THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

Effect of nitrate therapy in postmyocardialial infarction patients and in animal experimental models of obesity

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1. Introduction

The administration of nitrates for the treatment and prevention of angina pectoris has been known for more than a hundred years. First, Sir Lauder Brunton described the improving chest pain due to inhalation of amylnitrite in 1867, than William Murell was able to relieve of chest pain with nitroglycerin solution in 1879.

The vasodilatory effect of nitrates is more pronounced on the venous system and it reduces preload more substantially, compared to the afterload. These mechanisms will result in the reduction of: 1) the left ventricular filling pressure, 2) the dilatation of the ventricle and 3) the systolic wall tension. Consequently, the energy and oxygen demand of the myocardium is reduced, and the subendocardial blood flow improves. Previous investigations discovered that nitrates have vasodilatory effect on epicardial coronary arteries and on the so called resistency arteries, which directly improves myocardial perfusion this way. However, according to recent clinical trials, results on the mortality and efficacy of nitrate therapy in the daily clinical practice are still ambiguous. Following myocardial infarction, on the basis of international guidelines, nitrate therapy is primarily indicated in recurrent ischemic conditions. Two large clinical studies about nitrate treatment during and following myocardial infarction managed to prove benefit, in patients in reducing the number of cardiogenic shock and post infarction angina pectoris. However, the underlying mechanism of action is not completely understood at present.

More recent studies indicated that, in special pathological conditions (obesity), vascular response to nitrate therapy is substantially altered, influencing therapeutic effect this way. Overweight is defined as a BMI between 25 and 29.9, while obesity is defined as a BMI above 30. In the United States, 34 % of the population is overweighed and nearly 30% is regarded as obese. It is well-known, that obesity (which prevalence is rapidly rising in Hungary) substantially increases the risk of cardiovascular diseases. There is a special entity, involving multiple metabolic abnormalities, called the metabolic syndrome, which plays a crucial role in the pathogenesis of cardiovascular diseases. In metabolic syndrome, central obesity stands in the focus of pathological disorders, beside elevated blood sugar level, impaired glucose tolerance, lipid abnormalities like elevated triglycerol and low high density lipoprotein (HDL) level, and hypertension. According to the definition of the International Diabetes Federation, the diagnosis of metabolic syndrome can be set if at least two of the above mentioned metabolic disorders exist beside central obesity.

Presumably, circulatory failures in overweight patients can significantly contribute the development of ischemic heart disease and hypertension, and to the progression of cardiovascular morbidity and mortality. In obesity, the exact nature and mechanism of action of the altered coronary-vessel function is not fully clarified. Thus in obese patients the efficacy of nitrate therapy in coronary vessel vasodilatation to maintain proper tissue perfusion is still unclear.

2. Aim of the study

Based on the above mentioned problems with nitrate therapy the main objects of my scientific investigations were the followings:

1. To examine the effect of a 6-month transdermal nitrate therapy in post-myocardial infarction patients on the diastolic and systolic left ventricular function, viewed by echocardiography.

2. To examine the effects of nitrates and nitrogen oxide (NO) donors on the coronary microvessel vasodilatory response in an experimental animal model of obesity.

3. Methods

3.1. Patients recruitment and methods

Patients with diagnose of myocardial infarction and stable clinical conditions were enrolled in the present study, between the ages of 18-85 years. The enrollment of the study was carried out in a period between 1st March 2000 and 31st October 2000. The diagnosis of myocardial infarction was based on Troponin T (Qualitative, Roche Diagnostic Corporation, Indianapolis, IN) positivity and the presence of at least two of the following conditions:

- Chest pain lasting for more than 20 minutes and no reaction to sublingual nitrate,
- CK and/or CK-MB level increase above the double of the upper limit of the normal range,
- One mm or more ST elevation in at least two connected (representing the same area) standard leads, or 2 mm or more ST elevation in at least two thoracic leads,
- Development of new left bundle branch block.

Patients were regarded clinically stable if 1) there were no recurring angina episodes, 2) there was no pulmonary congestion, 3) no invasive intervention was planned and 4) the blood pressure was between 100-150/65-90 mm Hg. The exclusion criterias were as follows: 1)

chest pain requiring sublingual nitrate within 14 days of the onset of the infarction, 2) pulmonary congestion requiring diuretic treatment, 3) coronary intervention (Percutan Coronary Intervention, or Aorto-Coronary Bypass Graft), 4) onset of hypotension or hypertension, 5) right ventricular infarction, 6) intolerable side-effects to nitrate (headache, allergy), 7) presence of clinically significant aortic or mitral insufficiency, and 8) molsidomine treatment. During the 8 months enrollment period 92 patients were screened for eligibility to be included in the study. In 33 cases, conditions for exclusion were observed (in 8 cases recurring angina within the first two weeks after infarction, in 6 cases pulmonary congestion requiring diuretics, in 7 cases coronary intervention, in 2 cases severe hypotension, in 3 case hypertension, in 5 cases intolerable nitrate-induced headache and in 2 cases nitrate-related skin allergy).

Finally, 59 patients have been enrolled in the study and the data of these cases have been analyzed. All patients received intravenous nitroglycerine within the first 48 hours after infarction. Two therapy groups were then formed: one (nitrate group – 30 patients) received transdermal nitroglycerine treatment (0.2 mg/h) from the third day after infarction for 6 months. The other group was regarded as control (control group - 29 patients) and received nitrate free treatment after the 2^{nd} day of myocardial infarction for 6 months (the sublingual nitrate at the onset of angina was allowed in both groups). To avoid the development of nitrate resistance for the patients in nitrate group, the patch was applied at 7 a.m. and was removed at 19 p.m. each day. All other treatments - fibrinolysis, heparin, aspirin, beta-blockers and ACE inhibitors - corresponded to the usual clinical practice and there was no significant difference between the two groups.

During the randomization period, the patients with odd numbers were entered into the nitrate group, while the ones with even numbers were enrolled into the control group. Before randomization all patients provided their written consent to the study, which was performed with the approval of the local Ethics Committee. The study visits were performed at day 3, 14 and 42 (±2 days) and at 6 months (±2 weeks) following infarction (visit 1, 2, 3 and 4, respectively), which included detailed physical examination, 12 lead ECG and Doppler echocardiography. During echocardiography the E and A waves, and the deceleration time (DT) were measured (in all 4 visits). Moreover, the pulmonary venous flow (in all 4 visits), and the ejection fraction (EF, in 2-4 visits) were also defined. Wall motions, cavity diameters and valve functions were established according to the usual clinical practice. Two experts with good echocardiography practice performed the examinations independently of each other, using a HP (Hewlett-Packard Co Andover, MA) SONOS 2000 device, equipped with a

2,5 MHz transducer. Doppler sample volume was placed caudal to the mitral annulus between the tips of the mitral leaflets, where peak inflow velocity in early diastole was recorded. To minimize the influence of heart rate, DT was measured as the time between the peak E wave and the upper deceleration slope extrapolated to the baseline. Pulmonary venous flow recordings were obtained from the four-chamber view directed to the right upper pulmonary vein. Sample volume was obtained 1-2 cm into the pulmonary vein. The modified Simpson formula was used to define the ejection fraction. Examinations were performed between 10-12 a.m., and two independent experts examined each patient. The results of the two examinations were averaged and used for further evaluation.

The exercise tests were performed during visits 2 and 3 with a Marquette (ACU 002C Milwaukee, WI) treadmill. The first test was made according to Naughton protocol (as is usual with early infarctions – 14 day), while the second test was made according to the Bruce protocol .Both tests were carried out between 10-12 a.m. without interrupting the usual medical regimen.

All patients recorded the number of angina requiring nitrate in an issued patient's diary book and this was checked during visits. The measurement of blood pressure was performed in sitting position after 5 minutes of rest with a mercury manometer according to the method of Korotkoff. Pulse checking was done at the same time on the radial artery counting it for 1 minute.

3.2.Experimental animal model of obesity

Our experiments were conducted according to the protocol approved by the Ethical Committee of Animal Experiments of University of Debrecen. The experiments were all performed by experienced staff. We used male Wistar rats. Animals were kept in dark and light in 12-hours periods, with continuous water and food supply. Right after the end of the experiments, we performed active euthanasia by injecting 150 mg/kg sodium-pentobarbital intraperitone. Overweight was gained by feeding the animals with HFD (High Fat Diet; Testdiet; PMI Nutrition International). HFD animals (n=20) were fed with high fat (60) diet for 10 weeks. Rats in the control group (n=20) were kept on normal fat diet for 10 weeks either.

Analytical methods

Serum total cholesterol and glucose level was measured by colorimetric assay (Cobas Integra,

Roche). Serum insulin level was measured by radio immune-assay based method (BYK Sangtec).

Isolated micro-vessel technique

Experiments with isolated micro-vessels were performed on coronary arterioles (~120 µm). Pentobarbital was used to narcotise the animals. The surgically removed rat heart was put and fixed into a silicon based tray containing cold (0-4 °C, pH 7.4) Krebs solution. A 1-2 mm long segment of the intramuscular part of the septal artery was isolated thereafter, and put into organ-tray, containing cold oxygenised Krebs solution. One end of the arteriole was cannulated using microsurgical methods by the help of a Nikon, Eclipse 80i stereomicroscope. Red blood cells were removed afterwards with 20 millimetres of mercury perfusion pressure. Distal end of the arteriole was cannulated thereafter, and the original vessel-length was reset with a micro-screw. The preparation was put into an organ-bath with standard temperature (T=37°C, pH=7.4) solution. The organ tray was perfused with oxygenated (O_2:10%; CO_2:5%; N_2:85%) Krebs solution (perfusion flow: 40ml/min). Intraluminal pressure was then gradually raised to 80 millimeters of mercury using a special feedback pressure-control system (Living system), and this pressure was kept for 60 minutes while intraluminal pressure was measured continuously with pressure transducers. By the end of the incubation period, arterioles developed spontaneous myogenic tone. Internal luminal diameter was detected with a digital camera (CFW1310, Scion Corp) fixed to a Nikon Eclipse 80i video microscope, and measurements were performed with computer.

Examination of coronary microvessel vasodilatation

Primarily, we investigated vasodilatory response of coronary arterioles isolated from HFD animals and from control group, to different pharmacons with known, either receptor mediated or receptor-independent signalling. Vasoactive agents were administrated cumulatively, in proper end-concentrations into a defined volume (15 ml) basin, following development of myogenic tone. Concentration dependent vasodilatory effect of the certain agent was continuously registered, and cumulative dose-effect curves were made thereafter. Each dose-effect curves were followed by a minimum of 10 minutes wash-out period, during which arterioles regained their original myogenic tone. Dose-dependent effects of sodium-nitroprusside (10^{-9} – 10^{-6} mol/L) and NONO-ate (10^{-9} - 10^{-6} mol/L) as NO donors were examined. Vasodilatory responses to NO donors were also measured following a 30 minutes incubation period with the soluble guanilate-cyclase inhibitor oxadiazolo-quinoxaline (ODQ;

1 µmol/L). Finally, we measured vasodilatory response to increasing concentrations of the cell permeable cGMP-analogue 8-bromo-cGMP.

Western immunoblott

Coronary arteries were isolated from the heart of HFD and control animals, cleared of connective tissue then frozen in soluble nitrogen and store on -80 °C until further utilization. The isolated coronary segments were homogenised in 20 µl's of SDS-puffer, and boiled for 5 minutes. Proteins of the sample were separated with 8% SDS polyacrlymide gel-electrophoresis, and then transferred to nitrocellulose membrane. To identify the examined proteins, anti β1 subpart of soluble guanilate-cyclase antiobody (Sigma) and, as a laoading control, anti β-aktin IgG antibody (Abcam) were used. Signals gained with chemiluminescency were recorded by autoradiography. Optical density of the streaks were measured and quantified by Image J software.

Statistical analysis

All data were given as the mean±SD or mean±SEM. Categorical data were compared with Fischer's exact test. For continuous independent variables as age, angina pectoris and animal experimental data Student's t-test was used. Two-way (group x time) repeated-measures ANOVA was done for E, A, E/A, S, D, AR, EF, Stress-test, BP, HR variables. Post hoc analysis was carried out using the Tukey test. Changes in vessel diameter to different vasoactive agents were expressed proportionally compared to maximal dilatation. Two-sided statistical tests were performed at the 5 percent of significance. Statistical analyses were performed using the SAS statistical software package (version 6.12).

4. Results

4.1. Clinical results on patients with myocardial infarction

First, homogeneity of the two groups was examined. There was no statistical difference between the two treatment groups as regards to the examined risk factors – age, gender, hypertension, smoking, total cholesterol level, diabetes mellitus and previous myocardial infarction. Next the comparison of the outset values was carried out between the two treatment groups at visits 1 to 4. As regards to the flow velocities measured on the mitral valve plane (E, A wave, E/A ratio and DT) there was no difference between the two groups throughout the treatment period. Examination of the data revealed that there was no significant difference in the different mitral flow parameters within the individual groups, and

at the time of visit 4 (6^{th} month visit) there was no difference between the two groups either. The outset values of the pulmonary flow velocities (S and D waves) were not significantly different between the two groups at the time of visit 1. Examination of the changes in the S value during the period of the study, revealed a gradual and significant increase in both groups, however at visit 4 this elevation was statistically greater in patients receiving nitrate. The D value on the other hand, has shown a gradual and significant decrease in both groups during the study period. This decrease, similarly to the S value, was more pronounced in the nitrate group. The atrial reverse flow (AR wave) was identical in the two groups at visit 1 and then it was significantly reduced in both groups. In the case of this parameter again the changes were more pronounced in the nitrate group.

The intra- and interobserver variability values of the measurements of the mitral and pulmonary flow velocities were as follows 1) E wave of the mitral flow: 2±1 and 3±2 cm/s, respectively, 2) A wave of the mitral flow: 3±2 and 3±3 cm/s, respectively, 3) DT of mitral inflow 8±6 and 10±8 msec, respectively, 4) S wave of the pulmonary flow: 1.5±1 and 2±2 cm/s, respectively, and 5) D wave of the pulmonary flow: 1.8±1 and 2.3±2 cm/s, respectively. Based on the low intra- and interobserver variability values of the mitral and pulmonary flow velocities it is obvious that the observed significant differences are reflecting true changes between the two groups of patients.

The ejection fraction referring to systolic left ventricular function at visit 1 was statistically identical in the two groups. However, in the nitrate group a gradual and significant increase was noted for this parameter and at visit 4 significantly exceeded the value found in the control group (nitrate group 52.6±7.4% and control group 47.4±7.3%). The maximal ST depression during exercise test was statistically identical at visit 2 between the two groups. At visit 3 the maximal ST depression was significantly reduced in the nitrate group, compared to the value measured at visit 2, while the ST depression observed in the control group did not change significantly between visit 2 and 3

No statistically significant difference was noted in the number of angina during the study period between the two groups. Moreover, the systolic and diastolic blood pressure values and the heart rate did not differ significantly between the two treatment groups As regards to the mortality, due to the small number of patients (and deaths during the study period) no statistical analysis was carried out. In the nitrate group 2 patients died (one sudden death at home, and one traffic accident), in the control group 3 patients died (one apoplexy, one pneumonia and one acute left ventricular failure).

4.2. Results gained in animal experimental models of obesity

Effect of high fat diet to different measured parameters

We investigated vasodilatory response of coronary arterioles in experimental models of obesity. Following a 10-week period of nutrition with high fat diet (HDF), total body mass and retroperitoneal adipose-tissue mass was significantly higher than in the control population, that was kept on normal diet. Among HFD animals, serum glucose, insulin and total cholesterol levels resulted to be significantly higher.

Vasodilatation of coronary arterioles in response to different NO donors

Vasodilatory response to different NO donors, like sodium nitroprusside (SNP) and NONO-ate, resulted to be significantly higher in HFD vessels compared to vasodilatory response in the control group arteries. There was no difference in the degree of vasodilatation induced by the cell-permeable cGMP-analogue 8-bormo cGMP between HFD animals and the control group. Thereafter, we examined vasodilatory response to SNP and NONO-ate in the presence of the soluble guanilate-cyclase (sGC) inhibiting oxadiazolo-quinoxaline (ODQ). ODQ decreased vasodilatory response of isolated coronary arterioles equally in the HFD and control groups.

Results with western immunoblott

We performed western immunoblott analysis of coronary arterioles isolated from the heart of HFD and control animals. We could not prove any significant difference in the sGC β1 subunit expression of coronaries between the two groups.

5. Discussion

Aim of the nitrate treatment in myocardial infarction is to reduce the arterial mean pressure with 10 percent in normotensive patients and 30 percent in hypertensive patients, while the systolic blood pressure should remain above 80-90 mmHg. Many clinical studies examined the long-term effectiveness of nitrates on the left ventricular function after acute myocardial infarction. One of the first trials was carried out by Judgutt et al., who examined captopril, nitrate and combination of the two compounds in the case of Q-wave anterior myocardial infarction compared with placebo. Both strategies improved the asynergy, the expansion of infarction, the left ventricular diameter, the thickening of infarction, the ejection fraction, the number of aneurysm and the mortality data comparing with placebo. The combination of the two drugs further improved the clinical results. Two great, multicentre studies, the GISSI-3

and the ISIS-4 have dealt with the influence of nitrates and ACE inhibitors on mortality. In GISSI-3 after intravenous nitroglycerin transdermal nitrate applied during 6 weeks, in ISIS-4 long acting isosorbid-5-mononitrate tablets administered. In these two studies involved almost 77000 patients did not eventually prove significant decrease of mortality in nitrate groups, but as a tendency, a small-scale decrease could be observed in 6 week mortality (in case of 1000 patients this decrease was 2.1 in ISIS-4, and 3.9 in GISSI-3 study). In addition in GISSI-3 study the number of post-infarction angina and the occurrence of cardiogenic shock decreased significantly ($p=0.03$ and $p=0.009$). Meantime the combination of nitrate and lisinopril reduced mortality with 17 percent ($p=0.02$). In ISIS-4 the first 48 hours mortality was significantly smaller in the nitrate group ($p<0.0001$) as compared to the placebo. These data refer to the advantageous effects of nitrates.

In the 1999's ACC/AHA Acute Myocardial Infarction Guideline the intravenous nitroglycerin has Class I (absolute indication) recommendation in the first 48 hours in the case of heart failure, large anterior infarction, persistent ischemia and hypertension. After 48 hours nitrate has been recommended only in recurrent angina and persistent lung edema. Transdermal nitrate form is suggested only in recurrent ischemia as a Class II/a (indicated) recommendation. Opinions differ on the prolonged nitrate treatment following myocardial infarction. Although, large multicenter randomized studies (GISSI-3 and the ISIS-4) have shown a minor effect upon nitrate treatment to decrease mortality, but its measure did not reach the level of statistical significance. However, in order to promote a better quality of life (symptom relief) for the patients, in the absence of any contraindications the prolonged transdermal or oral formulations of the nitrates may be used after infarction (even in the case of no complains).

On the basis of our studies we concluded, that the continuously administered nitroglycerin therapy:

1) Significantly improved systolic left ventricular function (EF) and systolic vena pulmonary flow (S) measured by echocardiography
2) Significantly reduced diastolic flow velocity detected in pulmonary vein, and atrial reverse flow (D, AR),
3) thus significantly increased the S/D ratio,
4) and significantly decreased maximal ST depression upon the 6 week's exercise test.

Interestingly, examination of the traditional echocardiographic parameters (E/A ratio and DT) characterizing the left ventricular diastolic function did not reveal any statistical difference between the nitrate-treated and control groups. Explanations for this discrepancy could be that 1) the pulmonary venous flow indices are more sensitive indicators of the diastolic function than are E/A or DT and 2) the nitrates exert a more pronounced effect on preload than on afterload.

Regarding the number of registered angina episodes during the 6 months period of treatment, a small, but not significant reduction has been observed in the nitrate group. No statistically significant difference has been detected in heart rate and in either the systolic, or diastolic blood pressure during the 6 months period of treatment between the two groups. In consequence of to the relatively low numbers of enrolled patients and deaths during the study period (2 deaths in the nitrate group and 3 in the control group), statistical evaluation of the mortality data was not carried out. However, no sign of an excess mortality was observed upon nitrate treatment.

How can nitrates preserve the left ventricular function (post-infarction remodeling) following myocardial infarction? The beneficial effects are most probably complex. The histological recovery after a myocardial infarction involves collagen deposition and the formation of strong scar tissue in the infarcted zone to resist distension and wall dilation. However, the insufficiency of these defensive mechanisms in a majority of the cases means that the early remodeling leads to the local dilation and expansion of the involved area within a few days after the infarction. This process can eventually give rise to overall ventricular dilation and an elevated diastolic wall tension in both the infarcted zone and the intact area. Nitrates protect the left ventricular function and prevent the extension of the infarcted area (and the development of an aneurysm) by lowering the ventricular wall tension and preserving the collagen matrix in the necrotic zone. Moreover, it has been shown that nitrates exert a protective effect on the myocardium following stunning and reperfusion damage. The anti-ischemic properties of nitrates have also been demonstrated in our study, since a significant reduction was noted in the maximal ST segment depression in response to the stress test in the nitrate group as compared to the controls.

In summary it can be concluded that the prolonged nitrate treatment following myocardial infarction resulted in a more preserved left ventricular function as evidenced by echocardiography (even in symptoms free patients). These limited observations warrant further studies on the beneficiary effect of nitrates on the left ventricular function and in the

future the authors plan to extend this examination on a larger number of patients, employing novel echocardiographic techniques (e.g. tissue Doppler echocardiography).

In the second part of our experiments, we investigated the functional alterations of coronary microvessels, in case of HDF nutrition induced obesity and in metabolic syndrome. It is well-known fact, that tissue perfusion is primarily determined by the diameter of small arteries and arterioles. Considering coronary circulation, where oxygen supplementation is essential, impaired arteriolar vasodilatation can result in major consequences in myocardial perfusion. Several studies provided data, that endothel-dependent vasodilatation in arterioles, isolated from different tissues, was impaired in obesity. These impairments were proven in cerebral arterioles by different authors using skeletal muscle or mesenterial arteries. In a previous publication we have shown that in animals kept on HDF nutrition, synthesis of endothelial NO is both reduced in coronary vessels and in peripheral skeletal muscle vessels. Our recent findings however indicate, that coronary NO sensitivity is increased in animals kept on HFD nutrition. This may suggest that there are such adaptive mechanisms located in the wall of coronary capillaries, which activation might have a role in preserved vasodilatatory response of coronary arterioles.

Interestingly, in accordance with our findings, more papers were recently released in this topic, which stated that in experimentally induced obesity, coronary artery vasodilatation can be preserved or even enhanced. These results indicate, that in case of obesity and insulin resistance, certain but yet not known adaptive mechanisms can be activated to compensate the damaged vasodilatation and maintain proper myocardial perfusion. Since endothelium derived NO has a crucial role in maintaining coronary arteriole luminal diameter, in the lack of proper compensatory mechanisms, blood flow would decrease. Therefore, our goal was to identify those certain adaptive processes that can maintain agonist-induced vasodilatory responses of the coronary arterioles. Since we obtained significantly greater vasodilatory response in HFD coronary arterioles, using direct NO donors, like sodium nitroprusside and NONO-ate, we might conclude, that in HFD coronary microvessels, sensitivity of arteriolar smooth muscle to NO is increased. Further investigations revealed that there was no significant difference in the direct cGMP dependent vasodilatation between the two groups, since the cell-permeable, stable cGMP analogue, the 8-bromo-cGMP induced vasodilatation was equal in both groups. On this basis, we concluded that the reason for the increased NO sensitivity may be the increased production of cGMP. These conclusions were confirmed by the fact, that in the presence of soluble guanilate-cyclase inhibitor ODQ, sodium-nitropusside and NONO-ate induced vasodilatation did not differ in the two groups. Hence, inhibition of

sGC decreased both NO donor induced vasodilatation to the same level, diminishing the difference between the two groups we experienced without inhibitor. The reason for the increased production of cGMP could be the overexpression of soluble guanilate cyclase, thus we measured the amount of soluble guanilate cyclase ß1 protein with Western immunoblott. These measurements however, did not reveal any difference in the expression of sGC ß1 subunit between the two animal groups. In conclusion, our results showing no changes in the protein expression of the sGC ß1-subunit, along with the findings that 8-bromo-cGMP-evoked coronary dilations were similar in lean and obese rats, suggest a primary role for an enhanced activity of sGC enzyme in the coronary arterioles of obese rats.

Our recent results are in accordance with the ones of Brandes et al. (Hypertension 2000; 35:231-236), who showed compensatory increase of soluble guanilate cyclase in the absence of endothelial NO synthesis, in eNOS knockout mice. Mechanisms, however, responsible for the increased activity of soluble guanilate cyclase are not fully clarified yet.

In summary, the findings of our experimental study suggest that in coronary arterioles of high-fat diet-induced obese rats, an increased activity of sGC leads to the enhanced sensitivity of smooth muscle cells to NO, a mechanism that may contribute to preserved coronary arteriolar dilations. We suggest that this mechanism could contribute to the early adaptation of coronary arterioles for providing adequate blood flow to meet the enhanced metabolic demand due to obesity.

6. Summary

Main objects of my experiments were as follows: 1) to examine the effect of 6-month transdermal nitrate treatment on the diastolic and systolic left ventricular function following myocardial infarction, and 2) to examine the effects of nitrates and nitrogen oxide (NO) donors on the coronary microvessel vasodilatory response in an experimental animal model of obesity.

On the basis of my investigations, the following novel conclusions may be drawn.

Long-term nitrate treatment following myocardial infarction:

1) significantly improved left ventricular systolic function measured by echocardiography;

2) significantly increased systolic vena pulmonary flow;

3) significantly reduced vena pulmonary diastolic flow and atrial reverse flow;

4) significantly reduced the level of ST-depression on 6-week's ergometry test.

According to our results, we are convinced that the appropriateness of nitrate therapy following myocardial infarction is justified (even in patients with no complaints).

In overweight animals kept on high fat diet we have found that: 5) sensitivity of coronary micro-vessels to nitrate donors and to NO was increased. 6) The increase in sensitivity was associated with the enhanced activity of vascular smooth muscle soluble guanilate cyclase. We hypothesize, that these mechanisms might have a role in maintaining coronary microcirculation in obesity. Moreover, the increased nitrate sensitivity of coronary vessels might explain the beneficial cardiovascular effects of nitrate therapy in obesity and in other pathological conditions (however, further investigations are still required to confirm these findings).

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