Detrimental effects of papaverine on the human internal thoracic artery

Yu-Jing Gao, MD^a
Homer Yang, MD^a
Kevin Teoh, MD^b
Robert M. K. W. Lee, PhD^a

Teoh, Yang, Gao, Lee (left to right)

Objective: We sought to determine the effects of papaverine on human and canine internal thoracic artery function and structure.

Methods: Vascular function was assessed with wire myography, and apoptosis was examined with confocal microscopy in arteries stained with ApopTag.

Results: Acetylcholine-induced endothelium-dependent relaxation in phenylephrine-precontracted arteries was significantly impaired by papaverine treatment in both human and canine internal thoracic arteries (maximal relaxation: $68.35\% \pm 7.13\%$ vs $47.5\% \pm 9.32\%$ in human arteries and $74.8\% \pm 5.5\%$ vs $34.3\% \pm 8.5\%$ in canine arteries) but not by incubation with acidified saline solution (pH 3.9, which is equivalent to the pH of 10^{-2} mol/L papaverine solution) in canine internal thoracic arteries. Contraction of human internal thoracic arteries to phenylephrine or to U46619 was not significantly affected by papaverine treatment and neither was the contraction of canine internal thoracic arteries to phenylephrine. Total apoptotic endothelial and smooth muscle cells were significantly greater in papaverine-treated human and canine internal thoracic arteries.

Conclusions: Papaverine impairs endothelial function and triggers apoptosis of endothelial and smooth muscle cells of human and canine internal thoracic arteries. The long-term consequence of this impairment on vascular function is not known. Until this question is answered, it will be prudent to use other vasodilators that are less damaging to the internal thoracic artery for cardiac surgery.

From the Departments of Anaesthesia^a and Surgery,^b McMaster University, Hamilton, Ontario, Canada.

Supported by a grant-in-aid from the Heart and Stroke Foundation of Ontario to R. Lee (T 4414).

Received for publication April 26, 2002; revisions requested July 8, 2002; revisions received Sept. 12, 2002; accepted for publication Oct 17, 2002.

Address for reprints: Robert M. K. W. Lee, PhD, Department of Anaesthesia (HSC-2U3), McMaster University, 1200 Main S West, Hamilton, Ontario, Canada, L8N 3Z5 (E-mail: rmklee@mcmaster.ca).

J Thorac Cardiovasc Surg 2003;126:179-85

Copyright © 2003 by The American Association for Thoracic Surgery

0022-5223/2003 \$30.00 + 0

doi:10.1016/S0022-5223(03)00040-0

Coronary artery bypass grafting (CABG) is widely accepted for myocardial revascularization of patients with extensive coronary heart disease.¹ The internal thoracic artery (ITA) is a better graft conduit than veins because of its long-term survival and rare atherosclerosis.^{2,3} An analysis of hospital discharge data across Canada between 1992 and 1995⁴ and in Ontario from 1981 to 1995⁵ consistently found a decrease in mortality after CABG. This is despite the fact that there was a steady increase in the mean age of patients undergoing the procedure, with more of these patients having other coexisting conditions, such as acute myocardial infarction, pulmonary disease, and diabetes.⁵

One problem associated with the use of ITAs in CABG is the occurrence of perioperative vasospasm, which might result in myocardial infarction and postoperative morbidity.⁶ Intraoperatively, it is noted that the ITA might be in spasm during or after dissection. The release of various vasoconstricting substances during CABG, which might include sympathetic amines (epinephrine and norepinephrine) and thromboxane A₂, and a decrease in the levels of vasodilators, such as endothelium-derived relaxing factor, atrial natriuretic factor, bradykinin, and prostaglandin E₂,⁷ have been suggested to be among the contributing factors in the genesis of

Figure 1. Effects of papaverine treatment on the relaxation effects of acetylcholine (ACh) on human (A) and canine (B) ITAs and the influence of acidified saline (pH 3.9) on acetylcholine-induced relaxation in canine ITAs (C). * $P < .05$, ** $P < .01$ versus respective control (human: $n = 4$ for control and $n = 12$ for treated; dog: $n = 3$ for control and $n = 5$ for treated).

the vasospasm of the graft artery, but the real cause of this vasospasm is poorly understood.

Various vasodilators have been used to prevent vasospasm during CABG. Papaverine, a benzyloisoquinoline alkaloid, is a widely used agent well known for its nonselective smooth muscle relaxant action. Although the efficacy of papaverine for preventing graft spasm has been reported,^{8,9} some studies have found that papaverine caused cultured saphenous vein endothelial cells to lose their viability,¹⁰ caused degeneration of smooth muscle cells of rat cerebral arteries,¹¹ or abolished the endothelium-dependent relax-

ation response to acetylcholine.¹² These reports suggest that papaverine might have detrimental effects on vascular tissues, but the limitations of these studies are that the nature of papaverine-induced endothelial injury has not been characterized, and the cause of vascular damage caused by papaverine solution remains unclear. One report has attributed the damage to the low pH of papaverine solution.¹³

In our laboratory we previously found that in cultured endothelial and vascular smooth muscle cells, papaverine induced apoptosis, but the low-pH solution did not.¹⁴ However, whether similar events occur in vivo during CABG is not clear. Experiments with cultured cells might be limited by the change in phenotype, reactivity, or both of these cells during passage in an artificial culture environment. Such changes might make these cells more vulnerable to papaverine. The purpose of the present study is to investigate the effects of papaverine on human ITAs from the standpoints of vascular function and apoptosis. Canine ITAs were also used to supplement the studies on human ITAs. We report here that papaverine impairs ITA endothelial function and induces apoptosis of human ITA endothelial and smooth muscle cells.

Methods

Human ITA segments were obtained from patients undergoing CABG. Consent from each patient was obtained before the operation, and we followed the institutional guidelines on the use of human tissues. From each patient, a piece of the terminal segment, which is generally discarded during operations, was collected in oxygenated physiologic salt solution (PSS) with the following composition: NaCl, 119 mmol/L; KCl, 4.7 mmol/L; $MgSO_4 \cdot 7H_2O$, 1.17 mmol/L; KH_2PO_4 , 1.18 mmol/L; N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid, 8 mmol/L; $NaHCO_3$, 25 mmol/L; glucose, 11.1 mmol/L; and $CaCl_2$, 2.5 mmol/L, with antibiotic-antimycotic at 4°C. Care was taken to ensure minimal handling of the ITA during the operation and on subsequent transportation to minimize damage. The terminal segments of the ITAs were dissected with the connective tissues attached, and the ITAs were handled by using the connective tissues. The ITAs were transported to our laboratory within 60 minutes on ice. Most of the ITAs were used the same day. Three of these segments (2 treated and 1 control segment) were stored in the oxygenated PSS at 4°C and used the following morning. Maximum storage time was 16 hours. We did not notice any difference in response between ITAs that were stored overnight versus those that were studied the same day. This is consistent with our findings in rat mesenteric arteries, in which we found that these arteries can be stored for up to 48 hours without affecting their functional responses to agonists.

The time between papaverine application and the collection of the graft ranged from 15 to 45 minutes, depending on the complexity of the operation. Papaverine was diluted in Plasmalyte and used at a concentration of 1.7 mmol/L (pH 6.17) or diluted in normal saline and used at a concentration of 17.0 mmol/L (pH 3.83). Papaverine was applied in ambient temperature on the adventitial side of ITAs with either a soaked gauze or by spreading the solution with a 23-gauge needle. In some cases of CABG, a small piece of the distal segment of the ITA was obtained before the surgeon applied the

Figure 2. Effects of papaverine treatment on phenylephrine-induced contraction in human (A) and canine (B) ITAs (human: $n = 4$ for control and $n = 11$ for treated; dog: $n = 4$ for control and $n = 4$ for treated).

vasodilator, and this served as our control vessel. The experimenters were blinded to the type of papaverine treatment to the ITA, so that any effects of papaverine on the ITA function and morphology were correlated after the experiments were done.

Functional Studies

After removal of the connective tissues, the ITA segments were cut into 4- to 5-mm-long rings. A wire myograph system was used to study the contraction and relaxation of the ITA. Generally, 2 to 4 rings were obtained from each ITA segment, and each ring was used to test one agent, so that in the analyses, n represents the number of patients from which the samples were obtained. The resting tension was set at 4 g. We have established in our preliminary experiments that this is the optimal tension for the human

Figure 3. Effects of papaverine treatment on U46619-induced contraction of human ITA (A) and contraction of canine ITA to U46619 (B) (human: $n = 3$ for control and $n = 14$ for treated; dog: $n = 4$).

ITA. After equilibration for at least 60 minutes, the ITA was challenged with 100 mmol/L KCl twice at an interval of 30 minutes. Endothelium-dependent relaxation to acetylcholine was assessed in phenylephrine (10^{-6} mol/L to 3×10^{-6} mol/L)-precontracted vessels (approximately 80% of the maximal contraction) and was expressed as a percentage of the maximal relaxation by using sodium nitroprusside (3×10^{-3} mol/L). The contractile properties of the ITA to phenylephrine and U46619 were expressed as a percentage of KCl (100 mmol/L)-induced contraction.

Canine ITAs were obtained from mixed-breed pound-source dogs. The care of the animals was in accordance with the guidelines of the Canadian Council on Animal Care. ITAs were obtained from the dogs after achievement of sodium pentobarbital anesthesia. Papaverine solution or acidified saline or normal saline solution was injected into the lumen of the vessel, with all branches and both ends of the vessel tied off with surgical sutures to prevent leakage from the vessel. After 1 hour of incubation at room temperature, the vessels were washed twice in PSS with the ties at the 2 ends loosened and then cut into rings 4 to 5 mm in length for functional studies.

Figure 4. Confocal images of human ITA stained with the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling assay: **A** and **D**, ITA treated with papaverine; **B** and **E**, control arteries; **C** and **F**, positive control treated with DNase I. Apoptotic endothelial and smooth muscle cells appear as bright dots. **LM**, Lumen of the vessel.

Detection of Apoptotic Cells in the ITA

Segments of the ITA were prepared in sterilized conditions and then incubated with cell culture media (Dulbecco's modified Eagle's medium; Gibco BRL, Life Technologies, Inc, Rockville, Md) supplemented with 10% fetal bovine serum, 2.5 $\mu\text{g/mL}$ fungizone, 50 $\mu\text{g/mL}$ gentamicin, 2 mmol/L glutamine, and 0.5 mmol/L N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid in a humidified incubator (37°C, 5% carbon dioxide, and 95% air). Twenty-four hours after incubation, the ITA segments were fixed with 4% neutral buffered formaldehyde for 1 hour under a consistent pressure of 160 mm Hg for human ITAs and 80 mm Hg for canine ITAs at room temperature. The application of pressure facilitated quantification of apoptotic cells by ameliorating folding of endothelial cells. To detect the apoptotic vascular smooth muscle cells, digestion with elastase (40 U/mL) and collagenase (300 U/mL) for 1 hour or more was carried out to facilitate the penetration of the reagents into the vessel wall. Apoptotic cells were stained with the ApopTag in situ apoptosis detection kit, with some modifications,¹⁵ and viewed with a confocal microscope (Carl Zeiss LSM). Arteries treated with DNase I (15 KU/mL for 30 minutes) to induce DNA fragmentation were used as positive controls.

Quantification and Statistical Analysis

A confocal microscope was used to quantify the number of apoptotic cells. Optical cross-sections of the ITA at a thickness of 10 μm were cut. The images were analyzed by using Sicon Image software (National Institutes of Health).¹⁶ The total number of

apoptotic endothelial cells was calculated from an area of $2.5 \times 10^4 \mu\text{m}^2$ for both human and canine ITAs. The number of apoptotic smooth muscle cells in the vessel was counted in a volume of $0.5 \times 10^7 \mu\text{m}^3$ for human ITAs and $7 \times 10^7 \mu\text{m}^3$ for canine ITAs. The values are expressed as means $\pm$ SEM. In the contractility study the concentration causing 50% of the maximal response (EC_{50}) was estimated by fitting each concentration-response curve. Statistical analyses were performed with SigmaStat software (SPSS, Inc). Two-way repeated-measures analysis of variance was used to determine any significance between the treatment and control groups. Post hoc unpaired *t* tests were used to identify the concentrations at which statistically significant differences occurred.

Chemicals

Acetylcholine, DNase I, phenylephrine, papaverine hydrochloride, sodium nitroprusside, and U46619 were purchased from Sigma Chemical Co; ApopTag plus the fluorescein indirect immunofluorescence kit was purchased from Intergen Co; and Dulbecco's modified Eagle's medium was purchased from Gibco BRL.

Results

Effects of Papaverine on Endothelium-Dependent Relaxation

Endothelium-dependent relaxation was tested with acetylcholine in phenylephrine-precontracted vessel rings. As shown in Figure 1, human ITAs treated with papaverine

Figure 5. Confocal images of canine ITA stained with the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling assay: **A and D**, ITA treated with papaverine; **B and E**, control arteries; **C and F**, positive control treated with DNase I. Apoptotic endothelial and smooth muscle cells appear as bright dots. **LM**, Lumen of the vessel.

(1.7–17.0 mmol/L) during grafting and canine ITAs incubated with papaverine (10 mmol/L) showed significantly less relaxation to acetylcholine: the maximal relaxation (control vs treated) values were $68.35\% \pm 7.13\%$ versus $47.5\% \pm 9.32\%$ in human ITAs and $74.8\% \pm 5.5\%$ versus $34.3\% \pm 8.5\%$ in canine ITAs. There was no difference between ITAs from human subjects and dogs in their response to acetylcholine with or without papaverine treatment. Acidified saline (pH 3.9) did not affect the relaxation of canine ITA to acetylcholine significantly (maximal relaxation: $81.1\% \pm 7.3\%$ for control and $74.8\% \pm 7.4\%$ for acidified saline-treated ITAs). We did not detect any significant difference among the human ITAs as a result of the different methods used to apply papaverine during CABG.

Effects of Papaverine on Smooth Muscle Contractility

Phenylephrine contracted human and canine ITA in a concentration-dependent manner with EC_{50} s of $1.15 \pm 0.19 \mu\text{mol/L}$ ($n = 4$) and $0.85 \pm 0.13 \mu\text{mol/L}$ ($n = 4$), respectively. Human and canine ITAs treated with papaverine showed similar EC_{50} s ($5.1 \pm 0.19 \mu\text{mol/L}$ [$n = 11$] and $1.01 \pm 0.18 \mu\text{mol/L}$ [$n = 4$]) and percentage contractions to phenylephrine as those of the control vessels (Figure 2).

U46619, a thromboxane A₂/prostaglandin H₂ receptor agonist, dose-dependently contracted human ITAs in the range of 3×10^{-10} mol/L to 10^{-6} mol/L with an EC_{50} of 5.02 ± 2.2 nmol/L ($n = 3$). Papaverine treatment did not significantly affect the EC_{50} (4.4 ± 1.1 nmol/L, $n = 14$) and the percentage of contraction to U46619 in human ITAs (Figure 3, A). Canine ITAs were less responsive to U46619 than human ITAs (Figure 3, B).

Assessment of Apoptotic Endothelial and Smooth Muscle Cells

In control arteries only a few apoptotic cells were detected in some samples. There was no difference between control ITAs from human subjects and dogs in the number of apoptotic cells in the endothelium and smooth muscle cells. Human or canine ITAs treated with papaverine showed a significantly higher incidence of apoptotic endothelial and smooth muscle cells than their respective control segments (Figures 4–6).

Discussion

The main purpose of this study was to determine whether the use of papaverine during CABG affects endothelial and smooth muscle function, induces apoptosis in human ITA

A: Human

B: Dog

Figure 6. Summary of papaverine-induced apoptosis in human (n = 4 for control, n = 9 for treated) and canine (n = 4 for control, n = 6 for treated) ITAs. **P* < .05, *P* < .01 versus respective control.**

grafts, or both. We found that papaverine impairs endothelium-dependent relaxation and induced prominent apoptosis of endothelial and smooth muscle cells. The impaired endothelium-dependent relaxation might be related to the induction of apoptosis in endothelial cells.

The effects of papaverine on endothelium function have been studied in human subjects, but the reports were inconsistent. Hillier and colleagues¹⁷ found that intraluminal papaverine treatment during CABG did not impair endothelium-dependent relaxation, whereas Schyvens and associates¹² found that papaverine treatment abolished endothelium-dependent relaxation of the graft artery. In the human radial artery He¹⁸ also found that papaverine abolished acetylcholine-induced relaxation. In this study we found that human ITAs treated with papaverine showed an

attenuated relaxation response to acetylcholine compared with that of untreated control vessels. A similar result was obtained with canine ITAs when 10^{-2} mol/L papaverine was administered by means of intraluminal injection. Therefore, in contrast with previous findings, our results showed that papaverine attenuated but did not abolish endothelium-dependent relaxation of human ITAs.

One previous study has suggested that the acidity of papaverine solution might be the cause of damage to vascular cells.¹³ Papaverine hydrochloride is dissolvable only in acidic condition (pH 3.9, 10^{-2} mol/L), and it is known that extracellular acidity lowers intracellular pH, as shown in polymorphonuclear leukocytes.¹⁹ However, our results with canine ITAs showed that treatment with acidified saline solution, which is of the same pH as papaverine solution, did not affect acetylcholine-induced relaxation. Therefore, it could be concluded that papaverine itself, and not the low pH of its solution, induces endothelial cell damage.

The mechanism for this endothelial function impairment by papaverine might be the genesis of apoptosis of the endothelial cells. Consistent with what we have found in our previous study with cultured cells,¹⁴ here we found that papaverine induced a prominent genesis of apoptosis in the endothelial cells of human and canine ITAs. It is well known that endothelium contributes to the regulation of vascular tone by releasing vasorelaxing agents, such as nitric oxide, prostacyclin, or constrictor agents, such as endothelin. Because cells that undergo apoptosis will cease their normal function and will either detach from the vessel or be engulfed by neighboring cells, the initiation of apoptosis will affect the function of the endothelium. Papaverine might induce apoptosis (1) by increasing the intracellular level of cyclic adenosine monophosphate by slowing down or inhibiting the decomposition of cyclic adenosine monophosphate,²⁰⁻²² (2) by acting as a calcium antagonist,^{23,24} and (3) interrupting mitochondrial function.²⁵

The effect of papaverine on vessel contractility was also examined in this study. We elected to use phenylephrine and U46619 as agonists to simulate sympathetic nerve activation and local release of thromboxane A2. In human ITAs U46619 is a stronger agonist than phenylephrine both in potency and sensitivity, whereas in canine ITAs contraction to phenylephrine was stronger than that to U46619. This is probably a result of species difference. Surprisingly, despite the presence of apoptotic smooth muscle cells caused by papaverine treatment in both human and canine ITAs, the contractility of the ITA to these agonists was not affected. A study by Chester and coworkers²⁶ using intraluminal administration of papaverine also found that papaverine did not affect the contractility of the ITA. The influence of papaverine on smooth muscle contractility is different from that on the endothelium, where an association could be made between an impairment of endothelium-dependent

relaxation response and the presence of apoptotic cells caused by papaverine treatment. This difference might be related to the relative abundance of smooth muscle cells in a vessel wall compared with the endothelial cells, which consist of only one cell layer, so that the functional consequence of the damage is more immediate and obvious with endothelial cells than with smooth muscle cells. The contractility of smooth muscles to some circulating contractile agents, including norepinephrine, might increase as a result of the loss of endothelial cells.^{27,28} Furthermore, phagocytosis of apoptotic endothelial cells by their neighbors²⁹ could expose smooth muscle cells directly to vasoactive agents in the blood stream because of the loss of the endothelial protective barrier.³⁰ Therefore, the overall contractile response will be affected by both endothelial dysfunction and smooth muscle cell apoptosis, depending on which is the prevailing factor. The long-term consequence of the damage to smooth muscle cells in relation to vessel wall function is unknown and remains to be studied. However, because the maintenance of the integrity of vascular cells is important for their functions, it is essential that we seek out other vasodilators that are less damaging to the ITA for cardiac surgery. The use of verapamil plus nitroglycerin solution is one of the possible alternatives.¹⁸

In summary, we found that papaverine impairs endothelial function and induces apoptosis of endothelial and smooth muscle cells of human and canine ITAs. Because the integrity of graft tissue is important for its function, we suggest that it will be prudent to use other vasodilators that are less damaging to the ITA for cardiac surgery.

We thank Ms Mary Helen Blackall for her assistance in the collection of the ITAs from surgical patients; Drs L. Abouzahr, I. Cybulsky, A. Lamy, L. Semelhago, and J. Gunstensen for providing us with the ITAs obtained during their cardiac operations; Ms S. Stead for her technical assistance; and Dr E. E. Daniel for the use of his equipment.

References

1. Kurlansky PA, Dorman MJ, Galbut DL, Moreno NL, Traad EA, Carrillo RG, et al. Bilateral internal mammary artery grafting in women: a 21-year experience. *Ann Thorac Surg.* 1996;62:63-9.
2. Lytle BW, Loop FD, Cosgrove DM, Ratliff NB, Easley K, Taylor PC. Long-term (5 to 12 years) serial studies of internal mammary artery and saphenous vein coronary grafts. *J Thorac Cardiovasc Surg.* 1985; 89:248-58.
3. Loop FD, Lytle BW, Cosgrove DM, Stewart RW, Goormastic M, Williams GW, et al. Influence of the internal mammary graft on 10 year survival and other cardiac events. *N Engl J Med.* 1986;314:1-6.
4. Ghali WA, Quan H, Brant R. Coronary artery bypass grafting in Canada: national and provincial mortality trends, 1992-1995. *CMAJ.* 1998;159:25-31.
5. Tu JV, Wu KY. The improving outcomes of coronary artery bypass graft surgery in Ontario, 1981 to 1995. *CMAJ.* 1998;159:221-7.
6. Sasson L, Cohen AJ, Hauptman E, Schachner A. Effect of topical vasodilators on internal mammary arteries. *Ann Thorac Surg.* 1995; 59:494-6.
7. Downing SW, Edmunds LHJ. Release of vasoactive substances during cardiopulmonary bypass. *Ann Thorac Surg.* 1992;54:1236-43 [see comments].
8. Vilandt J, Kjaergard H, Aggestrup S, Andreassen JJ, Olesen A. Intraluminal papaverine with pH 3 doubles blood flow in the internal mammary artery. *Scand Cardiovasc J.* 1999;33:330-2.
9. Dregelid E, Heldal K, Resch F, Stangeland L, Breivik K, Svendsen E. Dilation of the internal mammary artery by external and intraluminal papaverine application. *J Thorac Cardiovasc Surg.* 1995;110:697-703.
10. Rubens FD, Labow RS, Meek E, Bedard E, Gill IS, Dudani AK, et al. Papaverine solutions cause loss of viability of endothelial cells. *J Cardiovasc Surg (Torino).* 1998;39:193-9.
11. Yoshimura S, Hashimoto N, Goto Y, Sampei K, Tsukahara T, Iihara K. Intraarterial infusion of high-concentration papaverine damages cerebral arteries in rats. *Am J Neuroradiol.* 1996;17:1891-4.
12. Schyvens CG, Owe-Young RA, Spratt PM, Mundy JA, Farnsworth AE, Macdonald PS. Papaverine abolishes endothelium-dependent dilatation of human internal mammary arteries in vitro. *Clin Exp Pharmacol Physiol.* 1997;24(3-4):223-8.
13. Cooper GJ, Gillot T, Parry EA, Kennedy A, Wilkinson GA. Papaverine injures the endothelium of the internal mammary artery. *Cardiovasc Surg.* 1995;3:553-5.
14. Gao YJ, Stead S, Lee RMKW. Papaverine induces apoptosis in vascular endothelial and smooth muscle cells. *Life Sci.* 2002;70:2675-85.
15. Dickhout JG, Lee RM. Apoptosis in the muscular arteries from young spontaneously hypertensive rats. *J Hypertens.* 1999;17:1413-9.
16. Dickhout JG, Lee RMKW. Increased medial smooth muscle cell length is responsible for vascular hypertrophy in young hypertensive rats. *Am J Physiol.* 2000;279:H2085-94.
17. Hillier C, Watt PA, Spyt TJ, Thurston H. Contraction and relaxation of human internal mammary artery after intraluminal administration of papaverine. *Ann Thorac Surg.* 1992;53:1033-7.
18. He G-W. Verapamil plus nitroglycerin solution maximally preserves endothelial function of the radial artery: comparison with papaverine solution. *J Thorac Cardiovasc Surg.* 1998;115:1321-7.
19. Leblebicioglu B, Walters J. Alkaline conditions accelerate polymorphonuclear leukocyte apoptosis in vitro. *Infect Immun.* 1999;67:2019-21.
20. Beavo JA. Cyclic nucleotide phosphodiesterases: functional implication of multiple isoforms. *Physiol Rev.* 1995;75:725-47.
21. Leszczynski D, Zhao Y, Luokkamaki M, Foegh ML. Apoptosis of vascular smooth muscle cells. Protein kinase C and oncoprotein Bcl-2 are involved in regulation of apoptosis in non-transformed rat vascular smooth muscle cells. *Am J Pathol.* 1994;145:1265-70.
22. Hamet P, Richard L, Dam TV, Teiger E, Orlov SN, Gaboury L, et al. Apoptosis in target organ of hypertension. *Hypertension.* 1995;26:642-8.
23. Kimura M, Kobayashi S, Tadano K, Kimura I. Effects of papaverine on the relative distribution of ⁴⁵Ca ion in subcellular fractions of dog biliary muscles. *Biochem Pharmacol.* 1984;33:281-6.
24. Martikainen P, Isaacs J. Role of calcium in the programmed death of rat prostatic glandular cells. *Prostate.* 1990;17:175-87.
25. Morikawa N, Nakagawa-Hattori Y, Mizuno Y. Effects of dopamine, dimethoxyphenylethylamine, papaverine, and related compounds on mitochondrial respiration and complex I activity. *J Neurochem.* 1996; 66:1174-81.
26. Chester AH, Panda R, Borland JAA, Tadjkarimi S, Schyns CJ, O'Neil GS, et al. Effects of mode of application of papaverine on the contractile response of the internal mammary artery. *Br J Surg.* 1994;81:527-31.
27. Chamiot-Clerc P, Renaud JF, Safar ME. Pulse pressure, aortic activity, and endothelium dysfunction in old hypertensive rats. *Hypertension.* 2001;37:313-21.
28. Karlsson C, Bodelsson G, Bodelsson M, Stjemquist M. Endothelium-derived prostanoids reduce 5-hydroxytryptamine-induced contraction in human uterine artery. *Hum Reprod.* 1998;13:1947-51.
29. Milligan CE, Schwartz LM. Programmed cell death during animal development. *Br Med Bull.* 1997;53:570-90.
30. Walia M, Sormaz L, Samson SE, Lee RM, Grover AK. Effects of hydrogen peroxide on pig coronary artery endothelium. *Eur J Pharmacol.* 2000;400(2-3):249-53.