electron microscopic when appropriate) studies be performed. The presence of characteristic immunofluorescence patterns, such as IgA deposition in Henoch-Schönlein purpura, linear IgG deposition in Goodpasture's syndrome, and irregular immunoglobulin and complement deposition in SLE, help provide a specific diagnosis. When the lung is clinically involved, surgical lung biopsy almost always provides definitive pathologic evidence. If obtained, the clinician, surgeon, and pathologist must coordinate the management of the sample and collect tissue in saline for culture, frozen tissue for immunofluorescence, and formalin-fixed tissue for standard hematoxylin and eosin staining (3, 6-9)

CONCLUSION

Despite the considerable advances made in the diagnosis and management of the small-vessel vasculitides, the morbidity and mortality of these disorders remains high. A cytoplasmic ANCA study is both sensitive and specific for ANCA-associated granulomatous vasculitis in the appropriate clinical setting. A perinuclear ANCA study is neither sensitive nor specific, but it is suggestive of the presence of CSS and microscopic polyangiitis.

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ACTUALITĂȚI ÎN VASCULITA PULMONARĂ

REZUMAT

Vasculitele pulmonare sunt afecţiuni inflamatorii neinfecţioase care afectează în special vasele de sânge ale plămânului, de la artera pulmonară la capilarele alveolare şi sunt de obicei associate cu vasculită sistemică. Prezenţa vasculitei pulmonare poate să fie sugerată la examenul clinic de hemoragie pulmonară difuză, glomerulonefrită acută, sinuzită cronică recidivantă sau rinoree. Vasculita pulmonară se poate întâlni izolată, fără implicarea altor organe. Testele serologice cuprind anticorpii anticitoplasma polimorfonuclearului neutrofil pattern-ul citoplasmatic şi perinuclear, care se utilizează pentru diagnosticul diferenţial.

Cuvinte cheie: vasculita pulmonară, boli autoimune, fiziopatologie, date clinice şi de laborator

AMIODARONE ASSOCIATED OPTIC NEUROPATHY, IS IT A REAL THING?

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ABSTRACT
The purpose of our study was to assess the ophthalmologic impact of amiodarone treatment, especially amiodarone associated optic neuropathy (AAON). We included in our study 256 clinical cases with ocular changes following amiodarone therapy. All patients included in our study had a history of complete amiodarone loading dose of 11-12g, with a minimum of 4 months therapy. All patients were on chronic use of amiodarone medication (200-400 mg/daily). The Macaluso *et al* criteria were used to diagnose AAON. Amiodarone optic neuropathy is characterized by insidious onset of visual loss, protracted disc edema (for months), and bilateral (usually simultaneous) involvement. We included in our study 256 patients (136 men, 120 women) treated with amiodarone. The average age of the patients was 45±16 years. The duration of amiodarone medication varied from four months to six years. The ocular complication were represented by cornea verticillata, anterior subcapsular lenticular opacities, amiodarone induced neuropathy. After interrupted or discontinued amiodarone therapy 75% of the cases of amiodarone keratopathy were reversible. The most frequent ocular complication from amiodarone treatment is cornea verticillata. Despite the fact that amiodarone induced optic neuropathy is rare, the screening for diagnosis is important because of clinical impact. We believe that opthalmological surveillance is mandatory in all patients on long term amiodarone therapy, and those patients should undergo a complete ocular examination every 6 months. If the diagnosis is suspected we should discontinue amiodarone. The decision regarding the safety of discontinuing the amiodarone is best left to the cardiologist.
Key words: amiodarone treatment, optic retinopathy

INTRODUCTION

Amiodarone is a class III antiarrhythmic drug used especially in the treatment of severe arrhythmias, refractory to other medication, such as malignant ventricular tachycardia and atrial fibrillation. The widespread use of this drug has increased after approval, by the American Heart Association, for its use in advanced cardiac life support protocols (1).

Potential adverse effects include corneal microdeposits (70-100%) (2), optic neuropathy/neuritis (≤1%-2%), blue-gray skin discoloration (4%-9%), photosensitivity (25%-75%), hypothyroidism (6-10%), hyperthyroidism (0.9%-2%), pulmonary toxicity (1%-17%), and hepatotoxicity (elevated enzyme levels, 15%-30%; hepatitis and cirrhosis, <3%). A range of neuropsychiatric adverse effects may also occur. The most common are tremor and ataxia (3%-35%, depending on dose and duration of therapy). Peripheral neuropathy is uncommon (0.3% annually) but may be severe, requiring dose reduction or discontinuation of therapy. Insomnia, memory disturbances, and delirium have been reported as well (3-6).

Amiodarone has also been reported to cause optic neuropathy, which may result in permanent visual loss. The clinical presentation of amiodarone induced optic neuropathy may be similar to that of non-arteritic anterior ischaemic optic neuropathy (NAION) (7-10). Casual relationship between amiodarone and optic neuropathy is subject of intense controversy and the pathophysiology is unknown (11,12).

PURPOSE

The purpose of our study was to assess the ophthalmologic impact of amiodarone treatment, especially amiodarone associated optic neuropathy (AAON).

MATERIAL AND METHODS

We included in our study 256 clinical cases with ocular changes following amiodarone therapy. All patients included in our study had a history of complete amiodarone loading dose of 11-12g, with a minimum of four months therapy. All patients were on chronic use of amiodarone medication (200-400 mg/day). Exclusion criteria included evidence of an alternative systemic disorder that could explain optic neuropathy and insufficient clinical information. We recorded for each patient age, sex, amiodarone dose, time of amiodarone therapy, the interval between initiation of treatment and onset of visual symptoms. All patients had a complete ocular examination with a particular attention given to slit-lamp biomicroscopy and fluorescein angiography. Our study is a retrospective one and we didn't obtain informed consent. We used the Macaluso *et al* (13) criteria to diagnose AAON. Amiodarone optic neuropathy is characterized by insidious onset of visual loss, protracted disc edema (for months), and bilateral (usually simultaneous) involvement. In contrast, NAION is characterized by acute unilateral visual loss with resolution of disc edema over several weeks.

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RESULTS

We included in our study 256 patients (136 men, 120 women) treated with amiodarone. The average age of the patients was 45±16 years. The duration of amiodarone medication varied from four months to six years. The ocular complications were represented by cornea verticillita (amiodarone keratinopathy-243 patients, 95% - Figure 1), anterior subcapsular lenticular opacities (56 patients, 21.8% - Figure 2), amiodarone induced neuropathy (4 patients, 1.56% - Figures 3 and 4). From patients with amiodarone keratinopathy 47 (18.35%) was associated with anterior lenticular opacities. We found bilateral asymmetric lesion of cornea vericillita in 170 patients (66.4%). After interrupted or discontinued amiodarone therapy 75% of amiodarone keratopathy were reversible.

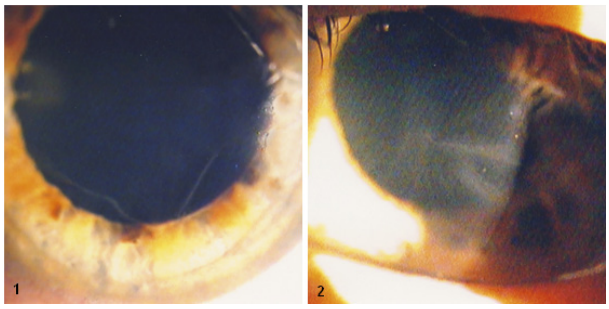

Fig.1. Cornea verticillata (amiodarone keratopathy); **Fig.2.** Anterior lenticular opacities

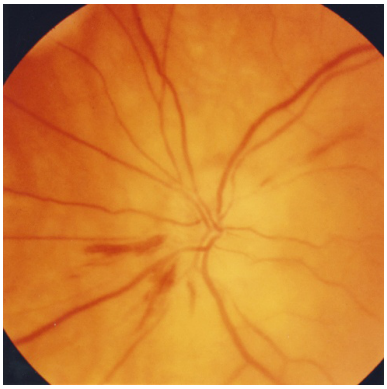

Fig.3. Amiodarone associated optic neuropathy-retinophotography

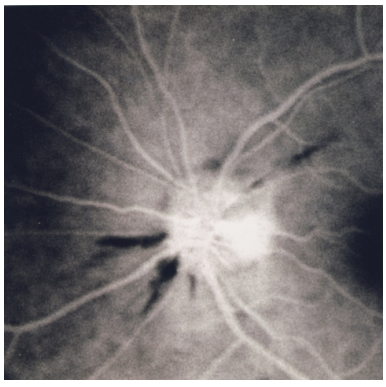

Fig.4. Amiodarone associated optic neuropathy-fluorescein angiography

DISCUSSION

Despite the fact that ultrastructural changes have been observed in nearly all ocular tissues in patients taking amiodarone, most patients treated with the drug do not develop visual symptoms. During therapy, intracytoplasmic lamellar deposits occur in the cornea, lens, retina, and optic nerve. Although nearly 100% of patients on amiodarone develop a cornea verticillata (amiodarone keratopathy), this finding only infrequently produces symptoms of glare, halos, or blurred vision (14,15). The keratopathy is probably dose and duration of treatment-dependant, with usual onset after one or more months, and slowly disappearing from the cornea if amiodarone is discontinued. The consensus in the literature is that it does not disturb vision. We found in our study that 95% patients developed cornea vericillata after at least four months of treatment; in 75% of cases, a resolution after discontinuing the drug was observed. Of great interest is that amiodarone is involved in causing an optic neuropathy, findings that are indistinguishable from those of non-arteritic anterior ischemic optic neuropathy (NAION), which is frequent in normal people older then 50 years. The incidence of amiodarone-related optic neuropathy was estimated by Feiner *et al* (7) at 1.76% over the preceding 10 years versus 0.3% in an age-matched population not using the drug. We used the criteria develop by Macaluso *et al* to distinguish between amiodarone induced neuropathy and non-arteritic anterior ischaemic optic neuropathy. Using these criteria we found amiodarone associated neuropathy in 4 patients (1.56%), similar to other reports. Johnson *et al* (16) recently reported a clinical spectrum of amiodarone-induced optic neuropathy based on an observational case series. They classified amiodarone-associated optic neuropathy in five categories: insidious onset (43%); acute-onset (28%); retrobulbar (13%); increased intracranial pressure (8%) and delayed-progressive onset (8%), although overlap in clinical presentation with NAION may occur. The fact that we use Macaluso ctiteria of AAON can be a limitation of our study, but it offers a more accurate diagnosis. Amiodarone has a long half life (21-78 days) therefore the optic disc swelling may persist for weeks to months after the medication is stopped. The diagnosis is certain only after discontinuing the drug, slow resolution of optic disc swelling and improvement in visual function.

CONCLUSION

The most frequent ocular complication from amiodarone treatment is cornea verticillata. Despite the fact that amiodarone induced optic neuropathy is rare, the screening for diagnosis is important because of clinical impact. We believe that opthalmological surveillance is mandatory in all patients on long term amiodarone therapy, and those patients should undergo a complete ocular examination every 6 months. If the diagnosis is suspected we should discontinue amiodarone. The decision regarding the safety of discontinuing the amiodarone is best left to the cardiologist.

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NEUROPATIA ASOCIATA TRATAMENTULUI CU AMIODARONA – ESTE O REALITATE?

REZUMAT

Scopul studiului nostru a fost de a evalua impactul oftalmologic al tratamentului cu amiodaronă, în special neuropatia optică asociată cu amiodarona (AAON). Am inclus în studiul nostru 256 de cazuri clinice cu modificări oculare în urma terapiei cu amiodaronă. Toţi pacienţii incluşi în studiul nostru aveau un istoric de doză completă de amiodaronă de 11-12g, urmând terapia de cel puţin 4 luni. Toţi pacienţii utilizau cronic amiodaronă (200-400 mg/zi). Pentru a diagnostica AAON s-au utilizat criteriile enunţate de Macaluso *et al*. Neuropatia optică indusă de amiodaronă este caracterizată prin instalare insidioasă a pierderii vedererii, edem de disc prelungit (timp de luni de zile) şi implicare bilaterală (de obicei simultană). Am inclus în studiul nostru 256 de pacienţi (136 de bărbaţi, 120 de femei) trataţi cu amiodaronă. Vârsta medie a pacienţilor a fost de 45±16 ani. Durata administrării medicaţiei a fluctuat între patru luni şi şase ani. Complicaţiile oftalmologice au fost reprezentate de cornea verticillata, opacităţi lenticulare subcapsulare anterioare, neuropatie indusă de amiodaronă. După întreruperea sau terminarea terapiei cu amiodaronă, 75% dintre cazurile de keratopatie au fost reversibile. Cea mai frecventă complicaţie oftalmologică în urma tratamentului cu amiodaronă este cornea verticillata. În ciuda faptului că neuropatia optică indusă de amiodaronă este rară, screeningul pentru diagnostic este important din cauza impactului clinic. Considerăm că supravegherea oftalmologică este obligatorie la toţi pacienţii care urmează terapie cu amiodaronă pe termen lung; aceşti pacienţi ar trebui să fie supuşi unui examen oftalmologic complet o dată la 6 luni. Dacă se suspectează acest diagnostic, tratamentul cu amiodaronă trebuie suprimat. Este bine ca decizia cu privire la siguranţa încetării tratamentului cu amiodaronă să fie luată de cardiolog.

Key words: tratament cu amiodaronă, retinopatie optică