Experimental Medicine

The Role of Rosuvastatin in the Correction of Dyslipoproteinemia and Endothelial Dysfunction in Patients with Stable Angina

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ABSTRACT. Despite great achievements of modern medicine, cardiovascular pathologies (CVD) still remain the leading cause of mortality in many countries of the world. Endothelial dysfunction and dyslipidemia are one of the main reasons of coronary artery sclerosis.

The aim of the present work was to study the hypolipidemic effect of lipid-correcting preparation-Rosuvastatin (Crestor), and its endothelium dysfunction controlling effect in patients with stable angina pectoris.

We studied 69 patients (46 males/23 females, age range 43-61 years, mean age 56±5.2 years) with stable angina pectoris. Mean indices of T-C, HDL-C, LDL-C, TG and Endothelin-1 (ET-1) were: 270.46±26.69 mg/dl, 33.42±3.21 mg/dl, 183.06±22.4 mg/dl, 274.84±33.74 mg/dl and 0.62±0.12, respectively. Patients were treated with 10 mg Rosuvastatin once daily for 2 weeks everyday and then day after day for 3 months. Besides routine examinations, flow-mediated vasodilatation of brachial artery, according to the changes of its diameter after 2-min occlusion, was performed prior to and 24-h post Rosuvastatin administration.

Analyses of the data obtained revealed statistically significant difference between the results obtained pre- and post-treatment initiation (2 weeks and 3-month treatment course): for T-C, HDL-C, LDL-C, TG and ET-1 post-treatment levels are: 152.74±19.94 mg/dl, 35.46±5.84 mg/dl, 87.28±18.04 mg/dl, 162.08±19.63 mg/dl ($P<0.001$) and 0.55±0.05 ($P<0.01$), respectively. Statistically significant difference was revealed in flow-mediated vasodilatation degree of brachial artery, between pre- (7.171±0.71 %) and post-treatment (10.20±0.96 %) indices, ($P=0.18$).

Data obtained indicate that 2-week administration of Rosuvastatin (10 mg/daily) has a significant clinical and lipid-modifying effect, and does not cause statin-specific negative effect even after 3-month treatment course. Both single administration and 2-week treatment with Rosuvastatin decreases levels of ET-1 in peripheral blood. 24-h post single dose administration we observed increase in the degree of velocity of flow-mediated vasodilatation of brachial artery, this increase was statistically significant and registered in all the patients. *© 2008 Bull. Georg. Natl. Acad. Sci.*

Key words: *dyslipidemia, endothelial dysfunction, flow-mediated vasodilatation.*

Introduction

Despite great achievements of modern medicine, cardiovascular pathologies (CVD) still remain the leading cause of mortality in many countries of the world. According to the data published by the WHO 1/3 of deaths is caused by CVD (17 million of people die annually) [1].

The leading risk factor for developing CVD is elevated level of cholesterol in blood; 60-70% of total cholesterol in blood is transported by low-density lipoprotein cholesterol (LDL-CH). Data of clinical trials indicate that decrease in LDL-CH levels significantly reduces the risk of CVD development and this reduction is statistically significant [2]. This statement is the basis of modern recommendations for the treatment of pathologies

related to atherosclerosis. According to modern clinical recommendations [3] the levels of LDL-CH in patients with CAD must not exceed 2.6 mmol/l. Based on the results of HPS (Heart Protection Study) and PROVE IT (Pravastatin or Atorvastatin Evaluation and Infection Trial). Cholesterol National American Educational Program, together with American Association of Cardiology and American College of Cardiology recommended lower target levels of LDL-CH-<1.8mmol/l. If lifestyle modification does not provide target levels of LDL-CH in patients with cardiovascular pathologies, statins must be administered. Positive effects of statins in CVD is determined by various mechanisms [4]. Despite the fact that decrease in LDL-CH, due to inhibition of reduction in 3-hydroxy-3-methylglutarin coenzyme A-reductase, is thought to be the main mechanism of action of the preparations belonging to this class, it is still not possible to explain various effects only by its hypolipidemic qualities [5]. Decrease in endothelin-1 (ET-1) expression is achieved via influence on vascular endotheliocytes. There are also data on the inhibiting effect of statins on thrombocyte aggregation [6] and monocyte adhesion to the endothelium surface and their anti-oxidative [7] and anti-inflammatory effect, which is the most pronounced in their effect on C-reactive proteins. It is thought that some playotropic effects may be revealed before hypolipidemic effect develops, and the mentioned effect is associated more strongly with the reduction of blood lipids [8].

Endothelial dysfunction and dyslipidemia are one of the main reasons of coronary artery sclerosis [9-11]. It is well known that oxidative stress [12] plays an important role in the development of endothelial dysfunction and atherosclerosis.

One of the major factors responsible for endothelial dysfunction appears to be a reduced stability of the endothelial enzyme generating NO, endothelial nitric oxide synthesis (eNOS). Statins prevent hypoxia-induced down-regulation of eNOS in human endothelial cells by stabilizing eNOS mRNA, leading to an increase in NO production by these cells [13,14]. Although impaired endothelial NO activity is a key variable in the development of endothelial dysfunction and atherosclerosis in several authors' opinion [15], vascular reactivity is very complex and involves many substances besides NO. Indeed, normal vascular tone and changes of vascular diameter are dependent on a fine balance between vasodilating and vasoconstricting substances. One of the important vasoconstrictor regulators of the vasculature is the peptide ET-1 which is synthesized in endothelial cells. Increased tissue ET-1-like immunoreactivity was found inactive coronary atherosclerotic plaques indicating that this peptide might contribute to the exaggerated vasoconstriction associated with acute coronary syndromes [16]. On the other hand, circulating ET-1 levels were found to be elevated in patients with both advanced atherosclerosis and coronary endothelial dysfunction. The importance of ET-1 in the very early stages of coronary atherosclerosis is highlighted by several studies demonstrating that endothelin receptor antagonists prevent the development of endothelial dysfunction in experimental hypercholesterolemia and hypertensive humans [17]. In the performed experiment (mice), vascular ET-1 production is inhibited by endothelium-derived NO [18].Thus, according to the results, statins may attenuate ET-1 synthesis indirectly by increasing NO bio-availability. On the other hand, in 1998 [19], it was demonstrated that both atorvastatin and simvastatin reduce the synthesis of ET-1 in aortic endothelial cells in vitro. In several trials it is demonstrated that the observed effect of statins on ET-1 synthesis is independent of their impact on NO bioavailability, supporting the hypothesis that the inhibition of isoprenoid synthesis is the main mechanism of action involved in the regulation of ET-1 production by statins. Given the involvement of ET-1 in the development of endothelial dysfunction and the growing evidence for a contribution of ET-1 to various cardiovascular disease states [20-23], these findings suggest a possible role for statins in the therapy of disease states associated with elevated ET-1 levels [24-27].

Today, when a large scale of statin preparations is available, it is important to select them individually, taking into consideration their tolerability, safety and dose-dependent effectiveness. Only such conditions could guarantee safety of the drug, when it should be administered for a substantial time.

The aim of the present work was to study the hypolipidemic effect of lipid-modifying preparation-Rosuvastatin (Crestor), on patients with stable angina pectoris, when it was administered for a short time (2 weeks), every day and every second day for a 3-month period; to assess undesirable side effects of Rosuvastatin, specific for statins, and its endothelium dysfunction controlling effect in patients with stable angina pectoris.

To achieve the aims stated above, the following objectives were set: To evaluate the hypolipidemic effect of 2-week and 3-month dose-dependent therapy with Rosuvastatin in patients with stable angina pectoris, using clinical and laboratory data.To determine endothelin-1 concentrations in peripheral blood of patients with stable angina pectoris prior to, 24-h post Rosuvastatin administration and post therapeutic course. To assess the degree of brachial artery flow-mediated vasodilatation in patients with stable angina pectoris prior to and post treatment (24-h post Rosuvastatin administration). To determine endothelin-1 concentrations in peripheral blood of healthy volunteers (Control group) prior to and post treatment (24-h post Rosuvastatin ad-

ministration). To reveal correlation between the concentration of endothelin-1 in peripheral blood and degree of brachial artery flow-mediated vasodilatation.To study the degree of brachial artery flow-mediated vasodilatation in healthy volunteers (Control group) prior to and 24-h post administration of 10 mg Rosuvastatin.

Design and Methods of the Study: 69 patients (46 males/23 females, age range 43-61 years, mean age 56±5.2 years) and 19 healthy volunteers (19 males/7 females, age range 44-63 years, mean age 51±7 years) were enrolled in the study. Patients were subdivided into 2 groups (Gr): Gr.1 – patients with stable angina pectoris, I-II f.c; Gr.2- healthy volunteers. In total, 69 patients with stable angina pectoris, I-II f.c (46 males/23 females, age range 43-61 rears, mean age 56±5.2 years) were enrolled in the study (Table 2). They comprised Gr.1. Coronary heart disease (CHD), post-MI cardiosclerosis were registered in 13 patients. Echocardiography and X-ray examinations revealed heart enlargement, ejection fraction 48%-40% (mean EF 45±4.1%). On ECG of all patients with post-MI cardiosclerosis Q-wave was registered. In all the cases post-MI dysfunctions were associated with angina of effort, I-II f.c. Arterial hypertension (AH) was observed in 27 of patients selected (AH, I-II stage, GNC VII). Fifteen patients had type 2 diabetes mellitus (DM) in anamnesis. Ventricular extra systoles Lawn II-III grade were registered in 15 cases. In 2 cases tachycardia episodes were observed. Angioplasty was performed in 1 case. Disease duration in the study group was 1 to 6 years (mean 29±0.9 years). Healthy volunteers (Gr.2, n=19, 12 males/17 females, age range 44-63 years, mean age 51±7 years) felt practically healthy and had no signs of any pathologic process at the moment of inclusion. Echocardiography and X-ray examinations did not reveal heart enlargement, EF was not < 55% (mean EF 60±3.1%). Indices of lipid profile were within the normal range. Gr. 1 patients together with non-medicamentous treatment, were treated with B-blockers, peripheral vasodilators, Ca-antagonists, anti-aggregates and Rosuvastatin.

Gr. 1 patients were separated into 2 sub-groups (Sgr.): Sgr. 1a, (n=35) - the patients were treated with 10 mg of Rosuvastatin once daily for 3 months.

Sgr. 1b (n=34) - the patients were treated with 10 mg of Rosuvastatin once daily for 2 weeks (to achieve target levels), then the drug was administered every second day for the following 3 months (Table 3).

Clinical and laboratory data were assessed prior to and post (2 weeks and 3 months) treatment initiation.

Gr.1 - in 22 patients (who did not get peripheral vasodilator, Ca-antagonists and B-blockers 2 weeks prior to the inclusion in the study) degree of brachial artery flow-mediated vasodilatation was measured after 2-min occlusion of the artery according to the changes in the artery diameter (difference between the brachial artery diameter before and after ischemia (D2-D1)=Δ abc, divided by the initial diameter of the artery (D1), Δ%=Δ abc/D1).

These measurements were performed before the treatment with Rosuvastatin and 24-h post administration of the single dose of the drug. Endothelin-1 (ET-1) concentrations in peripheral blood were measured simultaneously.

Patients' histories were collected at the hospital, complaints were evaluated. ECG, echocardiography and X-ray examinations; clinical, diagnostic and laboratory tests and investigations were performed.

Recommendations of the Canadian Association of Cardiologists were used for diagnosis of coronary artery disease.

Beside routine examinations, ET-1 concentrations in peripheral blood were measured using immune-enzyme method (ELISA). Tests were performed at the Immunological Laboratory "Test".

The study was based on double-blind, observation trial principles. The study was carried out by 3 participants–lab. assistant and a person who processed the data achieved, had no information on the goals of the study, its awaited outcomes and study participants, the third participant assessed the condition (disease), while having no information on the results of immunophenotyping of peripheral blood.

Assessment of the Lipid Profile: Blood samples were obtained after 12-h fasting period before dietary treatment was initiated and one month post its initiation; samples were also obtained 2 weeks and 3 months post therapy on-set. TCH and TG were measured by the enzyme method, LDL-CH levels were calculated using Fridwald's equation: LDL-CH= TCH -(TG/5+HDL-CH). HDL-CH levels were determined using lipoprotein fraction precipitation; atherogenity index was calculated using Klimov's equation: AI=(TCH-HDL-CH)/HDL-CH.

Assessment of Endothelin-1 Concentration: Immune-enzyme analyzer (Cayman Diagnostics) was used to measure concentrations of Endothelin-1 in peripheral blood. Multi-scan immune-enzyme reader (Labsystem, Finland) was used to register the results obtained. Three persons participated in the study–lab. assistant, operator and a person who assessed the disease, using the recommendations of the Canadian Association of Cardiologists.

Assessment of the Brachial Artery Endothelial Function: Assessment of the brachial artery endothelial function was performed using reactive hyperemic test, with standard methods of Celermajer. After patient's 10-minute horizontal disposition, the location of brachial artery was performed by a transducer of 5 mghc frequency (TOSHIBA SSH-140A Ultrasonography System), along the piece, 2-15cm from the internal surface of the elbow, in triplex regime (B-regime, colored carting of the

flow, spectral analysis of frequency). Brachial artery basic diameter and the maximal velocity of the arterial blood-flow were measured before occlusion. Then sphygmomanometer mange was attached at the upper third of the shoulder and after having measured arterial blood pressure, to induce enhanced blood-flow, 2-minute occlusion of the artery was performed with 50mmHG more pressure than the systolic one. As soon as air was released from the mange, blood-flow velocity was measured during 15 seconds (reactive hyperemic phase) in the artery, and brachial artery diameter was measured during the following 60 seconds. Brachial artery diameter was measured from the front wall of the artery adventitia/media boundary to the back wall media /adventitia boundary, very near to the anatomic marker, selected by the investigator. Average value of three measurements was accounted. Endothelium-dependent vasodilatation was assessed by percent changes of diameter to the basic data: $(D_2\text{-}D_1)$ /D_1 X100%, where D_1 and D_2 are brachial artery diameters before and after occlusion, respectively. The normal value of endothelium-dependent vasodilatation was considered 8-11%.

Evaluation of the results: The study revealed that 2-week administration of Rosuvastatin at daily dose of 10 mg did not cause negative statin-specific side-effects, no toxic liver dysfunction was registered. Proteinuria was not observed either, patients did not complain of taste changes and/or pathologic processes of gastro-intestinal origin, etc.

Two-week administration of Rosuvastatin to patients with stable angina pectoris and dyslipoproteinemia resulted in statistically significant decrease in TCH, LDL-CH, TG and increase in HDL-CH levels. The same results were observed after relatively long (3 months) administration of Rosuvastatin. Clinical data of the patients post 3-months treatment improved (ECG, reduction in angina episodes and obligatory dose of peripheral vasodilators in patients who were receiving the drug.)

The study showed that administration of 10 mg Rosuvastatin daily is effective in patients with stable angina pectoris and results in improvement of clinical parameters and indices of lipid metabolism. When Rosuvastatin was administered every second day (at a dose 10 mg) no lipid modifying effect of the drug was achieved.

Peptide Endothelin-1 (ET-1), synthesized by endothelial cells, supports intensification of vasoconstriction, which is associated with an acute coronary syndrome. On the other hand, increased circulatory levels of ET-1 were observed in acute atherosclerosis, as well as in coronary endothelium dysfunction. The role of ET-1 at early stages of coronary atherosclerosis is well-documented in different studies.The results of our study revealed that there was statistically significant difference in ET-1 levels in peripheral blood between patients with stable angina, I-II f.c and healthy volunteers. These values were higher in Gr. 1 patients (stable angina) than in Gr. 2 (Controls).

The study showed that Rosuvastatin reduces cocentrations of ET-1 in peripheral blood and this decline is statistically significant. Such effect was observed both after 2-week therapeutic course and single (14h) administration of the drug. This may be explained by the effect of Rosuvastatin on NO.

It is important to stress that a single dose of Rosuvastatin (24h post drug administration) increases the degree of velocity of flow-mediated vasodilatation of brachial artery and this increase is statistically significant. These changes were observed in all the patients, besides, we registered statistically significant decrease in ET-1 24-h post drug administration though, the results obtained in the study, do not reveal any correlation between ET-1 levels in peripheral blood and the degree of flow-mediated vasodilatation of brachial artery and blood flow velocity, these changes cannot be explained only by the shifts in ET-1 concentrations, and the mechanism of the changes is more complex and multifactorial.

Conclusions: Data obtained indicate that 2-week administration of Rosuvastatin (10 mg/daily) has significant clinical and lipid-modifying effects, and does not cause statin-specific negative effect even after 3-month treatment course. Both single administration and 2-week treatment with Rosuvastatin decreases levels of ET-1 in peripheral blood. 24-h post single dose administration we observed increase in the degree of velocity of flow-mediated vasodilation of brachial artery, this increase was statistically significant and registered in all the patients. In patients with stable angina pectoris (Georgian population) ET-1 concentrations in peripheral blood do not correlate with the degree of velocity of flow-mediated vasodilation of brachial artery. Administration of Rosuvastatin every second day at a dose of 10 mg causes quantitative increase in lipid metabolism components, when compared to 2 week (10 mg/daily) treatment with the drug, though this difference was not statistically significant.

ექსპერიმენტული მედიცინა

როზუვასტატინის როლი დისლიპოპროტეინემიისა და ენდოთელიუმის დისფუნქციის კორექციაში სტაბილური სტენოკარდიის დროს

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მიუხედავად მედიცინაში არსებული მიღწევებისა, გულ-სისხლძარღვთა სისტემის დაავადებები მაინც რჩება სიკვდილის ძირითად მიზეზად მსოფლიოს მრავალ ქვეყანაში. კორონაროსკლეროზის ძირითად მიზეზს ენდოთელური დისფუნქცია და დისლიპიდემია წარმოადგენს. სტატიის ავტორთა კვლევის მიზანს წარმოადგენდა ლიპიდმაკორეგირებელი პრეპარატის — როზუვასტატინის (კრესტორი) ჰიპოლიპიდემიური და ენდოთელიუმის დისფუნქციის მაკორეგირებელი ეფექტის შესწავლა სტაბილური სტენოკარდიის მქონე პაციენტებში. ავტორებმა შეისწავლეს სტაბილური სტენოკარდიის მქონე 69 პაციენტი (46 მამაკაცი/23 ქალი, ავადმყოფთა საშუალო ასაკი შეადგენდა 56±5.2 წელს). სქ, მსლქ, დსლქ, ტგ და ენდოთელინ-1-ის საშუალო მაჩვენებლები შესაბამისად იყო: 270.46±26.69 მგ/დლ, 33.42±3.21 მგ/დლ, 183.06±22.4 მგ/დლ, 274.84±33.74 მგ/დლ და 0.62±0.12; პაციენტები იღებდნენ 10 მგ როზუვასტატინს ერთხელ დღეში ყოველდღე 2 კვირის განმავლობაში და შემდეგ დღეგამოშვებით 3 თვის მანძილზე. გარდა რუტინული კვლევებისა, ხდებოდა მხრის არტერიის ნაკადდამოკიდებული ვაზოდილატაციის ხარისხის განსაზღვრა 2 წთ-იანი ოკლუზიის შემდგომ ცვლილებების მიხედვით, როზუვასტატინის მიღებამდე და მიღებიდან 24 სთ-ის შემდეგ. მიღებული შედეგების დამუშავების შედეგად გამოვლინდა სტატისტიკურად სარწმუნო განსხვავება მკურნალობამდე და მკურნალობის შემდეგ მიღებულ მონაცემებს შორის როგორც 2-კვირიანი, ასევე 3-თვიანი დღეგამოშვებითი მკურნალობის ფონზე. მკურნალობის შედეგად სქ, მსლქ, დსლქ, ტგ და ენდოთელინ-1-ის დონე შესაბამისად შეადგენდა: 152.74±19.94 მგ/დლ, 35.46±5.84 მგ/დლ, 87.28±18.04 მგ/დლ, 162.08±19.63 მგ/დლ (P<0.001) და 0.55±0.05 (P<0.01). სტატისტიკურად სარწმუნო იყო ასევე მხრის არტერიის ნაკადდამოკიდებული ვაზოდილატაციის ხარისხის ცვლილება პრეპარატის მიღებამდე (7.171±0.71 %) და მიღების შემდეგ (10.20±0.96 %),(P=0.18). ჩატარებული კვლევის შედეგებმა გვიჩვენა, რომ სტაბილური სტენოკარდიის პაციენტებში (I-II ფ.კ.) როზუვასტატინს ყოველდღიური 2-კვირიანი მიღებისას (10 მგ 24 საათში) გააჩნია მნიშვნელოვანი კლინიკური და ლიპიდმაკორეგირებელი ეფექტი და არ იწვევს სტატინებისათვის დამახასიათებელ უარყოფით ეფექტებს 3-თვიანი მკურნალობის შემდეგაც. როგორც პრეპარატის ერთჯერადი, ასევე 2-კვირიანი მიღების შემდეგ ჩატარებული კვლევის შედეგებმა აჩვენა, რომ როზუვასტატინი ამცირებს პერიფერიულ სისხლში ენდოთელინ-1-ის კონცენტრაციას. როზუვასტატინის ერთჯერადი მიღების შემდეგ (პრეპერატის მიღებიდან 24-სთ-ის შემდეგ) ადგილი ჰქონდა მხრის არტერიის ნაკადდამოკიდებული ვაზოდილატაციის ხარისხისა და სიჩქარის სტატისტიკურად სარწმუნო ზრდას, რასაც სტაბილური სტენოკარდიის მქონე პაციენტებისათვის დიდი კლინიკური მნიშვნელობა აქვს.

REFERENCES

1. *S.H. Grundy, B. Cleeman, et al.* (2004), Circulation, **110**: 227-239.
2. *J. Shepherd, S.M. Cobbe, I. Ford, et al.* (1995), N. Engl. J. Med., **333**: 1301-1307.
3. *G. De Backer, E. Ambrosioni* (2002), Eur. Heart J., **24**: 1601-10.
4. *N. Kipshidze, Sh. Chumburidze* (2001), Achievements and Problems of Int. Medicine, **II**: 13-23.
5. *N. Kipshidze, Sh. Chumburidze* (2000), Georgian Medical News: 18-24.
6. *G. Dangas, J.J. Badimon, D.A. Smith et al.* (1999), J. Amer. Coll. Cardiol., **33**: 1294-1304.
7. *C. Cardilo, U. Campia, Ch. Rilcoyne, et al.* (2002), *Circulation*, **105**: 452–456.
8. *O. Hernandez-Perera, D. Perez-Sala, E. Soria, et al.* (2000), J. Clin. Invest., **101**: 2711–19.
9. *W. Castelli* (1996), Lipids. – Atherosclerosis, **124**: Suppl: S1-S9.
10. *C.P. Canon, E. Braunwald, C.H. McCabe et al.* (2004), N. Engl. J. Med., **350**: 1495-1504.
11. *D. S. Celermajer* (1997), J. Amer. Coll. Cardiol., **30**: 325–333.
12. *C. Napoli, L. O. Lerman* (2001), Mayo Clin. Proc., **76**: 619–631.
13. *U. Laufs, V.L. Fata, J.K. Liao* (1998), Circulation, **97**: 1129–1135.
14. *A. Brouet, P. Sonveaux, C. Dessy, et al.* (2001), Circ. Res., **89**: 866–873.
15. *C. Napoli, L.J. Ignarro* (2001), *Coron Artery Dis.*, **9**: 257–264.
16. *A.M. Zeiher, H. Goebel, V. Schächinger, et al.* (1995), Circulation, **91**: 941–947.
17. *P.J.M. Best, C.J. McKenna, D. Hasdai, D.R. Jr. Holmes, A. Lerman* (1999), Arterioscler. Thromb. Vasc. Biol., **19**: 2769–75.
18. *C. Boulanger, T.F. Lüscher* (1990), J. Clin. Invest., **85**: 587–590.
19. *R. Wever, E. Stroes, T.J. Rabelink* (1998), Atherosclerosis, **137** (Suppl): S51–S60.
20. *M. Barton, W. Kiowski* (2001), Curr. Hypertens. Rep., **3**: 322–330.
21. *M. Barton, C.C. Haudenschild, L.V. D'Uscio, et al.* (1998), Proc. Natl. Acad. Sci. USA, **95**: 14367–14372.
22. *P.J.M. Best, C.J. McKenna, D. Hasdai, D.R. Jr Holmes, A. Lerman* (1999), Circulation, **99**: 1747–1752.
23. *A. Bobik, A. Grooms, J.A. Millar, A. Mitchell, S. Grinpukel* (1990), Amer. J. Physiol., **258** (3 pt. 1): C408–C415.
24. *S. Dimmeler et al.* (2001), J. Clin. Invest., **108**:391, 397. doi:1172/JCI200113152.
25. *M. Endres, U. Laufs, Z.H. Huang, et al.*(1998), Proc. Natl. Acad. Sci. USA, **95**: 8880–8885.
26. *O. Feron, C. Dessy, J.P. Desager, et al.* (2001), Circulation, **103**:113–118.
27. *M. Fukunaga, T. Yura, K.F. Badr* (1995), J. Cardiovasc. Pharmacol., **26** (suppl 3): S51–S52.

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