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CUPRINS

REVIEW

STEM CELLS FOR HEART REPAIR

CARMEN TATU, GABRIELA TANASIE, FLORINA BOJIN, RF TATU, DANIELA PUSCASIU, CARMEN BUNU

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ABSTRACT

Cell therapy has emerged as an attractive therapeutic modality to repair damaged myocardium. A variety of stem and progenitor cell populations could be used for cardiac repair. Each cell type has its own profile of advantages, limitations, and practicability issues in specific clinical settings. Cells differ markedly in regard to their site of origin as well as to their anatomy and function, as characterized by surface markers, transcription factors, and expressed proteins. They also differ in their ability to form one or more differentiated cell types.

Key words: mesenchymal stem cells, endothelial progenitor cells, hematopoietic stem cells, embryonic stem cells

INTRODUCTION

Cardiac repair can be considered as the outcome of three major processes: replacement (tissue transplant), rejuvenation or restoration (activation of resident cardiac stem cells or other stem cells via paracrine or autocrine mechanisms; modulation of apoptosis, inflammation, angiogenesis, or metabolism), and regeneration (progenitor or stem cell engraftment forming differentiated myocytes)(6,7). These different entities may be interlinked in that modulation of myocardial injury, may also benefit subsequent therapy directed at myocardial regeneration. In preclinical and clinical studies, a variety of cells have been considered as candidates for cell repair therapy. Cells differ markedly in regard to their site of origin as well as to their anatomy and function, as characterized by surface markers, transcription factors, and expressed proteins. They also differ in their ability to form one or more differentiated cell types(7). The bone marrow acts as a reservoir, housing diverse types of progenitor cells (e.g., endothelial progenitor cells, mesenchymal stem cells, and hematopoietic stem cells), which are differentially regulated by growth factors and cytokines, affecting their retention, self-renewal, cell-cycle status, and mobilization (5). These distinct subsets of progenitor cells are multipotent, although more committed, arising from a parent stem cell, and developmentally, hold a hierarchy between stem cell and a fully differentiated adult cell. On the contrary, stem cells define a class of multipotent or pluripotent cells fully capable of self-renewal and clonal expansion. Although, literature is replete with instances of both autologous stem and progenitor cell therapies for the treatment of ischemic cardiomyopathies and their improved therapeutic outcomes have been widely accepted, these trials have led to a less precise use of terms to identify multipotent cells and true stem cells. Myocardial infarction engenders an irreversible loss of cardiomyocytes. It has been reported that a typical human infarct involves a loss of 1–1,5 billion cardiomyocytes with a consequent loss in myocardial cell mass (approximately one third of the total volume) (1,5). Therefore, progenitor cell therapies that can contribute to an increase in the number of viable and functional cardiomyocytes population behold considerable clinical significance. As a consequence, many stem and progenitor cell types have been subjected to challenges to demonstrate a cardiomyocytes-differentiation potential. Cell therapy has emerged as an attractive therapeutic modality to repair damaged myocardium. The recent identification of various bone marrow-derived adult stem or progenitor cells capable of contributing to tissue regeneration has opened the possibility that these cells can be used to repair damaged heart tissue (8). Although bone marrow transplantation has long been used in the treatment of hematologic diseases, bone marrow also has stem or progenitor cells that can give rise to nonhematopoietic cells, including endothelium, neural cells, skeletal myoblasts, cardiomyocytes, and epithelium of lung, gut, and skin. Various bone marrow cells have been examined for their ability to repair myocardial damage, and initial animal and limited human studies to date have shown therapeutic benefit. Controversy exists about the fate of transplanted cells and the therapeutic mechanism of bone marrow cell therapy in the heart.

A variety of stem and progenitor cell populations could be used for cardiac repair. Each cell type has its own profile of advantages, limitations, and practicability issues in specific clinical settings (23).

Endothelial Progenitor Cells (EPCs)

EPCs have originally been defined by their cell surface expression of the hematopoietic marker proteins CD133 and CD34 and the endothelial marker vascular endothelial growth factor receptor-2, and their capacity to incorporate into sites of neovascularization and to differentiate into endothelial cells in situ (23). Endothelial progenitor cells are a subpopulation within the bone marrow that can promote vasculogenesis leading to vessel regrowth, thereby improving oxygenation to tissue after damage. Preclinical trials indicate that endothelial progenitor cells contribute to 1–25% of vessel formation after ischemic injury for several diseases. Additionally, endothelial progenitors may secrete growth factors and paracrine signals that prevent cardiac myocyte

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cell death. These cells are capable of vascular differentiation and secretion of growth factors promoting angiogenesis and increasing blood supply to the damaged heart (3). Improved neovascularization can play a crucial role in rescuing the failing ischemic myocardium. Transdifferentiation of EPCs and whether this transdifferentiation plays a main role in the therapeutic benefit of EPCs in recovering cardiovascular function is controversial. Endothelial progenitor cells are potential candidates for cardiovascular repair, as they have been shown to mediate neovascularization at the infarct border zone. Bone marrow–derived hemangioblasts and peripheral blood–derived monocytes can differentiate into EPCs, which in turn can differentiate into functional endothelial cells (5). Soon after the discovery of EPCs, it was realized that tissue ischemia and cytokines can mobilize EPCs, thereby contributing to neovascularization of the ischemic tissue (21). Several recent studies demonstrated transdifferentiation of human peripheral blood CD34+ cells into endothelial lineage cells with incorporation into blood vessels (8,18). However, other investigators claim that bone marrow-derived cells do not transdifferentiate or incorporate into vessel walls (8,26). These discrepancies may be due to the difference in cell types, the use of different animal models used to look for transdifferentiation, or the application of less or more rigorous criteria to define transdifferentiation. Considering these controversies, more-sophisticated methods with rigorous criteria, such as z-stacked confocal imaging and long-termin vivo tracking, are needed to elucidate the role of transdifferentiation of EPCs or more fundamentally to address the nature and identity of EPCs. Therapeutic mechanisms of EPCs in cell therapy in vivo also remain to be better characterized.

Other studies have demonstrated that EPCs can mediate vasculogenesis under diverse pathophysiologic conditions like wound healing, limb ischemia, and after myocardial infarction, under the influence of the released cytokines (5). These EPCs are characterized by their ability to take up acetylated low-density lipoprotein and are CD34+, CD133+, Flk-1+. Quite interestingly, EPCs share many common characteristics and overlapping expression of surface markers with diverse cell types. For instance, EPCs derived from the bone marrow as well as the circulating EPCs demonstrate the expression of endothelial markers Flk-1, Tie-2, VE-cadherin, CD34, CD146, and E-selectin, just as do the mature circulating endothelial cells. Moreover, the uptake of acetylated LDL, a property associated with EPCs, can be mimicked by monocytes and certain hematopoietic cells. However, despite these complexities related to the purification and characterization of EPCs (22), EPCs are the most commonly studied candidates in clinical trials for reendothelialization and neovascularization of the myocardium. Differentiation of stem cells into functional cardiac myocytes is a main goal of stem cell therapy.

Mesenchymal Stem Cells (MSCs**)**

MSCs represent a rare population of CD34- and CD133- cells present in bone marrow stroma, 10-fold less abundant than hematopietic stem cells and other mesenchymal tissues. MSC are present in adult tissues including the bone marrow and adipose tissues, MSCs are characterized by an absence of hematopietic stem cells markers. They can be isolated and expanded easily and, can transdifferentiate into functional cardiomyocytes and a variety of other cells, resulting in an improvement in left ventricular function and remodeling. MSCs can readily differentiate into osteocytes, chondrocytes, and adipocytes. Differentiation of MSCs to cardiomyocyte-like cells has been observed under specific culture conditions and after injection into healthy or infarcted myocardium in animals. The magnitude of transdifferentiation is currently under investigation, but accumulating evidence supports the active engraftment and differentiation of transplanted human MSCs within the healing myocardium in sheep (7). This finding has been corroborated in vitro, with cardiogenic MSC guidance demonstrating a capacity for sarcomerogenesis and electromechanical coupling. Noninvasive multimodality imaging indicates that therapy after myocardial infarction with allogeneic MSCs promotes active cardiac regeneration in vivo. When injected into infarct tissue, MSCs may enhance regional wall motion and prevent remodeling of the remote, noninfarcted myocardium. Little is known about the effects of MSCs on myocardial perfusion (23). It is interesting to note however, that cultured MSCs secrete angiogenic cytokines, which improve collateral blood flow recovery in a murine hind limb ischemia model. One group of reasercher demonstrated that, in a model of murine hindlimb ischemia, MSC transplantation enhanced tissue repair via secretion of multiple cytokines, including vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), and placental growth factor (PlGF), rather than incorporation of MSCs into new or remodeling tissues (8,11,12). Others reported that MSC viability can be enhanced with gene transfection (8,17) with greater therapeutic effect on the injured myocardium. Recently, it was reported that direct MSC injection by using catheter is safe, reduces fibrosis, and augments myocardial regeneration (8). The advantage of MSCs for potential clinical application lies in the ease of acquisition and isolation, high expansion capability in culture, and low immunogenicity. The low immunogenicity can render MSCs more-attractive candidate cells for treatment of myocardial infarction because of their capability for modulating inflammatory reactions in acute myocardial infarction and the utility of the allogeneic approach. This extensive capacity for clinical scale expansion in vitro has facilitated the development of preclinical models as well as clinical studies designed to assess the safety, feasibility, and efficacy of MSC transplantation in a variety of diseases. However, the use of animal serum for culture expansion restricts the growth of pilot clinical trials with MSCs in humans (8). Because MSC clones can be expanded in vitro, and reportedly have a low immunogenicity, they might be used in an allogeneic setting in the future. They can also modulate immune responses. It has been argued that these multipotent MSCs can mediate an immunosuppressive effect. Although MSCs do express MHC class I, they lack the B7 costimulatory molecule and escape recognition via both CD4+ T helper (naive and memory) and CD8+ cytotoxic T cell subsets. Moreover, MSCs can induce a bias toward T helper 2 rather than T helper 1 (two stable differentiation states of T helper that produce different lymphokines and different effector functions

via divergent signaling mechanisms), owing to reduced IFN-g secretion (4,5,13). Surprisingly, the expression of surface markers associated with lymphocyte activation, like CD25, CD38, and CD69, have been shown to decrease in the presence of MSCs (5,13). Therefore, they can inhibit lymphocyte proliferation by B-cell mitogens. Adipose tissue derived from the embryonic mesenchyme contains MSCs and endothelial progenitor cells as well as adipose cells. Experimental data suggest that adipose tissue – derived cells may transdifferentiate into cells with the characteristics of cardiomyocytes and perhaps blood vessels, or at least neovascular tissue. Adipose cells are attractive because access to them is easy and they are not in short supply in most societies. Nonetheless, additional characterization and demonstration of efficacy in animal models are needed before a clinical role for these cells can be established (7).

Hematopoietic Stem Cells (HSCs)

HSCs probably define a class of the oldest progenitor cells that are still in use for cell-based therapeutics. Initial studies using HSCs can be traced back to the 1970s. A hematopoietic stem cell (HSC) is a cell that can self-renew and give rise to all blood cells. The transplantation of a single HSC can constitute all hematopoietic cells in an organism, and a HSC fulfills the requirements for being a stem cell (i.e., selfrenewal capacity and potential to generate differentiated progeny)(8). Commonly identified by the expression of CD34+ and CD133 cell surface antigens, HSCs have been studied extensively and used clinically for bone marrow transplant for a variety of hematologic disorders. Cells capable of assuming an endothelial phenotype have been identified in the blood and bone marrow. Endothelial progenitor cells, a heterogenous population of cells that also reside predominantly in the bone marrow, likely promote neovascularization by secreting proangiogenic growth factors and by stimulating re-endothelialization; both functions could contribute to vascular homeostasis and perhaps myogenesis (7,19). The potential regenerative role of these cell types is based on findings in experimental animal models, not on clinical experience. The use of HSCs to repair an infarcted myocardium in a murine model has been investigated (8,16). Recent studies have suggested the possibility that specific stem cell populations derived from murine bone marow could regenerate most of the key elements of myocardium and ameliorate ischemic cardiac dysfunction. Studies involving an injection of Lin-/c-Kit+ HSCs in the periinfarct area of female C57BL/6 mice showed de novo cardiac regeneration (68% of the infarct volume) (16).

The more-versatile potential of bone marow-derived HSCs has been documented with the use of side-population cells (8) and with lineage-negative, c-kit–positive cells (cells that express a specific protein known as a tyrosine kinase, which adds phosphate groups to the amino acid tyrosine in other proteins); both of which differentiated into endothelial cells, vascular smooth muscle cells, and cardiomyocytes in a murine myocardial infarction model (15,16). In another attempt, intravenous injection of GFP-tagged Lin-Scal+ HSCs in murine model revealed intercellular connections of the administered cells with the resident cardiomyocytes. In the same study, Lin-CD34+CD38- human HSCs demonstrated intercellular connections with the resident cardiomyocytes in immunodeficient mice (NOD/SCID) (9,16). However, subsequent studies failed to show any differentiation of the labeled HSCs to cardiomyocytes or endothelial cells in the infarcted myocardium (16). It remains to be determined whether bone marrow cells or cell lines from other sources will be favored for myocyte formation in the future. Unless the efficacy of existing bone marrow cell preparations is substantially enhanced, attention will likely shift to other sources of cells. However, other studies suggested that HSCs do not readily acquire a cardiac phenotype in the injured heart, despite the improvement in ventricular function in the HSC-transplanted group (15). These studies were all performed with murine HSCs, and currently no studies have been performed with the human counterpart of these stem cells. In terms of clinical application, the lack of these HSCs in bone marrow and the unavailability of culture-expansion techniques may limit the therapeutic application of HSCs in ischemic heart diseases.

Embryonic Stem Cells (ES)

The embryonic stem cell, derived from the inner mass of the developing embryo during the blastocyst stage, has the greatest potential for organ regeneration and is the prototypical stem cell (7,20). ESCs posses all the qualities of a prototypical stem cell: clonal expansion, self-renewal and multipotentiality (5). Embryonic stem (ES) cells are totipotent stem cells. Under specific culture conditions, ES cells differentiate into multicellular embryoid bodies containing differentiated cells from all three germ layers including cardiomyocytes; however, their innate aptitude for pluripotent proliferation also presents an increased risk of teratoma (7). Transcriptional and functional profiling of human ES cell (hESC)-derived cardiomyocytes revealed a similarity to those found in 20-week fetal heart cells, thereby appearing as a strong candidate for the in vivo replacement of apoptotic cardiomyocytes in the failing myocardium (2,5). Human ES cell–derived cardiomyocytes display structural and functional properties of early-stage cardiomyocytes that couple electrically with host cardiomyocytes when transplanted into normal myocardium (10,23). In electrophysiologic studies of ESC-cardiomyocytes exhibit diverse electrophysiologic signatures that include cells with distinct nodal/pacemaker-, atrial, and ventricular-like action potential properties (25). In theory, infinite numbers of cardiomyocytes could be obtained from human ES cell clones. In animal models of experimental myocardial infarction and nonischemic cardiomyopathy, embryonic stem cell transplant has resulted in a remarkable improvement in cardiac function and structure, and the cells appear to be electrically integrated (7,24). At least at early stages of in vitro maturation, ESC-cardiomtocytes exhibit electrophysiologic characteristics of primitive myocardium, including a comparatively low action potential upstroke velocity and a depolarized maximum diastolic potential, but these parameters appear to transition toward somewhat more mature values with increasing duration in culture (25). Voltage-clamp studies indicate that several cardiac-specific

currents are present in these cells, including fast sodium current, L-type calcium current, pacemaker currents, as well as transient outward and inward rectifier potassium currents (14,25).

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CELULELE STEM SI REGENERAREA CARDIACA

REZUMAT

Terapiile celulare au fost dezvoltate ca o modalitate atractiva de a repara miocardul afectat de diverse injurii. O mare varietate de populatii de celule stem si celule progenitoare ar putea fi utilizate pentru repararea cardiaca. Fiecare dintre aceste tipuri celulare prezinta avantaje, limitari si aspecte legate de aplicarea practica in anumite circumstante clinice. Aceste celule prezinta diferente semnificative in ceea ce priveste locul de origine, anatomia si functionalitatea, fiind caracterizate prin prezenta markerilor de suprafata, a factorilor de transcriptie si a expresiei proteice. Un alt aspect care le diferentiaza este capacitatea acestora de a forma unul sau mai multe tipuri celulare diferentiate.

Cuvinte cheie: celule stem mezenchimale, celule progenitoare endoteliale, celule stem hematopoetice, celule stem embrionare

APELIN INVOLVEMENT ON ANGIOTENSIN II – INDUCED VENOCONSTRICTION IN OBESE RATS

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ABSTRACT

Apelin (AP), the endogenous ligand of the recently de-orphanized APJ receptor is a relatively newly identified adipokine. Despite this high level of expression of both AP and APJ in the vascular wall, the vasomotor effects of AP are still under investigation. This study was undertaken to evaluate comparatively the *in vitro* effects of apelin on angiotensin II (Ang II) – induced constriction of various veins from normal *vs*. obese rats. Rings of the inferior vena cava (IVC), portal vein (PtV), femoral vein (FV), and pulmonary vein (PV) from male Sprague Dawley rats (control rats), obese prone (OP) rats and obese resistant (OR) rats were mounted between wires and isometric contraction induced by Ang II (1μM) was measured. These results showed that reactivity for Ang II was increased in IVC and FV in OR. The AP13 treatment decreased the Ang II- induced contractions by more than 30% on IVC and PtV from OR. On OP, the F13A treatment increased the contraction induced by Ang II on all studied veins. These results suggested that AP could play protective roles by antagonizing Ang II – induced venoconstriction.

Key words: apelin, angiotensin II, veins, obesity, rats

INTRODUCTION

The last decades has brought many data about white adipose tissue capacity to secrete hormones, named adipokines, which could be involved on the regulation of vascular reactivity and the modulation of local inflammatory responses (1). Taking into account the published studies about the obesity roles in the development of pulmonary diseases we studied the apelin implications on Ang II contractile effects on pulmonary veins from male Sprague Dawley rats (control rats), obese prone (OP) rats and obese resistant (OR) rats.

We have recently shown that apelin (AP), the endogenous ligand of the recently de-orphanized APJ receptor and a newly identified adipokine, could have vasodilatatory and NO-dependent effects (2). Even more, apelin modulated vasoconstrictor tone in rat pulmonary vessels (3) and reduced the lipopolysaccharide-increased pulmonary permeability in rats (4).

This study aims to investigate the role of apelin/APJ receptors system in agonist-mediated pulmonary venoconstriction in normal and obese rats, in order to accomplish a greater understanding of the involvement of adipokine in the regulation of pulmonary vascular tone.

MATERIAL AND METHODS

The experiments were conducted in Sprague Dawley rats (control rats), the OP-CD (Obese Prone) and the OR-CD (Obese Resistant) that were obtained from Charles River Laboratories (Wilmington, MA, USA). The animals were kept under conventional laboratory conditions of temperature, humidity and light, and allowed free access to water and standard rat chow (for control rats) or a high fat diet (D12266B, 32.5% fat, Research Diets, NJ, USA). Rats were given daily intraperitoneal injections of AP13 or F13A (100 nmol/kg) for 2 weeks (5). After finishing the protocol, the inferior vena cava (IVC), portal vein (PtV), femoral vein (FV), and pulmonary vein (PV) veins were quickly removed, cleaned and cut into 1-2 mm wide rings as previous described (6,7). Individual rings were then mounted in a MYO-01 MYOGRAPH SYSTEM (Experimetria LTD., Budapest, Hungary) and changes in vessel tension were recorded and analyzed by ISOSYS data acquisition system (Experimetria LTD., Budapest, Hungary). The tissue organ bath contained the Krebs–Henseleit solution containing (mM): NaCl 118, KCl 4.8, CaCl2 2.5, MgSO4 1.6, KH2PO4 1.2, NaHCO3 25, glucose 5.5. The Krebs-Henseleit buffer was maintained at 37°C, and bubbled continuously with a mixture of 95% O2 and 5% CO2 (pH=7.2–7.4). A resting tension of 0.5g for JV, FV and PtV, and 0.2g for PV was applied to each ring and then allowed to equilibrate for 45-60 minutes before starting the experiment. The bathing medium was renewed every 15 minutes. After the equilibration period, vessel rings were initially stimulated twice with 40 mM KCl as a standard stimulus. The functional integrity of the endothelium was assessed by testing the degree of relaxation produced by adding 10 μM acetylcholine (ACh) to KCl pre-contracted rings. The rings that produced less than 70 % relaxation in response to ACh were discarded. After re-equilibration, the rings were stimulated with the Ang II (1 μM) and the peak contractile responses were measured. Results are expressed as percentage of control contraction induced by 40 mM KCl (mean ± S.E.M, n=6). The statistical significance was tested using one-way

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analysis of variance (ANOVA), completed by the Bonferroni method (SigmaStat software, Jandel Corporation). p<0.05 was considered statistically significant.

Ang II (rat), KCl, ACh, were all obtained from Sigma-Aldrich Inc. (Germany). Apelin and the antagonist Apelin-13 (Ala13, F13A) were purchased from Phoenix Europe GmbH (Germany). All the other compounds used were of analytical grade.

RESULTS

The F13A treatment amplified the Ang II – induced contractions on IVC from control rats (88.78±5.71 vs. 62.97±7.91) and OP rats (96.18±7.16 vs. 68.72±6.02). On OR rats Ang II contractille effects were higher (90.84±7.25 vs. 62.97±7.91) as compared with control rats. IVC from AP13 treated OR rats had a lower contractile response to Ang II as compared with untreated OR rats (48.51±10.34 vs. 90.84±7.25).

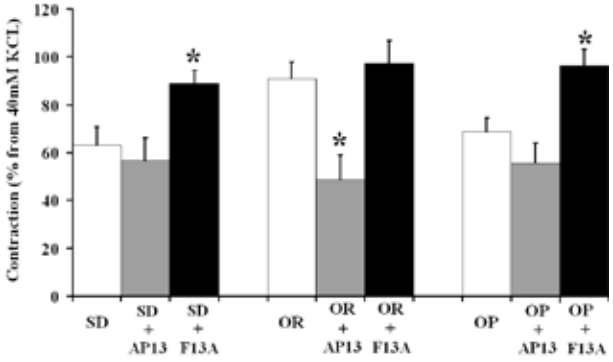

Fig. 1. Contractions induced by Ang II on IVC from control rats (SD), obese resistant (OR) rats and obese prone (OP) rats untreated or treated with AP13 (SD+AP13, OR+AP13, OP+AP13) or F13A (SD+F13A, OR+F13A, OP+F13A). ***:** p<0.05 as compared with SD from the same protocol.

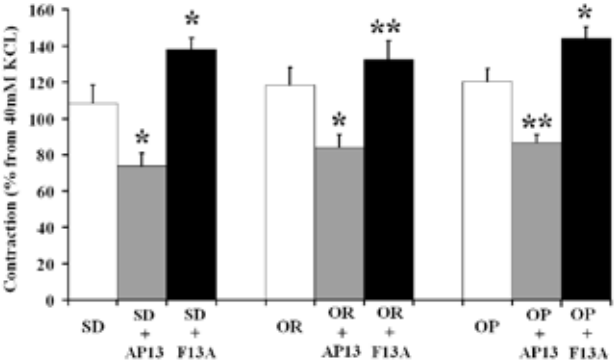

Fig. 2. Contractions induced by Ang II on PtV from control rats (SD), obese resistant (OR) rats and obese prone (OP) rats untreated or treated with AP13 (SD+AP13, OR+AP13, OP+AP13) or F13A (SD+F13A, OR+F13A, OP+F13A). ***:** p<0.05 and ******: p<0.01 as compared with SD from the same protocol.

Both the AP13 treatment and F13A treatment significantly decreased (by an average of 30%) and increased (by an average of 20%), respectively, the Ang II – induced contractions on portal vein from SD, OR and OP rats.

On FV (Figure 3), there are significant differences between Ang II – induced contractions on OR *vs*. SD rats (45.42±3.61 vs. 21.11±2.18) and on OP *vs*. OR rats (20.28±3.14 vs. 45.42±3.61). The F13A treatment increased the Ang II effects on OP rats (41.21±5.11 vs. 20.28±3.14).

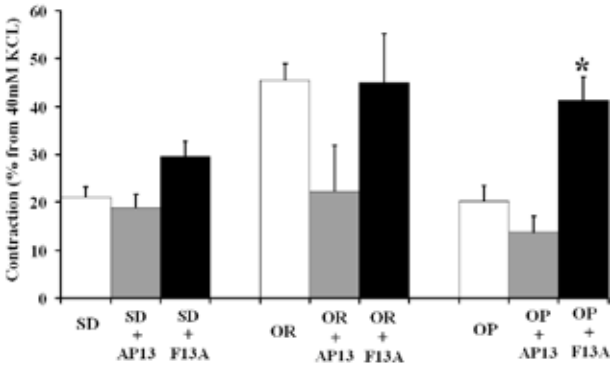

Fig. 3. Contractions induced by Ang II on FV from control rats (SD), obese resistant (OR) rats and obese prone (OP) rats untreated or treated with AP13 (SD+AP13, OR+AP13, OP+AP13) or F13A (SD+F13A, OR+F13A, OP+F13A). ***:** p<0.05 as compared with SD from the same protocol.

Neither AP13 nor F13A modified the Ang II – induced contractions on PV (Figure 4) of SD rats. On the contrary, on OR rats the AP13 treatment and F13A treatment significantly decreased (by more than 50%) and increased (by than 30%), respectively. On OP rats only the F13A treatment significantly modified the Ang II – induced contractions (28.55±2.41 vs. 20.90±1.38). On the other hand, even if there was no difference between Ang II – induced contractions on OP and OR rats, after the AP13 treatment, the responses of PV to Ang II was significantly lower on OR rats as compared with OP rats (10.50±1.24 vs. 19.82±1.79).

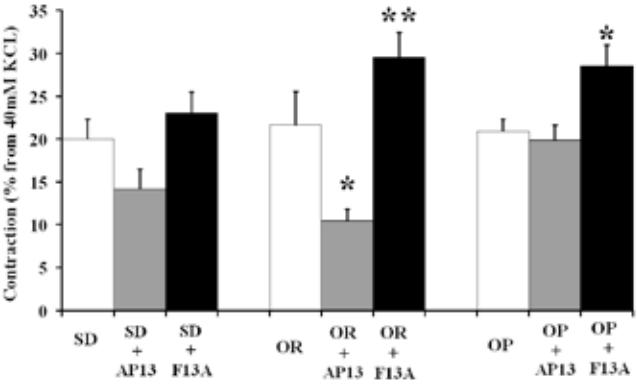

Fig. 4. Contractions induced by Ang II on PV from control rats (SD), obese resistant (OR) rats and obese prone (OP) rats untreated or treated with AP13 (SD+AP13, OR+AP13, OP+AP13) or F13A (SD+F13A, OR+F13A, OP+F13A). ***:** p<0.05 and ******: p<0.01 as compared with SD from the same protocol.

DISCUSSIONS

Apelin is the endogenous ligand of recently de-orphanised APJ receptors. In rat tissues, the APJ receptor and apelin mRNAs were found in the lung, heart, skeletal muscle, kidney, brain and liver (8). Even more, *in situ* hybridisation histochemistry studies revealed intense APJ receptor gene expression in the parenchyma of the lung (9). In rats, the highest expression of APJ mRNA was detected in the lung, suggesting that APJ and its ligand play an important role in the pulmonary system (10). Until now there are no references about

functional importance of AP – APJ pulmonary system. Considering our data about NO-dependent inhibitory effects of AP13 on Ang II-induced venoconstriction, we previous showed that AP13 could stimulate NO synthesis in both airways and pulmonary vessels (11). More recently, apelin has been described as an adipocyte-secreted factor (adipokine), markedly up-regulated in obesity. By acting as circulating hormone or paracrine factor, adipokines are involved in physiological regulations (fat depot development, energy storage, metabolism or eating behavior) or in the promotion of obesity-associated disorders as vascular dysfunctions (12).

Taking into account (i) the increased of AP level in obesity (12) (ii) the functional importance of tissue synthesized AP (3) and (iii) the impact of veins dysfunction on vascular beds circulation (13,14) we comparatively assessed the isolated veins reactivity to Ang II on control rats, OR rats and OP rats untreated or treated with either AP13 or F13A (the blocker of APJ receptors).

The Ang II – induced contractions were significantly increased on OP and OR rats, as compared with SD rats, only for FV (Figure 3). Either APJ receptors stimulating or APJ receptors blocking prevent differences between OP/OR rats and SD rats, emphasizing importance of AP/APJ receptors system in modulation of obesity – induced modulation of FV reactivity. Administration of AP13 decreased Ang II induced contraction of FV and PV (on OR rats) and PtV (on all experimental models). Blocking of APJ receptors by ip administration of F13A increased the venous reactivity for all studied veins but only on OP rats. On control (SD) rats the F13A effects were significantly only for IVC (Figure 1) and PtV (Figure 2). On OR rats the F13A effects were significantly only for PtV (Figure 2) and PV (Figure 4). These data suggested that on OP rats were the AP levels are increased (12); the AP/APJ receptors system could have a vasorelaxant role. For PtV, the AP13 or F13A effects are significantly on all studied experimental models sustaining the importance of the AP/APJ receptors system in regulation of portal circulation.

Taking into account the well known contribution of obesity in the progression of various venous diseases (15, 16) our data could suggest the involvement of apelin/APJ receptor system in dysregulation of venous tone and reactivity as a new component of obesity related induced vascular diseases.

CONCLUSION

These results suggested that AP/APJ system could play protective roles by antagonizing Ang II – induced venoconstriction mainly on obese rats. The functional importance of AP/APJ system on venous tone regulation is determined by vascular beds studied.

Acknowledgements

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IMPLICAREA APELINULUI ÎN VENOCONSTRICȚIA INDUSĂ DE ANGIOTENSINĂ II LA ŞOBOLANII OBEZI

REZUMAT

Apelinul (AP), ligand endogen al receptorilor APJ recent de-orfanizaţi este o adipokină nou identificată. Cu toate că s-a evidenţiat la nivelul peretelui vascular o expresie crescută a apelinului şi a receptorului său, APJ, efectele vasomotorii ale apelinului nu s-au descifrat în totaltate. Acest studiu a fost efectuat pentru a evalua comparative efectele *in vitro* ale AP asupra veoconstricţiei indusă de angiotensina (Ang II) îndiferite teritorii venoase de la şobolanii normali *vs*. obezi. Inele de venă cavă inferioară (VCI), venă portă (VPt), venă femurală (VF) şi venă pulmonară (VP) de la şobolani Sprague Dawley (lot de control), şobolani predispuşi la obezitate (OP), şobolani rezistenţi la obezitate (OR) au fost montate în miograf şi a fost măsurată contracţia izometrică indusă de Ang II. Rezultate obţinute au arătat că efectele venomotorii ale Ang II au fost mai crescute la nivelul VCI şi VF la OR. Tratamentul cu AP13 a scăzut contracţia indusă de Ang II cu mai mult de 30% la nivelul VCI şi PtV. La OP, tratamentul cu F13A a crescut contracţia indusă de Ang II în toate inelele venoase studiate. Aceste rezultate sugerează că AP poate avea un roluri protective prin antagonizarea venoconstricţiei indusă de Ang II.

Cuvinte cheie: apelin, angiotensina II, vene, obezitate, şobolani

CONTRIBUTION OF INTRAPULMONARY RENIN ANGIOTENSIN SYSTEM ON MODULATION OF RAT PULMONARY ARTERIES REACTIVITY

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ABSTRACT

Published data sustain the involvement of urotensin II (U_II) in the pathogenesis of pulmonary vascular diseases. Taking into consideration the importance of renin angiotensin system (RAS) into regulation of pulmonary vessels reactivity we investigated if the modulation of intrapulmonary RAS could modify the vasomotor effects U_II on rat pulmonary artery (RPA) rings from ovalbumin sensitized (OSR) or pulmonary hypertensive (PHR) rats. U_II induced contractions were recorded and data obtained from OSR and PHR were compared with those from normal rats (NR). In order to modulate intrapulmonary RAS, the losartan, chymostatin and amastatin were administered by tracheal instillation. The U_II – induced contractions are higher on RPA from OSR (with at least a fifth) and PHR (with more than 30%) as compared with NR. The blocking of intrapulmonary RAS by tracheal administration of losartan decreased the differences between OSR or PHR and NR. Reducing of angiotensin degradation significantly amplified U_II contractile effects on both experimental models. These data sustain the implication of intrapulmonary RAS on increasing reactivity of pulmonary arteries during some pathological situations and suggest possible positive effects of intrapulmonary administrated angiotensin II type 1 receptor antagonists on pulmonary vessels diseases.

Keywords: urotensin II, angiotensin, pulmonary artery, asthma, pulmonary hypertension, rats

INTRODUCTION

Urotensin-II (U_II), a peptide isolated from fish urophysis, more than four decades ago, is the endogenous ligand of the mammalian recently deorphanized receptor GPR14. The initial studies proclaimed the U_II as the "new endothelin" (1).

U_II has potent vasodilator and vasoconstrictor actions and is synthesized in the pulmonary endothelium (2). Plasma and lung tissue homogenate levels of U_II (1) and expression of U_II by pulmonary arterial endothelial and smooth muscle cells (3) are elevated in different models of pulmonary hypertension. U_II can stimulate proliferation of vascular smooth muscle cells and vascular remodeling processes that, in conjunction with enhanced vasoconstriction, are involved in the pathogenesis of pulmonary vascular diseases (4).

There has been accumulating evidence which suggests the interaction among the renin angiotensin system (RAS) and U_II could have important functional implications (5, 6). On isolated rat aortic rings with intact adventitia and endothelium the vasoconstrictor effects of U_II and angiotensin (Ang) II have synergistic vasoconstrictor effects (5, 6). On the oher hand, Ang II mediates U_II increased cell proliferation and intracellular reactive oxygen species levels in rat cardiac fibroblasts (8).

Based on the well known roles of the RAS on diseased pulmonary vessels (9, 10) and the already discussed collaboration between U_II and RAS we investigated if and to what degree the blockade of RAS could modulate the vasomotor effects U_II on pulmonary artery rings from OVA sensitized rats (OSR) and pulmonary hypertensive rats (PHR). To modulate the intrapulmonary RAS losartan (LOS, an AT1 receptor blocker), chymostatin (CST, a chimase inhibitor) and amastatin (AMA, an aminopeptidase inhibitor) were given by tracheal instillation.

MATERIALS AND METHODS

The experiments were conducted in age-matched male Wistar rats with body weights of 200-250 g housed under standard laboratory conditions, with free access to standard rodent chow and tap water. Three groups of animals were used: (1) untreated control rats (NR, n=18), (2) OVA sensitized rats (OSR, n=18), (3) rats with monocrotaline – induced pulmonary hypertension (PHR, n=18). This study was approved by the Ethics Committee of the University of Medicine and Pharmacy "Grigore T. Popa" Iasi and it was performed according to the Helsinki convention for the use and care of animals, and to the European Communities Council Directive 609 of 24 November 1986.

Sensitization: The rats were sensitised against OVA by s.c. and i.p. injection of 0.2 mL physiological saline, containing 100 mg OVA and 8 mg aluminium hydroxide. The protocol was repeated 2 weeks later (8). *In vitro challenge*: After 7 days, before starting administering of studied substances, vascular rings were pre-treated with OVA (100 mg/mL) (11).

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Monocrotaline(MCT) – induced pulmonary hypertension. To induce pulmonary hypertension, the rats from PHR group received a subcutaneous injection of MCT (60 mg/kg body wt) as previous described (12).

Modulation of intrapulmonary RAS: In the last week of experimental protocol (third week for OSR or the forth week for PHR or in the week before euthanasia for NR) the LOS (0.1mM, a blocker of angiotensin II type 1 (AT1) receptors), CST (0.1mM) or AMA (0.1mM) were administered by tracheal instillation (50microL/day, once every two days), as previous described (13).

Wire myography. Rats were decapitated and exsanguinated. The first branches of rat pulmonary arteries (RPA) were rapidly removed, cleaned and cut into rings, 1-2 mm wide. The endothelium was removed mechanically by gently rubbing the luminal surface with a tungsten wire. Individual rings were then mounted between tungsten wires in a MYO-01 MYOGRAPH SYSTEM (Experimetria LTD., Budapest, Hungary) and changes in vessel tension were recorded and analyzed by ISOSYS data acquisition system (Experimetria LTD., Budapest, Hungary). The tissue organ bath contained the Krebs–Henseleit solution containing (mM): NaCl 118, KCl 4.8, CaCl2 2.5, MgSO4 1.6, KH2PO4 1.2, NaHCO3 25, glucose 5.5. The Krebs-Henseleit buffer was maintained at 37°C, and bubbled continuously with a mixture of 95% O2 and 5% CO2 (pH=7.2–7.4). A resting tension of 0.7 g for RPA was applied to each ring and then allowed to equilibrate for 90 minutes before the start of the experiment. The bathing medium was renewed from 15 to 15 minutes. After the equilibration period, vessel rings were stimulated twice with 40 mM KCl as a standard stimulus. Endothelium absence was certified by absence of 1microM acetylcholine (ACh) – induced relaxation on KCL precontracted rings. After re-equilibration (60 min.) the dose response curves of U_II – induced contractions were constructed.

Expression of results and statistical analysis: The results are expressed as the percentage of the control contraction (KCl, 40 mM; mean ± S.E.M.). Statistical significance was tested using one-way analysis of variance (ANOVA), completed with Bonferroni method (SigmaStat software, Jandel Corporation). $p<0.05$ was considered statistically significant.

KCl, U_II, LOS, CST, AMA, OVA, aluminium hydroxide and MCT were all obtained from Sigma (Sigma-Aldrich Inc., St. Louis MO). All the other compounds used were of analytical grade.

RESULTS

U_II cumulative doses (0.1 nM – 1 μM) induced dose – dependent contractile effects on RPA rings (Emax = 83.86±4.70%; -logEC50 = 8.52±0.03) from NR (Figure 1). The U_II – induced contractions were significantly higher on RPA rings from both OSR (Emax = 103.59±1.26%; -logEC50 = 8.51±0.06) and PHR (Emax = 110.00±4.86%; -logEC50 = 8.86±0.06). Even more, the contractile responses of RPA from PHR were significantly higher than from OSR for U_II doses between 5nM and 50nM: 15.58±0.77 vs. 9.82±0.94 ($p<0.001$), 33.44± 1.41 vs. 26.13±2.21 ($p<0.05$), 67.17±4.60 vs. 52.21±3.32 ($p<0.01$), 95.26±4.09 vs. 78.27±3.52 ($p<0.01$), 106.67±3.80 vs. 91.41±3.13 ($p<0.01$).

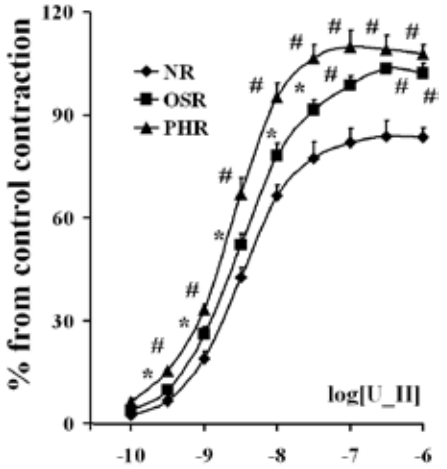

Fig. 1. Contractile effects of urotensin II (U_II, cumulative doses 0.1nM - 1 μM) on rat pulmonary arteries rings from normal rats (NR), ovalbumin sensitized rats (OSR) and rats with pulmonary hypertension (PHR). Data are presented as percentage from control contraction with 40 mM KCl. *: $p<0.05$, ^: $p<0.01$, #: $p<0.005$ as compared with NR.

The losartan treatment did not modify the U_II - induced dose – dependent contractions (Emax = 81.64±3.69 %; -logEC50: 8.48±0.01) on NR (figure 2). LOS treatment reduced the contractile effects on OSR (Emax = 96.68±2.68%; -logEC50:8.34±0.01) and PHR (Emax = 101.16±1.21%; -logEC50:8.30±0.24). Even if on untreated rats the differences between OSR and PHR versus NR were significantly for almost all doses, after LOS treatment only for 50nM, 0.1microM, 0.5microM, 1 microM the contractille responses are higher on OSR (82.37±2.67, $p<0.01$; 89,39±3,20, $p<0.05$; 96,68±2,68, $p<0.01$; 94,96±0,85, $p<0.001$) and PHR (84.80±5.98, $p<0.05$; 94.94±4.51, $p<0.005$; 101.16±1.21, $p<0.005$; 99.61±1.67, $p<0.001$) as compared with NR (70.11±3.12; 71.89±4.97; 81.64±3.69; 81.40±1.89). Another interesting finding is the decreasing of U_II efficacy on LOS – treated OSR (4.59nM *vs*. 3.09nM) and LOS – treated PHR (4.96nM *vs*. 1.38nM) as compared with untreated OSR and PHR.

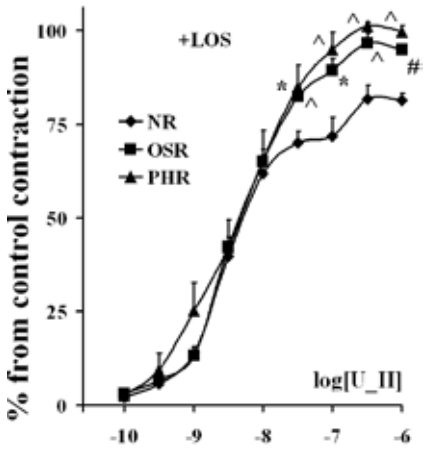

Fig. 2. Losartan (LOS) treatment effects on contractions induced by urotensin II (U_II, cumulative doses 0.1nM - 1 μM) on rat pulmonary arteries rings from normal rats (NR), ovalbumin sensitized rats (OSR) and rats with pulmonary hypertension (PHR). Data are presented as percentage from control contraction with 40 mM KCl. *: $p<0.05$, ^: $p<0.01$, as compared with NR.

The inhibition of chymostatin – sensitive Ang II formation did not significantly modify the U_II – induced contractions on RPA from NR (Emax: 83.81±3.13; -logEC50: 8.50±0.09), OSR (Emax: 98.03±2.85; -logEC50: 8.37±0.07) and PHR (Emax: 102.69±3.97; -logEC50: 8.64±0.11) as compared with untreated rats. On the other hand, the treatment with CST lowered the differences between contractile effects of U_II on OSR versus NR.

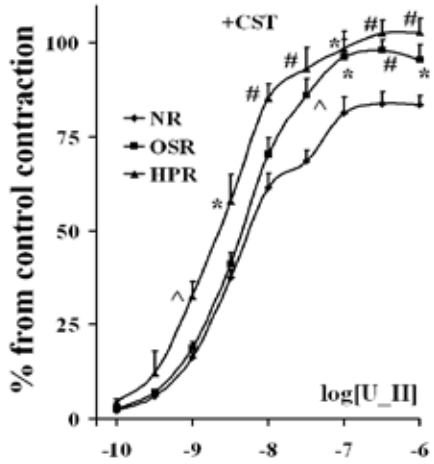

Fig. 3. Chymostatin (CST) treatment effects on contractions induced by urotensin II (U_II, cumulative doses 0.1nM - 1 μM) on rat pulmonary arteries rings from normal rats (NR), ovalbumin sensitized rats (OSR) and rats with pulmonary hypertension (PHR). Data are presented as percentage from control contraction with 40 mM KCl. *: p<0.05, ^: p<0.01, as compared with NR.

Decreasing of pulmonary Ang II degradation by amastatin tracheal instillation increased the U_II contractile effects in all rings. Even on NR after the AMA treatment, the U_II – induced contractions (Emax: 98.46±3.72%; -logEC50: 8.52±0.06) are significantly elevated for doses higher than 10nM. There are no more significant differences between OSR (Emax: 121.94±2.03%; -logEC50: 8.81±0.18) and PHR (Emax: 124.35±4.48%; -logEC50: 8.96±0.17) after AMA treatment. As expected, the EC50 for U_II is reduced too, 1.52nM on OSR and 1.09nM on PHR.

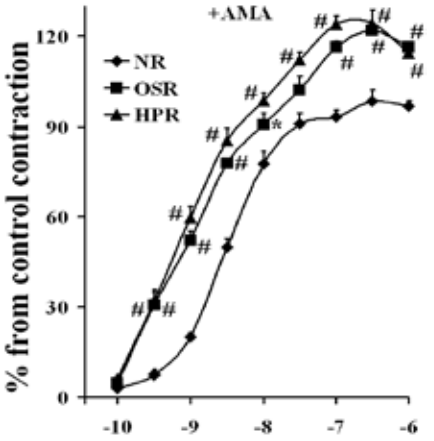

Fig. 4. Amastatin (AMA) treatment effects on contractions induced by urotensin II (U_II, cumulative doses 0.1nM - 1 μM) on rat pulmonary arteries rings from normal rats (NR), ovalbumin sensitized rats (OSR) and rats with pulmonary hypertension (PHR). Data are presented as percentage from control contraction with 40 mM KCl. *: p<0.05, ^: p<0.01, as compared with NR.

DISCUSSIONS

Urotensin-II (U_II) has been considered as the most potent mammalian vasoconstrictor identified so far (3, 4). But, the vasoconstrictor activity of U_II was dependent upon the anatomical origin of the vessel studied and the species from which it was isolated (14). U_II was a potent vasoconstrictor of rat main pulmonary arteries; the U_II contractile effects were increased in the absence of the endothelium or in presence of N^G-nitro-L-arginine methyl ester (L-NAME) (15, 16). The U_II – induced contractions were powerful on rat main and small pulmonary arteries from chronic hypoxic rats as compared with normal rats (16). On the other hand, published results about the U_II effects on human pulmonary artery are unclear. U_II does not induce vasoconstriction of isolated human pulmonary arterial or in lung preparations (17), but in human small pulmonary arteries U_II was a potent vasodilator (18).

Taking into account the endothelium dependence of U_II effects and different degree of endothelial disease on OSR *versus* PHR we used on this paper only data from RPA without endothelium. Our results showed that in absence of endothelium, U_II could induce powerful contractions on OSR and PHT than in NR (fig. 1). As compared with published data (15, 16), our results clearly present the dose dependent vasoconstrictory activity of U_II.

Both experimental models used by us, the OVA sensitization and MCT – induced PHT, are producing approximating the same effects (but different degree) on pulmonary arteries: increase reactivity to vasoconstrictor agonists and initiate the vascular inflammation (followed by endothelial disease and vascular remodeling) (7,19,20).

As we supposed based on published data, the U_II contractile effects on RPA were modified by the modulation of intrapulmonary RAS. The LOS, CST and AMA were delivered by tracheal instillation in order to be active mainly at pulmonary level (21).

Blocking AT1 receptors decreased the U_II contractile effects on OSR and PHR. In experimental models used in this study, OSR and PHR, both the efficacy and potency of U_II are lower (figure 1). These results confirm the hypothesis that, during some pathological circumstances, the intrapulmonary RAS could sustain the U_II reactivity of pulmonary arteries. These results are agreed with published data about the benefic effects of AT1 blockade on pulmonary arterial disease (22, 23).

Taking into account our previous results about chimase –dependent Ang II formation on vascular walls of pulmonary arteries from OSR we delivered intratracheally the CST. The only modification that we observed on CST – treated rats as compare with untreated rats was the increase of EC50 on OSR. So, the existence of multiple ways to synthesize Ang II (24) prevents the CST treatment effects on the U_II contractile effects.

Even if we can not significantly modify the U_II – induced contractions by decreasing Ang II formation, the inbition of aminopeptidases and subsequently decrease of Ang II degradation had significantly amplificatory effects on U_II – induced contractions. On this case, we obtain an increase of Emax to U_II even on

RPA from NR (the EC50 did not modify). On RPA from OSR and PHR, after CST treatment, both the Emax and EC50 (with 50% for OSR) were increased.

CONCLUSION

The isolated pulmonary arteries from PHR and OSR are reacting more powerfully to U_II as compared with vessels obtained from NR. Blocking of intrapulmonary RAS by intratracheal administration of LOS decreased U_II-induced contractions on OSR and PHT and the reducing of Ang II degradation had opposite effects. So, our data sustain the implication of intrapulmonary RAS on increasing reactivity of pulmonary arteries during some pathological situations and suggest possible positive effects of local blocade of AT1 receptors on pulmonary vessels diseases.

Acknowledgements

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PARTICIPAREA SISTEMULUI RENINA ANGIOTENSINA IN MODULAREA REACTIVITATII VASELOR PULMONARE

REZUMAT

Datele publicate susţin participarea urotensin II (U_II) in patologia vaselor pulmonare. Luând în considerare importanţa sistemului renină angiotensină (SRA) în reglarea reactivitaţii vaselor pulmonare am investigat posibila influenţă a modularii activitaţii SRA intrapulmonar asupra efectelor contractile ale U_II la nivelul inelelor de arteră pulmonară (IAP) de la şobolani sensibilizaţi la ovalbumină (SSO) şi şobolani cu hipertensiune pulmonară (SHP). Contracţiile induse de U_II au fost înregistrate şi datele obţinute de la SSO şi SHP au fost comparate cu cele de la şobolani normali (SN). Losartan, chimostatin si amastatin au fost administrate prin intubaţie traheală pentru a modula SRA intrapulmonar. Contracţiile induse de U_II sunt mai mari la nivelul IAP de la SSO şi SHP comparativ cu SN. Blocarea SRA intrapulmonar prin administrarea intratraheală de losartan reduce diferenţele dintre SSO sau SHP şi SN. Scăderea degradării angiotensinelor amplifică semnificativ efectele semnificative ale U_II în ambele modele experimentale. Aceste date susţin implicarea SRA intrapulmonar în creşterea reactivităţii arterelor pulmonare în unele situaţii patologice şi susţin efecte benefice ale administării pulmonare a antagonistilor receptorilor angiotensinei II de tip 1 (AT1) în afecţiuni ale arterelor pulmonare.

Cuvinte cheie: urotensina II, angiotensina, artera pulmonara, astm, hipertensiune pulmonara, sobolani

OXIDATIVE STRESS IN CHRONIC VENOUS HYPERTENSION – INSIGHTS FROM AN ANIMAL MODEL

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ABSTRACT

Objective: Endothelial disorders of the veins are considered to play a role in the pathogenesis of primary varicose veins and oxidative stress is implicated in these disorders. With this aim, we investigated the levels of metabolic markers of oxidative stress after creating partial venous obstruction on an experimental animal model.

Methods: Chronic venous hypertension was produced by partial ligation of the superficial and common femoral vein in Wistar rats (2 groups: group 1- ligation of the superficial femoral vein, n=30 animals,10 animals/lot; group 2 – ligation of the common femoral vein, n=30 animals,10 animals/lot). At specified intervals blood was collected from the retroorbital sinus of the animals. We investigated the levels of malondialdehyde, carbonilated proteins, glutathion, hydrogen donors and thiol radicals in the study groups and compared the results with a control group.

Results: There was statistically significant difference between the study groups and the control group concerning the levels of oxidative stress markers. Plasma levels of malondialdehyde and carbonilated proteins were found higher than in control group at each time point for both study groups.Total antioxidant capacity of the blood was found at decreasing levels compared with controls.

Conclusions: The current model of induced venous hypertension demonstrates early changes in the oxidative status of study animals not only in the local area but also in the sistemic circulation.

Key words: oxidative stress, free oxygen radicals, antioxidants, inflammation, chronic venous insufficiency.

INTRODUCTION

Chronic venous disease (CVD) is common. Its manifestations include varicose veins, skin changes such as dermatitis, hyperpigmentation and lipodermatosclerosis and chronic leg ulcers. The pathogenesis of chronic venous disease is still unclear. The mechanisms regulating varicose vein development and the subsequent skin sequelae seen in chronic venous disease (CVD) have been investigated recently. Despite the diversity of signs and symptoms associated with the disease, it seems likely that they are related to venous hypertension (1). Valvular incompetence is the most important cause of venous hypertension. It seems that initial valvular lesion is followed by reflux in the superficial venous system and dilation of superficial veins (2). Other authors suggest that valvular damage occurs secondary to the initial venous wall lesion generating venous hypertension (3). Recent findings suggest that inflammatory processes are involved in the structural remodeling in venous valves and in the vein wall, leading to valvular incompetence and the development of varicose veins. Vein wall remodeling occurs during the inflammatory reaction produced by hypertension. Venous hypertension alters the laminar shear stress and stimulates the interaction between leucocytes and endothelium which initiates migration, adhesion and activation of white blood cells. There has been shown infiltration of valve leaflets and the venous wall by leukocytes (monocytes and tissue macrophages) in all valve specimens from patients with CVD and in none from controls (4). The leukocyte activation is accompanied by the expression of integrins and by synthesis and release of many inflammatory molecules, including proteolytic enzymes, leukotrienes, prostaglandin, bradykinin, free oxygen radicals, cytokines, and possibly other classes of inflammatory mediators (5). Neutrophils, mast cells, tissue macrophages, endothelial cells and fibroblasts are believed to play an important role in the initiation of the inflammatory response in CVD, being responsible for the production of free oxygen radicals (6,7) Vein wall remodeling is likely to involve the complex interplay of a range of factors, including an altered ratio between metalloproteinases (MMPs) and their tissue inhibitors (TIMPs), and elevated levels of cytokines and growth factors favor an alteration of the extracellular matrix (8). Among the mediators of vessel wall damage free oxygen radicals play an important role, acting through two mechanisms. The first is the oxidation of cell membrane components, followed by endhotelium damage, which eventually leads to increased vascular permeability. The second mechanism mechanism is the chemotaxis and activation of leucocytes and platelets (9). As these mechanisms work together, the inflammatory reaction perpetuates itself, cell damage is amplified and venous stasis is increased (10).

Studies in humans can generally give information only about

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processes operating in established disease, whereas the very early stages may be helpful in understanding pathogenesis and trigger mechanisms. Animal models have a role to play in this regard (11). The aim of this study is to investigate the levels of metabolic markers of oxidative stress after creating partial venous obstruction on an experimental animal model.

MATERIAL AND METHODS

We produced venous hypertension by partial ligation of the common or superficial femoral vein under general anesthesia. The experiments were performed on 2 groups (30 animals/group) of white Wistar rats, males, with a mean weight of 200 grams, from the University of Medicine Animal House. A third group of 10 animals was used as control. Each group was divided in three lots, 10 animals/lot, based on three different time points for collecting blood samples.

Group I – ligation of the superficial femoral vein.

- Lot 1 – 4 weeks
- Lot 2 – 8 weeks
- Lot 3 – 10 weeks

Group II – ligation of the common femoral vein.

- Lot 4 – 1 week
- Lot 5 – 2 weeks
- Lot 6 – 3 weeks

Blood samples were collected from the retroorbital sinus of the rats at different time points as described earlier and conserved by freezing at -80 degrees until examination. Laboratory tests were performed at the Physiology Department in the Laboratory for Oxidative Stress Study. We investigated the levels of malondialdehyde (MDA)(12), carbonilated proteins (PC) (13), glutathion (GSH)(14), hydrogen donors (DH)(15) and thiol radicals (SH)(14) in the study groups and compared the results with a control group.

Operating protocol included the following stages:

- the day of the surgery animals were prevented from food and water
- each animal was given general anesthesia (Ketamine 80mg/kg and Xylasine 8mg/kg Ketamine/ Xylazine = 2/1, intramuscular) with supplemental doses when needed
- an incision was performed on the medial surface of the groin, from the inguinal ligament to the knee and the femoral vessels were exposed. We dissected the superficial, common femoral vein. We placed a suture on the common femoral vein just above the confluence of the superficial and profound femoral veins, we also ligated the superficial epigastric vein in order to prevent back flow; we ligated the superficial femoral vein just below the confluence using monofilament sutures (8-0). Monofilament sutures (4-0) were used as a guide in order to prevent the total ligation of the veins and to ensure a restant blood flow
- the skin incision was closed, and the rats were given intensive postsurgical care
- the rats were housed in a light-cycle controlled flow hood, and maintained with standard pellet diet and water ad libitum.

All the studied rats were in the custody of the Physiology Department Animal House and the experiments were performed respecting international rules concerning experiments on animals.

Statistical methods:

- Values were expressed as percentage calculated from control values
- Mean ± standard deviation as centrality and dispersion indexes
- Non-parametric tests: Mann-Whitney U Test, Kruskall-Wallis Test, Spearman r calculus.

RESULTS

1. Analysis of the oxidative stress markers in the first group, with ligation of the superficial femoral vein (Table I)

MDA expressed higher levels when compared with the control lot (Figure 1) and the values were found significantly higher in the second and third lot (p=0.000, p=0.000)(Table II).

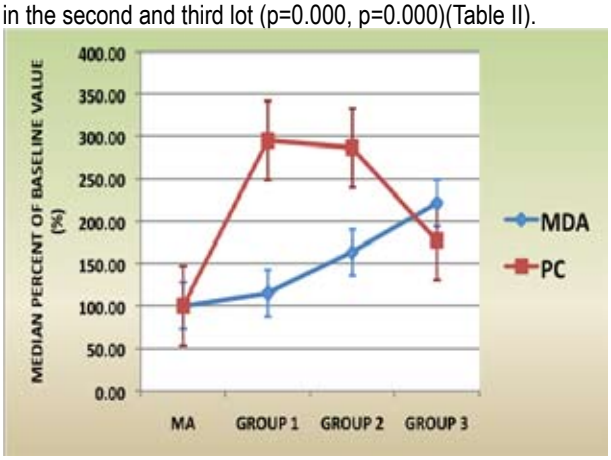

Fig. 1. Protein carbonyl values in groups included in our experiment

Regarding the protein carbonyl we found increased values compared to those from controls and the peak was found in the first lot (Figure 1). All detected values were significantly higher than those found in controls (p=0.000, p=0.000, p=0.000)(Table II).

The dynamical assessment of the values indicated a decrease of PC values, but they still remain significantly higher than controls (p=0.87, p=0.000, p=0.001). MDA values were found rising in dynamics (p=0.001, p=0.000, p=0.01) (Table V).

The antioxidant capacity of the blood presented lower levels then those found in control groups (Figures 2 and 3). All SH detected values were found significantly lower than control values (p=0.004, p=0.003, p=0.002)(Table II). Total antioxidant capacity of the blood, expressed by DH values was significantly lower in all studied lots when compared to controls (p=0.001, p=0.049, p=0.001) (Table II). GSH detected levels were higher than in control group. When we performed the analysis between studied lots we remarked a significant decrease in the antioxidant

capacity in dynamics (Table V)

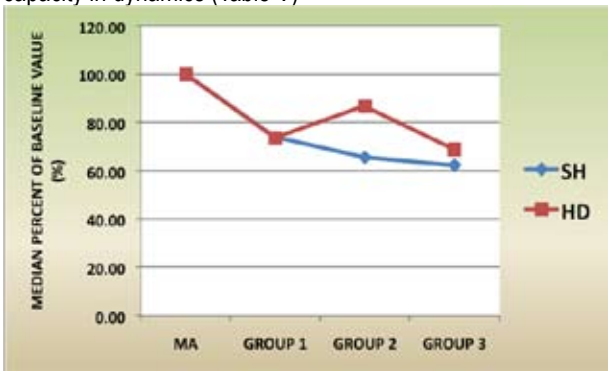

Fig. 2. Antioxidant capacity of the blood – SH and HD

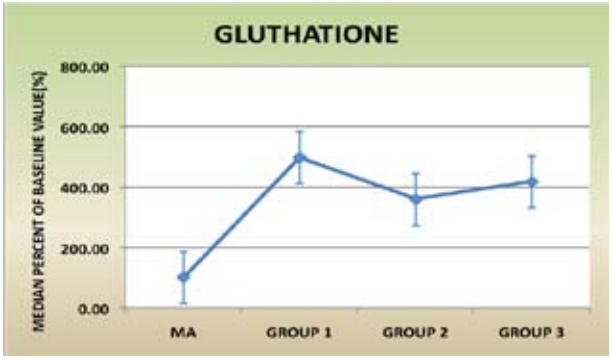

Fig. 3. Gluthatione levels in all groups considered for our experiment

2. Analysis of the oxidative stress markers in the second group, with ligation of the common femoral vein (Table III)

We found significant higher levels of MDA only in the third week after vein ligation (p=0.000) (Table IV), for the first two weeks the values were lower than those from controls (Figure 4). Concerning PC, all values were found higher then controls with a peak in the second week after surgery (p=0.000) (Table IV) (Figure 4).

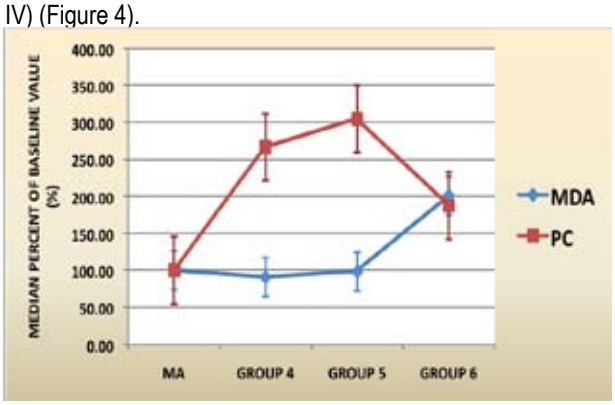

Fig.4. Oxidative stress markers in all groups considered for our experiment – MDA and PC

The analysis between lots confirmed that in dynamics after the initial lower values of MDA, without statistical significance (p=0.7), the ulterior values were significantly rising (p=0.000, p=0.002), with linear increase. Regarding PC levels the second lot expressed a nonsignificant rise compared to the first one (p=0.22), and the second and third lots had significantly lower values than the first one (p=0.005, p=0.001) but their values were still higher than those found in controls (Table VI).

Regarding the antioxidant capacity, SH levels were found lover in all study groups compared to controls (Figure 5), with statistical significant values in the first two weeks after ligation (p=0.002, p=0.002)(Table IV) but there was no significant difference between study lots in dynamics (Table VI).

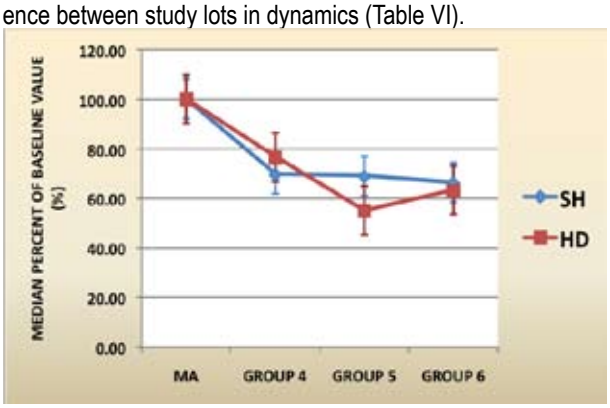

Fig.5. Antioxidant capacity – SH and HD levels

The total antioxidant capacity of the blood, reflected in DH values showed a significant decrease in the first two weeks (p=0.002, p=0.000) and a slightly increase in the third week, but without statistical significance when compared in dynamics with the previous lot (p=0.25) (Table VI).

When compared between lots, SH values expressed a significant lower value in dynamics (p=0.004, p=0.001)(Table VI).

3. Analysis of the oxidative stress markers between the two study groups: ligation of the superficial femoral vein versus ligation of the common femoral vein (Table VII, Table VIII)

We found significantly increased levels of MDA in the second group (p=0.000). PC values were lower in the group with ligation of the common femoral vein compared to the first group (p=0.001).

Antioxidative defense was found significantly lower in the second group, regarding all three studied markers: SH (p=0.01), DH (p=0.016), GSH (p=0.001).)(Table VII, Table VIII).

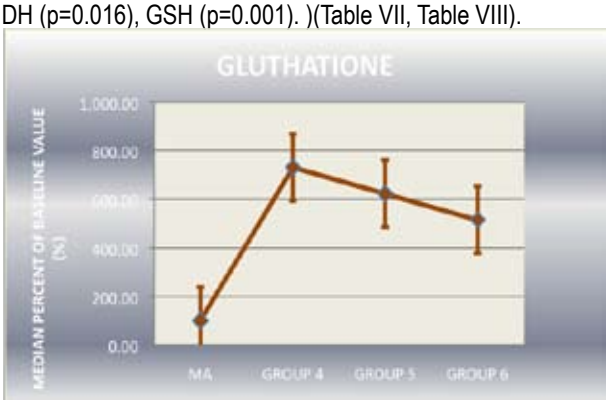

Fig.6. Gluthatione levels in all groups considered in our experiment

Table I. Comparative analysis between lots with ligation of superficial femoral vein and controls

Lot	MDA	PC	SH	DH	GSH
	Mean/SD	*Mean/SD*	*Mean/SD*	*Mean/SD*	*Mean/SD*
Controls	1.25 ± 0.3	0.74 ± 0.06	0.19 ± 0.05	37.17 ± 5.6	2.88 ± 1.12
Lot 1	1.44 ± 0.22	2.18 ± 0.41	0.14 ± 0.01	27.26 ± 3.73	14.33 ± 0.41
Lot 2	2.04 ± 0.23	2.11 ± 0.43	0.12 ± 0.08	32.15 ± 3.85	10.33 ± 1.05
Lot 3	2.78 ± 0.55	1.30 ± 0.11	0.11 ± 0.01	25.55 ± 5.49	12.02 ± 1.8

Table II. Statistical significance

Lot	MDA	PC	SH	DH	GSH
	p	*p*	*p*	*p*	*p*
Controls					
Lot 1	0.15	0.000	0.004	0.001	0.000
Lot 2	0.000	0.000	0.003	0.049	0.000
Lot 3	0.000	0.000	0.002	0.001	0.000

Table III. Comparative analysis between lots with ligation of common femoral vein and controls

Lot	MDA	PC	SH	DH	GSH
	Mean/SD	*Mean/SD*	*Mean/SD*	*Mean/SD*	*Mean/SD*
Controls	1.25 ± 0.3	0.74 ± 0.06	0.19 ± 0.05	37.17 ± 5.6	2.88 ± 1.12
Lot 4	1.14 ± 0.1	1.96 ± 0.44	0.13 ± 0.01	28.44 ± 2.55	20.23 ± 8.19
Lot 5	1.23 ± 0.85	2.25 ± 0.46	0.13 ± 0.02	20.46 ± 6.41	15.14 ± 1.27
Lot 6	2.52 ± 0.42	1.38 ± 0.18	0.12 ± 0.02	23.48 ± 3.89	12.24 ± 1.71

Table IV. Statistical significance

Lot	MDA	PC	SH	DH	GSH
	p	*p*	*p*	*p*	*p*
Controls					
Lot 4	0.88	0.000	0.002	0.002	0.000
Lot 5	0.4	0.000	0.002	0.000	0.000
Lot 6	0.000	0.000	0.01	0.001	0.000

Table V. Dynamical assessment between lots with ligation of superficial femoral vein

Lot	MDA	PC	SH	DH	GSH
	p	*p*	*p*	*p*	*p*
Controls					
Lot 1 vs lot 2	0.001	0.87	0.023	0.013	0.000
Lot 1 vs lot 3	0.000	0.000	0.01	0.40	0.005
Lot 2 vs lot 3	0.01	0.001	0.49	0.007	0.023

Table VI. Dynamical assessment between lots with ligation of common femoral vein

Lot	MDA	PC	SH	DH	GSH
	p	*p*	*p*	*p*	*p*
Controls					
Lot 4 vs lot 5	0.7	0.22	0.65	0.004	0.007
Lot 4 vs lot 6	0.000	0.005	0.13	0.001	0.000
Lot 5 vs lot 6	0.002	0.001	0.36	0.25	0.001

Table VII. Comparative analysis between lots with ligation of superficial femoral vein, common femoral vein and controls

Lot	MDA	PC	SH	DH	GSH
	Mean/SD	*Mean/SD*	*Mean/SD*	*Mean/SD*	*Mean/SD*
Controls	1.25 ± 0.3	0.74 ± 0.06	0.19 ± 0.05	37.17 ± 5.6	2.88 ± 1.12
Lot 1	1.44 ± 0.22	2.18 ± 0.41	0.14 ± 0.01	27.26 ± 3.73	14.33 ± 0.41
Lot 6	2.52 ± 0.42	1.38 ± 0.18	0.12 ± 0.02	23.48 ± 3.89	12.24 ± 1.71

Table VIII. Statistical significance

Lot	MDA	PC	SH	DH	GSH
	p	*p*	*p*	*p*	*p*
Lot 1 vs lot 6	0.000	0.001	0.01	0.016	0.001

DISCUSSIONS

Numerous experimental studies using animals were performed, most of them indicating the levels and activity of leucocytes, MMPs, cytokines and adhesion molecules. Only few studies were concerned about the role of free radicals in the development of local venous damage. All reviewed data revealed the role of inflammatory response in chronic venous disease. Many questions need to be answered, such as: Are the free oxygen radicals responsible for activation the inflammatory cascade, or they are only a consequence of the activation of white blood cells and their interaction with the endothelium? Increased production of free oxygen radicals after venous hypertension and stasis are the result of hypoxia or of inflammation? What is the role of free radical scavengers in the treatment of CVD?

CONCLUSIONS

1. The current model of induced venous hypertension demonstrates early changes in the oxidative status of study animals not only in the local area but also in the sistemic circulation.

2. The increase of the venous pressure that we induced by ligating the superficial femoral vein is followed by increased levels of oxidative stres markers and decreased values of antioxidant markers.

3. The higher increase of the venous pressure in the lover limb that we induced by ligating the common femoral vein determined a more agressive oxidative stress, that appeard in early stages after the surgery, as well as an important decrease of the antioxidative defence capacity of the blood.

4. Comparing the two procedures we performed we can conclud that the more important the obstructed blood vessel is for the physiology of the lower limb circulation, the more important and agressive is the damage of the oxidative/antioxidative balance.

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STRESUL OXIDATIV IN INSUFICIENȚA VENOASĂ CRONICĂ – MODEL EXPERIMENTAL

REZUMAT

Obiectiv: Alterarea endoteliului venos se consideră a juca un rol important în patogeneza bolii varicoase. Stresul oxidativ pare a fi implicat în producerea tulburărilor endoteliale. Pornind de la această premisă am investigat nivelurile markerilor stresului oxidativ după crearea experimentală a unei obstrucţii venoase parţiale pe un model animal.

Metodă: Hipertensiunea venoasă cronică a fost obţinută prin crearea unor ligaturi parţiale pe vena femurala superficială şi comună la şobolani rasa Wistar. Experimentele s-au efectuat pe două grupuri de animale: grupul I - ligatură de venă femurală superficială, n=30 animale, 10 animale/lot; grupul 2 – ligatură de venă femurală comună, n=30 animale, 10 animale/lot. La intervale de timp specificate s-a recoltat sânge din sinusul retroorbital şi s-au determinat nivelurile serice de malondialdehidă, proteine carbonilate, glutation, donori de hidrogen şi grupări tiol. Rezultatele au fost analizate între loturi precum şi faţă de un lot martor.

Rezultate: S-au obţinut diferenţe semnificative statistic între loturile studiate şi lotul martor în ceea ce priveşte nivelurile markerilor de stres oxidativ, cu creşteri semnificative ale malondialdehidei şi proteinelor carbonilate, insoţite de scăderea capacităţii antioxidante.

Concluzii: Hiperpresiunea venoasă creată prin ligaturi parţiale ale venelor mari ale membrelor inferioare de şobolan determina amplificarea stresului oxidativ nu doar la nivel local ci şi în circulaţia generală.

Cuvinte cheie: Stres oxidativ, radicali liberi, insuficienţă venoasă cronică, hiperpresiune venoasă, antioxidanţi

EXTENDED SPECTRUM BETA LACTAMASE PRODUCING *KLEBSIELLA PNEUMONIAE* STRAINS ISOLATED FROM INTENSIVE CARE UNIT

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ABSTRACT

Aims: The aim of our study was to determine the prevalence of ESBL producing germs, especially of *Klebsiella* strains, isolated from patients hospitalised in Intensive Care Unit (ICU), and their associated resistance patterns.
Methods: Identification of germs and extensive antimicrobial tests (by dilution antimicrobial susceptibility tests) were performed by the Vitek2 system (bioMerieux France). For ESBL detection we performed the Vitek ESBL test (AST-GN27 cards), which includes cefotaxime and ceftazidime, alone and in combination with clavulanic acid. **Results:** From 835 pathological samples we isolated 548 microbial strains with nosocomial potential. 285 from these strains were represented by *Enterobacteriaceae*, from which, 48,07% (137 strains) were extended spectrum beta-lactamase (ESBL) producers. The highest percentage was noticed in the case of ESBL producing *Klebsiella pneumoniae*- 78 strains (56,93%).
Conclusions: The routine detection of the beta-lactam resistant phenotypes *Klebsiella pneumoniae* clinical isolates is particularly important because of therapeutical problems related to acquired resistance in this species.
Keywords: *Klebsiella pneumoniae*, infection, resistance phenotypes

INTRODUCTION

The introduction of the third-generation cephalosporins into clinical practice in the early 1980s was heralded as a major breakthrough in the fight against beta-lactamase-mediated bacterial resistance to antibiotics. These cephalosporins had been developed in response to the increased prevalence of beta-lactamases in certain organisms, for example, ampicillin hydrolyzing TEM-1 and SHV-1 beta-lactamases in *Escherichia coli* and *Klebsiella pneumoniae* (1).

Beta-lactamases are most commonly classified according to two general schemes: the Ambler molecular classification scheme and the Bush-Jacoby-Medieros functional classification system (2, 3, 4). The Ambler scheme divides beta-lactamases into four major classes (A to D). The basis of this classification scheme rests upon protein homology (amino-acid similarity), and not phenotypic characteristics. In the Ambler classification scheme, beta-lactamases of classes A, C and D are serine beta-lactamases. In contrast, the class B enzymes are metallo-beta-lactamases. The Bush-Jacoby-Medeiros classification scheme groups beta-lactamases according to functional similarities (substrate and inhibitor profile). There are four main groups and multiple subgroups in this system. This classification scheme is of much more immediate relevance to the physician or microbiologist in a diagnostic laboratory because it considers beta-lactamase inhibitors and beta-lactam substrates that are clinically relevant.

A commonly used working definition is that the ESBLs are beta-lactamases capable of conferring bacterial resistance to the penicillins, first-, second-, and third-generation cephalosporins, and aztreonam (but not the cephamycins or carbapenems) by hydrolysis of these antibiotics, and which are inhibited by beta-lactamase inhibitors such as clavulanic acid (3).

OBJECTIVES

The aim of our study was to determine the prevalence of ESBL producing germs, especially of *Klebsiella* strains, isolated from patients hospitalised in Intensive Care Unit (ICU), and their associated resistance patterns.

METHODS

We collected from ICU (County Hospital, Timisoara, Romania), between 01.03.2009-04.12.2009 a total number of 835 samples (538 bronchoalveolar aspirates, 101 urines, 3 peritoneal fluid, 4 cerebrospinal fluid, 16 blood, 173 wound secretions), from 207 patients with various diagnosis, the most prevalent ones being polytrauma, cerebral stroke, neoplasia, acute abdomen, burns.

Isolation on conventional culture media and identification of germs were performed at the hospital laboratory. Confirmation of identification tests (Vitek2 system-bioMerieux France), as well as extensive antimicrobial tests (by dilution antimicrobial susceptibility tests) were performed at the University

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Microbiology Department's laboratory. For ESBL detection we performed the Vitek ESBL test (AST-GN27 cards), which includes cefotaxime and ceftazidime, alone and in combination with clavulanic acid. A predetermined reduction in the growth of the cefotaxime or ceftazidime wells containing clavulanic acid, compared with the level of growth in the well with the cephalosporin alone, indicates a positive result. Sensitivity and specificity of the method exceed 90% (5).

RESULTS

From 835 samples (bronchoalveolar fluids, wound secretions, urines, etc.) we isolated 548 microbial strains with nosocomial potential (Figure1, Tables I and II).

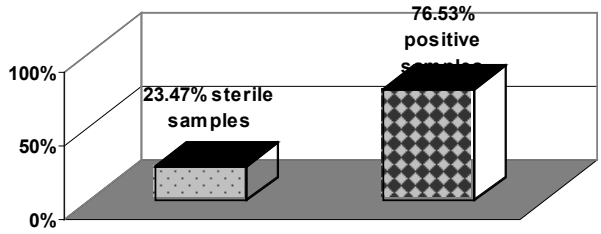

Fig. 1. Positive pathological samples

Table I. *Klebsiella pneumoniae* in positive samples

Pathological samples	Positive samples		*Klebsiella pneumoniae*	
bronchoalveolar aspirates	514	95.53%	117	22.76%
urines	32	31.68%	14	43.75%
wound secretions	84	48.55%	6	7.14%
peritoneal fluid	3	100%	-	-
cerebrospinal fluid	1	25%	-	-
blood	5	31.25%	-	-

Table II. Isolated germs from ICU department

ISOLATED GERMS	Strains		MDR strains	
	No.	%	No.	%
S. aureus	139	25.36	MRSA 61	43.88
E.coli	66	12.04	ESBL 21	31.81
Klebsiella pneumoniae	137	25.00	ESBL 78	56.93
Klebsiella oxytoca	7	1.27	ESBL 3	42.85
Pseudomonas aeruginosa	51	9.30	Carba-penems resistant 22	43.13
Acinetobacter baumannii	75	13.68	Carba-penems resistant 44	58.66
Other *Enterobacteriaceae*	75	13.68	ESBL 35	47.94

285 from these strains were represented by *Enterobacteriaceae*, from which, 48, 07% (137 strains) were extended spectrum beta-lactamase (ESBL) producers. The highest percentage was noticed in the case of ESBL producing *Klebsiella pneumoniae*- 78 strains (56,93%). The majority of these strains were involved in respiratory and urinary tract nosocomial infections and all of them have associated other resistance phenotypes as well (table 3).

Table III. Resistance phenotypes in *Klebsiella pneumoniae* isolates

Klebsiella pneumoniae	137	78 ESBL 56.93%	**ESBL**+ KTGANt+cross resistance Fq + TE^R+SXT^R	19 24.35%
			ESBL+ impermeability cephamycins + KTG+ partially resistant Fq + TE^R	4 5.12%
			ESBL+ KTGANt+cross resistance Fq + TE^R	11 14.11%
			ESBL + KTANt + cross resistance Fq	22 28.21%
			ESBL (CTX-M) + KTGNt + partially resistant Fq +SXT^R	3 3.84%
			ESBL+ KTGANt+cross resistance Fq	14 17.94%
			ESBL+ impermeability cephamycins+ partially resistant Fq + TE^R+SXT^R	3 3.84%
			ESBL+TE^R+SXT^R	1 1.28%
			ESBL+SXT^R	1 1.28%

Legend: ESBL-extended spectrum beta-lactamase, K-resistance to kanamycine, G-resistance to gentamycine, T- resistance to tobramycine, A-resistance to amikacin, Nt-resistance to netilmicin, Fq-fluoroquinolone, SXT-trimethoprim-sulfamethoxazole,TE-tetracycline

DISCUSSIONS

In our study 78 strains (56,93%), from 137 *Klebsiella pneumoniae* strains, were extended spectrum beta-lactamase producing.

There is a considerable geographical difference in the occurrence of ESBLs in the European countries. Within countries, hospital-to-hospital variability in occurrence may also be marked (6). In a SENTRY worldwide surveillance program report, ESBL phenotypes were detected in 45% of *K. pneumoniae* strains from Latin America, 23% from the western Pacific, 23% from Europe, 8% from the United States, and 5% from Canada (7).

The first ESBL was identified in Germany in 1983; since then, over 200 variants of the clavulanic acid-inhibited form of the enzyme have been described worldwide. The most common extended-spectrum phenotypes arise from point mutations in the bla_{TEM}, bla_{SHV}, or bla_{CTX} genes resulting in alterations of the primary amino acid sequence of the enzyme (8). Since these genes are generally found on plasmids, many of the organisms that harbor ESBLs also are resistant to other classes of antibiotics, such as aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol, and sulfonamides (9). Many strains were multiresistant, being sensitive only to imipenem (7).

In this study, all ESBLs have been associated with other

resistance patterns like: resistance to quinolones, trimethoprim-sulphamethoxazole, aminoglycosides or tetracycline. Only 7 strains (8,97%) were cefoxitin resistance and we did not isolate any strain with imipenem resistance.

CONCLUSIONS

1. The routine detection of the beta-lactam resistant phenotypes *Klebsiella pneumoniae* clinical isolates is particularly important because of therapeutical problems related to acquired resistance in this species. It can help clinicians prescribe empiric antibiotic therapy and can help microbiologists monitor trends in antimicrobial resistance, such as the dissemination of ESBLs.

2. Carbapenems remain active against all our ESBL producing *Klebsiella pneumoniae* strains. This information should be considered when prophylactic therapy is recommended in this department.

3. Alteration of antibiotic susceptibility breakpoints may become necessary but need

to be carefully considered in combination with pharmacokinetic, pharmacodynamic and clinical data.

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TULPINI DE *KLEBSIELLA PNEUMONIAE* PRODUCĂTOARE DE BETA LACTAMAZE CU SPECTRU EXTINS IZOLATE DINTR-O SECȚIE DE TERAPIE INTENSIVĂ

REZUMAT

Scop: În studiul nostru am urmărit determinarea prevalenţei tulpinilor producătoare de BLSE, în special a celor din genul *Klebsiella*, izolate de la pacienţi internaţi într-o secţie de Terapie Intensivă.

Metode: Pentru identificarea germenilor şi testarea sensibilităţii la antibiotice am utilizat sistemul automat Vitek2 (bioMerieux France). Detecţia tulpinilor producătoare de BLSE s-a realizat cu ajutorul cardurilor AST GN27 pentru sistemul Vitek 2, care includ testarea simultană la cefotaxim şi ceftazidim asociate sau nu cu acid clavulanic.

Rezultate: Din 835 produse patologice am izolat 548 tulpini microbiene cu potenţial nosocomial. 285 dintre aceste tulpini au fost reprezentate de *Enterobacteriaceae*, din care, 48,07% (137 tulpini) au fost producătoare de beta-lactamaze cu spectru extins (BLSE). Cel mai mare procent de tulpini BLSE a fost înregistrat pentru *Klebsiella pneumoniae*- 78 tulpini (56,93%).

Concluzii: Detectarea de rutină a fenotipurilor de rezistenţă la beta-lactamine pentru tulpinile de *Klebsiella pneumoniae* izolate este importantă datorită problemelor terapeutice create de rezistenţa dobândită pentru această specie.

RISK ASSESSMENT STUDY ON OCCUPATIONAL METHYL-ETHYL KETONE EXPOSURE

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ABSTRACT

The study assesses the presence of methyl-ethyl-ketone in a factory producing polyurethanic foam and estimates the potential health risk associated to its inhalation. Values obtained at the workplace were compared to legal occupational exposure limits (OELs) and to the maximal exposure values not involving health risks (HBEL - health-based exposure limits). The hazard index was calculated (HI). When reported to OELs, a maximal HI of 1.2 is observed in case of 15 minutes exposure and of 1.48 in case of 8 hours exposure. When reported to HBEL, the hazard index is much higher than the cut-off of 1, even when the lowest recorded value is taken into account. Thus, HI values are in the range of 4.47 and 21.35 for records on 15 minutes, whereas for 8 hours exposure HI is between 5 and 17.41. Even though there is no risk for acute effects, the possibility of some chronic effects imperatively requires the identification of factors which favour concentration increase in the occupational environment.

Key words: occupational exposure limits, health-based exposure limits, hazard index

INTRODUCTION

Methyl-ethyl-ketone (MEK) is used as a solvent in the application of protective coatings (varnishes) and adhesives (glues and cements), in magnetic tape production, in smokeless powder manufacture, in the dewaxing of lubricating oil, in vinyl film manufacture, and in food processing. Its use as a component in adhesives used to join PVC pipes is a potential route for entry of the chemical into potable water (1). It is also commonly used in paint removers, cleaning fluids, acrylic coatings, pharmaceutical production, and colorless synthetic resins, and as a printing catalyst and carrier (2). MEK has been detected as a natural component of numerous foods, including milk, nuts, cheese, bread and nectarines (1,9,21). MEK is also found in tobacco smoke and volatile releases from building materials and consumer products (1).

Short-term inhalation exposure (4 hours) to MEK under experimental conditions at or near 200 ppm (590 mg/m^3) does not appear to pose an increased risk of neurologic or irritation symptoms (4,5,6,7).

Several occupational studies examined the effects of chronic exposure to MEK. Freddi et al. reported that MEK exposure was associated with slightly, but not statistically significant, reduced nerve conduction velocities (distal axonopathy) and other symptoms such as: headache, loss of appetite and weight, gastrointestinal upset, dizziness, dermatitis, and muscular hypotrophy, but no clinically recognizable neuropathy (8). The human case reports and some studies provide limited and equivocal evidence that repeated exposure to MEK in the workplace increases the hazard for persistent neurological impairment (13,14). The available occupational studies are limited by inadequate characterization of exposure, multiple solvent exposure, and study design problems.

Although there is some suggestion of increased risk for some cancers (including bone and prostate) and multiple solvent exposures that includes MEK, there is no clear evidence for a relationship between these cancers and MEK exposure alone. According to EPA's (United States Environmental Protection Agency) draft revised cancer guidelines, the hazard descriptor "data are inadequate for an assessment of human carcinogenic potential" is appropriate for MEK (17,18).

The present study assesses the presence of MEK in occupational environments and estimates the potential health risk associated to its inhalation. For this, the targeted objectives were:
- determining MEK concentration at the workplace;
- comparing obtained levels to limits stated by various organisms;
- evaluation of chronic carcinogenetic and non-carcinogenetic risks determined by occupational MEK exposure.

MATERIAL AND METHODS

The study was performed in a polyurethanic foam producing factory, during the period between 2001 and 2005, as a part of an occupational harmful agents exposure assessment programme. The investigated departments were as follows: foaming, foam processing, injection equipments, rigid, casting, confections, valuing, mechanical-energetically workshop, technical quality control department, research department.

Substance detection procedures were performed according to methodological specifications edited by the Ministry of Health (10,11,12,22). MEK was determined by spectrophotometry, using an UV absorption spectrophotometer.

MEK concentrations are presented as point concentrations ($C_{15\ min}$, resulting from collections made within 15 minutes) and weighted mean concentrations ($C_{8\ hours,}$ representing mean

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exposure during an 8 hours working period). Result analysis was made by:

1. Comparing obtained concentrations with

- OELs (loccupational exposure limits) in Romania, according to present legislation (23);

- RfC (inhalation reference concentration). This is defined as the concentration to which human population may be exposed by inhalation, during a lifetime, without a considerable risk regarding non-carcinogenetic effects (19,20) and it is included in IRIS-EPA monographs (Integrated Risk Information System - United States Environmental Protection Agency)

- HBEL; health-based exposure limits (limits of exposures without health risk). HBEL was calculated by the Santa Clara Centre for Occupational Safety and Health (SCCOSH) using the methodology for environmental risk assessment, developed by the United States Environmental Protection Agency (EPA) (16).

2. Calculation of the hazard index (HI), for the assessment of chronic non-carcinogenetic effects, by reporting the concentrations of substances detected at the workplace to limits imposed by the Organisms involved in environmental and workplace protection. Hazard index higher than 1, represents a high health risk for chronic effects.

RESULTS

Chemical laboratory tests performed on samples collected in the factory producing polyurethanic foam revealed the presence of MEK in one of the 10 investigated departments, namely the department of casting. The limits of detected values are presented in Table I.

Table I. MEK concentrations detected in the factory producing polyurethanic foam (mg/m^3)

Substance	No. positive samples	C_{15min}		C_{8hours}	
		Min. value	Max. value	Min. value	Max. value
MEK	36	76	363	85.3	296

For occupational risk assessment determined by the presence of MEK, values were reported to concentrations which certain authorities or legal provisions regard as without risk or posing a minimal risk (Table II).

Table II. Exposure limits stated by various Public Regulatory Organisms in the field (mg/m^3)

Substance	OELs 15min	OELs 8hours	RfC	HBEL noncancer	HBEL cancer
MEK	300	200	5	17	-

Note: OELs – occupational exposure limits (Romania); RfC (inhalation reference concentration) – concentration without chronic non-carcinogenetic effects in the general population; HBEL (health-based exposure limits) – exposure limits without occupational health risk.

Table III presents the hazard index (HI), calculated with reference to various norms, established by different Organisms. When reported to occupational exposure limits OELs, a hazard index slightly over 1 is observed. On the contrary, in case of reporting to HBEL, the hazard index is well over the cut-off of one, even when the lowest recorded concentration value is taken into account. Thus, HI values for records on 15 minutes are between 4.47 and 21.35 and for the 8 hours exposure, it is between 5 and 17.41. Reporting to RfC shows high values over the unitary limit for the hazard index.

Table III. The hazard index (HI) calculated according to exposure limits stated by various Public Regulatory Organisms in the field

Substance	HI_{OELs}		HI_{RfC}		HI_{HBEL}	
	15 min	8 hours	15 min	8 hours	15 min	8 hours
MEK	0.25-1.21	0.42-1.48	15.2-72.6	17.06-59.2	4.47-21.35	5-17.41

Note: OELs – occupational exposure limits (Romania); RfC (inhalation reference concentration) – concentration without chronic non-carcinogenetic effects in the general population; HBEL (health-based exposure limits) – exposure limits without occupational health risk.

DISCUSSION

Even if present scientific evidence do not support with certainty the harmful effects of MEK, the precaution principle and risk assessment are imperative. This position is justified by the percent of samples in our study with concentration values over the occupational exposure limits (OELs) stipulated by Romanian legislation. These are limits of time weighted mean, for a specified period (8 hours or on short term – maximum 15 minutes) of the air concentration of the chemical agent, in the breathing area of the worker (23). Analysing the results obtained by our study, from the perspective of these norms, we found that, even though $OELs_{15min}$ was over the highest limit in 8.33% of the samples, $OELs_{8hours}$ presented values over the limit in 33% of cases, thus revealing the risk of chronic exposure.

Limits of occupational exposure are the highest legally acceptable values for human exposure to a toxic substance. Very many of these values are very close to the maximal acceptable values, with a small safety margin for the risk of long term effects (3). General population exposure to the same air pollutant is much more strictly regulated. The proof is the RfC value which is 40 times lower than $OELs_{8\ hours}$ for MEK.

Santa Clara Centre for Occupational Safety and Health (SCCOSH) considers that exposure of workers to toxic substances must not be higher at the workplace than in any other ordinary place where exposure is possible. For this reason, values corresponding to a very low disease risk in an occupational environment were calculated for a series of substances starting from the IRIS, HEAST and California EPA databases and adjusting RfC (inhalation reference concentration) by changing the exposure time in occupational exposure (8 hours/day, 240 days/year, for a 40 years period) (16). Analysing these data, the high difference between legal provisions and health protection levels may be observed.

Risk assessment is the scientific, systematic characterization of potential adverse effects resulting from human exposures to risk agents or situations. Risk assessment is the basis for regulating quality criteria, for elaborating standards and decision making according to local realities (15).

For characterization of the risk of acute effects the point (momentary) exposure is used, whereas for chronic non-carcinogenetic effects is advisable to take into account the mean exposure during a period of time. Our results show that the hazard index calculated by reporting to HBEL recorded values much higher than the limit of 1, even in cases of the lowest concentrations detected. This aspect suggests a very high risk for chronic non-carcinogenetic diseases. In the case of calculation by reporting to $OELs_{15min}$, a maximal IH value of 1.2 is recorded, with no acute pathology problems. It may be observed that val-

ues for momentary exposure do not differ very much from those determined by medium exposure, fact which may be considered an alarm signal for the exposed staff members.

As for carcinogenetic effects, there is no assessment methodology in the case of MEK, as there are not enough data supporting the carcinogenicity of this substance and thus, URL (Unit Risk – the additional number of cancer cases determined by inhalator exposure to a concentration unit during a lifetime).

CONCLUSIONS

The study identifies a critical point in the technological process regarding MEK exposure. Even though pollution with this substance is limited, identification of factors favouring the increase of its occupational concentration is mandatory.

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STUDIU DE EVALUARE A RISCULUI DETERMINAT DE EXPUNEREA LA METIL-ETIL-CETONĂ ÎN MEDIUL PROFESIONAL

REZUMAT

Studiul evaluează prezenţa metil-etil-cetonei într-o întreprindere de producere a spumelor poliuretanice şi estimează riscul potenţial pentru sănătate asociat cu inhalarea acesteia. S-au comparat valorile obţinute la locul de muncă cu valorile limită de expunere profesională (VLE) prevăzute de legislaţie şi cu valorile limită de expunere fără risc pentru sănătate (HBEL - health-based exposure limits). S-a calculat indicele de hazard (IH). Când raportarea s-a făcut la valorile limită de expunere profesională (VLE), se observă un indice de hazard de maximum 1,2 în cazul expunerii pe 15 min şi 1,48 în cazul expunerii pe 8 ore. În cazul raportării la HBEL, indicele de hazard depăşeşte cu mult pragul de 1, chiar dacă se ia în calcul valoarea minimă a concentraţiei înregistrate. Astfel, valorile IH se situează la înregistrările pe 15 minute între 4,47 şi 21,35 iar pentru expunerea timp de 8 ore, acesta este cuprins între 5 şi 17,41. Deşi nu există riscul unor efecte acute, posibilitatea efectelor cronice impune identificarea factorilor care favorizează creşterea concentraţiei în mediul ocupaţional.

Cuvinte cheie: valori limită de expunere profesională, valorile limită de expunere fără risc pentru sănătate, indice de hazard

MAJOR FACTORS INFLUENCING LIFE QUALITY – RATIONAL NUTRITION AND FITNESS

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ABSTRACT

In the present paper we revealed the synergic link between rational nutrition and fitness, for the prevention, improvement and treatment against obesity. Foods represent one of the most important environmental factors influencing human health. They have the peculiarity of continuous and slow action upon the human body, influencing physical, psychological development and work capacity. Together with rational nutrition, physical activity is a major factor participating in the accomplishment of a balanced lifestyle management, preventing some risk behaviors. Medical statistics show that the number of obese individuals is constantly increasing. Overweight trigger the increased risk for many diseases such as diabetes mellitus, high blood pressure, atherosclerosis and especially ischemic cardiopathy, gallbladder diseases and even some forms of cancer. Fitness is presented as a health related concept connected to keeping and promoting health, with reference to the beneficial effects upon the organism.

Key words: rational nutrition, obesity, fitness

INTRODUCTION

The human body develops and maintains its complex structure by a permanent substance exchange with the environment. From the environment, man receives the oxygen required for burning processes occurring in living cells, as well as *nutritional factors* or *trophins* from which the energy needed for maintaining body functions and activities is obtained, and also reparatory and reconstruction materials used for repairing used tissues or for growth during childhood. Every second, within the human body, millions of cells die and are born.

Aliments represent one of the most important factors of the environment influencing human health. They characteristically act continuously and slowly upon human organisms, influencing physical, psychological development and work capacity. From foods entering the composition of a daily portion, the body only keeps and uses a certain percent, the rest being eliminated. This percent of foods needed for the organism to maintain life and work capacity is designated as nutritive factors or trophins. The richer in nutritive factors the more useful and precious an aliment is for man.

All nutritional factors needed by the organism are now known, as well as the foods containing them and the respective proportions, their chemical composition, and the necessary quantity during various physiological states or environmental conditions. Because no food contains all nutritional factors needed by the organism, the necessity for a complete and varied nutrition, also known as rational nutrition, becomes imperative (1).

For maintaining the energetic balance, physical activity is indispensable, being the most effective way to consume ingested calories. Studies show that physical exercises are especially useful for maintaining a normal body weight, together with an adequate diet.

A beneficial physical activity program may be achieved by intensive walking sessions of 30-60 minutes a day. The movement may also be performed in more rounds of 15-30 minutes. Other forms of aerobic physical exercise may be used, such as gardening, running, bicycle riding, swimming, and fitness.

Sport is recommendable, especially for young people and children predisposed to obesity, it being extremely useful in preventing obesity. The exclusive preoccupation of obese people for food may be replaced with something else, such as sports. These individuals must not be isolated from their peers because the company of other young people creates relations and preoccupations susceptible of distracting the attention from the obsessive need for sweets and candies. When regularly practiced, sport may have effects against the accumulation of fat tissue and it may also create a new preoccupation and a different rhythm of living which may replace sedentary life and the overrating of pleasures provided by meals. The possibilities of practicing sports must be used starting during childhood, especially in the case of children of obese families. Different preoccupations, such as artistic ones, may also distract the attention from the perpetual obsession of feeding. They do not pose the disadvantage of sports, namely the danger of stimulating the already increased appetite of the obese or obesity predisposed individual, due to physical efforts which increase the calories and food needs, implicitly (2).

OBESITY. OBESOGENIC RISK FACTORS. OBESITY RISKS

Obesity is the excessive increase of the fat tissue mass, manifested by the body weight increase with more than 20% of

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the normal weight. Body weight values between the upper limit of the ideal weight and the inferior limit of obesity are considered as overweight or excess weight. Statistics show that the number of obese persons is continuously increasing, especially in developed societies.

Genetical factors may play a major role in the occurence of obesity or in the increased susceptibility to its occurence. In many severe obesity forms, starting during childhood, the total number of adipocytes is increased.

Among changeable factors, the first and most obvious is overfeeding, i.e. the use of an excessive quantity of food. It has been demonstrated that an increase of the calories intake leads to the increase of the fat deposits. A second important factor is the lack of physical activity, a sedentary, inactive lifestyle. The low energy consumption at work and during free time highly contributes to the occurrence or increase of the genetic misbalance. In the occurrence of obesity, an important role is played by psychological, family and socio-professional factors. For some people, food ingestion represents a psychological support, a way of coping with the problems they are confronted with or with various negative emotions. On the other hand, the family environment may constitute the transmission route for some wrong habits and attitudes towards foods. Sedentary professions, the increased use of cars, the excessive TV watching indirectly exerts a significant influence on the alarming spread of obesity. One of the rather frequently incriminated factors is alcohol consumption. This is due to the excess of calories that it determines, and which usually is added to a food excess.

Extra kilos cause an increased risk for many diseases, such as diabetes mellitus, arterial hypertension, aterosclerosis in general and ischemic heart disease in particular, gallbladder diseases and even some forms of cancer. In fact, not only the degree of ponderal excess, but also the regional distribution of the fatty tissue influences to an important extent the risk of disease. Obesity is frequently accompanied by the premature wear of joints and by varicose veins disease of the lower limbs. Unfortunately, the estetic inconvenience cannot be excluded either. Very often, obese people suffer emotionally due to their stigmatization by society. Last but not least, the physical and social handicap plays a role. Many obese people reach the point when they cannot help themselves, they move with great difficulty or are even in the impossibility to move and suffer intensely due to their isolation from the rest of the community (3).

FITNESS AND ITS EFFECTS

Fitness is a health related concept which refers to its maintaining and promoting. Being healthy does not only mean not being ill. Many people are content if nothing hurts, but health does not only refer to the physical, but also to the psychical, mental and social status. Moreover, if the aging process cannot be stopped, it can certainly be slowed down.

The favorable effects of sports on the health of the individual and community and on human activity in general, are known and scientifically proven. Many studies describe the mechanisms by which physical exercise influences the functions and systems of the human body. "The European Chart of Sport", in article 2, defines sport as being *"the total of physical activities aiming at: the expression or improvement of physical condition (fitness) and of mental wellbeing; construction of social relationships; obtaining sport results in competitions at various levels"* (4).

Regularly practiced physical activity determines maintaining or improving of the structure of various tissues and organs (muscles, tendons, heart), improves functions and counterweights deteriorations occurring due to inactivity, sedentary life and aging.

The term fitness defines physical condition, and it is quasi-similar with health. When speaking of health producing effects of fitness, reference is made to positive psychological changes as well as to obvious and beneficial influences on some diseases, best known being the diseases of vertebral disks, endocrine and especially cardiovascular diseases. In practice, because it best responds to the effect of physical effort, the ischemic heart disease must constitute the main objective of any motion based programme, regardless of age, gender and of the way physical activity is being practiced on an individual basis or under specialized supervision. The beneficial effects of fitness are described on various apparatuses and systems:

- The cardiovascular apparatus: increases the quantity of blood which the heart can pump; increases the quantity of blood in the blood vessels; blood becomes more fluid and circulates easier through arteries and veins (beneficial in arteriosclerosis, ischemic heart disease, arterial hypertension).
- The respiratory apparatus: the lung becomes able to ventilate a larger quantity of air per minute (helps in chronic lung diseases).
- The skeletal muscles: increases the force, resistance and power; muscles"dissolve" more slowly with aging (helps in lumbar diseases, fractures produced by falling, in elderly people).
- The adipose tissue: decreases the total fat mass around viscera.
- The glucidic metabolism: increases the capacity of the muscle to take glucose from the blood (helps in diabetes).
- The lipidic metabolism: increases the capacity of the muscle to take fats from the blood and to use them for procuring energy (helps in arteriosclerosis)
- The defense function of the organism: the capacity of the immune system to respond to biotic and abiotic aggressions improves.
- Digestive processes: the intestinal transit improves, eliminating constipation.
- The nervous system: the reaction speed and the promptitude of responses to various stimuli are improved (helps in fractures by falling, in elderly persons).
- The cognitive functions: the reaction speed and promptitude of responses to various stimuli improve.
- The psycho-social behavior: the self image, professional efficacy, family behavior improves and the state of wellbeing and joy of living occur (helps in depression and anxiety).

Fitness is not synonym with body building. It shares some

exercises with body building but the work intensity and volume are different. Fitness means a good physical condition and it includes: walking, running, cycling, stretching, exercises on various devices, relaxation and stretching methods, all performed under the guidance of a sports teacher (5,6).

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FACTORI DE INFLUEȚĂ MAJORI AI CALITĂȚII VIEȚII – ALIMENTAȚIA RAȚIONALĂ ŞI FITNESSUL

REZUMAT

În cadrul acestei lucrări am evidenţiat legătura sinergică dintre alimentaţia raţională şi fitness, în vederea prevenirii, ameliorării şi combaterii obezităţii. Alimentele reprezintă unul din cei mai importanţi factori ai mediului extern care influentează sănătatea omului. Ele au particularitatea de a acţiona continuu şi lent asupra omului, influenţându-i dezvoltarea fizică, psihică şi capacitatea de muncă. Alături de alimentaţia raţională, activitatea fizică este un factor care participă la realizarea unui management echilibrat al modului de viaţă, prevenind unele comportamente cu risc. Statisticile medicale arată că numărul de obezi este în continuă creştere. Kilogramele în plus antrenează cu sine creşterea riscului faţă de numeroase boli cum ar fi diabetul zaharat, hipertensiunea arterială, ateroscleroza în general şi cardiopatia ischemică în particualar, bolile vezicii biliare şi chiar anumite forme de cancer. Fitnessul este prezentat ca fiind un concept legat de sănătate, care se referă la menţinerea şi promovarea acesteia, menţionându-se efectele benefice asupra organismului.

Cuvinte cheie: alimentaţie raţională, obezitate, fitness

PREVALENCE OF EXTENDED SPECTRUM BETA LACTAMASE PRODUCING KLEBSIELLA PNEUMONIAE STRAINS ISOLATED FROM THE HOSPITAL AND COMMUNITY

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ABSTRACT

Bacterial multidrug resistant (MDR) it is more and more frequently described not only in hospitals but in community as well, representing an alarming phenomenon for the modern medicine through the drastically restriction of therapeutic options. We isolated from ICU (Timisoara Emergency Clinical County Hospital), between March 2009 – December 2009, 137 Klebsiella pneumoniae strains, from a total number of 548 microbial strains, and between January 2009- November 2009 we isolated from community (processed at the level of Timisoara Bioclinica Laboratory) 303 Klebsiella pneumoniae strains, from the total number of 3002 microbial strains.

All the Klebsiella pneumoniae strains were phenotyped, with the help of automatic VITEK 2 compact system, using VITEK 2 GP/GN identification cards and AST cards for antimicrobial sensitivity tests. For ESBL detection we performed the Vitek ESBL test (AST-GN27 cards).

In both, hospital and community, Klebsiella pneumoniae showed the highest percentage of ESBL production, followed by other enterobacteria.

Keywords: ESBL, Klebsiella pneumoniae, phenotype

INTRODUCTION

Since first reported in Europe in the early 1980s, extended-spectrum beta-lactamases (ESBLs) have spread worldwide. When producing these broad-spectrum plasmid-encoded enzymes, organisms become highly effective at inactivating penicillins, most cephalosporins, and aztreonam. Mainly produced by *Klebsiella* spp, ESBLs have been isolated worldwide in different species, most of them belonging to the Enterobacteriaceae. ESBL-producing bacteria can appear as in vitro susceptible to beta-lactams by conventional laboratory methods, making the laboratory diagnosis problematic. [1]

In a SENTRY worldwide surveillance program report, ESBL phenotypes were detected in 45% of *K. pneumoniae* strains from Latin America, 23% from the western Pacific, 23% from Europe, 8% from the United States, and 5% from Canada [2].

MATERIAL AND METHOD

We isolated from ICU (Timisoara Emergency Clinical County Hospital), between March 2009 – December 2009, 137 *Klebsiella pneumoniae* strains, from a total number of 548 microbial strains, and between January 2009- November 2009 we isolated from community (processed at the level of Timisoara Bioclinica Laboratory) 303 *Klebsiella pneumoniae* strains, from the total number of 3002 microbial strains.

Samples were sent to the Microbiology Department Laboratory's of the University of Medicine and Pharmacy "Victor Babes" Timisoara, where have been processed. All the samples were cultured on Columbia 5% sheep blood agar (bioMerieux) and selective media Mac Conkey (BioRad). Identification of *Klebsiella pneumoniae* strains was generally based on morpho-tinctorial character (gram negative bacilli), cultural and biochemical tests (API 20E galleries) and phenotyped, with the help of automatic VITEK 2 compact system, using VITEK 2 GP/GN identification cards and AST cards for antimicrobial sensitivity tests.

For ESBL detection we performed the Vitek ESBL test (AST-GN27 cards), which includes cefotaxime and ceftazidime, alone and in combination with clavulanic acid. A predetermined reduction in the growth of the cefotaxime or ceftazidime wells containing clavulanic acid, compared with the level of growth in the well with the cephalosporin alone, indicates a positive result. Sensitivity and specificity of the method exceed 90% [3].

RESULTS AND DISCUSSION

From the total of 548 bacterial strains with nosocomial potential isolated from ICU, 264 were multidrug resistant (MDR) strains. Distribution of bacterial strains was: 139 *S.aureus*, 137 *Klebsiella pneumoniae,* 75 *Acinetobacter baumannii*, 66 *E.coli*, 51 *Pseudomonas aeruginosa*, 7 *Klebsiella oxytoca* and 73 other enterobacteria (Figure 1).

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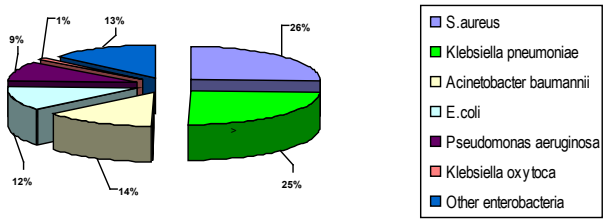

Fig. 1. Microbial strains isolated from ICU

283 from these strains were represented by *Enterobacteriaceae*, from which, 48.40% (137 strains) were ESBL producers. The highest percentage was noticed in the case of *Klebsiella pneumonia* - 78 strains (56.93%).

Table I. Resistance phenotypes in *Klebsiella pneumoniae* isolated from ICU

Klebsiella pneumoniae	137	78 ESBL 56.93%	**ESBL**+ KTGANt+cross resistance Fq + TE^R+SXT^R	19 24.35%
			ESBL+ impermeability cephamycins + KTG+ partially resistant Fq + TE^R	4 5.12%
			ESBL+ KTGANt+cross resistance Fq + TE^R	11 14.11%
			ESBL + KTANt + cross resistance Fq	22 28.21%
			ESBL (CTX-M) + KTGNt + partially resistant Fq +SXT^R	3 3.84%
			ESBL+ KTGANt+cross resistance Fq	14 17.94%
			ESBL+ impermeability cephamycins+ partially resistant Fq + TE^R+SXT^R	3 3.84%
			ESBL+TE^R+SXT^R	1 1.28%
			ESBL+SXT^R	1 1.28%

Legend: ESBL-extended spectrum beta-lactamase, K-resistance to kanamycine, G-resistance to gentamycine, T- resistance to tobramycine, A-resistance to amikacin, Nt-resistance to netilmicin, Fq-fluoroquinolone, SXT-trimethoprim-sulfamethoxazole,TE-tetracycline

In the community, from the total of 3002 bacterial strains isolated, 435 were MDR strains and the distributions were: 1778 *E.coli*, 809 *S.aureus*, 303 *Klebsiella pneumoniae*, 103 *Pseudomonas aeruginosa*, 9 *Acinetobacter baumannii* (figure 2).

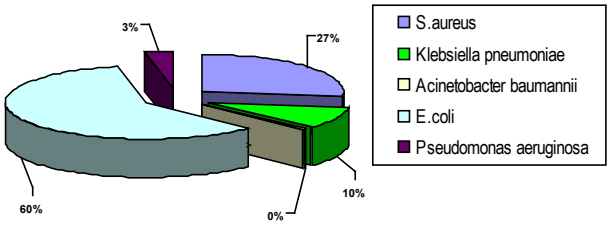

Fig. 2. Microbial strains isolated from community

In the community 2081 from isolated strains were represented by *Enterobacteriaceae*, from which, 10.90% (227 strains) were ESBL producers. The highest percentage was also noticed in the case of *Klebsiella pneumonia* - 107 strains (35.31%).

Table II. Resistance phenotypes in *Klebsiella pneumoniae* isolated from community

Klebsiella pneumoniae	303	107 ESBL 35.31%	**ESBL**+ impermeability cephamycins + KTGNt + TE^R+SXT^R	10 9.34%
			ESBL+ impermeability cephamycins + KTGANt	11 10.28%
			ESBL+ impermeability cephamycins + KTGANt + TE^R+SXT^R	6 5.60%
			ESBL+ impermeability cephamycins + G^R + TE^R	6 5.60%
			ESBL+ impermeability cephamycins + TE^R+SXT^R	1 0.94%
			ESBL+ impermeability cephamycins + G^R + TE^R + SXT^R	1 0.94%
			ESBL+ impermeability cephamycins + G^R	1 0.94%
			ESBL+ impermeability cephamycins + SXT^R	2 1.87%
			ESBL+ impermeability cephamycins + G^R + SXT^R	1 0.94%
			ESBL + G^R + TE^R + SXT^R + cross resistance Fq	18 16.81%
			ESBL+ G^R + TE^R + SXT^R	7 6.54%
			ESBL+ KTGNt + TE^R + SXT^R	7 6.54%
			ESBL+ KTGANt + TE^R+ SXT^R	6 5.60%
			ESBL+ G^R + TE^R	3 2.80%
			ESBL+ G^R + SXT^R	4 3.73%
			ESBL+ TE^R+ cross resistance Fq	5 4.67%
			ESBL+ TE^R	2 1.87%
			ESBL+ G^R + partially resistant Fq	3 2.80%
			ESBL+ G^R	2 1.87%
			ESBL+ TE^R+ SXT^R	2 1.87%
			ESBL - wild type	2 1.87%
			ESBL+ SXT^R	1 0.94%
			ESBL+ TA^R	1 0.94%
			ESBL+ KTGANt + SXT^R	1 0.94%
			ESBL+ KTGANt	1 0.94%
			ESBL+ KTGNt + TE^R	1 0.94%
			ESBL+ KTGNt + SXT^R	1 0.94%
			ESBL+ KTGNt	1 0.94%

Legend: ESBL-extended spectrum beta-lactamase, K-resistance to kanamycine, G-resistance to gentamycine, T- resistance to tobramycine, A-resistance to amikacin, Nt-resistance to netilmicin, Fq-fluoroquinolone, SXT-trimethoprim-sulfamethoxazole,TE-tetracycline

ESBLs are a rapidly evolving group of beta-lactamases which share the ability to hydrolyze third-generation cephalosporins and aztreonam yet are inhibited by clavulanic acid. Typically, they derive from genes for TEM-1, TEM-2, or SHV-1 by mutations that alter the amino acid configuration around the active site of these beta-lactamases. This extends the spectrum of beta-lactam antibiotics susceptible to hydrolysis by these enzymes.

An increasing number of ESBLs nor TEM or SHV lineage, have recently been described. The presence of ESBLs carries tremendous clinical significance. The ESBLs are frequently plasmid encoded. Plasmids responsible for ESBL production frequently carry genes encoding resistance to other drug classes (for example, aminoglycosides).[4]

In this study, all ESBLs have been associated with other resistance patterns like: resistance to quinolones, trimethoprim-sulphamethoxazole, aminoglycosides or tetracycline.

CONCLUSIONS

1. In both, hospital and community, *Klebsiella pneumoniae* showed the highest percentage of ESBL production, followed by other enterobacteria.
2. These *Klebsiella pneumoniae* strains have been isolated from bronchoalveolar fluids, wound secretions, urines.
3. Bacterial MDR it is more and more frequently described not only in hospitals but in community as well, representing an alarming phenomenon for the modern medicine through the drastically restriction of therapeutic options.
4. Because of the very limited remaining alternatives for treatment and ESBLs significant prevalence worldwide, infection control remains the best way to deal with this bacterial resistance mechanism.
5. The increasing frequency of ESBL-producing enterobacteria among hospitalized patients is an important problem for both microbiologists and clinicians, because of the difficulty in the correct detecting, reporting, and treating of the infections caused by these organisms.

Acknowledgments

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PREVALENTA TULPINILOR DE KLEBSIELLA PNEUMONIAE PRODUCĂTOARE DE BETA LACTAMAZE CU SPECTRU EXTINS IZOLATE DIN SPITAL SI COMUNITATE

REZUMAT

Bacteriile multirezistente sunt tot mai frecvent descrise nu doar în spital ci şi în comunitate reprezentând pentru medicina modernă un fenomen alarmant ce reduce drastic posibilităţile de tratament. Am izolat în perioada martie – decembrie 2009 de pe secţia de Terapie Intensivă a Spitalului Clinic Judeţean un număr de 137 tulpini de Klebsiella pneumoniae din totalul de 548 tulpini microbiene, iar în perioada ianuarie – noiembrie 2009 am izolat din comunitate (Laboratorul Bioclinica) un număr de 303 Klebsiella pneumoniae din totalul de 3002 tulpini.

Toate tulpinile de Klebsiella pneumoniae au fost identificate şi fenotipate cu ajutorul aparatului VITEK 2.

Atât în spital cât şi în comunitate, tulpinile de Klebsiella pneumoniae au reprezentat procentul cel mai mare de tulpini betalactamazoproducătoare cu spectru extins(ESBL), fiind urmată de alte enterobacterii.

Cuvinte cheie: ESBL, Klebsiella pneumoniae, fenotip de rezistenţă

NORMAL, EARLY AND DELAYED PUBERTY

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ABSTRACT

Puberty is defined as the period during which reproductive capability is acquired. Normal puberty starts around 10 years, girls having the tend to start puberty earlier than boys. Puberty lasts 3-4 years, with the transition from one stage to another, almost every year. Chronological pubertar evaluation is made with reference to Tanner stages, and biological evaluation, depending on bone age. Precocious puberty is defined as puberty before the normal age for the population, under 8 years old for girls and under 9 years old for boys. It affects about 4-5% of girls and is about five times more commonly seen in girls than boys. Delayed puberty is defined as no pubertal changes in a girl aged 13 years old or boys aged 14 years old, or failure of progression of puberty over 2 years. It affects about 2% of the population.

Keywords: puberty, chronological and biological evaluation

Normal puberty

Puberty is defined as the period during which reproductive capability is acquired. Frequently, the terms puberty and adolescence are used interchangeably. Puberty is different from adolescence which is the whole period of growth from childhood to adult and is expanding more than the period of puberty, between 10-19 years old.

There is a wide range of normality in puberty. Normal puberty starts around 10 years old, whereas girls tend to start puberty earlier than boys. Physical manifestations of puberty, in particular the emergence of secondary sexual characters, begin to occur at 11 years old to 50% of girls and 11 ½ years old to 50% of boys. Regular manifestation in female puberty, breast growth, it is more visible than the first signs of male puberty, increased testicles. Increase in height during puberty in girls occurs earlier, while in boys is installed later, with a difference of two years between the two sexes in the sequence of events in time (6, 11).

Puberty lasts 3-4 years, with the transition from one stage to another, almost every year. Puberty vary greatly not only as the match in time, but even in length. For example, 50% of girls need 4 years to go through all stages of puberty, some need only 18 months, while others need more than five years. Installation of the beginning of puberty can't predict the duration of puberty (8). Pubertar evaluation is usualy made with reference to Tanner stages (9, 10).

Assessment of the biological age of the child, as opposed to chronological age, is performed using the bone age. This uses assessment of epiphyses on an X-ray of the left hand and allows assessment of remaining growth and correlates better with pubertary stages than chronological age. Puberty is manifested in both sexes by the development of pubic and axillary hair; in girls, between the ages of 8 and 14 years old, by growth of the breasts and menstruation; in boys, between the ages of 9 and 15 years old, by growth of the penis, testes and scrotum, deepening of the voice, initiation of spermatogenesis, and by growth of facial hair.

Increase of height and other body dimensions, begin at boys one year after testicular growth, and reach maximum about a year after that, on average at the age of 14 years old. In girls, height increase starts once with breasts development, sometimes even before, and only when it passes the tip of height growth, menarche occurs (5).

The body changes that occur with puberty are due to the action of the sex hormones. These sex hormones include testosterone and other androgens for both sexes, together with oestrogen and progesterone in girls. The secretion of these hormones is from the testes in boys and ovaries in girls and to a lesser extent from the adrenal glands in both sexes. Some oestrogen is also produced in both sexes from peripheral conversion of sex hormones. Secretion of sex hormones is under the control of gonadotrophins, released from the anterior pituitary gland at the base of the brain. There are two gonadotrophin hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH).

The release of gonadotrophin hormones from the anterior pituitary gland is in turn controlled by gonadotrophin-releasing hormone(GnRH) from the hypothalamus. A negative feedback loop prevents excess hormone production. If there is sufficient sex hormone circulating, then this feeds back to both the pituitary gland and hypothalamus to reduce the amount of FSH and LH. At birth, the circulating levels of FSH and LH are similar to those of an adult. Soon after birth, however, both GnRH secretion and

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levels of circulating FSH and LH reduce to very low levels. As a child progresses towards puberty, the hypothalamus starts to secrete pulses of GnRH. The frequency and size of these pulses increase with time until there is constant secretion of GnRH, as in adulthood (3).

Puberty is a time of great physical and emotional change and can be a difficult time in any family. It can cause a great deal of anxiety both to the child and the parents if puberty does not progress as expected and is either too advanced or delayed for the age of the child. This makes assessment and management of disorders of puberty particularly challenging.

Precocious puberty

Precocious puberty results mostly from the precocious activation of the gonadotropic axis. Most physicians consider that onset of pubertal development before the age of 8 years old in girls and 9 years old in boys as precocious puberty and impose at least a clinical and bone age evaluation by a paediatric endocrinologist. The major concern in precocious puberty is the underlying condition, and central nervous system or gonadal neoplasm have to be formally excluded as a first step in the diagnosis. A secondary concern is height, since precocious puberty leads to accelerated growth, accelerated bone maturation and ultimately reduced stature. Precocious puberty affects about 4-5% of girls and is about five times more commonly seen in girls than boys (7). Precocious puberty is heterogeneous and strict criteria should be used to define it, both in terms of age and in terms of potential for progression (8).

Central or true precocious puberty

Premature activation of the hypothalamic – pituitary – gonadal axis results in a gonadotrophin-driven early puberty. In all cases, refer for further investigation to exclude hypothalamic tumours and other central nervous system pathology. No pathological cause for this pattern of precocious puberty is absent in 50 – 60% of males and 90% of females (1). In these individuals, there may be a family history of precocious puberty. Obesity may also be involved in development of precocious puberty in girls (2).

Pseudoprecocious puberty

Pseudoprecocious puberty, or gonadotrophin-independent precocious puberty, accounts for 20% of all cases of precocious puberty. There is an increased level of sex hormones in the absence of excess FSH or LH.

Causes of pseudoprecocious puberty:
- sex hormones secreting tumors
- hepatoma, hepatoblastoma
- congenital disorders
- familial male precocious puberty
- McCune-Albright syndrome
- hypothyroidism.

Social effects of precocious puberty

Children who are taller than other children of the same age will often be treated as older than they actually are. This can result in children being punished or teased for behaviour appropriate to their age or considered stupid or behind when in fact they are attaining expected developmental milestones (5). Children with precocious puberty may show more awareness of sexual parts and masturbate which can create embarrassment, especially in adults, and confusion in the child. Underarm deodorants and more frequent baths and hair washing, may be necessary as sweating and body odour can be a problem. All children want to look and act like friends of their own age. Although early puberty can give a child distinct physical advantages when playing many sports, being different from other children of the same age can lead to embarrassment for the child, teasing or bullying, and social exclusion that can lead to poor self-esteem. Parents have to face their own reactions to the changes in their child which can be alarm, distress, distaste, guilt and confusion (4).

Delayed puberty

Delayed puberty is defined as no pubertal changes in a girl aged 13 years old or boy aged 14 years old, or failure of progression of puberty over 2 years. It affects about 2% of the population.

The most common cause of delayed puberty in boys is constitutional delay, more than 50%. 80% of girls have a pathological cause for delayed puberty. Pathological causes can be divided into those caused by failure of the ovaries or testes also termed primary or hypergonadotrophic hypogonadism and those caused by failure of stimulation of normal gonads to produce sex hormones termed secondary or hypogonadotrophic hypogonadism (6).

Hypergonadotrophic delayed puberty

There are many possible causes of testicular or ovarian failure. Lack of sex hormone production results in low levels of circulating sex hormones, reduced negative feedback on the hypothalamus and pituitary, and high levels of circulating FSH and LH.

Causes of testicular and ovarian failure are:
- Klinefelter syndrome
- testicular dysgenesis syndrome
- absent or undescended testes
- testicular cancer, leukaemia
- epidemic parotidite
- testicular injury
- chemotherapy, glucocorticoids therapy
- Turner syndrome
- ovarian tumours
- polycystic ovarian syndrome.

Hypogonadotrophic delayed puberty

If the hypothalamus does not secrete enough GnRH or the pituitary gland does not secrete enough LH or FSH, then the normal gonads will not be stimulated to secrete sufficient sex hormones for pubertal changes to occur. Biochemically,

circulating levels of sex hormones, FSH and LH are all reduced. Depending on whether the underlying cause of the problem is in the hypothalamus or pituitary, GnRH levels may be reduced or increased.

Causes of hypogonadotrophic delayed puberty are:

- sporadic or familial constitutional delay
- destructive tumours or lesions of the hypothalamus or pituitary
- hypothalamic syndromes such as Prader – Willi syndrome or Laurence – Moon – Biedl syndrome
- thyroid disease, hyperthyroidism, rarely hypothyroidism
- suppression of the hypothalamic – pituitary – gonadal axis as a result of excess secretion of hormones negatively feeding back onto that axis — hyperprolactinaemia, Cushing's syndrome, hyperthyroidism or congenital adrenal hyperplasia
- serious systemic illnesses , including chronic renal failure, blood disorders such as thalassaemia and sickle cell disease, Crohn's disease
- malnutrition due to food shortage, anorexia nervosa or malabsorption, for example as a result of cystic fibrosis or coeliac disease
- excessive exercise particularly at gymnasts (12).

Social effects of delayed puberty

Many children, particularly boys, have great difficulty in coping with delayed puberty. They may be considerably shorter, and appear much younger, than their friends. Later on their lack of sexual development may cause them embarrassment and make them feel left out. In some, this may result in immature behavior, while in others, it may manifest as aggressive and/or antisocial behavior. The reactions of others to the child may also be affected by delayed puberty. Children who are small are often treated according to their size and not their age. Parents may become hyperprotective. Immature aspect and incapability to join in with group activities may then lead to further exclusion by the child's peer group. The small teenagers can be a natural target for bullying and sometimes, inappropriate skills are developed to compensate in order to survive with decline of self-esteem (5).

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PUBERTATEA NORMALĂ, PRECOCE ŞI ÎNTÂRZIATĂ

REZUMAT

Pubertatea este definită ca perioada în care este dobândită capacitatea reproductivă. Pubertatea normală începe în jur de 10 ani, fetele având tendinţa de a începe pubertate ceva mai devreme decât baieţii. Pubertatea durează 3-4 ani, cu trecerea de la o etapă la alta, aproximativ în fiecare an. Evaluarea pubertară cronologică este efectuată cu referire la etapele Tanner, iar evaluarea biologică, în funcţie de vârsta osoasă. Pubertatea precoce este definită ca pubertatea înainte de vârsta puberală normală pentru populaţie, sub 8 ani pentru fete şi sub 9 ani pentru băieţi. Ea afectează aproximativ 4-5% dintre fete şi este de cinci ori mai frecventă la fete decât la băieţi. Pubertatea întârziată este definită ca lipsa modificărilor pubertare la fetele de 13 ani sau băieţii de 14 ani, sau eşecul de progresie al pubertăţii de peste 2 ani. Afectează aproximativ 2% din populaţie.

Cuvinte cheie: pubertate, evaluare cronologică şi biologică