

Recombinant Streptokinase for the Treatment of Thrombotic Disorders

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INTRODUCTION

Coronary thrombolysis is currently under investigation as a means to reduce myocardial infarct size, and several efforts have been undertaken to develop fibrin-specific thrombolytic agents.¹

Recently, several studies have been conducted with streptokinase (Sk) and as compared with plasminogen activators in acute myocardial infarction.²⁻⁶ Fibrinolytic agents are capable of dissolving or promoting the liquefaction of fibrin filaments and, consequently, the resorption of thrombi.

Thrombolysis with Sk is now established as an effective therapy for improving survival^{7,8} and preserving left ventricular function.^{2,9,10} Furthermore, from the comparative studies, there appears to be little difference in left ventricular function or predischARGE patency rates whether Sk or recombinant tissue type plasminogen activator (rtPA) is used. Nor do mortality data dictate a preference for one agent over another.¹¹ However, the use of recombinant streptokinase (rSk) for the treatment of acute myocardial infarction and other thromboses has not been reported.

In this paper we present a review of the data obtained from the characterization and use of non-recombinant Sk preparations, and present the results obtained in our laboratory in the characterization, animal testing, and clinical trials with rSk produced from *Escherichia coli*.

CLONING AND EXPRESSION OF RECOMBINANT STREPTOKINASE

The streptokinase gene used in these studies was isolated by the Polymerase Chain Reaction (PCR) from a strain of *Streptococcus equisimilis* group C. Its nucleotide sequence differs from previously reported genes (Estrada *et al.*, manuscript in preparation). The Sk gene was expressed in *E. coli* (strain W3110) under the control of the very strong and regulable triptophan (trip) promoter, carried on a multicopy plasmid (pEKG-3^{12,13}).

The rSk expression was studied using 5 L fermenters (Marubishi, Japan). The maximal rSk expression level, corresponding to 25% of total cellular proteins, was obtained after 14 h of culture. At this time the plasmid copy number was also studied and a

maximum of 420 copies/cell was obtained. The level of expression for this scale was 300 mg of Sk per liter of culture.

CLINICAL TRIALS WITH rSk

Since 1955, when the first reports appeared on the use of Sk,¹⁴ considerable information has been accumulated about its efficacy in treating thrombosis and embolisms in the arterial and venous system.^{14,15} Furthermore, recent data indicate that, in acute myocardial infarction (AMI), Sk gives the same result as other plasminogen activators like rtPA.¹¹ The results obtained with the use of rSk in AMI were the same than those reported for non-recombinant Sk.^{16,17} Studies were conducted following the patients by angiography or in comparison with non-recombinant Sk preparations (Awelysin, Germany).

In one study, 18 patients (age 34–69 years, median ($M = 54.5$); 17 males and 1 female) were treated with intracoronary rSk, and in 12 (67%) of them thrombolysis was achieved as demonstrated by angiography. The rSk doses varied from 100 000 IU to 600 000 IU (median, $M = 283\,000$ IU (this study was conducted by Prof. LLerena's team at the Institute of Cardiology and Cardiovascular Surgery in Havana).

In the second study, 18 patients with no contraindications to thrombolytic therapy were admitted to the intensive care unit in The Hermanos Almejeiras Hospital in Havana (the study was conducted by Prof. Torres and Prof. Toruncha). Patients were pre-medicated with hydrocortisone (100 mg), lidocaine, heparin (50 mg), and aspirin (250 mg). Streptokinase (Awelysin, Germany; 1 500 000 IU) or rSk (1 500 000 IU) were administered in 250 ml 5% Dextrose in 1 hour. Heparin (50 mg every 6 h) and oral aspirin (250 mg daily) administration were maintained until the end of the hospital stay. The results indicated a high percent of thrombolysis for both groups with no significant differences between them (TABLE 1). Some characteristics of the patients and

TABLE 1. Intravenous Administration of Recombinant Streptokinase and in Comparison with a Non-recombinant Preparation (Awelysin, Germany) for the Treatment of AMI: Characteristics of the Patients and Results Obtained by Echocardiography

Characteristics of Patients	rSK ($n = 10$)	Awelysin ($n = 10$)
Age mean (range)	54 (39/63)	48 (34/65)
Sex (M/F)	9/1	7/1
Hypertension	2	2
Smokers	9	5
Diabetes	0	1
Obesity	1	2
Previous infarct	0	2
Infarct with/without Q	9/1	8/0
Duration of symptoms minutes (range)	249 (355–175)	265 (360–180)
Infarct area before/14 days after thrombolytic therapy.	5.3 (3.6)/ 7.9 (4.1)	5.1 (2.6)/ 7.8 (3.9)
(Silvester score mean SD)		

the results obtained by ecocardiography are summarized in TABLE 1. In this study, it was noted that the recombinant drug is probably less immunogenic (FIG. 1).

In the treatment of deep vein thrombosis (DVT), where Sk has been used with success,¹⁸ preliminary results with the rSk were also satisfactory. Recombinant Sk was used for the treatment of two patients with DVT. Diagnosis for patient No. 1 (F, 54 years old, wt-45 kg) and patient No. 2 (M, 37 years old, wt-58 kg) were of DVT of left lower extremity of 6 days duration and DVT of left upper extremity of 3 days duration, respectively.

The patients were put in a monitored bed, and baseline coagulation data were obtained. A dose of 220 000 IU of rSk was administered by infusion over 1 h, followed by an infusion of 110 000 IU rSk per h for 72 h. After rSk infusion, a heparin maintenance infusion was started and continued over 5 days; a treatment with oral warfarin was also started.

At the end of the treatment, a venography was conducted showing complete (patient No. 1) or partial (patient No. 2) thrombolysis. No major complications were found, and only a short (48 hours) febrile reaction was recorded in both patients (this study was conducted by Prof. Charles-Edouard at the Institute of Angiology in Havana).

In clinical application, side effects are rarely observed with highly purified Sk preparation, providing that contraindications have been taken into account. With a high concentration of antibodies to Sk, there may be allergic reactions.¹⁶

In long-term Sk therapy, there was a drop in the level of fibrinogen in the blood, and less frequently, in the levels of factors V and VIII. Hemorrhages may result from hypofibrinogenemia.

In Sk therapy there may be a reduction in the levels of acidic-glycoprotein, haptoglobulin, β A-globulin, A-globulin, and macroglobulin as a result of the general proteolytic effect of the forming plasmin and its union with macroglobulin. On the other hand, the drop in arterial pressure of administration of Sk, especially after rapid injection of the initial dose, is explained by the activation of the kinin-forming system.¹⁶ In the clinical trials conducted with the rSk side effects were generally not observed.

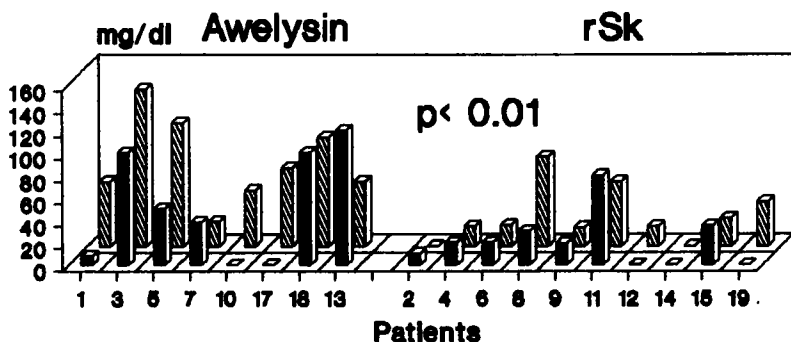


FIGURE 1. Antibody levels against Sk before and five days after treatment in AMI. The levels of antibody in the serum of patients were determined by Elisa using anti-streptokinase antibodies.

CONCLUDING REMARKS

During the last 40 years, much effort has been put in the application of anticoagulant and fibrinolytic agents for the treatment of thrombosis and embolisms in the arterial and venous system. In the Center for Genetic Engineering and Biotechnology in Cuba we have developed a new thrombolytic agent capable of dissolving fibrin clots. The product is a recombinant streptokinase obtained from a transformed *E. coli* strain (W3110). The rSk expression was scaled from 5 L fermenters to 100 L fermenters (Chemap, Switzerland). The maximal expression of the rSk for this scale was 100 mg per liter of culture. The rSk was purified with high recovery until homogeneity. The final product is a preparation with high specific activity, DNA and *E. coli* protein free, sterile and lyophilized powder. The drug has been successfully assayed for the treatment of acute myocardial infarction and deep vein thrombosis among other afflictions.

In conclusion, this new recombinant thrombolytic agent can be obtained via a highly productive process, and the product has more than 98% purity. Its safety and efficiency in clinical trials have been indicated in small pilot studies, and the recombinant product could be less immunogenic than the non-recombinant.

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