

# LOCAL EFFECT OF THE INFRASOUND TO THE ENDOTHELIN, THROMBOMODULIN AND INTERLEUKIN-8 LEVELS RELEASING FROM THE ARTERIES AT PATIENTS SUFFERING FROM ARTERIOSCLEROSIS IN THE LOWER EXTREMITIES

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## Arteriosclerosis:

The arteriosclerosis obliterans is an endemic disease of our ages. It may have an influence to the whole arterial system, consequently it is a generalized disease. The exact reason of its development is unknown. Intima injury, hormonal activity, hypertony, thrombocyte metabolism, prostaglandine, plasmolipid metabolism and control, mechanical reasons and inflammation take part in its etiology.

Risk factors are: smoking, hypertension, diabetes mellitus, hyperlipidaemia, hyperhomocysteinaemia, the way of living, genetics, old age and male sex. Approximately half of the arteriosclerosis obliterans cases manifest themselves on the lower extremity. At the beginning segmental localization is typical. According to the symptoms (cold, atrophic and atrophic skin on the leg, atrophied muscle, claudication intermittens, a state of rest pain, missing pulse, necrosis, ulcer, gangraena) it can be divided to stages.

Stage classification according to Fontaine:

- I. objectively demonstrable blood circulation disorder without subjective complaint
- II. claudicatio intermittens (in case of walking distance more than 200 meters)
- III. claudicatio intermittens (in case of walking distance less than 200 meters)
- IV. a state of rest pain
- V. a state of rest pain and necrosis (gangraena, ulcer)

According to the present state of medical science it is inpreventable and the „cure” of it is only possible by vascular surgery methods. As to patients suffering from obliterative vascular disease of lower extremities the surgical solution may result a significant improvement both in walking distance and in the state of rest pain and as well as in the recovery of the extremital gangrenous processes. In our country surgical solution can be taken into consideration at about 20 per cent of the patients because in many cases the injured vascular system, respectively, the general status of the patient doesn't even or already make the surgical intervention possible. So at 70-80 per cent of the patients only conservative methods come into question such as medical therapy, (calcium antagonistic, aspirin, pentoxifyllin, etc.), the curative gymnastics, balneotherapy, haemodilution, carbonic bath. According to literary data in the stage of Fontaine II. the advantages of invasive procedures aren't unanimous contrary to the conservative therapy. Consequently, interest in the non-invasive procedures is understandable. Such an alternative physiotherapeutic method is the local sound wave treatment.

## Sound wave treatment

Certain researches have proved by now that sounds of low frequency are able to influence in the living organism the speed of the anaphylactoid reaction, activity and regenerative

ability of the liver cells, permeability of the erythrocyte membrane. They slow down the progression of myopia in childhood through the morphological changes of the vascular membrane of the eye, moderate the erectile dysfunction of atherosclerotic origin and may have analgetic and circulation supporting effect. Using sound of determined frequency and intensity locally has an effective influence on the progression of the peripheral arteriosclerosis. It reduces complaints and increases walking distance.

Local sound wave therapy makes possible these favourable and up to the present slightly examined effects. It may successfully complement the conservative general treatment under determined indication (Fontaine II. stage). It hardly has contraindication. It is uninvasive, painless, of short period, easy to reach, repeatable at any time. It doesn't require bed-patient background, by effect can be left out of consideration.

During our research we studied the local effect of the sound wave on endothelial cells by demodulating the endothelin, soluble thrombomodulin and IL-8 levels of the plasma before and after treatment.

### Materials and methods

The applied equipment is the Sonomat 4000 generating a sound of 60-80 Hz frequency being an intensity of 40-60 decibel auditory sensation. The appliance used for treatment contains a treating head furnished with wave creating signal source. It is joined to the central unit which is in connection with two parameter measuring apparatus (heart action, skin temperature) by inserting an adjusting stage. At the beginning of the treatment electroencephalograph electrodes and skin thermometer are put on the patient as well as the treating heads are fastened to the soles of the patient. The signal source being in the treating head passes the sound waves generated by the central unit, alternately, sometimes to the one and sometimes to the other sole of the patient in a given phase of the systole. During the treatment the control panel continuously measures and records the heart rhythm and temperature of the body surface to be treated and changes the amplitude and frequency of the output sign in inverse position with it. Period of the treatment is 1x15 minutes.

Choosing criterion of our patients, without age and sex restriction, was claudication being in the stage of Fontaine II/a. Patients undertook the test voluntarily, free of charge, following by an internal examination and getting detailed information. The test was reliably successful in case of 8 female and 11 male persons (whose average age was 64 years).

To measure endothelin, thrombomodulin and IL-8 levels we used plasma gained from the vein of the dorsum of foot directly before and after the treatment. We immediately froze the plasma containing EDTA and as well as the citrated one to  $-70^{\circ}\text{C}$  and stored them this way. After that the Regional Immunological Laboratory of the Medical Clinic III. of DEOEC prepared the determinations by ELISA method. We evaluated before and after treatment data using paired T-trial ( $P<0,05$ ).

The thrombomodulin is a glycoprotein molecule which is an integrant element of the membrane of the endothelial cell being on its endovascular surface. It is the key molecule of one of the most important anticoagulant mechanism of the intact endothelium. It is complex with the thrombin and so activates the protein-C which, conversely, will irreversibly inactivate the VIII. and V. factors most important elements of the haemostasis. The IL-1 and TNF- $\alpha$  inhibit their expression and synthesis. An endothelial injury is indicated if the level of its soluble form increases in the plasma.

The endothelins are peptides consisting of 21 amino acids being de novo synthesized and separated from a preproprotein by the endothelial cell, having an effect through the ET-a-b-c receptors. PL-C activation, later on IP<sub>3</sub> and DAG release and increase of the IC calcium concentration transmit their effects in the vascular system.

In small concentration (normal value: 0,2-0,7 fmol/ml) endothelin has a vasodilative effect, in greater quantity it has an intensive vasoconstrictive effect.

Vascular smooth muscle and fibroblast proliferation, increase of the collagen production (c-myc, c-fos genic activation)  $\Rightarrow$  indicate its role in atherogenesis.

It plays a part in the smooth muscle proliferation induced by the mechanical stress and in neointima genesis (during endothelial destruction its plasma level increases).

The effects inducing synthesis: hypoxia, shear stress, hyperglycaemia, oxLDL, adrenalin, erythropoietin, thrombin, Ang-II, ÁVP, TGF- $\beta$ , TNF- $\alpha$ , IL-1-2

Its synthesis is impeded by: ANP, PGI<sub>2</sub>, EDRF

Structurally homologous 8-12 KDa polypeptides constitute the IL-8 family. The IL-8 is produced by activated T-lymphocytes, activated peripheral leukocytes, endothelial cells, fibroblasts, thrombocytes, keratinocytes. It has a chemotactical effect on Neu and Ly.

The IL-8 reduces the symptoms of inflammation through the impediment of IL-1 while it promotes the symptoms of inflammation by activating leukocytes and by increasing the permeability of microarteries, consequently it is proinflammatorical. It increases the IC Ca level, rises the genic expression, the receptor expression, causes respiratory exploison.

#### 4. oldal

##### Thrombomodulin

Deviation of the before (32,45 ng/ml) and after (32,53 ng/ml) treatment average concentrations isn't significant (P=0,8)

##### Endothelin

The average of after treatment concentrations (0,30 fmol/ml) is significantly higher (P=0,04) than that of the before treatment concentrations (0,27 fmol/ml). The increase remains in the normal concentration domain (0,2-0,7 fmol/ml).

#### 5. oldal

##### Interleukin-8

The average of the after treatment concentration (19,3 pg/ml) is significantly lower (P=0,02) than the before treatment one (23,4 pg/ml).

#### Discussion:

As I mentioned above the increase of the soluble form of the thrombomodulin measured in the plasma indicates endothelial injury. So the constancy of its concentration reinforces the endothelial protective nature of the infrasound.

Small increase of the endothelin level remaining within the normal domain indicates a vasodilative effect, namely in small concentration (normal domain: 0,2-0,7 fmol/ml) the endothelin is vasodilating. Besides the endothelin doesn't influence the progression of the arteriosclerosis through the sound.

Decrease of the IL-8 level reinforces the hypothesis of its inflammation reductive and pain soothing effect.

In our study we drew the attention to an alternative – applicable besides the internal medical therapy - physiotherapeutic treatment of arteriosclerosis obliterans in the lower extremity which may be effective at patients being in Fontaine II. stage.

#### 6. oldal

Examining the effect of the sound wave treatment on endothelium we found that it presumably doesn't cause endothelial injury and it has some vasodilative and inflammation decreasing effect.

It is reinforced by the favourable experiences of the patients. Further prospective clinical examinations are needed to clear the part of the method playing in the therapy.

#### 7. oldal

##### Arteriosclerosis:

- Endemic disease
- Generalized
- About half of it manifests itself on the lower extremity

Stage classification according to Fontaine:

- I. objectively demonstrable blood circulation disorder without subjective complaint
- IIa. claudicatio intermittens (in case of walking a distance more than 300 meters)
- IIb. claudicatio intermittens (in case of walking a distance less than 300 meters)
- III. state of rest pain
- IV. state of rest pain and necrosis (gangraena, ulcer)

- Medical treatment of it only with vascular surgery methods (20 %)
- Solely conservative therapy comes into question in 70-80 % of the cases
- The local sound wave treatment is an alternative physiotherapeutic method.

#### 8. oldal

##### Sound wave treatment

- Many physiologic effect of it is known
- It is inflammation decreasing and circulation improving
- It increases the walking distance in claudicatio
- Practically it has no contraindication
- It isn't invasive
- It is painless
- It is of short period
- It is within easy reach, repeatable at any time
- It doesn't require bed-in background
- It may complement the internal therapy

#### 9. oldal

##### Materials and methods

- The applied equipment is the Sonomat 4000
- Time of the treatment is 1x15 minutes
- Patients being in Fontaine II/a stage of claudicatio
- ELISA
- Paired T-trial
- The thrombomodulin
- The endothelins
- The IL-8

#### 10. oldal

The concentration of thrombomodulin before and after treatment

- The deviation of before (32,45 mg/ml) and after (32,53 mg/ml) treatment average concentrations isn't significant ( $P=0,8$ ).

#### 11. oldal

The concentration of endothelin before and after treatment

- The average of after treatment concentrations (0,30 fmol/ml) is significantly higher ( $P=0,04$ ) than that of the before treatment one (0,27 fmol/ml). The increase (0,2-0,7 fmol/ml) remains in the normal concentration domain.

#### 12. oldal

The concentration of Interleukin-8 before and after treatment

The after treatment average concentration (19,3 pg/ml) is significantly lower ( $P=0,02$ ) than the before treatment one (23,4 pg/ml).

#### 13. oldal

Discussion:

- The increase of the soluble form of thrombomodulin measured in the plasma indicates endothelial injury. The constancy of its concentration reinforces the endothelial protective nature of the low frequency sound.
- A small increase of the endothelin level within the normal domain indicates a vasodilative effect. Endothelin has a vasodilative effect in small concentration (normal domain: 0,2-0,7 fmol/ml).
- Decrease of the IL-8 level reinforces the hypothesis of its inflammation reductive and pain soothing effect.

#### 14. oldal

Discussion:

- In our study we drew the attention to an alternative physiotherapical treatment of arteriosclerosis obliterans in the lower extremity which may be effective at patients being in Fontaine II. stage.
- Testing the effect of the sound wave treatment on endothelium we found that it doesn't cause endotheliocyte injury and it has certain vasodilative and inflammation reducing effect.
- Favourable experiences of the patients also reinforce it.
- Further prospective clinical tests are needed to clear the place of the method playing in the therapy.