Vasodilatory Effect and Endothelial Integrity in Papaverine- and Milrinone-Treated Human Radial Arteries

P. Rudzinski1*, P. Wegrzyn1*, G.J. Lis3, J. Platek1, J. Konstanty-Kalandyk1, R. Nosalski1, T. Mikolajczyk2, M. Jasinska1, G. Pyka-Fosciak3, T. Guzik1, J.A. Litwin3, R. Korbut3, J. Sadowski1

1Department of Cardiovascular Surgery and Transplantology, John Paul II Hospital, Jagiellonian University Medical College, Cracow, Poland; 2Department of Internal and Agricultural Medicine, Jagiellonian University Medical College, Cracow, Poland; 3Department of Histology, Jagiellonian University Medical College, Cracow, Poland; 4Department of Pharmacology, Jagiellonian University Medical College, Cracow, Poland

Prevention of the vasospasm is an important aspect of coronary artery bypass grafting (CABG) with the use of radial artery (RA) as the conduit. We compared the effect of two phosphodiesterase inhibitors papaverine and milrinone on vasodilation and endothelial integrity of human RA segments harvested from 20 CABG patients. Vasodilatory effect of the drugs were assessed by organ bath technique in RA rings precontracted with KCl and phenylephrine. Endothelial integrity was evaluated by CD34 immunofluorescence in frozen sections. Vasorelaxation induced by papaverine was significantly greater as compared to that induced by milrinone (90.47%±10.16% vs. 78.98%±19.56%, p<0.05). Similarly, pretreatment with papaverine more strongly inhibited the contractile response of RA rings to KCl (6.0±8.0 mN vs. 26.7±21.5 mN, p<0.001). Papaverine was also superior to milrinone in the preservation of endothelial integrity (75.3%±12.9% vs. 51.8%±18.0%, p<0.02). In conclusion, papaverine seems to be more suitable than milrinone for prevention of vasospasm in radial artery conduits used for CABG.

Key words: coronary artery bypass grafting, radial artery, vasospasm, papaverine, milrinone, endothelium

*Authors equally contributed to the study
The organ bath experiments were carried out according to the method described previously (10, 11). In short, the organ chamber was filled with 5 ml of the Krebs-Henseleit buffer, containing 120 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO\textsubscript{4}, 1.2 mM KH\textsubscript{2}PO\textsubscript{4}, 2.5 mM CaCl\textsubscript{2}, 25 mM NaHCO\textsubscript{3}, and 5.5 mM glucose, at 37°C. The vessel rings mounted between two hooks were equilibrated for one hour and then passively strained to the baseline value of 20 mN (stabilization). The rings were precontracted with 20 mM KCl (concentration used for cardioplegy during CABG) followed by increasing concentrations (10\textsuperscript{-9} to 10\textsuperscript{-2}M) of phenylephrine (PE). Next, the rings were immersed in solutions of milrinone (Corotrope, Sanofi-Aventis, 0.4 mg/ml) or papaverine (papaverinum hydrochloricum, Polfa, 1 mg/ml) and the vasodilatory effect was measured after 10, 20, 30, 40, 50 and 60 min. This was followed by another treatment with KCl for 10 min to assess the spasm-preventing effect of the studied vasodilators. Stabilization of the rings at 20 mN in the buffer was performed before each treatment to keep the constant baseline of the measurements. The measurements were recorded using AcomiPC (Siemens, Germany) software. The organ bath procedure is illustrated in Fig. 1.

**Table 1. Clinical characteristics of patients.**

<table>
<thead>
<tr>
<th>Characteristics of the examined patients</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63.05±8.65</td>
</tr>
<tr>
<td>Male</td>
<td>17 (85%)</td>
</tr>
<tr>
<td>Female</td>
<td>3 (15%)</td>
</tr>
<tr>
<td>Atherosclerosis risk factors: smoking</td>
<td>6 (30%)</td>
</tr>
<tr>
<td>arterial hypertension</td>
<td>10 (50%)</td>
</tr>
<tr>
<td>diabetes mellitus</td>
<td>8 (40%)</td>
</tr>
<tr>
<td>myocardial infarction</td>
<td>13 (65%)</td>
</tr>
<tr>
<td>mean blood pressure [mmHg]</td>
<td>135.3±11.1</td>
</tr>
<tr>
<td>body mass index [kg/m\textsuperscript{2}]</td>
<td>28.87±3.94</td>
</tr>
<tr>
<td>CCS scale: I</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>10 (50%)</td>
</tr>
<tr>
<td>III</td>
<td>9 (45%)</td>
</tr>
<tr>
<td>IV</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>5 (25%)</td>
</tr>
</tbody>
</table>

The arterial rings were immersed for 20 min in PBS (controls) or in solutions of papaverine or milrinone at the same concentrations as those used in organ bath experiments and fixed for 24 hours in 4% buffered formalin. They were next thoroughly washed with PBS and frozen. The influence of vasodilators on endothelial integrity was studied by immunofluorescence in 10 µm thick cryostat sections. Each ring was serially sectioned and every 20\textsuperscript{th} section was used for immunohistochecmical examination. After preincubation with 5% normal goat serum for 40 min, sections were incubated overnight with endothelial cell marker, mouse monoclonal anti-CD34 antibody (dilution 1:50; Novocastra, Newcastle, UK) in a humid chamber at room temperature. Next, sections were washed extensively in PBS and incubated for 90 min with goat anti-mouse Cy-3-conjugated antibody (dilution 1:400; Jackson IR, West Grave, PA). Cell nuclei were counterstained with DAPI (Sigma, Saint Louis, MO, USA). Sections were washed three times in PBS and mounted in glycerol/PBS solution (pH=8.6). Negative controls were performed by omitting the primary antibodies during the first incubation.

Sections were examined under Olympus BX50 light/fluorescence microscope. Images were recorded using DP-71 digital CCD camera (Olympus, Japan) coupled to IBM P-class computer equipped with AnalySIS-FIVE\textsuperscript{®} (Soft Imaging System GmbH, Munster, Germany) image analysis system. The endothelial integrity was expressed as the mean percentage of the lumen perimeter immunopositive for CD34, calculated from measurements carried out in at least five sections of each sample.

**Statistical analysis**

All results obtained by organ bath method were expressed as means±S.D. For comparison of vasodilatory effect of papaverine and milrinone, Student’s t-test and Mann-Whitney test were used. Results of endothelial integrity measurements were expressed as means±S.D. and analysed using Student’s t-test. P values <0.05 were considered significant.

**RESULTS**

The vasorelaxation of precontracted radial arteries during incubation in the examined drugs is shown in Fig. 2. The mean...
vasorelaxation was stronger in vessel rings incubated in papaverine than in milrinone. After 30 min of incubation the difference reached statistical significance (papaverine: 90.47±10.16%, milrinone: 78.98±19.56%, p<0.05) which was maintained for further 20 min (Fig. 2). After 50 min vasorelaxation induced by papaverine and milrinone was 97.48±3.29% and 91.15±13.62%, respectively, p<0.05. Similarly, papaverine more effectively inhibited vasoconstriction induced by KCl (6.0±8.0 mN for papaverine vs. 26.7±21.5 mN for milrinone; p<0.001, Fig. 3).

Control (untreated) segments of RA showed complete endothelial lining. The endothelial integrity was better preserved after treatment of RA rings with papaverine (75.3±12.9%) as compared with milrinone (51.8±18.0%, p<0.02) (Fig. 4).

DISCUSSION

The efficient reperfusion of cardiac muscle during the first 24 hours after surgery is critical for successful coronary artery bypass grafting. Hence, the grafted vessel should not undergo vasospasm and moreover, it should remain resistant to vasoconstrictive agents (e.g. KCl) administered to patient in this period. Several vessel types: internal thoracic artery, radial artery, gastroepiploic artery, inferior epigastric artery and saphenous vein are used as conduits (12). The radial artery is easily accessible, but it should be pretreated with vasodilator agents because of its tendency to vasospasm. The agents employed include nitrates and their derivatives, calcium channel blockers, alpha-blockers and phosphodiesterase inhibitors (3, 13). The representatives of the latter group, papaverine and milrinone have been tested in various experimental and clinical setups, but there is no clear agreement as to which one is better in terms of vasodilatory effect and preservation of endothelial lining.

Our results show that papaverine exerts a stronger vasodilatory effect on radial artery segments and also more effectively inhibits their contractile response to KCl and phenylephrine. Although milrinone has been found to be a potent vasodilator of human radial artery (6, 7), its efficacy was compared with that of papaverine only in case of internal thoracic
artery, where it was a more effective vasodilator than papaverine (14). This discrepancy can result from different response of internal thoracic artery and radial artery to phosphodiesterase inhibitors. It has been demonstrated that human arteries from different regions of the body: gastroepiploic arteries, internal thoracic arteries and radial arteries show different sensitivity to phosphodiesterase-3 inhibitors including milrinone. It can be attributed to differences in affinities of the inhibitors for various PDE-3 isoforms present in vascular myocytes (15, 16).

The grafted bypass conduit should retain relatively intact endothelial lining to prevent blood clotting which could lead to decreased patency or occlusion of the vessel. Moreover, endothelium-derived nitric oxide (NO), one of the most potent vasodilators, contributes to the regulation of the vascular tone and the bioavailability of graft endothelial NO seems to be important for graft patency and clinical outcome (17). In this context, effect of vasodilatory agents used for preventing vasospasm on endothelial integrity of the radial artery is another important factor to be considered. Several studies demonstrated papaverine-induced endothelial damage revealed by morphological observations under scanning electron microscope (18) and by functional assays of endothelium-dependent relaxation (19, 20). In our experiments papaverine better preserved the endothelial integrity of radial artery than milrinone, although partial endothelial denudation was observed after pretreatment with both agents. It certainly has a clinical significance, since impaired endothelial function lowers duration of the graft (21), moreover, endothelium-dependent relaxation in the radial artery is superior to that observed in other conduits, such as internal thoracic artery and saphenous vein (22). The damaging effect of milrinone on vascular endothelium has not been studied before and its vasorelaxant activity is endothelium-independent, since removal of the endothelium did not affect vasodilatory effect of this agent (11). It seems that milrinone induces even more endothelial injury than papaverine, but this observation should be confirmed by assessment of endothelial function in milrinone-treated vessels. The endothelium damaging effect seems to be an unfavorable property of PDE inhibitors studied, since other vasodilator agents, such as calcium blockers nicardipine and verapamil as well as irreversible alpha-adrenoreceptor antagonist phenoxbyzenzamine, used in combination with nitroglycerin, have been reported to effectively protect the endothelial function of the grafted radial artery (4, 18, 23).

In conclusion, vasodilatory efficacy of the examined agents and their effect on endothelial integrity show that papaverine seems to be more suitable than milrinone for prevention of vasospasm in radial artery conduits used for coronary artery bypass grafting. However, from the clinical point of view it should be mentioned that short duration of papaverine action limits its use to intraluminal injection during the perioperative period, whereas milrinone exerts a beneficial vasodilatory and inotropic effects also when employed postoperatively in long-term infusion (24).

Acknowledgements: The study was supported by statutory grant K/ZDS/000962 from the Jagiellonian University Medical College to P.R. and by a Welcome 02/2009 grant from the Foundation for Polish Science.

Conflict of interests: None declared.

REFERENCES


Received: April 12 2012
Accepted: January 24, 2013

Author’s address: Dr. Piotr Wegrzyn, Department of Cardiovascular Surgery and Transplantology, Jagiellonian University Medical College, 80 Pradnicka Street, 31-202 Cracow, Poland.

E-mail: peter-wegrzyn@o2.pl