Effects of recombinant staphylokinase on coronary thrombosis in Chinese experimental miniature swine

LIU Jian-Xun[1], SHANG Xiao-Hong, FU Jian-Hua, LI Xin-Zhi, WANG Gang (*Department of Basic Medicine Science, Xiyuan Hospital, China Academy of Traditional Chinese Medicine, Beijing 100091, China*)

KEY WORDS recombinant proteins; miniature swine; coronary thrombosis; thrombolytic therapy

ABSTRACT

AIM: To study the effects of recombinant staphylokinase (r-Sak) on coronary thrombosis, cardiac ischemia, and myocardial infarction in Chinese experimental miniature swine. **METHODS**: Endarterium was injuried and coronary thrombi were formed gradually through direct electrical stimulation on the coronary artery of Chinese experimental miniature swine. Effects of r-Sak *in vitro* were studied through coronary pathological section, microkinematography, multi media graphic analysis, epicardial electrogram mapping, myocardium histochemical stain, serum creatine phosphokinase (CPK), and hemorheology. **RESULTS**: r-Sak showed a remarkable effect on coronary thrombus. Compared with the control group, the two dosage groups, r-Sak 0.45 mg/kg and 1.35 mg/kg, reduced the transverse section area of coronary thrombus ($P < 0.01$), lessened the degree and range of cardiac ischemia ($P < 0.01$), decreased the myocardial infarction area ($P < 0.01$), the activity of CPK and blood viscosity ($P < 0.05$), restrained platelet adhesion and aggregation, and reduced fibrinogen concentration ($P < 0.05$). **CONCLUSION**: r-Sak could dissolve coronary thrombus obviously and lessen the pathologic reaction of myocardium.

INTRODUCTION

Cases of coronary heart disease (CHD) have increased constantly in the past few years and acute myocardial infarction (AMI) has become the major disease that imperils human life. Coronary thrombosis is the major reason for AMI[1]. In the early period of AMI (in 3 - 6 h), the thrombolytic therapy can dissolve the blocking thrombus and reperfuse the ischemic myocardium, then save the agonal necrotic myocardia or minify the necrotic ranges and it have achieved rather satisfying curative effect. Whereas, it is difficult to obtain data such as the nicety time of coronary thrombosis, the thrombolysis degree of thrombolytic, and the effect on myocardial ischemia and infarct area after coronary reperfusion while doing clinic research, and they are important on judging curative effect of thrombolytic. Recombinant staphylokinase (r-Sak) is one of the profibrinolytic activators made by recombination technique[2]. In addition, the cardiac function, coronary artery distribution, physical characteristic, and chemical characteristic of miniature swine are similar to human, so they are the perfect experimental animal to study cardiovascular system drugs. In this study, we use Chinese experimental miniature swine, cultivated by Beijing Agricultural University, to study the effects of r-Sak on coronary thrombosis, cardiac ischemia, and myocardial infarction.

MATERIALS AND METHODS

Animal Chinese experimental miniature swine (♀ and ♂, from multiplying farm of Beijing Agricultural University) weighing 14.6 kg ± 2.0 kg. At the onset of the study they were employed and allocated to four treatment groups: animals received normal saline (NS) 1 mL/kg ($n = 6$), animals treated with urokinase (UK) 10 000 IU/kg ($n = 5$), and animals treated with recombinant staphylokinase 0.45 mg/kg ($n = 5$) and 1.35 mg/kg ($n = 5$).

Drugs and reagents Recombinant staphylokinase (2 mg per branch), provided by Chengdu Di-ao Pharmaceutical Co, Ltd, Chinese Academy of Science (batch № 940401); urokinase (10 000 IU per branch), manufactured by Shanghai Biochemistry Pharmaceutical Factory; 2 % lidocaine hydrochloride parenteral solution (10 mL per branch, batch № 931103), produced by the

[1] Correspondence to LIU Jian-Xun.
Phn Fax 86-10-6288-6691. E-mail jianxun@ht.rol.cn.net
Received 2001-08-06 Accepted 2002-02-25

ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Jun; 23 (6)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

Third Pharmaceutical Factory of Beijing and normal saline (batch № 940911128) provided by Tianjin Animo Co, Ltd.

Animal model Briefly, animals were anesthetized with pentobarbital sodium (50 mg/kg), their hearts were exteriorized through a left thoracotomy at the 4th intercostal space. The pericardium was opened and a pericardial bed was made[3]. The end section of left anterior descending branch of coronary artery was isolated. Stimulating pole was placed underneath the coronary artery, while another pole (reference electrode) was placed underneath the epicardium from the stimulating pole 0.6 mm, and the two poles were connected with positive electrode and cathode of SS-102J model isolator of electronic stimulator SEN-3201 respectively[4]. Epicardial eletrogram pole (twenty points) was placed at the ventricular surface[5]. Direct current, 2.5 V, 1 mA, stimulated coronary artery for 30 min, and epicardial electrocardiograms were obtained under anesthesia to document the development of infarcts during the period. Epicardial electrocardiograms ST segment rose evidently when coronary thrombus formed. Fifteen minutes later, epicardial electrocardiograms were recorded and blood was drawn out at auricula sinistra. Experimental drugs and normal saline were trickled *via* vein channel, and epicardiogram maps were recorded after 15, 30, 45, 60, 90, 120, and 180 min. ST segment elevated more than 2 mV as criterion to calculate the degree of myocardial ischemia (total mV of ST segment elevating, Σ-ST) and myocardial ischemic scope (total point number of ST segment elevating, N-ST)[6].

Area of thrombus and infarct The record was finished at 180 min. Then the heart was took out, washed with normal saline, and weighed immediately. The stimulating segment coronary artery was stripped and fixed in the 10 % formaldehyde solution, then was made paraffin section, stained with HE. Under the ligation line, the ventricle was transversely divided into five equal thickness pieces, then they were infiltrated with nitroblue-tetrazolium (N-BT) staining solution at 25 ℃ for 15 min. The pathological change was observed by camera and photographic system. The maximum thrombus area was selected, and video graphicprint was done through UP-811 type video graphic printer. The mean percentage of thrombus area in blood vessel, the infarct area, and the percentage of heart ventricle and the entire heart were calculated with multimedia color pathological image analytical system (MPIAS-500)[6].

Hemorheology The whole blood viscosity of different shears (1.92 s^{-1}, 9.16 s^{-1}, 38.11 s^{-1}, 192.0 s^{-1}) was determined by ELD type viscometer. The round-tree type, expansion type and aggregate number were observed and casted with SONY telecamera microscope. Hematokrit was determined by KUBOTA KH120 Ⅱ type hematokrit set. Platelet adhesion rate, erythrocytic sedimentation rate, plasma ratio viscosity, and the content of plasma fibrinogen were checked up.

Statistical analysis Data were expressed as $x \pm s$. Comparison among groups was assessed for significance by analysis of variance, comparison between groups was assessed for significance by t-test.

RESULTS

Thrombolysis effect of r-Sak on coronary artery Compared with control group, r-Sak 0.15 mg/kg, 1.35 mg/kg, and UK groups all reduced thrombus area significantly, though there was significant difference compared with UK group ($P < 0.01$, Tab 1). Observed with microscope, there were typical thrombi in the coronary artery compartment of the normal saline group. The thrombi were made up of red thrombus, white thrombus, and mixed thrombus. They arranged compactly, whereas there were interspaces between individual thrombus. Proportion of thrombus in atrium was considerable, even vascular compartment was completely obstructed by thrombus. Most coronary artery endothelial cells disappeared and muscular layer turned thinner. Compared with control group, thrombus was relatively loose in UK group, and parts were lysised and there was partial lacuna. Moreover, to r-Sak 0.45 mg/kg and 1.35 mg/kg groups, thrombi in the coronary compartment were loose and fragmentation, and lumen space was aggrandizement obviously (Tab 1, Fig 1-4).

Tab 1. Effect of r-Sak on coronary thrombosis. $x \pm s$. $^cP < 0.01$ ***vs* normal saline treated group.** $^tP < 0.01$ ***vs* urokinase treated group.**

Groups	Dose	n	Thrombus area/vascular compartment area (%)
Normal saline	1 mL/kg	6	93 ± 3
Urokinase	10 000 IU/kg	5	80 ± 6^c
r-Sak	0.45 mg/kg	5	56 ± 12^{ct}
r-Sak	1.35 mg/kg	5	31 ± 10^{ct}

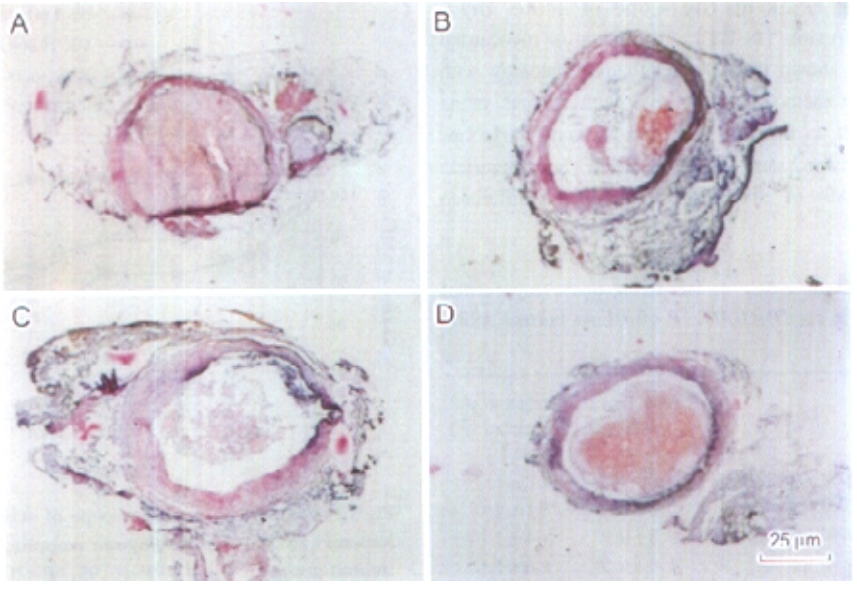

Fig 1. Thrombolysis effect of r-Sak on coronary artery. (A) Normal saline group showed there were typical thrombi in the coronary artery compartment; (B) Urokinase group showed thrombus was relatively loose, and parts were lysised and made partial lacuna; (C) r-Sak 0.45 mg/kg treated group; (D) r-Sak 1.35 mg/kg treated group showed thrombi in the coronary compartment were loose and fragmentation, and lumen space was aggrandizement obviously. HE stain. ×40.

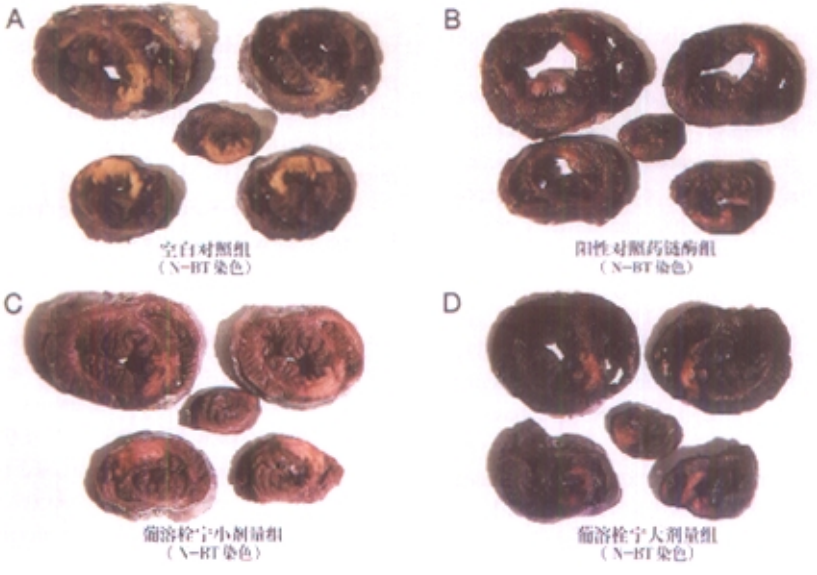

Fig 2. The effect of r-Sak on the scope of acute myocardial infarction. (A) Normal saline 1 mL/kg; (B) Urokinase 10 000 IU/kg; (C) r-Sak 0.45 mg/kg; (D) r-Sak 1.35 mg/kg. N-BT stain.

ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Jun; 23 (6)
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Effect of r-Sak on the scope of acute myocardial infarction (N-BT) The scope of myocardial infarction was shown through quantitative histology with N-BT staining method. Compared with normal saline group, r-Sak 0.45 mg/kg, 1.35 mg/kg and UK groups reduced infarction areas both of heart and ventricle evidently. All of them had significant difference (Tab 2, Fig 2).

Tab 2. Effect of r-Sak on scope of acute myocardial infarction. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* **normal saline treated group.**

Groups	Dose	n	Infarct area/ heart (%)	Infarct area/ ventricle (%)
Normal saline	1 mL/kg	6	4.8 ± 1.6	9.8 ± 1.7
Urokinase	10 000 IU/kg	5	2.5 ± 0.7^b	7.1 ± 2.0^b
r-Sak	0.45 mg/kg	5	2.5 ± 0.7^b	6.4 ± 1.6^c
r-Sak	1.35 mg/kg	5	1.8 ± 0.9^c	4.6 ± 1.6^c

Effect of r-Sak on the scope of myocardial ischemia (N-ST) After thrombus had formed, there were 17.2 ± 2.9 (n = 21) mapped points that showed myocardial ischemia in 20 epicardial electrogram mapping points. In other words, the section that the stimulated coronary artery distributed appeared extensive ischemia. At 120 min after medication, the N-ST of normal saline group had no significant change, however, UK 10 000 IU/kg, r-Sak 0.35 mg/kg, and 1.35 mg/kg groups reduced the N-ST evidently. The epicardial electrogram mapping points which showed myocardial ischemia decreased from 16 ± 3, 17.2 ± 2.6, and 18 ± 3 mapping points to 11 ± 4, 12.6 ± 2.0, and 11 ± 5 mapping points, respectively. They lowered (39 ± 3) %, (26 ± 3) %, and (39 ± 4) % respectively. Compared with pre-medication ($P < 0.05$) and normal saline group ($P < 0.01$), they were significantly different (Fig 3).

Effect of r-Sak on the degree of myocadial ischemia (∑-ST) Through coronary thrombosis, ST segment (epicardial electrogram mapping) rised evidently. Fifteen minutes later, compared with normal state, it rised to (147 ± 63) mV (n = 21, ∑-ST). As time went on, it had the trend of alleviating lessening naturally. Ninety minutes after medication, ST segment of normal saline group began to alleviate. It reduced from (166 ± 460) to (118 ± 21) mV by (27 ± 3) %. Compared with pre-medication, it was a significant difference ($P < 0.05$). Whereas 15 min after medication, the degree of myocardial ischemia of r-Sak 1.35 mg/kg group had descended obviously. Compared with pre-medication and control group, the differences were significant ($P < 0.01$). Thirty minutes later, the degree of myocardial ischemia of UK and r-Sak 0.45 mg/kg groups descended evidently too ($P < 0.05$). The ∑-ST of UK, r-Sak 0.45 mg/kg, and 1.35 mg/kg groups reduced by (55 ± 6) %, (63 ± 6) %, and (76 ± 8) % respectively 120 min later ($P < 0.01$, Fig 4).

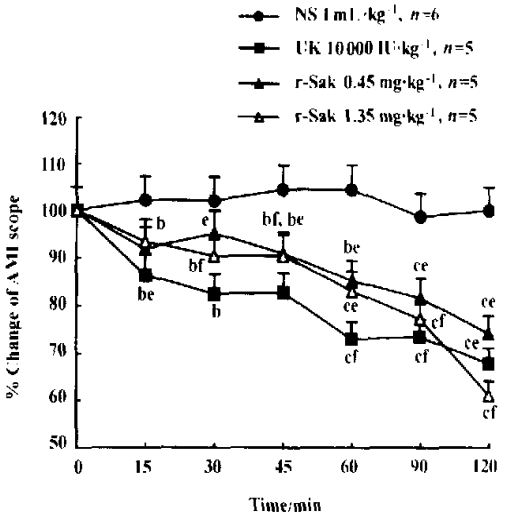

Fig 3. Comparison between scope of acute myocardial ischemia (N-ST) (epicardiogram mapping) in different treated groups. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* **normal saline treated group.** $^eP < 0.05$, $^fP < 0.01$ *vs* **itself pre-medication.**

Effect of r-Sak on serum creatine phosphokinase (CPK) activity The serum CPK activity increased as the prolonging of coronary thrombosis. It reached the maximum at 120th min, and fell at 180th min. The serum CPK activity of normal saline group increased by (101 ± 9) % 180 min after medication. Both UK and r-Sak 1.35 mg/kg groups could inhibit the extravasation of CPK obviously ($P < 0.05$). At the same time, CPK activity only increased by (42 ± 5) % and (24 ± 3) % respectively ($P < 0.05$ *vs* NS group). r-Sak 0.45 mg/kg group had the trend to inhibit the extravasation of CPK (Fig 5).

Effect on hemorheology Blood viscosity of low shear (1.92 s^{-1}) of normal saline group rose continually, it achieved peak (34 mpa·s ± 7 mpa·s, n = 6) 30 min after medication. It was significant difference

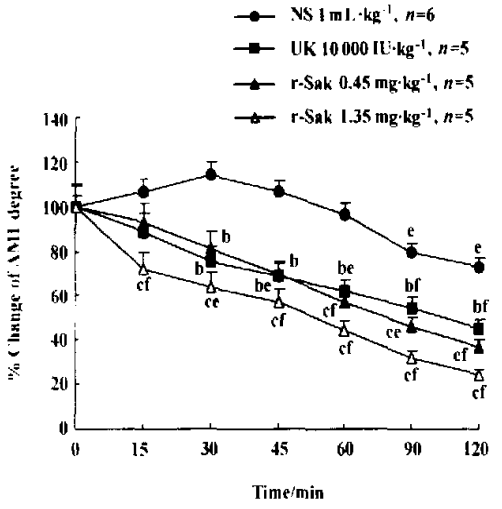

Fig 4. Effect of r-Sak on the degree of acute myocardial ischemia (Σ-ST, epicardiogram mapping). $\bar{x} \pm s$. $^bP<0.05$, $^cP<0.01$ *vs* normal saline treated group. $^cP<0.05$, $^fP<0.01$ *vs* pre-medication itself.

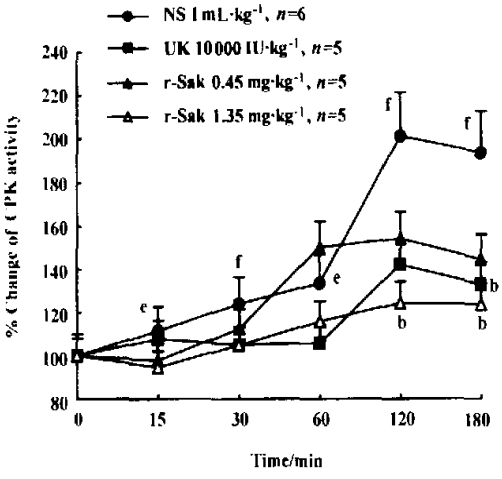

Fig 5. Effect of r-Sak on serum CPK activity in Chinese experimental miniature swine. $\bar{x} \pm s$. $^bP<0.05$ *vs* normal saline treated group. $^cP<0.05$, $^fP<0.01$ *vs* pre-medication itself.

compared with pre-medication (26 mpa·s ± 10 mpa·s, $n=6$, $P<0.01$). r-Sak could restrain the hoist of blood viscosity evidently. It was significant difference (r-Sak 1.35 mg/kg group, 20 mpa·s ± 3 mpa·s, $P<0.01$, $n=5$) compared with normal saline group. Furthermore, r-Sak could act till 180 min after medication.

Plasma viscosity, erythrocytic sedimentation rate, and hematocrit of the different treated groups had no significant change. Thirty, 60, and 120 min after medication, r-Sak 1.35 mg/kg group reduced the content of plasma fibrinogen evidently [3.9 g/L ± 0.5 g/L, 3.6 g/L ± 0.5 g/L, and 3.8 g/L ± 0.7 g/L ($n=5$) *vs* 4.6 ± 0.6 g/L, 4.4 g/L ± 0.8 g/L, and 4.8 g/L ± 0.8 g/L of normal saline group($n=6$), $P<0.05$].

Platelet adhesion rate of normal saline group changed from 31 % ± 6 % ($n=6$, pre-medication) to 39 % ± 5 % and platelet aggregation number changed from 50.0 ± 2.8 ($n=6$, pre-medication) to 55 ± 6 (60 min after medication, $P<0.05$). r-Sak 1.35 mg/kg restrained platelet adhesiveness and platelet aggregation evidently in the course of myocardial ischemia. Platelet adhesion rate of r-Sak 1.35 mg/kg group was 31.1 % ± 1.7 % ($n=5$) and platelet aggregation number was 46.8 ± 1.1 ($n=5$) respectively (60 min after medication, $P<0.05$, *vs* NS group).

DISCUSSION

r-Sak is one of the profibrinnolytic activators made by recombination technique[2]. It translates non-active plasminogen into plasmin. Afterwards, plasmins dissolute fibrin, but they have nonsignificant action on the components of normal hemostatic system such as fibrinogen, coagulation factor-Ⅴ and Ⅷ, *etc*, and hardly cause hemorrhagic tendency and side effect of the like[7-9]. Sak combined with plasminogen in the proportion of 1 : 1. Non-active plasminogen-Sak compound was formed, then active plasmin-Sak compound were brought out at rate-limiting step. Plasminogen-Sak compound exposed lysine active site of plasminogen molecule structure. Plasminogen turned into plasmin, thereby, plasmin played fibrinolytic activity[10,11]. At normal state, Sak could not activate fibrinolytic system in the blood. Plasmin-Sak compound combined with fibrin at lysine combined site when they met fibrin of the thrombus. Then it showed significant activity and sequentially digested fibrin[12,13].

Randomized patency trial in 102 patients shows that a double-bolus staphylokinase dose of 15 mg induces complete and sustained coronary artery recanalization rates without associated systemic fibrinogen breakdown in two thirds of patients with acute myocardial infarction[14]. r-Sak could reduce euglobulin lysis time (ELT), increase fibrin degradation products (FDP) evidently, but could

ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Jun; 23 (6)
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not change fibrinogen[15]. Compared with control group, r-Sak clearly shows amendatory effect on myocardial ischemia and myocardial infarction. Furthermore, r-Sak 0.35 mg/kg and 1.35 mg/kg dosage groups excelled UK group obviously.

Thrombosis is quite a complex pathophysiologic course. Platelet play key action during thrombosis. Platelet adhesiveness, platelet activator, and platelet aggregation are the key steps of thrombosis[16]. Platelet activation promotes coronary atherosclerosis, induces and aggravates myocardial infarct. The result shows that r-Sak could reduce blood viscosity of specific shear, platelet adhesiveness and platelet aggregation; and could restrain the forming of plasma fibrinogen. Thus, it could confront thrombosis and protect myocardial cell.

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重组葡激酶对中国实验小型猪冠脉血栓作用的研究

R96 A

刘建勋[1]，尚晓泓，傅建华，李欣志，王 刚
(中国中医研究院西苑医院基础医学研究室，北京 100091，中国)

关键词 重组蛋白质类；雏型猪；冠状动脉血栓形成；血栓溶解疗法

目的：观察重组葡激酶(r-SaK)对中国实验小型猪冠状动脉血栓、心肌缺血、心肌梗塞的影响. **方法**：直接电刺激中国实验小型猪冠状动脉造成动脉内膜损伤，逐渐形成冠脉内血栓. 运用冠状动脉病理切片、显微成像、多媒体图象分析、心外膜电图、心肌组织化学染色、血清生化酶学检查、血液流变学等多种试验手段，研究了 r-SaK 静脉给药对冠脉血栓的溶栓作用. **结果**：r-SaK 对冠脉血栓有显著的溶栓作用，与对照组比较，r-SaK 两个剂量组均能明

显缩小冠脉血栓横切面积($P<0.01$)，减轻心肌缺血程度和范围($P<0.01$)，缩小梗塞区($P<0.01$)，降低CPK活性和血液粘度($P<0.05$)，抑制血小板粘附、血小板聚集及纤维蛋白原的形成($P<0.05$)。**结论**：r-SaK对冠状动脉血栓具有明显的溶栓作用，并可对抗心肌缺血、心肌梗塞等病理反应。

(责任编辑 吕 静)

The 11th international conference on ulcer research (ICUR-2003)

2003 September 15 – 20 Dubrovnik, Hotel Croatia, Cavtat, Croatia

Info: P Sikiric MD, PhD
Organization Committee Chairman
Department of Pharmacology
Medical Faculty University of Zagreb
Salata 11, POB 916
10 000 Zagreb, Croatia
Phn +385 1-456-6833
+385 1-456-6834
Fax +385 1-492-0050
E-mail sikiric@mef.hr