THE ROLE OF NEUROPEPTIDES IN THE PATHOMECHANISM OF ATHEROSCLEROSIS

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1. INTRODUCTION

In the 21st century, one of the greatest health care challenges in the world is the high mortality rate caused by cardiovascular diseases. Atherosclerosis, the most important risk factors of which – according to our present knowledge – are the consequences of life in the civilised world (bad diet, lack of exercise, obesity, smoking), is closely related to the so-called metabolic syndrome. This syndrome may occur as a complex syndrome or in the form of separate entities, such as obesity, type 2 diabetes mellitus, hypertension, and dyslipidemias. High total serum cholesterol levels due to high LDL cholesterol, especially when accompanied by low HDL-C levels, are only one step away from cardiovascular collapse. Cholesterol intake with food and endogenenous cholesterol synthesis in the cells is key issues in respect of the development of atherosclerosis. Apart from disruptions in cholesterol homeostasis, calcium imbalance and the increased production of free radicals also play significant roles in the formation of an atheromatous plaque. As a result of this, during the last 25-30 years, atherosclerosis research gradually became focused on cells, and researchers studying atherosclerosis laid down the foundations of our present knowledge about receptors and genetic regulations. Upon choosing the subject of my PhD theses, I became enthusiastically interested in this field of research, which, by that time, had significant traditions at the 1st Department of Internal Medicine, University of Debrecen. Our basic principle was that using the theoretical results recently achieved in the field of cell research relating to cardiovascular diseases, we examined the practical significance of individual theoretical results in ex vivo experiments on white blood cells obtained from patients.

1.1. The effect of neuropeptides (angiotensin II, leptin) on the formation of free radicals of white blood cells in control subjects and in patients with hypercholesterolemia (HC)

The superoxide anion production generating effect of both neuropeptides we chose is well known, and their atherogenic effect is also due to this feature. It should be noted that certain receptors of both angiotensin II (Ang II) and leptin (AT1 and LpRb), which are expressed on monocytes and, in the case Ang II, are also expressed on the surface of neutrophils. Both Ang II and leptin belong to the proinflammatory cytokine I

superfamily. Consequently, their stimulating effect, apart from the Gi protein- PLC-Ins(1,4,5)P3 pathway, also involves signalling pathways, such as the PI3 kinase, MAP kinase, PKC, ERK1/ERK2, as a result of which they can have a regulating effect on gene expression in the cells' nucleus. On the other hand, it has been known for more than 10 years now that the mevalonate cycle, through the prenylation of Rac1/Rac2 small GTPase belonging to the Rho superfamily, is an important regulator of NADPH oxidase activation. It is also known that in atherosclerosis, the therapeutic effect of HMG CoA reductase enzyme inhibitor statins in atherosclerosis is, in part, secondary to the inhibition of free radical formation triggered by stimulus. Numerous, thus far unresolved issues emerged on the basis of literature data. Do the two neuropeptides generate the same effect in the phagocytic cells of control individuals and patients with HC? Are the signal pathways of the signals altered in the case of destructive metabolic diseases of the whole body? How do the phagocytic cells of patients suffering from different entities of the metabolic syndrome respond to the two neuropeptides? If superoxide anion production consists of a statin sensitive and a statin resistant part, then what is their proportion in the cells of patients with HC?

1.2. Connection between the formation of free radicals induced by neuropeptides in different entities of the metabolic syndrome and the damage of cell membrane

It is a fact known for a long time that in the circulation of patients with cardiovascular diseases, typical alterations occur in the membrane of white blood cells, red blood cells and thrombocytes. These changes are, first of, all due to the transformation of the so-called "rafts" consisting of cholesterol and sphingolipids that are present in the membrane. These lipid rafts contain numerous G protein-coupled receptors, such as those of the chemotactic peptide FMLP, Ang II and leptin. The main issue with transformation is that in atherosclerosis, the cholesterol content of lipid rafts of cell membranes increases, in the lipid rafts of the cell membranes, and it may alter the signalling of cytokines and cytokine type receptors. In the development of the metabolic syndrome nearly all theories pay attention to obesity with great detail, more concretely to visceral obesity. It has been known for more than fifty years now that fatty tissue, as an endocrine organ, controls food intake. Quite unusually, the adipocytes of visceral fatty tissue work as an endocrine organ. Some of the hormones – or factors – produced by them occur

exclusively or, at least in an assessable amount, in the fatty tissue, while, for example, the proinflammatory cytokines produced by them are products of immune competent cells that are present in the fatty tissue. The possibility of a close relationship between calcium metabolism and obesity has emerged recently. 1,25-dihydroxycalcitriol, the level of which increases in obesity, increases Ca2+ flow into the cells, and also into adipocytes, through its specific receptors. At the same time, high $[Ca^{2+}]i$ levels can simulate the effect of agouti protein regulated by the agouti gene, which means that it can significantly inhibit lipolysis in adipocytes and increase the activity of fatty acid synthase (FAS). These phenomena together result in the increase of fatty tissue quantity. On the other hand, it is also known that the formation of free radicals has a severe damaging effect on ion and especially on Ca^{2+} transport, as it can damage nearly all enzymes or channels in the cell membranes. Calmodulin, which is a basic component of the calmodulin-dependent Ca^{2+}-ATPase enzyme, is especially sensitive to the effect of superoxide anions. This enzyme is the only one that is able to remove unnecessary Ca^{2+} from cells with a non-conductive membrane. It is undoubted that oxidation stress, membrane alternations and ion transport are related to one another in such a complex way that the order of sequence of their effects on one another is very difficult to clarify. However, we did not find any data relating to how membrane rigidity, the Ca^{2+} signal triggered by agonist stimulus or the release of superoxide anion and leukotriene develop in different entities of the metabolic syndrome.

1.3. The effect of leptin on endogenous cholesterol synthesis of human monocytes

The fact, known from the literature and mentioned above, that in phagocytic cells stimulated by cytokines, the production of superoxide anion and leukotriene can be reduced by inhibiting the HMG CoA reductase, called the attention to the finding that the activity of the mevalonate cycle is also increased by the effect of stimulation. This fact is also notable since one of the end products of the mevalonate cycle is cholesterol itself. On the other hand, it is also known that cytokines stimulate SCAP gene expression in HepG2 and mesangial cell lines, and it also stimulates SCAP synthesis in the endoplasmic reticulum of the cells. The excessive presence of SCAP results in a condition, when SCAP remaining after the binding of cholesterol molecules is able to form a compound with SREBP2, and this compound is transported to Golgi, where

SREBP2 becomes activated. All the above called our attention to the possibility that leptin, the receptor of which has the same characteristics as cytokine receptors, is able to affect the endogenous cholesterol synthesis of monocytes.

2. OBJECTIVES

1.) Through the in vitro use of different inhibitors, we intended to compare the signalling pathways of those two peptides (angiotensin II and leptin) in leukocytes of control subjects and patients with atherosclerosis, which also participate in the pathomechanism of atherosclerosis. Furthermore, we intended to clarify the dependence of superoxide anion and leukotriene production generated by different agonists, on the mevalonate cycle in white blood cells of healthy individuals and patients.

2.) We intended to examine the relationship between the formation of free radicals triggered by neuropeptide stimulus in the cells of patients with metabolic syndrome and the damage of cell membranes. We also planned to clarify whether leptin does play a role in the development of intracellular Ca^{2+} homeostasis disturbance in obesity.

3.) Our goal was to clarify whether leptin is able to affect endogenous cholesterol synthesis in human monocytes obtained from circulating blood, and if yes, what the probable signalling pathway is.

3. METHODS

3.1. Patients: In the control groups, we obtained blood, in part, from healthy volunteering health care employees, and, in part, from voluntary blood donors with the help of the Blood Donor Station. We asked patients seeking consultations at the Department of Metabolic Diseases at the 1st Department of Internal Medicine to donate blood samples. It was difficult to gather the different entities of metabolic syndrome and to select patients with HC, whose LDL receptor defect was ruled out by performing a preliminary test, nevertheless, the endogenous cholesterol synthesis of resting monocytes was already higher than that of control monocytes. The tests needed for the demographic data of controls and patients with HC were performed at the Institute of Clinical Biochemistry and Molecular Pathology of DEOEC. During the course of performing an individual experiment, venous blood samples (10-15 mL) were taken at 4-5-day intervals

from 6-8 patients with HC and 3-4 control subjects. The inter-assay coefficient was below 15%. The Ethics Committee of DEOEC granted permission to our experiments.

3.2. Isolation of cells, in vitro conditions: Monocytes and neutrophils were isolated with the usual method of gradient centrifugation, and further purification of monocytes was performed using the Kumagai method. All tests were performed under sterile conditions, incubation took place in a CO_2 thermostat (37ºC, 5% CO_2, 95% humidity).

3.3. Inhibitors used for the clarification of signalling pathways: Generally, cells were suspended in HBSS, and after preliminary experiments, the following agonists were used for stimulation: 10 nM angiotensin II (Serva), 100 ng/mL leptin (Sigma), 10 nM formyl-Met-Leu-Phe (Serva), 100 nM PMA (Sigma), 1 μM A23187 (Sigma). The inhibitors were used in the following concentrations and for the following time periods: PLC-inhibitor 5 μM neomycin (Sigma) for 60 minutes, Gi protein inhibitor 100 ng/mL pertussis toxin (Sigma) for 120 minutes, intracellular Ca^{2+} translocation inhibitor 1.0 μM thapsigargin (Sigma) for 60 minutes, 1μM losartan (Merck) inhibiting the AT1 receptor of angiotensin II for 20 minutes, 1 μM PD123319 (Sigma and Aldrich) inhibiting the AT2 receptor for 20 minutes, PKC inhibitor 1.0 μM H-7 (Sigma) for 60 minutes, PI3 kinase inhibitor 20 nM wortmannin (Sigma) for 30 minutes, MAP kinase inhibitor 50 μM PD98059 (Sigma) for 30 minutes, the HMG CoA reductase inhibitor 5 μM fluvastatin (Merck) and 20 μM lovastatin for 60 and 120 minutes respectively., 25 μM 25-hydorxycholesterol (Sigma) in a methyl-β-cyclodextrin compound inhibiting the formation of the SCAP-SREBP compound for 120 minutes. Finally, in order to inhibit Ca^{2+}-influx, cells were incubated in Medium V for 60 minutes. Medium V contained 10 μM verapamil (Sigma) and 3 mmol/l of EGTA in Ca^{2+}-free HBSS.

3.4. Applied methods

3.4.1. Superoxide anion: spectrophotometric determination of the reduction of ferricytochrome C (Sigma) inhibited with superoxide dismutase.

3.4.2. Arachidonic acid cascade: determining the release of radioactive arachidonic acid products from cells filled with [^{14}C] arachidonic acid, triggered by different stimuli.

3.4.3. Leukotriene determination: using reverse HPLC technique, it was carried out at 280 nm in the ultraviolet range (UV-VIS, using L-4250 detector).

3.4.4. [Ca^{2+}]i: using Indo1/AM, with spectrofluorimetric method (Hitachi, F-4500), at 405 and 485 nm, and at 37º C, during constant mixing.

3.4.5. Ins(1,4,5)P3: With the help of an internal standard, with reverse phase ion-pair chromatographic method. After fractioning, the radioactivity values were determined in a Packard 2200 CA liquid scintillation counter.

3.4.6. Protein kinase C: the integration of ^{32}P from [^{32}P]ATP into histone III-S was measured.

3.4.7. Membrane fluidity: 1,6-diphenyl-1,3,5-hexatreine (DPH, Sigma) dispersion was used for fluorescent polarisation; the fluorescent polarisation of the cells was measured with a Hitachi F4500 spectrofluorimeter, with the help of a polarisation filter at 37º C (excitation at 355, while emission at 430 nm).

3.4.8. Cholesterol synthesis: we measured the integration of [^{14}C] acetate into the cholesterol fraction of monocytes.

4. RESULTS AND DISCUSSION

4.1. **Controlling free radical formation generated by neuropeptides:** Leptin in human monocytes, while Ang II in human granulocytes and monocytes, depending on the concentration of neuropeptides, are able to increase superoxide anion production, the release of arachidonic acid products and leukotriene C4 and B4 synthesis. In HC cells stimulated with neuropeptides, the formation of free radicals is significantly increased. This is obviously secondary to increased statin-sensitive free radical formation. In patients treated with fluvastatin, the reaction of neutrophils to stimuli became normal. The increased free radical formation of HC cells is due to the fact that the isoprenylation of Rac1/Rac2 through the mevalonate cycle plays a considerably more significant role in HC leukocytes than in control ones. The successful use of statin therapy is also due to this phenomenon. The signalling pathway in HC cells is altered, and in the case of Ang II and leptin, it is altered the same way. In the signalling of HC cells, the Ca^{2+}-influx through verapamil-sensitive Ca^{2+} channels, the diversion pathway leading through the activation of PI3 kinase and the Rac1/Rac2 activation play important roles in the activation of NADPH oxidase and phospholipase A_2 responsible for the explosion of the

arachidonic acid cascade. To summarize the above results: in HC cells, if a stimulus originates from either the AT1 or the LpRb receptor, the classic Gi protein → phospholipase C → Ins(1,4,5)P3 → Ca^{2+} translocation signalling pathway does not work, and instead, the Ca^{2+}-signal transpires through verapamil-sensitive Ca2+ channels. Another signalling pathway is the Ins(3,4,5)P3 split by PI3 kinase, which is able to activate an isoform of phospholipase C, but the role of its down-stream regulation in HC cells is not significant.

4.2. The membrane-damaging effect of reactive oxygen species (ROS) occurring in stimulated leukocytes of patients with metabolic syndrome: In patients with metabolic syndrome (obesity, type 2 diabetes mellitus, hypercholesterolaemia), the membrane rigidity of their granulocytes, the activity of PKC attached to the membrane, the Ca^{2+} signal transpiring through verapamil-sensitive Ca^{2+} channel, moreover, the saturated fatty acid, lipid hydroperoxide and conjugated diene contents of the membrane are significantly higher. In contrast with this, the amount of cholesterol bound to the membrane was only higher in the membrane of patients with hypercholesterolemia. The increased superoxide anion release of granulocytes stimulated with Ang II, and the arachidonic acid metabolism in all three patient groups were the result of rearrangement of lipid rafts in the membrane and the increased rigidity of the membrane. As a "vicious circle", however, the increasing release of free radicals damages the cell membrane's condition in the patient groups. The high $[Ca^{2+}]i$ value of resting monocytes in obesity, as well, as the leptin-induced Ca^{2+} signal also change with respect to the shape of the time curve. The smaller peak appears later on in time and only normalises slowly, compared to the high basal level. Calculating the area under curve value (AUC value), we were able to evaluate the quantity of thapsigargin and verapamil sensitive Ca^{2+} signals in the intracellular processes induced by the agonists. According to our results, in monocytes of obese patients, as a result of increased ROS formation, the enzymes that ensure Ca^{2+} homeostasis became damaged, especially the thapsigargin-sensitive Ca-ATPase and the calmodulin-dependent Ca^{2+}-ATPase linked to SERCA, which ensures the extrusion of calcium from the cells. Preincubation with fluvastatin inhibiting HMG CoA reductase restored the original calcium homeostasis, partially through its anti-oxidant effect.

4.3. The effect of leptin stimulus on endogenous cholesterol synthesis of monocytes of controls and HC patients: According to our results leptin at a concentration of 10-100 ng/mL, under in vitro conditions, increases endogenous cholesterol synthesis in monocytes of both the control and HC patients, in contrast with this, in concentrations > 250 ng/mL cholesterol synthesis is reduced in control cells, while it's further increased in HC cells. However, the amount of cholesterol bound cell membranes is only increased in HC cells, following leptin treatment. At lower leptin concentrations, it is the Ca^{2+} signal, the PI3 kinase, the MAP kinase and the conventional PKC in HC cells that participate in the increase of cholesterol synthesis. These signal pathways, in a similar way to cytokines, may lead to SCAP gene amplification in the nucleus. In control monocytes, cholesterol synthesis suppression is clearly the result of H-7-sensitive conventional PKC activity, which confirms our earlier result, according to which, with H-7, the negative feedback reaction that can be generated with the administration of LDL, and, that inhibits endogenous cholesterol synthesis, can be suspended. As opposed to this, in HC monocytes, further increase of cholesterol synthesis may only be suspended with wortmannin that inhibits PI3 kinase, which finding, based on data from the literature, suggests that in this process the PI3 kinase → Ins(3,4,5)P3 → PKCξ → NFκB signalling pathway plays a role.

5. Summary

5.1. The neuropeptide-stimulated phagocytic cells of patients with metabolic syndrome have increased superoxide anion production compared to control cells. This increase can be explained by the enhancement in intensity of the mevalonate cycle triggered by stimuli, in which the isoprenylation and the Rac1/Rac2 activity play parts. Increased free radical formation in the cells of the patient groups may be inhibited, in vitro and in vivo, with statins**.**

5.2. The signalling of angiotensin II and leptin, which neuropeptides belong to the proinflammatory cytokine family, in granulocytes and monocytes do not deviate significantly from each other, but in metabolic syndrome and, especially, in cells of patients with HC, we found significant alterations. In the cells of the patients, the rapid

superoxide and leukotriene formation, triggered by a stimulus, is the result of opening of the verapamil-sensitive Ca^{2+} channels, while in the control group, Ca^{2+} is released from the intracellular stores into the cytosol.

5.3 In the cells of patients with metabolic syndrome not only does the mechanism of the cell membrane's Ca^{2+} channels change, but the membrane rigidity of resting cells is also increased, as is the membrane-linked protein kinase C activity, the saturated fatty acid, lipid hydroperoxide and conjugated diene content. As opposed to this, the amount of cholesterol bound to the membrane is only higher in the membrane of patients with hypercholesterolemia.

5.4. On the basis of our investigations, it may be determined that a proportion of the changes observed in the membrane is the result of increased free radical formation, while, as a "vicious circle", the changes observed in the membrane contribute to the increased free radical formation triggered pathogenic signalling. In obesity, the high $[Ca^{2+}]i$ value of resting monocytes and granulocytes, and the Ca^{2+} signal generated by leptin also change, with respect to the shape of the time curve. The smaller peak appears later on in time and only normalises slowly, as compared to the high basal level. However, considering the area under curve value (AUC) of the time curve measured for 6 minutes, we showed that the leptin-induced Ca^{2+} signal in the cells of obese patients was significantly higher than in the cells of the control patients due to the prolongation of the Ca^{2+} signal.

5.5. The increased free radical formation occurring in the patients' cells due to the effect of leptin stimulation, according to our findings, may be the cause of the pathogenic Ca^{2+} signal-curves, and of the increased $[Ca^{2+}]i$. The reason for this is the strong free radical sensitivity of the Calmodulin-dependent Ca^{2+}-ATPase, which, in cells with nonconductive membranes, is the only route to remove unnecessary Ca^{2+} from the cell. An important consequence in obesity therapy is the statin sensitivity of this phenomenon, as in adipocytes, the high $[Ca^{2+}]i$ significantly increases the size of fat cells, and, therefore, it also increases the amount of fatty tissue. With fluvastatin, under in vitro conditions, we were completely able to restore the Ca^{2+} homeostasis characteristics of control patients.

5.6. On the basis of the results presented in the first 2 points - according to which, in patients with HC, the increased free radical formation of granulocytes and monocytes stimulated with neuropeptides is the result of the intensity of the statin-sensitive mevalonate cycle, the question we had to solve was whether there is a significance of this increased biochemical cascade in the pathomechanism of atherosclerosis. Our raising this question was also supported by the result that we showed signalling pathways (PI3 kinase, MAP kinase, protein kinase C) related to free radical formation caused by neuropeptides that are well known to exert their effects in the nucleus as well. After these preliminary results, using the [^{14}C]acetate incorporation method, we investigated the endogenous cholesterol biosynthesis due to neuropeptide stimulus in monocytes of controls and patients with HC. On the basis of previous investigations, we only used the cells of those patients whose endogenous cholesterol synthesis of monocytes was higher than 13.1 pmol/60 min/10^7 cells, and whose negative feedback reaction was less than 45% following administration of 6 mmol/L LDL.

5.7. We showed that leptin in concentrations between 10-100 ng/mL, under in vitro conditions, increased endogenous cholesterol synthesis both in controls' and HC patients' monocytes, while in concentrations at > 250 ng/mL, it reduced cholesterol synthesis in control cells, while further increasing it in HC cells. However, the amount of cholesterol attached to the cell membrane is only increased in HC cells due to leptin treatment. In the case of smaller leptin concentrations, the Ca^{2+} signal, PI3 kinase, MAP kinase and, in HC cells, the conventional protein kinase C play roles in increasing cholesterol synthesis. These signal pathways in the nucleus, we assume, may lead to SCAP gene amplification, then via the SCAP-SREBP2 pathway to enhanced HMG CoA reductase synthesis. In the control monocytes, the suppression of cholesterol synthesis is clearly the result of the activity of the H-7-sensitive conventional protein kinase C.

5.8. As a final summary, in metabolic syndrome, but especially in granulocytes and monocytes of patients with HC, the neuropeptides closely related to cytokines lead to membrane changes and to disturbed Ca2+ homeostasis, as a result of the formation of free radicals that, as "vicious circle" further increases the NADPH oxidase and lipoxygenase activity acting through the mevalonate cycle. The fact that this process is statin-sensitive also raises the possibility of new statin applications.

6. NEW RESULTS

6.1. We were the first ones to show that Ang II and leptin having cytokine-like receptors in the monocytes and/or granulocytes of patients suffering from HC, increase ROS formation and this increase is the consequence of increased isoprenylation of Rac1/Rac2.

6.2. We were the first ones to show that the signalling pathways of Ang II and leptin change in the phagocytic cells of patients with HC, i.e., the canonical pathway does not work and the Ca^{2+} signal is the result of Ca^{2+}-influx occurring through the verapamil-sensitive Ca^{2+} channels.

6.3. We were successful in clarifying that in certain separate entities of the metabolic syndrome, the increased ROS formation is related to the rigidity of the membrane, but it does not depend on the amount of cholesterol attached to the membrane. The severity of membrane damage in the individual entities is the following: obesity< type 2 diabetes mellitus < hypercholesterolemia.

6.4. In monocyte-model experiments we were the first ones to show that in obesity, leptin may play a part in the rise of $[Ca^{2+}]i$, and the area under the leptin-created Ca^{2+} signal time curve (AUC) grew pathologically, the shape of the time curve changed. A new and important piece of data is that with fluvastatin ,in vitro, we were able to correct the time curve of the Ca^{2+} signal in obesity.

6.5. We were the first ones to describe that depending on the leptin concentration, it exerts a biphasic effect on the endogenous cholesterol synthesis in human monocytes: in a concentration of 10-100 ng/mL, it increases, while > 250 ng/mL, it inhibits the activity of the mevalonate cycle. In HC monocytes, leptin at concentrations of 10-500 ng/mL was only able to cause a cholesterol synthesis increase, and in these cells, the reverse cholesterol transport did not work either.

7. PUBLICATIONS

The publications serving as the basis of the thesis:

1. Seres I, Fóris G, Páll D, **Kosztáczky B**, Paragh G Jr. Varga Z, Paragh G. Angiotensin II-induced oxidative burst is fluvastatin sensitive in neutrophils of patients with hypercholesterolemia. (2005) Metabolism **54:** 1147-1154. (**IF:2.497**)
2. **Kosztáczky B**, Fóris G, Seres I, Balogh Z, Fülöp P, Koncsos P, Paragh G. Neuropeptides induced a pronounced and statin-sensitive dysregulation of mevalonate cycle in human monocytes of patients with hypercholesterolemia. (2006) Neuropeptides **40:** 309-316. (**IF: 2.789**)
3. Seres I, Fóris G, Varga Z, **Kosztáczky B**, Kassai A, Balogh Z, Fülöp P, Paragh G. The association between angiotensin II-induced free radical generation and membrane fluidity in neutrophils of patients with metabolic syndrome. (2006) J Membr Biol **214:** 91-98. (**IF: 2.112**)
4. **Kosztáczky B**, Fóris G, Paragh G Jr, Seres I, Zsíros E, Koncsos P, Balogh Z, Paragh G. Leptin stimulates endogenous cholesterol synthesis in human monocytes: New role of an old player in atherosclerotic plaque formation. (2007) Int J Biochem Cell Biol **39:** 1637-1645. (**IF:4.804**)
5. Balogh Z, Fóris G, **Kosztáczky B**, Paragh G Jr, Seres I, Zsíros E, Kónya G, Paragh G. The concentration dependent biphasic effect of leptin on endogenous cholesterol synthesis in human monocytes. (2007) Peptides (In Press) (**IF:2.701**)

Publications not directly linked to the topic of the thesis:

1. Arokoski JP, Hyttinen MM**,** Lapvetelainen T, Takács P, **Kosztáczky B**, Módis L, Kovanen V, Helminen H. Decreased birefringence of the superficial zone collagen network in the canine knee (stifle) articular cartilage after long distance running training detected by quantitative polarised light microscopy. (1996) Ann Rheum Dis **55**: 253-264. (**IF:5.767**)
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(**IF: 3.277**)

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1. I Seres, G Paragh**, B Kosztáczky**, T Kalmár, H.Z. Mirdamadi, A. Kassai, G. Fóris: Disturbed Ca^{2+} transport in neutrophils of obese patients. Atherosclerosis Supplements 2006. 7 (3): 229-229. (**IF:5.875**)

2. G. Fóris, I. Seres, **B. Kosztáczky**, P. Fülöp, Z. Balogh, G. Paragh: Angiotensin II caused a statin-sensitive dysregulation of mevalonate cycle in human monocytes. Atherosclerosis Supplements 2006. 7 (3): 571-572. (**IF:5.875**)

3. **B. Kosztáczky**, G. Fóris, I. Seres, A. Kassai, T. Kalmár, G. Paragh: Mevalonate cycle of human monocytes is disturbed by leptin in vitro. Atherosclerosis Supplements 2006. 7 (3): 577-577. (**IF:5.875**)

4. Fóris, G, Seres, I, **Kosztáczky, B.,** Balogh, Z, Varga, E., Paragh, Gy: Concentration-dependent effect of angiotensin II on the endogenous cholesterol synthesis in human monocytes Atherosclerosis Supplements 2007; 8 (1):29-30. (**IF:5.875**)

Abstracts not directly linked to the topic of the thesis

1. G. Paragh, G. Foris, G. Paragh, Jr., I. Serest, Z. Karanyi, P. Fulop, Z. Balogh, **B. Kosztaczky**, P. Kertai:Different anticancer effects of fluvastatin on primary hepatocellular tumors and metastases in rats. Atherosclerosis Supplements, 2005, 6 (1): 118-118. (**5.875**)

Lectures and posters at Hungarian and international conferences:

1. Andrea Kassai, Ildikó Seres, **Béla Kosztáczky**, Gabriella Fóris, György Paragh: The possible connection between angiotensin II-induced free radical generation and membrane fluidity in neutrophils of patients with metabolic syndrome
   Semmelweis Symposium, Inflammatory mechanisms in atherosclerosis - A critical appraisal, 3-4 November 2005, Budapest, Hungary

2. G. Fóris, **B. Kosztáczky**, Gy. Paragh: Angiotensin-II-induced failure of mevalonate cycle in human monocytes
   Semmelweis Symposium, Inflammatory mechanisms in atherosclerosis - A critical appraisal, 3-4 November 2005, Budapest, Hungary

3. Paragh G, Fóris G, Paragh Gy Jr, Seres I, Karányi Zs, Fülöp P, Balogh Z, **Kosztáczky B,** Teichmann F, Kertai P: Different anticancer effects of fluvastatin on primary hepatocellular tumors and metastases rats

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5. Fóris G., **Kosztáczky Béla**, Balogh Z., Seres I., Paragh Gy.: Új szempontok az angiotensin II atherogén szerpének megítélésében. MAT Soproni Kongresszus 2006. október

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7. Seres I., Paragh Gy., **Kosztáczky B**., Hossein Zamir Mirdamadi, Kassai A., Fóris G.: A CA2+ transzport zavara elhízott betegek neutrofiljeiben. MAT Soproni Kongresszus 2006. október

8. I Seres, G Paragh**, B Kosztáczky**, T Kalmár, H.Z. Mirdamadi, A. Kassai, G. Fóris: Disturbed Ca^{2+} transport in neutrophils of obese patients. Poster XIV International Symposium on Atherosclerosis Rome, Italy, 2006. június 18-22.

9. G. Fóris, I. Seres, **B. Kosztáczky**, P. Fülöp, Z. Balogh, G. Paragh: Angiotensin II caused a statin-sensitive dysregulation of mevalonate cycle in human monocytes. Poster XIV International Symposium on Atherosclerosis Rome, Italy, 2006. június 18-22.

10. **B. Kosztáczky**, G. Fóris, I. Seres, A. Kassai, T. Kalmár, G. Paragh: Mevalonate cycle of human monocytes is disturbed by leptin in vitro. Poster XIV International Symposium on Atherosclerosis Rome, Italy, 2006. június 18-22.

11. Fóris, G, Seres, I, **Kosztáczky, B**., Balogh, Z, Varga, E., Paragh, Gy: Concentration-dependent effect of angiotensin II on the endogenous cholesterol synthesis in human monocytes Poster 76th Annual European Atherosclerosis Society Congress 2007. jún.10-13, Helsinki, Finnország

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