Aprotinin impairs endothelium-dependent relaxation in rat aorta and inhibits nitric oxide release from rat coronary endothelial cells

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Abstract

Objective: Aprotinin, a non-specific serine protease inhibitor, reduces postoperative bleeding after coronary artery surgery. The mechanism of action for this ‘blood-sparing’ effect of aprotinin is only partially clarified. We therefore aimed to investigate the effect of aprotinin on the release of nitric oxide (NO), a vasodilator and antiaggregant factor, from rat coronary microvascular endothelial cells and on the NO-mediated endothelium-dependent relaxation of rat thoracic aorta. Methods: Endothelium-intact and endothelium-denuded thoracic aortic rings from Wistar rats (250–300 g) were suspended in organ chambers. Contractile and relaxant responses in the absence and presence of aprotinin (125, 250 and 500 KIU/ml) were recorded via a mechanotransducer. Coronary microvascular endothelial cells (CMEC) were isolated on a Langendorff system by collagenase perfusion of the hearts from the same rats. Calcium ionophore- (1 μM) induced release of NO from confluent cells was determined spectrophotometrically by measuring its stable metabolites, nitrite and nitrate, via Griess reaction. Results: Aprotinin selectively enhanced phenylephrine-induced contractions in endothelium-intact rat thoracic aortic rings, but not in the endothelium-denuded rings. The use of a nitric oxide synthesis inhibitor Nω-nitro-L-arginine methyl ester (100 μM) on endothelium-intact rings produced a similar increase in phenylephrine-induced contractions. KCl-induced contractions remained unaltered. Aprotinin inhibited acetylcholine-, calcium ionophore- and L-arginine-induced endothelium-dependent relaxations, but not sodium nitroprusside-induced endothelium-independent relaxation. Aprotinin had no significant effect on basal nitrite–nitrate release from CMEC, while it inhibited calcium ionophore-induced total nitrate accumulation in the supernatants. Conclusion: Aprotinin selectively impairs endothelium-dependent relaxation as well as basal NO availability in rat thoracic aortic rings and inhibits NO release from rat CMEC. This effect of the drug may contribute to its ‘blood-sparing’ action and may also account for the increase in perioperative restenosis risk observed in clinical practice during aprotinin therapy. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Arteries; Cell culture/isolation; Endothelial function; Nitric oxide; Vasoconstriction/dilation

1. Introduction

Aprotinin, a non-specific serine protease inhibitor, is currently used to reduce blood loss and blood need for transfusion after cardiac operations requiring cardiopulmonary bypass [1]. The mechanism of action for the observed ‘blood-sparing’ effect of aprotinin has only been partially clarified. Recently, a meta-analysis of clinical trials with aprotinin has shown that the drug decreases mortality, the need for rethoracotomy and the proportion of patients requiring blood transfusion [2]. Although the efficacy of aprotinin as a haemostatic agent is indisputable, its safety has been a matter of debate. Indeed, it has been reported that aprotinin might increase the risk of graft thrombosis and subsequently the incidence of perioperative myocardial infarction [3]. There have been some experimental observations suggesting that aprotinin may increase tendency to thrombosis in vitro. Indeed, it has been shown that aprotinin alters the ratio of 6-keto-prostaglandin F1α–tromboxane B2 and increases the production of...
von Willebrand factor in cultured human umbilical vein endothelial cells, leading to increased platelet aggregation with a tendency to plug particularly the small vessels [4,5]. The implication of these in vitro studies is that perioperative infarction during aprotinin treatment may occur not only through thrombosis of the bypass graft but also through thrombotic occlusion of poorly perfused areas of coronary microcirculation.

Endothelial cells play an important role in the regulation of normal microcirculation by releasing several vasoactive factors including nitric oxide (NO). NO is generated by the enzymatic activities of a group of enzymes called NO synthases (NOSs). Three main isoforms of NO have so far been described; the constitutive isoforms NOS-I and NOS-III, and the inducible isoform NOS-II (for a recent review, see Ref. [6]). It has been suggested that the production of NO is significantly increased during and after cardiopulmonary bypass [7]. The systemic endotoxemia that occurs with the institution of cardiopulmonary bypass is a potent stimulus for the release of proinflammatory cytokines, which induces NOS-II expression and production of constitutive NO is rather limited. A recent report has shown that aprotinin inhibits both NOS-I and NOS-II in whole rat brain homogenates; hence it is accepted as the first competitive protein inhibitor of NOS activity [13]. The goal of this study was therefore to investigate the potential effects of aprotinin on the in vitro release of constitutive NO in aortic rings and coronary microvascular endothelial cells (CMEC) isolated from healthy rats by vascular reactivity experiments and Griess reaction, respectively.

2. Methods

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the Animal Ethics Committee of Ege University, Izmir, Turkey. Hearts and thoracic aortas of male Wistar rats (250–300 g) were excised under light ether anaesthesia. The hearts were used for CMEC isolation, while the thoracic aortas of the same animals were used for vascular reactivity experiments.

2.1. Vascular reactivity studies

The aorta was cut into four 3–4-mm wide rings. In some rings, the endothelium was removed mechanically by inserting a small forceps into the lumen and gently rolling. In each experiment, endothelium-intact and endothelium-denuded rings (two of each) were suspended horizontally under a resting tension of 2.0 g in 30-ml organ chambers containing Krebs solution of the following composition in mM: NaCl, 118.30; KCl, 4.70; MgSO$_4$, 1.20; KH$_2$PO$_4$, 1.22; CaCl$_2$, 2.50; NaHCO$_3$, 25.00; glucose, 11.10 (pH 7.4 at 37°C, gassed with carbogen). Each ring was connected to a force displacement transducer for the measurement of isometric force, which was continuously displayed and recorded on-line on a computer via an eight-channel transducer data acquisition system.

After initial equilibration period of 60–90 min, ordinary Krebs solution was replaced with fresh Krebs solution containing 1 μM indomethacin to exclude relaxant effects that may be generated by prostanoids. All experiments were performed in the latter solution.

Endothelium-denuded rings were used to assess the contractile responses. The absence of endothelium was confirmed by the absence of relaxations to acetylcholine (ACh) (1 μM) in rings precontracted with a submaximal concentration of phenylephrine (PE) (0.3–1 μM). Contractile responses were generated by addition of either incremental concentrations of PE (0.003–30 μM) or a single concentration of KCl (40 mM). The Krebs solution contained 40 mM KCl, and was prepared by the equimolar replacement of NaCl by KCl.

Endothelium-intact rings were used for assessing endothelium-dependent and endothelium-independent relaxant responses. The rings were precontracted with PE (0.3–1 μM) and relaxations to ACh (0.003–30 μM), calcium ionophore A23187 (A23187) (0.003–3 μM), L-arginine (0.3–1000 μM), d-arginine (0.3–1000 μM) or sodium nitroprusside (SNP) (0.001–3 μM) were studied. KCl (40 mM) depolarization was also recorded on the endothelium-intact rings.

Both the endothelium-intact and endothelium-denuded rings were incubated with three different concentrations of aprotinin (125, 250 and 500 KIU/ml) for 15 min. The aprotinin used in this study was the commercially available form of the drug (Trasylol©, Bayer) which contained 1.4 mg/ml (10 000 KIU/ml) of the active compound in sterile 0.9% NaCl. To ensure that vasomotor responses obtained were not a direct consequence of NaCl overloading, the concentration of NaCl was increased in control rings to the same levels of those incubated with aprotinin. Hence, the final approximate concentrations of NaCl corresponding 125, 250 and 500 KIU/ml aprotinin application were 0.011, 0.023 and 0.045%, respectively.
Finally, in a series of experiments, PE concentration–response curves were generated in endothelium-intact rings in response to successive incubation with NaCl, aprotinin alone and aprotinin plus Nω-nitro-L-arginine methyl ester (L-NAME) (100 μM) to inhibit NOS-III activity.

2.2. Cell culture

CMEC were isolated as previously described [14]. Briefly, the hearts were mounted and perfused retrogradely on a constant-flow Langendorff system with 0.04% collagenase. The ventricles were chopped and collagenase digestion was quenched by the addition of bovine serum albumin to the perfusate. CMEC were obtained by sedimentation of myocytes and incubated in 0.01% trypsin at 37°C for the prevention of non-endothelial cell attachment. Cells were then activated by washing in calcium and suspended in Medium 199 supplemented with L-glutamine, fetal calf serum and antibiotic/antifungal agents. Cell suspensions were plated and incubated at 37°C under 5% CO₂. After 1-h incubation, unattached cells were washed off with saline and remaining cells were cultured to confluence. Cultured cells formed confluent monolayers with typical ‘cobblestone’ morphology within 5–7 days. For further culture, cells were trypsinized and subcultured.

Cells have previously been characterised as endothelial by the uptake of fluorescently labelled acetylated LDL [14]. The endothelial cells obtained using this method may include some endocardial endothelial cells, as well as some endothelial cells from larger coronary vessels. However, the statistical average (more than 90%) represents predominantly microvascular endothelial cells since the vast majority of cardiac endothelial cells are found in small vessels and capillary bed. Therefore, the number of endocardial endothelial cells would be negligible (0.0002%) [14].

Cells between passages 3 and 5 were used in the study. On the day of study, the cells were washed with Krebs-HEPES solution of the following composition in mM: NaCl 99.00, KCl 4.69, CaCl₂ 1.87, MgSO₄ 1.20, NaHCO₃ 25.00, K₂HPO₄ 1.20, NaHEPES 20.00 and glucose 11.10 (pH 7.4). Some of the cells were then incubated with A23187 (1 μM) for 1 h at 37°C in Krebs solution to stimulate NO release, while others were left untreated to serve as controls to determine basal NO release. To investigate the effect of aprotinin on NO release, CMEC were treated with aprotinin (125, 250 and 500 KIU/ml) for 16 h. The control cells incubated with 0.045% NaCl served as controls.

2.3. Measurement of nitrite/nitrate

NO release from cultured CMEC was determined by measuring the accumulation of its stable degradation products, nitrite and nitrate. Nitrate was detected after reduction to nitrite using nitrate reductase [15]. Briefly, the supernatant from each flask was collected after the incubation period and centrifuged to remove cells and particles. Nitrate was reduced to nitrite by equilibrating 100 μl of samples (supernatants and standards) in sodium phosphate buffer (pH 7.5) containing FAD (0.02 mM), NADPH (0.5 mM) and nitrate reductase (0.1 U/ml) in a final volume of 510 μl at room temperature for 90 min. Sodium nitrate (2–50 μM) dissolved in Krebs-HEPES solution was used as the standard. Total nitrite was then determined spectro-photometrically using the Griess reaction. The amount of total nitrite in each sample was calculated by linear regression using the absorbance of the sodium nitrate standards each day. The amount of total cellular protein in the respective flasks was determined by Lowry’s method after lysis with a buffer containing SDS 0.1% in 10 mM Tris, pH 7.4 [16]. Total nitrite accumulated in each flask was defined as μM per mg of protein in the corresponding flask.

2.4. Drugs

Aprotinin (Trasylol®) was a generous gift of Bayer (Turkey). The following drugs were from Sigma (USA): PE, ACh, A23187, L-arginine, D-arginine, SNP, indomethacin, collagenase, trypsin, bovine serum albumin, and sodium nitrate. KCl was from Merck (Germany). All cell culture materials were from Gibco BRL (Life Technologies, UK).

2.5. Statistical analysis

Results were expressed as mean±S.E.M. n numbers given throughout the paper denote the number of aortic rings isolated from different rats in vascular reactivity studies and the number of flasks used in cell culture studies. Concentration–response curves were fitted by non-linear regression with simplex algorithm, and Eₘₐₓ and pD₂ (−log EC₅₀) values were calculated using the software of the data acquisition system. Contractile responses were expressed as milligram tension developed, while relaxant responses were given as the percentages of PE precontraction. Comparisons of concentration–response curves were evaluated by two-way analysis of variance (ANOVA) for repeated measures. The differences between the means of total nitrite levels and tension developed to KCl were analysed by one-way ANOVA, as well as the means of Eₘₐₓ and pD₂ values.

3. Results

3.1. Vasoconstrictor responses

PE induced a concentration-dependent contraction in both endothelium-intact and endothelium-denuded rings. The maximum contractile response (Eₘₐₓ) and sensitivity
Aprotinin 125 KIU/ml (n = 14) incubated with 0.011% (A), 0.023% (B) and 0.045% NaCl (C).

**Fig. 1.** Effect of incubation with aprotinin (solid circles; A, 125 KIU/ml; B, 250 KIU/ml; C, 500 KIU/ml) and L-NAME (solid triangles, 100 μM) on the maximum contractile response (E_max, mg) and pD_2 values for phenylephrine in endothelium-intact and endothelium-denuded rat aortic rings.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Endothelium-intact</th>
<th>Endothelium-denuded</th>
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<tbody>
<tr>
<td></td>
<td>E_max</td>
<td>pD_2</td>
</tr>
<tr>
<td>NaCl 0.011% (n = 14)</td>
<td>897±75</td>
<td>7.3±0.1</td>
</tr>
<tr>
<td>NaCl 0.023% (n = 9)</td>
<td>990±70</td>
<td>6.9±0.1</td>
</tr>
<tr>
<td>NaCl 0.045% (n = 12)</td>
<td>998±98</td>
<td>6.9±0.2</td>
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<td>Aprotinin 125 KIU/ml (n = 14)</td>
<td>1181±46*</td>
<td>7.4±0.1</td>
</tr>
<tr>
<td>Aprotinin 250 KIU/ml (n = 9)</td>
<td>1572±174*</td>
<td>7.0±0.2</td>
</tr>
<tr>
<td>Aprotinin 500 KIU/ml (n = 12)</td>
<td>1532±162*</td>
<td>7.1±0.1</td>
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</table>

* Data are expressed as mean±S.E.M. n is indicated in brackets for endothelium-intact rings, while n = 7 for endothelium-denuded rings.

" P<0.05, endothelium-denuded versus endothelium-intact.

+ P<0.05, aprotinin versus NaCl.

(pD_2) for PE in endothelium-denuded rings were significantly higher than those in endothelium-intact rings (P<0.05, Table 1). Incubation of endothelium-intact or endothelium-denuded rings with aprotinin (125, 250 and 500 KIU/ml) did not change their basal tensions (data not shown), nor did it affect PE response in endothelium-denuded rings. However, in endothelium-intact rings, all concentrations of aprotinin significantly increased E_max without changing pD_2 values (P<0.05, Table 1). When endothelium-intact rings were incubated with L-NAME in the presence of aprotinin, the contractile response to PE was significantly increased only in rings incubated with 125 KIU/ml aprotinin (P<0.05, Fig. 1A). There were no statistically significant differences in PE contractility between the rings incubated with 250 or 500 KIU/ml aprotinin alone and the rings incubated with aprotinin plus L-NAME (Fig. 1B, C). Meanwhile, when concentration–response curves to PE in rings incubated with aprotinin plus L-NAME were compared to respective control curves, a profound increase in contractility and sensitivity was observed (Fig. 1, P<0.001) (pD_2 values; L-NAME 7.7±0.1 vs. 0.011% NaCl 7.3±0.1, P<0.05, n = 7; L-NAME 7.6±0.2 vs. 0.023% NaCl 6.9±0.1, P<0.01, n = 8 and L-NAME 7.6±0.1 vs. 0.045% NaCl 6.9±0.2, P<0.05, n = 6).

Table 2 shows the results of contractile responses to KCl depolarization (40 mM) in aprotinin-treated and corresponding NaCl-treated rings. The contractile response generated by KCl was significantly higher in all endothelium-denuded rings compared to the endothelium-intact

### Table 2

Effect of aprotinin (125, 250 and 500 KIU/ml) on the tension (mg) developed to KCl (40 mM) in endothelium-intact and endothelium-denuded rat aortic rings.

<table>
<thead>
<tr>
<th></th>
<th>Endothelium-intact</th>
<th>Endothelium-denuded</th>
</tr>
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<tbody>
<tr>
<td>NaCl 0.011%</td>
<td>672±68</td>
<td>1106±86&quot;</td>
</tr>
<tr>
<td>NaCl 0.023%</td>
<td>715±81</td>
<td>1008±89&quot;&quot;</td>
</tr>
<tr>
<td>NaCl 0.045%</td>
<td>769±126</td>
<td>1214±131&quot;</td>
</tr>
<tr>
<td>Aprotinin 125 KIU/ml</td>
<td>761±67</td>
<td>1021±62&quot;&quot;</td>
</tr>
<tr>
<td>Aprotinin 250 KIU/ml</td>
<td>1034±127</td>
<td>1154±119</td>
</tr>
<tr>
<td>Aprotinin 500 KIU/ml</td>
<td>716±98</td>
<td>1207±170&quot;</td>
</tr>
</tbody>
</table>

* Data are expressed as mean±S.E.M., n = 7 in all groups.

" P<0.05 and ** P<0.01, endothelium-denuded versus endothelium-intact.

![Fig. 1](https://academic.oup.com/cardiovascres/article-abstract/50/3/589/332305/592)
3.2. Vasodilator responses

Endothelium-intact rings precontracted with PE gave a concentration-dependent relaxant response to ACh (Fig. 2A), A23187 (Fig. 2B), L-arginine (Fig. 2C) and sodium nitroprusside, but not to D-arginine (data not shown). Aprotinin significantly decreased endothelium-dependent relaxations to ACh, A23187 and L-arginine at all concentrations studied (P<0.001, Fig. 2) with a significant decrease in pD₂ values (P<0.05 and P<0.01, Table 3). However, it did not affect the endothelium-independent relaxation to sodium nitroprusside (Eₘₐₓ values; 91.8±1.7, 93.0±2.3 and 95.8±4.3% for 0.011, 0.023 and 0.045% NaCl, respectively; 92.0±3.1, 93.4±5.7 and 92.8±3.2% for aprotinin 125, 250 and 500 KIU/ml, respectively; see Table 3 for pD₂ values).

3.3. Nitrite levels

Total nitrite accumulation in cultured CMEC is shown in Fig. 3. A23187 (1 μM) induced a significant nitrite accumulation in these cells (195.9±18.6 μM/mg protein), which was approximately two times the basal value (115.5±4.1 μM/mg protein) (P<0.001). Incubation of the cells with aprotinin (125, 250 and 500 KIU/ml) did not significantly change basal nitrite accumulation. However, it significantly inhibited A23187-induced accumulation of nitrite at all concentrations studied (P<0.01 and P<0.001).

4. Discussion

The results of the present investigation show that incubation of rat thoracic aortic rings with aprotinin, a serine protease inhibitor, resulted in an impairment of endothelial function. The observation that aprotinin enhanced PE-induced contractility in endothelium-intact rings, but not in the endothelium-denuded rings, suggests a prominent effect of the drug on endothelial cells, particularly on the synthesis and/or release of endothelium-derived relaxant factors (EDRFs). It has previously been demonstrated in rat aortas that the removal of endothelium augments contractions induced by alpha-adrenergic agonists and spontaneous release of EDRF is a functional antagonist of these contractions [17]. Thus, the significant increase in PE contractility in endothelium-intact rings after aprotinin incubation suggests not a mechanical, but a pharmacological inhibition of basal release of EDRF from endothelial cells. Hence, the possibility of reduced basal NO availability was tested by inhibiting NO release.
Table 3

Effect of aprotinin (125, 250 and 500 KIU/ml) on the pD₂ (–log EC₅₀) values for acetylcholine, calcium ionophore (A23187), L-arginine and sodium nitroprusside (SNP) in endothelium-intact rat aortic rings

<table>
<thead>
<tr>
<th></th>
<th>Acetylcholine</th>
<th>A23187</th>
<th>L-Arginine</th>
<th>SNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl 0.011%</td>
<td>7.2±0.1</td>
<td>7.1±0.1</td>
<td>5.1±0.2</td>
<td>7.8±0.2</td>
</tr>
<tr>
<td>NaCl 0.023%</td>
<td>7.1±0.1</td>
<td>7.3±0.1</td>
<td>5.0±0.1</td>
<td>7.5±0.1</td>
</tr>
<tr>
<td>NaCl 0.045%</td>
<td>7.0±0.1</td>
<td>7.0±0.2</td>
<td>5.8±0.4</td>
<td>7.4±0.2</td>
</tr>
<tr>
<td>Aprotinin 125 KIU/ml</td>
<td>6.7±0.1*</td>
<td>6.3±0.1*</td>
<td>4.8±0.3</td>
<td>7.5±0.2</td>
</tr>
<tr>
<td>Aprotinin 250 KIU/ml</td>
<td>6.5±0.2*</td>
<td>6.3±0.3**</td>
<td>4.4±0.2*</td>
<td>7.5±0.3</td>
</tr>
<tr>
<td>Aprotinin 500 KIU/ml</td>
<td>6.5±0.2*</td>
<td>6.5±0.2*</td>
<td>4.6±0.3*</td>
<td>7.5±0.1</td>
</tr>
</tbody>
</table>

* Data are expressed as mean±S.E.M., n is indicated in brackets.

Aprotinin has also been known to inhibit kallikrein, the enzyme necessary for the synthesis of bradykinin which is another endothelium-dependent NO generator. Inhibition of the activity of the bradykinin-generating protease (kallikrein) in vascular tissue with aprotinin may be expected to decrease the basal tone of the aortic rings; however, this was not the case, since aprotinin incubation did not change the basal tone. This observation suggests that the impairment in endothelial function with aprotinin is independent of kallikrein inhibition.
The findings in vascular rings indirectly represent the effect of aprotinin on macrovascular endothelial cells. However, the likely effect of the drug on the endothelial cells of microcirculation also requires investigation; if aprotinin impaired NO-mediated responses in myocardial microcirculation, the outcome of this effect would be more dramatic. Therefore, experiments based on the direct measurement of stable NO metabolites, namely nitrite and nitrate, in cultured CMEC were performed to confirm the data obtained from vascular reactivity experiments. Aprotinin did not change the basal release of NO in these cells; however, A23187-stimulated release of NO was inhibited. Therefore, it may be suggested that the inhibition is limited to Ca\(^{2+}\)-dependent activation of NOS-III. Possible factors such as a decrease in the enzymatic activity of NOS-III or an inhibition in NOS-III gene expression may be proposed to account for this impairment. It has previously been reported that some protease inhibitors of the chloromethylketone type prevent expression of NOS-II in vascular smooth muscle cells by blocking the activation of nuclear factor \(\kappa B\) (NF-\(\kappa B\)) [20]. Consistent with this concept, the promoter region of the NOS-III gene also contains a nuclear factor-1 (NF-1)-like binding site [21]. Therefore it is likely that inhibition of NOS-III expression by aprotinin, a protease inhibitor with pharmacologic properties similar to chloromethylketones, may probably result from the blockade of this region. The exact mechanism of action of aprotinin remains to be determined experimentally with further studies targeting NOS-III expression in endothelial cells.

As a serine protease inhibitor, the dose of aprotinin for inhibition of different enzymes varies, for instance 50 KIU/ml is required to inhibit plasmin and \(~200\) KIU/ml to inhibit kallikrein (for a brief review, see Ref. [22]). Clinical studies with aprotinin have revealed that low-dose protocols (140-mg intravenous loading dose, 140-mg pump prime and 35-mg/h intravenous constant infusion) result in plasma concentrations of more than 125 KIU/ml, while high-dose protocols (two-fold the doses given in low-dose regime) result in plasma concentrations of more than 250 KIU/ml [23]. Therefore, the concentrations of aprotinin in this in vitro study were adjusted within the clinical effective concentrations. In addition, the value of the inhibition equilibrium constant (\(K_i\)) for aprotinin binding to NOS-I and NOS-II in rat brain homogenates was reported to be 0.05 and 0.078 mM, respectively [13]. The concentrations of aprotinin used in this in vitro study correspond to \(~0.0025, 0.005\) and 0.01 mM, which are considerably lower than the \(K_i\) for aprotinin binding to NOS-I. Furthermore, aprotinin was reported to inhibit cytokin-stimulated nitrite release from cultured murine lung epithelial cell line, LA-4 at 500- and 1000-KIU/ml concentrations (which correspond 0.01 and 0.02 mM, respectively) and to inhibit cytokin-induced NOS-II expression at 1000 KIU/ml [9,10]. The difference in the inhibitory concentrations of aprotinin on different NOS subtypes may be explained by the difference in the source of the tissue used in these in vitro studies.

The results of our study reveal an in vitro inhibitory effect of aprotinin on the release of NO from endothelial cells. A compound having such an effect is expected to increase arterial pressure when given in vivo. However, it has been reported that infusion of aprotinin in doses up to 7000 KIU/ml failed to affect mean arterial pressure and carotid blood flow or hindlimb/pulmonary vascular resistance in rat [24]. Therefore, additional studies investigating possible in vivo and in vitro effects of aprotinin on different vascular beds should be carried out.

In conclusion, aprotinin was found to impair NO-mediated endothelium-dependent relaxation in rat thoracic aortic rings and inhibit NO release from cultured CMEC isolated from rat hearts. Since NO is a native endothelium-derived inhibitor of platelet aggregation, this effect of aprotinin may contribute to its ‘blood-sparing’ action. However, impairment of endothelial function and excessive inhibition of constitutive NO release from endothelial cells may result in substantial formation of thrombosis. Therefore, aprotinin-induced impairment in endothelial function may also be responsible for the increase in perioperative restenosis risk observed in clinical practice during aprotinin therapy.

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