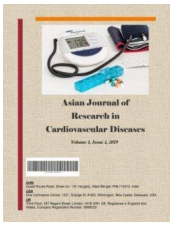

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A Comparative Study of Systemic Application vs. Topical Application of Aprotinin in Cardiac Surgery

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

The aim of this study was to make a comparative analysis between a regional and a systemic aprotinin use in coronary artery bypass grafting. The advantage of a topical aprotinin application is devoid of any systemic side effects. A randomized study & prospective in nature comprising ninety-seven patients was conducted. An average 5* 106 KIU aprotinin was given systemically to forty-nine patients and four doses of 1.25 times 106 KIU aprotinin in avg. were applied locally to forty eight patients by spraying the substance on the target (A. internal mamary region and pericardium). Markers for the inflammatory response, blood coagulation system, standard haematological markers were determined along with features of postoperative complications.

Patients having surgical bleeding, redo operations, neurological, bleeding disorders, hepatic and renal disorders were excluded. Sex, age, perfusion times, mortality, kidney failure and strokes were identical in both groups. Biochemical markers and clinical outcome demonstrated no significant differences between the generalised and localised applications. IL- 6 and serum elastase were found to be very higher (p= 0.1) within the topical group, but with a high variations in standard deviation in each patient. Our results suggest that there is absence of difference between the perioperative use of 5 106 KIU and systemically given aprotinin and 1.25 106 KIU which is locally applied aprotinin.

Keywords: Arotinin; cardiac surgery; patients; renal isufficiency.

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1. INTRODUCTION

Two studies as per references were able to show that small doses (5 105 to 1 106 KIU aprotinin) of topically applied aprotinin could reduce postoperative bleeding and also can reduce requirement of blood transfusion after the operation of coronary bypass grafting. 1,2 The aim of this study was to research whether topical application of aprotinin rather than the more conventional systemic application can be superior or at least give the same results regarding the reduction of postoperative blood loss. The advantage of a topical aprotinin application is seen in the fact that they devoid of systemic side effects (graft occlusions, cardiac infarcts, anaphylactic reactions following rapid infusions, disseminated intravascular coagulation in the case of lower hypothermia, worsening of kidney functioning) that might be caused by aprotinin.1,3,4 As well as the influence on postoperative blood loss, aprotinin is additionally credited with having an effect on postoperative sys-temic inflammatory response 5 ± 7.

2. METARIALS AND METHODS

One hundred patients designated for elective aorto- coronary bypass operation were listed for the study in a prospective and randomized way. Not included were patients who had been given ass 100, heparin i.v. or warfarin <5 days preoperatively, and also patients with known coagulopathies, con- genital or acquired thrombopenia, terminal renal insufficiency, ejection fraction <40%, signs of infec- tion and known oversensitivity to aprotinin. Three patients had to be removed from the study through rethoracotomy for surgery-caused postoperative bleeding, operations which had lasted > 5 h and renewed reperfusion. Of the remaining 97 patients, 48 received aprotinin in topical application (topaa) and 49 systemic aprotinin application (sysaa). Aver- age age of the topaa group was 66‹7 years and of the sysaa group 64‹9. The topaa group comprised 28 men/20 women and the sysaa group 35 men/14 women. The two groups were comparable with regard to all other demographical data (body weight [bw], height, and so forth) (Table 1). Group members receiving topical aprotinin application received 2.5×10^6 KIU aprotinin (Aprogen, Alkem-India) on the pericardium and in the mammaria region. The first application was done by spraying the substance, after preparation of the A. mammaria interna and opening of the pericardium, to the thoracic internal wall and intrathoracal. Then again applied for second time occurred after transfu- sion of protamine is complete, on the pericardium, the anastomosis area and into internal mammary artery region. In each case, the time aprotinin needed to take effect was 2 min. Following this, precise attention was paid to the correct removal of aprotinin by dirty suction and the direct elimination without cell-saver or coronary suction. The systemic aprotinin applica-tion group received 2*106 KIU aprotinin as a short infusion over 30 min, following the introduction of anaesthetic, 2£106 KIU aprotinin in the priming volume of the heart ±lung machine and 106 KIU aprotinin via a perfusor from the beginning of the operation with a flow rate of 5£105 KIU/h. Before being attached to the heart±lung machine (ECC), all patients received Three Hundred IU heparin/kg BW and Ten Thousand IU heparin in the priming volume of the ECC for anticoa- gulation. Attention was paid during the operation that the ACT lay between 400 and 800 s. At the comple-tion of the Cardio Pulmonary Bypass the heparin effect reversal was done with protamine at a ratio of 1:1 (0.3 mg/kg BW). Anaesthe- sia was introduced with 2 mg/kg BW sufentanil, 8.0 mg pancuronium as muscle relaxant and 0.08 mg/kg BW midazolam. Further anaesthetic application was with1.0±1.5 mg/kg BW/h sufentanil and 4.0 mg pancuro-nium pre-ECC. The Cardio Pulmonary Bypass machine having membrane oxygenator were connected with PVC tubes and primed with 1000 ml Ringer solution, 500 ml gelofusin, 20 ml sodium bicarbonate (8.4%) and 250 ml osmofundin (15%). The operations were carried out under slight hypothermia at an average temperature of 32.6°C. The following labo- ratory parameters were investigated: haemoglobin, haematocrit, leukocyte count, thrombocyte count (impedance method, Coulter Electronics), thrombin time, PTT, fibrinogen, AT III, FSP-D-dimers (STA tests, Boehringer Mannheim, Mannheim, Germany), prothrombin fragments 1+2 (ELISA, Boehringer Mannheim), factor VII:C (Immunochromtest1, Im-muno, Vienna, Austria), factor VIIa-rTF (Staclot Diagnostica Stago, AsnieÁres-Sur-Seine, France), tissue factor (ELISA, American Diagnostic, Greenwich), tis-sue factor pathway inhibitor (TFPI) (Activity Assay, American Diagnostic), PMN elastase, interleukin-6 (Immunoassay, Merck). The blood condition parame- ters and the PMN elastase were determined from EDTA plasma, interleukin-6 from serum and the coag- ulation parameters from citrated sodium plasma. The blood samples were taken at the following times: T1, baseline value deter- mination pre-anaesthesia; T2, 5 min

Table 1. Demographic and intra-operative patient data

	Topical aprotinin	Systemic aprotinin	p
Age (years)	66‹7	64‹9	ns
Body weight (kg)	78‹12	84‹15	ns
Operation length (min)	173‹42	178‹35	ns
Perfusion times (min)	64‹18	67‹19	ns
IMA preparation (n)	48	49	ns
Distal anastomoses (n)	2.97	3.11	ns
Heparin (IU)	35.221‹5.846	37.977‹5.168	ns
Protamine (mg)	23.953‹4.788	25.886‹4.179	ns

IMA: internal mammary artery

after complete heparinization; T3, 5 min after the start of CPB; T4, at the end of CPB; T5, arrival at ITU/ICU; T6, 24 h after the surgery. Over 24 h following the operation, blood loss and the giving of by the parameters erythrocyte concentrates and transfusion of blood products were documented. The first postoperative day was divided into four time intervals for the documentation of blood loss: interval 1, 0±2 h postoperative; interval 2, 2±6 h postoperative; interval 3, 6±12 h postoperative and interval 4, 12±24 h postoperative. The statistical eval-uation of the data was carried out via StatView1 software comprising utilization of the Student t test for independent variables. A p value <0.05 was taken as statistically significant.

3. RESULTS

No undesirable systemic or local side effects were observed with any of the patients in either study group. Regarding total blood loss over the first post- operative 24 h, a loss of 547‹259 ml blood was observed with the TopAA group, and a loss of 491‹217 ml (p=ns) with the SysAA group. Only in the first time interval (0±2 h postoperative) was there a difference in blood loss: 131‹107 ml for the TopAA group versus 68‹72 with the SysAA group (p<0.005) (Fig. 1). Regarding the infusion of erythrocyte concentrates, the TopAA group used 700‹283 ml for 15 patients versus 709‹193 ml for 11 patients in the SysAA group (p=ns). The hae-moglobin concentrations were identical in the postoperative course. No patients from either group received fresh frozen plasma (FFP), thrombocyte concentrate or coagulation preparations. The evalua- tion of results for the D-dimer indicated that no significant differences could be shown between the groups at any time. Before the introduction of anaes-thetic, the D-dimer in the TopAA group was 0.38‹0.36 versus 0.52‹0.60 mg/l for the SysAA group. Shortly before the end of ECC, the values were 0.57‹0.45 for the TopAA group versus 0.40‹0.36 mg/l for the SysAA group (p=ns).

Twenty-four hours postoperatively, levels of 0.59‹0.29 versus 0.70‹0.44 mg/l, respectively (p=ns), meant there were no group differences. The basic values for the prothrombin fragments 1+2 (normal value 0.32 ± 1.20 nmol/l) were 1.01‹0.38 for TAA group vs. 1.22‹0.72 nmol/l for the SAA group. The highest values founded on arrival at the ICU/ITU with 2.77‹1.32 in the AATop vs. 2.31‹0.64 nmol/l in the group of AASys. At all sampling times, p=ns. The average values were once again at their original level 24 h postoperative (Fig. 2). Tissue Factor Pathway Inhibitor (normal value 0.7±1.3 U/ml) rose to around double for both of this groups five minutes after completion of heparinization and stayed at this level (p<0.00005 in comparison to the basic values) at the time of complete heparinization. At the time of blood sample T5, i.e., after the giving of protamine, the results returned to their original level (Fig. 3). The factor VIIa-rTF reaction depend- ed on the TFP concentration: Baseline value for the TopAA group was 35‹18 and for the SysAA group 36‹21 mU/ml (normal value: 30±170 mU/ml). Five minutes following full heparinization both averages fell by approximately 75±80% (TopAA group 8‹5 versus SysAA group 7‹4 mU/ml). ECC haemodilu- tion did not reduce the values further (Fig. 4). On arrival at the intensive care unit, i.e., after the prot- amine application, the averages returned to their baseline values. At no time were significant differ- ences between topical and systemic applications seen. The progression curve for the thromboplastin time remained identical with the factor VIIa-rTF curve. In contrast, factor VII reduced in step wise manner by it's average values. The tested inflammatory markers (such as elastase, interleukin 6, leuko-cytes, C-reactive protein) showed an activation due to the extracorporal circulation and the perioperative tissue handling at the end of the

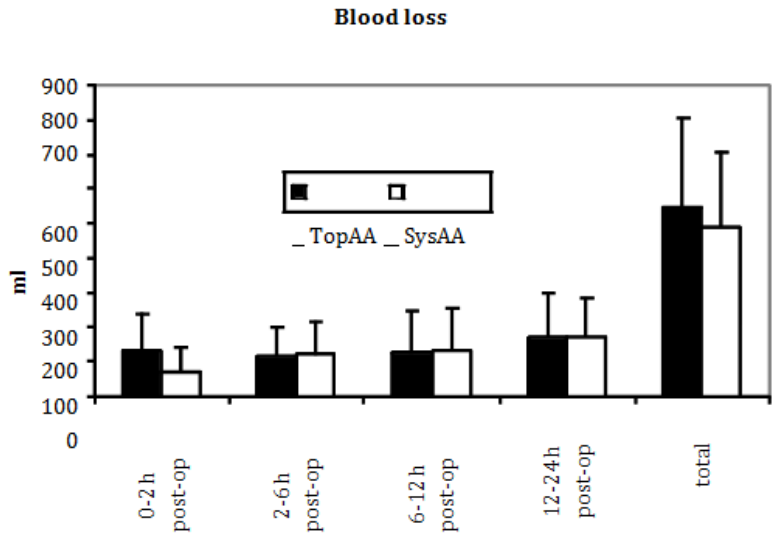

Fig. 1. Significant postoperative differences in blood loss withinthe first 2 h (*p*<0.05). There were no further differences observedin subsequent observation periods, this applied especially to theblood loss in total

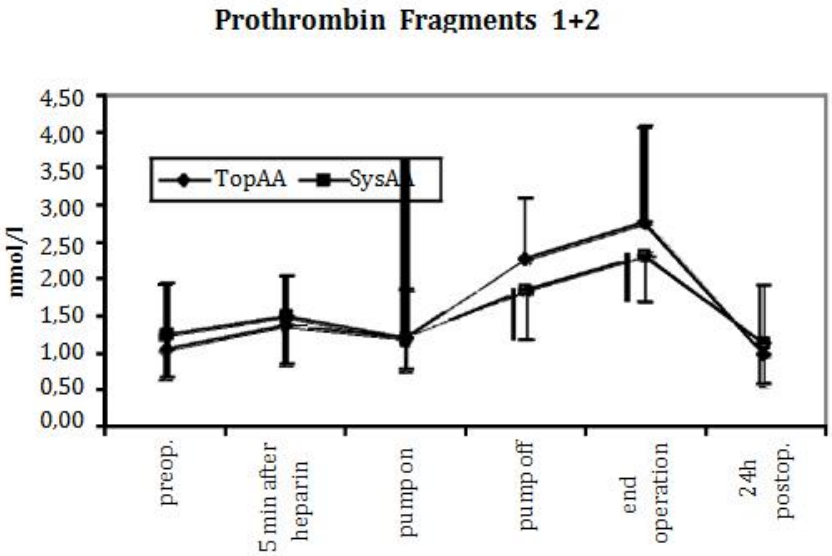

Fig. 2. The kinetics of prothrombin fragments 1+2 without evidence of group differences

procedures and recovered after 24 h. PNM elastase and interleukin 6 presented tendentially higher values in the local application groups, but without reaching a statisti- cally significant level (p=0.1). (Figs. 5 and 6) In the case of elastase and interleukin 6, high intra- individual ranges in values were recorded course, but there is no difference between the local and systemic applications.

C can then be activated to protein Ca. In that pro- tein C is a precursor of a serine protease, this can be locally inhibited by aprotinin. Furthermore, tissue-plasminogen activator (Tpa) which is secreted from the vessel endothelium is the most important activator regarding the matter of conversion of plasminogens into plasmin [1,2].

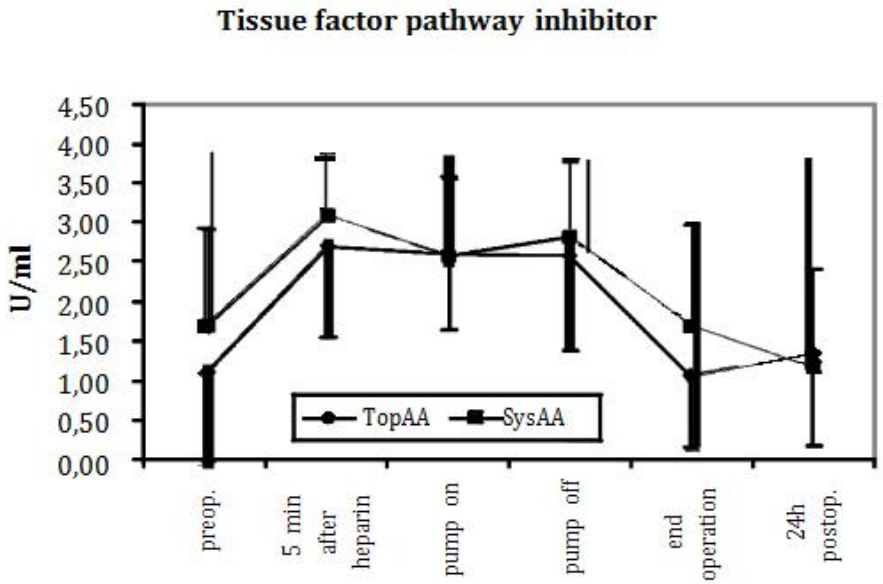

Fig. 3. The kinetics of tissue factor pathway inhibitor. No significant differences are evident at any sampling time

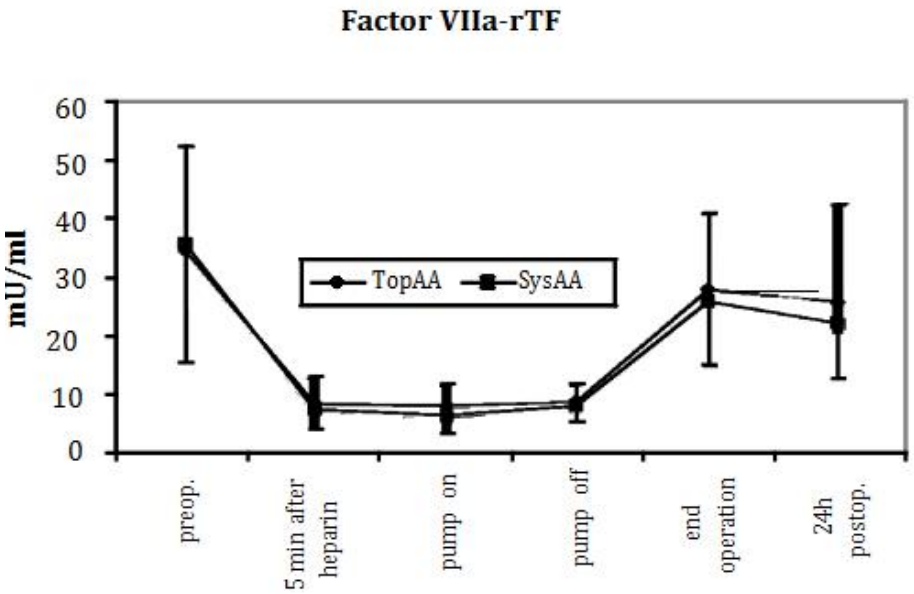

Fig. 4. The kinetics of factor VIIa-rTF. No group differences are evident. However, a highly significant extracorporeal circulation influence can be seen

Aprotinin also inhibits the serine protease plasmin, a controlling activator for the lysis of fibrin coagulation [3]. This means that topical applica- tion of aprotinin is an alternative therapy method to intravenous therapy. The advantage of a topical appli- cation is that it cannot lead to systemic side effects such as vein bypass occlusions, myocardial infarc- tion, `disseminated intravascular coagulation' in the with a high affinity and changes its enzymatic specificity [4]. Through this, the potent anticoagulant factor protein.

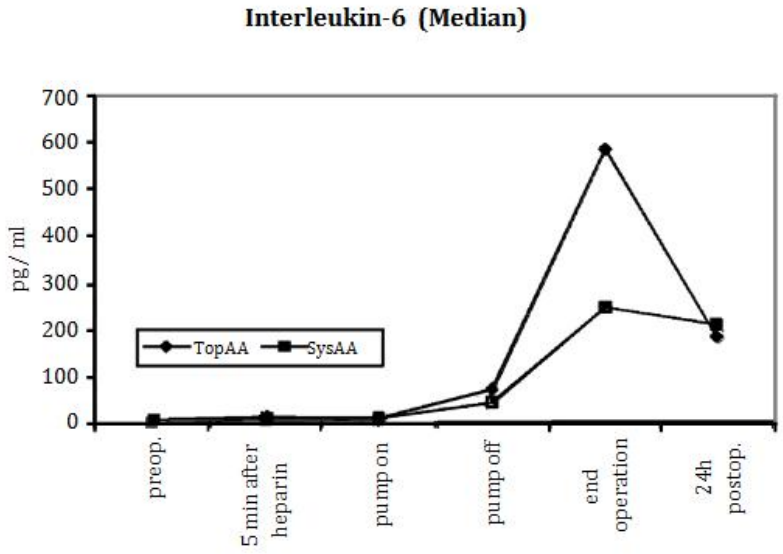

Fig. 5. Interleukin 6 showed an activation caused by the extracorporeal circulation and the operation trauma at the end of the procedure. Higher values in the local application group did not reach a statistically significant level (*p*=0.1). High standard deviations and individual ranges were recorded

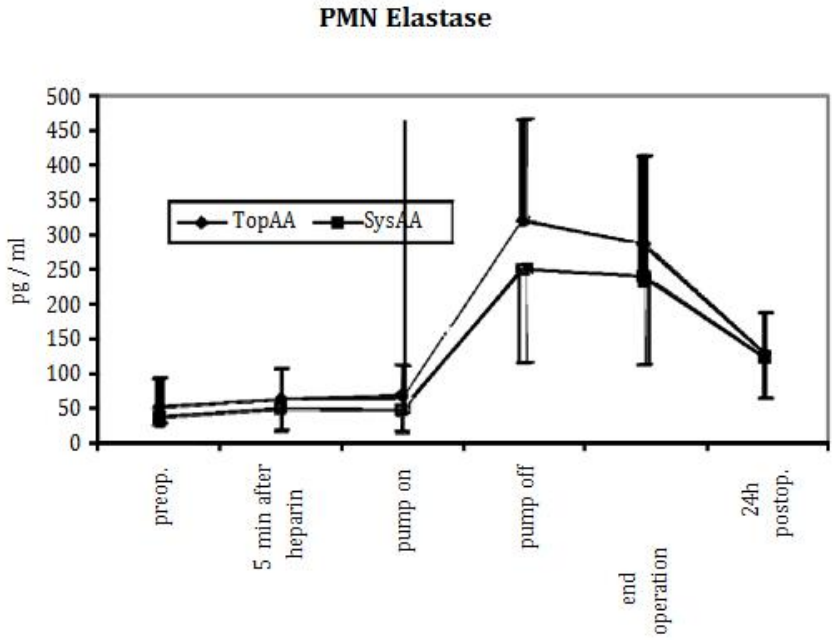

Fig. 6. Changes of polymorpho nuclear elastase in the perioperative

4. DISCUSSION AND CONCLUSION

Aprotinin is a serine protease inhibitor without specific action, which has as per studies can reduce blood loss. The inhibiting of the individual serine protease is strongly dependant on the concentration of the aprotinin in the blood.8 In this study, it could be shown that a topically applied high-dose applica- tion of aprotinin in open-heart operations has the same efficiency

with regard to reduction of blood loss and transfusion of blood products (PRBC, FFP, Platelets concentrates, and so forth) as systemic applications of aprotinin. During the postoperative recovery time, D-dimer kinetics were indifferent. Hyper- fibrinolysis can also be inhibited through topical aprotinin application. The mechanics of blood loss reduction when aprotinin is topically applied were explained by O'Regan et al. [5] in that the most impor- tant bleeding took place in the capillary regions of extracardiac tissue. The glycoprotein thrombomodulin on the endothelium of vessels which fixes thrombin case of deeper hypothermia and renal failure. 4 an important point is that membrane stabilization effects regarding platelets must be avoided with local appli- cation, and thrombocyte aggregation inhibitor must be applied in good time before the operation. With both forms of therapy, chances of allergic reaction to aprotinin can't be excluded. Whether re-occlusion of bypasses also occurs with topical application cannot be answered. For this, control angiographs would be necessary after the heart operation.3 TFPI, a Kunitz- type which inhibits serine protease, is a potent inhibitor of coagulation of the extrinsic blood coagulation system and, thus, a key for the feedback control of coagulation.13 TFPI is present in 3 different pools of the blood vessels. The largest pool is attached to the vessel endothelium surface and can be released from there by heparin.14 TFPI forms a complex with factor Xa. In the second stage this TFPI±factor Xa complex inhibits the VIIa factor±tissue factor complex (TFPI±factor Xa±factor VIIa ±tissue factor complex) through a quadrate for- mation. In this way, the extrinsic coagulation path is inhibited.13,14 The effect of heparin is dependant on the presence of the glycoprotein-a2- AT III, [2,6] & acentuates the formation of the thrombin ±antithrombin III complex (TAT) [7,1]. This is not the case with TFPI [8].

For anticoagulation during open-heart operations 300 IU/kg BW heparin is given before connection to the ECC. The data indicate that a definite release of TFPI occurs, independently, however, from the form of aprotinin application. The tissue factor±factor VIIa complex is inhibited by the high plasma concentra-tions of TFPI. The haemodilution due to ECC no longer plays a decisive role in the concentration reduction of factor VIIa. Prothrombin time (PT) shows the inhibiting effect of the tissue factor±factor VIIa complex, this being greatly extended (Five minutes after the heparin application). Following the application of protamine sulphate for heparin reversal, TFPI release is also halted. Due to this, the cumulative effect of anticoagulation from the TFPI to heparin16 has a special importance in open-heart operations. The activation of the inflammatory response on Cardio Pulmonary Bypass and the intraoperative tissue handlings are not influenced by the different forms of aprotinin application. The studies by O'Regan et al. [5] and Tatar et al. [9] mainly concerned on the effects of coagulation and blood loss after operations. There are no available comparisons with other studies on the effects of local aprotinin on the response of systemic inflammations. However, the positive influence of serine protease inhibitors on the kinetics of interleukin-6, interleukin-10 and elas-tase have been shown. 5 ± 7.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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