

INDIGENOUS RECOMBINANT STREPTOKINASE VS NATURAL STREPTOKINASE IN ACUTE MYOCARDIAL INFARCTION PATIENTS: PHASE III MULTICENTRIC RANDOMIZED DOUBLE BLIND TRIAL

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ABSTRACT

BACKGROUND: Streptokinase is the most widely used thrombolytic agent and can now be made using recombinant DNA technology. The present trial was initiated to assess an indigenous recombinant streptokinase (Shankinase, r-SK). **AIM:** To compare the efficacy and safety of indigenous recombinant streptokinase (Shankinase, r-SK) and natural streptokinase (Streptase, n-SK). **SETTINGS AND DESIGN:** Double blind, randomized, non-inferiority, multicentric, parallel study. **MATERIALS AND METHODS:** Patients of AMI < 6 hours of chest pain and 2 mm ST elevation in 2 contiguous chest leads V₁-V₆ or 1 mm in limb leads were randomized to receive 1.5 miu of either r-SK or n-SK. CK Peaking and decrease of ≥50% ST segment were used to assess reperfusion. **STATISTICAL ANALYSIS:** Difference in the groups was assessed by chi-square or paired t test as required. Probability value <0.05 was considered significant with 95% confidence interval. **RESULTS:** Overall 150 patients were recruited (96 r-SK group and 54 in n-SK group) and demographic and clinical profile of the groups was comparable. Reperfusion was seen in 68.2% (58) and 69.4% (34) patients in r-SK and n-SK groups respectively. Commonly seen adverse events were fever in 7 (8.5%), hypotension in 3 (3.6%), nausea in 2 (2.4%) patients. Minor bleeding were seen in 4 (4.8%) of patients. **CONCLUSION:** Indigenous recombinant Streptokinase (r-SK) is as efficacious as natural streptokinase (n-SK) in establishing reperfusion as assessed by non-invasive parameters with comparable side effect profile.

KEY WORDS: Streptokinase, Recombinant streptokinase, Myocardial infarction, Thrombolysis

INTRODUCTION

Over the last few years intravenous thrombolysis has become the standard

therapeutic approach for patients with acute myocardial infarction (AMI). Intervention with thrombolytic agents in acute myocardial infarction is an effective means of limiting

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myocardial damage. The reduction in short and long-term mortality rates caused by thrombolytics has been demonstrated in several large-scale clinical trials.^[1-3] Despite a slight difference in mortality found in the GUSTO trial, favoring tissue plasminogen activator (t-PA) when given as an accelerated infusion, the effects of natural streptokinase (n-SK) on the outcome of the patients with AMI has been similar to other thrombolytic agents and it remains the agent of choice in India as it is cheaper.^[4-6] Though the greatest benefit occurs when streptokinase infusion is initiated early after onset of symptoms, late benefit has also been observed in patients treated even after 24 hrs of onset of symptoms.^[1,7]

Presently all available streptokinase for use in India, are naturally derived and expensive, while Shankinase is the first indigenously manufactured streptokinase obtained by means of recombinant-DNA technology. The recombinant-DNA process entails the culturing of *E. coli* carrying appropriate plasmid DNAs encoding for recombinant natural-type streptokinase followed by cell-lysis, refolding of streptokinase to their biologically active states, and their isolation at approximately 98% purity by chromatographic means.

This study was conducted at 6 centers, with the objective of comparing the effects of recombinant (r-SK) and natural (n-SK) streptokinase on coronary reperfusion in patients with acute myocardial infarction (AMI) and also the side effects.

MATERIALS AND METHODS

The present comparative, double blind,

randomized, multicentric, parallel study between natural and recombinant Streptokinase was carried out in six hospitals across the country between January and June 2003. The medical division of Shantha Biotechnics, Hyderabad was responsible for coordinating and monitoring the study. An independent data monitoring safety board monitored all the events and serious adverse events regularly throughout the trial. The ethics committee at each hospital, and the Drug Controller General of India approved the protocol.

Study patients

Eligibility criteria: Patients with characteristic chest pain (ischemic origin) occurring for not more than 6 hours were considered for inclusion if there was 2 mm ST segment elevation in at least 2 contiguous chest leads or 1 mm elevation in standard limb leads. There was no gender restriction but patients below 65 years were enrolled.

Patients with previous or ongoing cerebrovascular disease, anticoagulant therapy, history of gastrointestinal hemorrhage or acute peptic ulcer, severe trauma, surgery, or invasive procedures in preceding 8 weeks, bleeding tendency, systemic BP (>180 / 110 mm of Hg), severe hepatic or renal failure, metastasizing malignancies and other terminal diseases, Streptokinase therapy within 5 days to 6 months, streptococcal disease within the previous 6 months, cardiogenic shock, allergy to Streptokinase or aspirin, proliferative diabetic retinopathy, infective endocarditis, and pregnancy or lactating were excluded.

Study procedure

Written informed consent was taken from the

patients who were randomized in 2:1 fashion (in favour of Shankinase r-SK) to receive 1.5 million units of recombinant Streptokinase (Shankinase, Shantha Biotechnics Ltd., India) or natural Streptokinase (Streptase, Hoechst-Roussel, Germany) diluted in 100ml saline through a peripheral vein over a period of 30-60 min. Intravenous hydrocortisone (100 mg) and oral aspirin (325 mg) was administered to all patients. The decision of administration of concomitant medications was left to the physician.

Objectives

Primary objective: To establish reperfusion and compare the reperfusion rates in both the groups.

Secondary objective: To establish and compare the safety profile in each group.

Outcomes: (Definitions of positive markers of coronary artery patency)

Maximal CK activity within 12 hr after the start of thrombolytic therapy was considered a positive marker of infarct related artery patency.^[8] A positive ST marker was defined as a reduction in ST segment elevation of $\geq 50\%$ within 90 mins after the start of thrombolysis.^[8] Pain reduction of more than 50% after 90 minutes of thrombolysis was also considered an indicator of reperfusion.^[7]

Determination of CK

Venous blood samples were collected before administration of Streptokinase and then again at 90 mins, 6 hrs, 12 hrs, 24 hrs and 36 hours after infusion. CK activity was estimated by Sigma Diagnostics Creatinine Kinase Kit (Procedure No. 47-UV, Sigma Diagnostics, US)

ECG assessment

Standard 12 lead ECG was done immediate before and 90 minutes after initiation of Streptokinase infusion and was assessed and interpreted by two different observers to avoid bias in reporting. Lead with maximum ST segment elevation was identified and level of ST segment elevation was measured immediately prior to and 90 min after initiation of therapeutic therapy.

Pain relief assessment

The percentage decrease in pain was recorded at 90 minutes after initiation thrombolytic therapy on a scale of 0-10 as perceived by the patient.

Other investigations

Serum samples were collected wherever possible for detecting anti-streptococcal antibodies. Pre discharge Echocardiogram was done to assess left ventricular function.

Liver function and renal function tests of all patients were carried before and after therapy. In case of bleeding episodes hemoglobin estimations were done to differentiate minor from major bleeds. Minor bleed was defined as bleeding not requiring transfusion and without hemodynamic compromise. Major haemorrhage was defined as bleeding requiring transfusion of blood or overt clinical bleeding (or documented intracranial or retroperitoneal hemorrhage) associated with a drop in hemoglobin of greater than 2 g/dl.

Statistical analysis

The sample size was determined as per regulatory requirements (schedule Y of drugs and cosmetics act 1940, where a study drug

has to be evaluated in at least 100 subjects). The difference in both the groups was assessed by the chi-square and paired t test wherever required. A probability value <0.05 was considered to be significant with 95% confidence interval. The SAS version 8.2 (SAS Institute Inc, Cary, NC, US) software was used to carry out the statistical analysis.

Randomization procedure

Block randomization was done by using computer generated codes using the SAS software version 8.2 (SAS Institute Inc, Cary, NC, US).

Random allocation procedures

To preserve the blinding of the study, only a minimum number of personnel at the clinical research department where the code was generated had seen the random assignment list. Sealed emergency codes were available to the independent clinical monitor. These codes, which revealed the subject's drug group when opened, were only allowed to be opened during the study only if the choice of follow-up treatment depended on the subject's therapy assignment. If a subject's therapy assignment was unblinded, the department was notified

immediately by telephone or fax. After the study the centers where the study was conducted returned all sealed and any opened codes.

RESULTS

Clinical data

Between January 2003 to June 2003, 150 were patients recruited in the study while 96 received recombinant 54 patients were administered natural streptokinase. As per protocol 85 out of 96 and 49 out of 54 patients were eligible for evaluation for efficacy while as per intention to treat all 150 were evaluated for safety. The organizational chart of the recruitment of the patients is represented in Figure 1. There was no significant difference in the demographic and clinical profile of patients in both the groups [Table 1]. Mean symptom-treatment interval was 3.6 ± 2.5 hrs and 3.8 ± 2.1 hrs for Shankinase and Streptase respectively.

Reperfusion

CK peaking less than or at 12 hours was seen in 83.5% (71) and 89.8% (44) patients in r-SK and n-SK groups respectively. The kinetics of

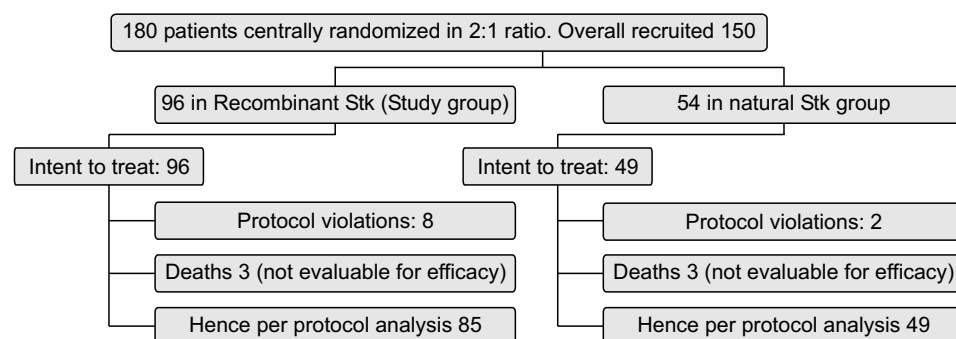


Figure 1: Patients and the recruitment scheme

Table 1: Demographic and clinical characteristics of patients

	Recombinant Streptokinase (%)	Natural Streptokinase (%)	'P' value
Age (year, mean \pm SD)	52.0 \pm 10.3	51.6 \pm 10.7	0.822
Sex (F: M)	1:6	1:5	NA
Diabetes Mellitus	23 (23.9)	14 (25.9)	0.843
Hypertension	25 (26.0)	14 (25.9)	0.929
Previous Angina	14 (14.6)	8 (14.8)	0.989
Smoking	48 (50.0)	28 (51.8)	0.926
Previous MI	5 (5.2)	1 (1.8)	0.303
Obesity	7 (7.3)	4 (7.4)	0.971
Symptoms treatment interval (Hrs)	3.6 \pm 2.5 (range 50 minutes to 5.5 hrs)	3.8 \pm 2.1 (range 45 min to 5.3 hrs)	0.466
Killips class			
I	85 (88.5)	45 (83.3)	0.435
II	7 (7.3)	7 (13.0)	0.435
III	2 (2.1)	2 (3.7)	0.435
IV	0	0	0.435

CK peaking at different intervals in both the groups is represented in Figure 2. ST segment resolution $\geq 50\%$ was observed in 81.2% (69) and 79.6% (39) patients in r-SK and n-SK group respectively. Pain resolution in 94.1% (80) and 93.9% (46) of patients in r-SK and n-SK group respectively. Reperfusion as assessed by the combination of CK peaking and ST resolution ≥ 50 was seen in 68.2% (58) and 69.4% (34) in r-SK and n-SK groups respectively. When assessed by all three parameters reperfusion rates were 69.0% (59) and 65.3% (32) in r-SK and n-SK respectively ($P=N.S.$) [Table 2].

Overall 47 serum samples were tested (27

from r-SK and 20 from n-SK group) for Anti-streptococcal antibodies. As is evident from the Table 3 presence of anti-streptococcal

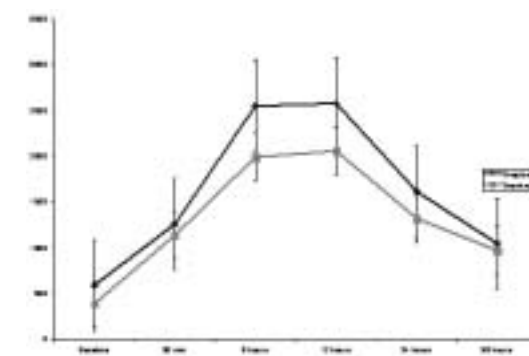


Figure 2: Mean CK values at different intervals in the two groups

Table 2: Efficacy parameters seen in both groups

Efficacy parameters	Percent efficacy		'P' value
	Recombinant Streptokinase % (n)	Natural Streptokinase % (n)	
CK Peaking ≤ 12 hours	83.5 (71)	89.8 (44)	0.293
ST resolution $\geq 50\%$	81.2 (69)	79.6 (39)	1.000
Pain resolution	94.1 (80)	93.9 (46)	1.000
CK and ST resolution	68.2 (58)	69.4 (34)	1.000
CK, ST resolution and pain relief	69.0 (59)	65.3 (32)	0.652

Table 3: Concomitant medication in both the groups

<i>Concomitant Medication administered during study</i>	<i>Recombinant Streptokinase N (%)</i>	<i>Natural Streptokinase N (%)</i>
Heparin	30 (31.2)	17 (31.4)
Beta blockers	15 (15.6)	21 (38.9)
ACE inhibitors	19 (19.8)	23 (42.6)
Angiotensin receptor blockers	1 (1.04)	0 (0)
Diuretics	6 (6.1)	6 (11.1)
Lipid lowering agents	20 (20.8)	15 (27.8)
Aspirin	30 (31.1)	25 (46.3)
Clopedogril	26 (27.1)	17 (31.5)
Morphine	1 (1.1)	1 (1.8)

antibodies did not interfere with the reperfusion, in either of the groups.

The occurrence of reperfusion arrhythmias was observed in 25% of patients in both the groups. The most commonly seen arrhythmias were accelerated idioventricular rhythm (AIVR) (38.8%), ventricular premature beats (38.8%) and ventricular tachycardia (22.2%).

Adverse Events

No significant differences were observed in the adverse event profile between the two groups although hypotension and fever were relatively common (Table 4). Hypotension in all the patients responded to inotropes and saline infusion after transient cessation of therapy.

Table 4: Adverse events seen in both the groups

<i>Adverse events</i>	<i>Drug related</i>	
	<i>Recombinant Streptokinase N (%)</i>	<i>Natural Streptokinase N (%)</i>
Hypotension	3 (3.6)	4 (7.2)
Fever	7 (8.5)	5 (9.2)
Rigors	3 (3.6)	0
Nausea vomiting	2 (2.4)	3 (5.4)
Bleeding		
Major	0	1 (1.8)
Minor	4 (4.8)	3 (5.4)
Angioedema	0	1 (1.8)
Anaphylaxis	0	1 (1.8)

Fever of moderate grade and was accompanied by rigors in three patients in r-SK group. All patients were given antipyretics and responded to therapy. Nausea and vomiting which was seen in 2 and 3 cases respectively in the two groups was controlled by administration of antiemetics.

Overall eight bleeding episodes were reported of which only one was major bleeding in the n-SK group, the remaining 7 were minor events 4 and 3 events in the r-SK and n-SK groups respectively. One patient who had a major gastrointestinal bleeding needed blood transfusion he also had Thrombocytopenia, which subsided on stoppage of heparin. Of these four events in the r-SK group two patients had bleeding at the site of venipuncture, which later healed without any need for medication, one patient had gastrointestinal bleeding, who was managed conservatively, while another patient had an episode of gum bleed followed by haemoptysis, which did not recur. Of the three events reported from the n-SK group two were cases of minor oozing at the site of vein puncture, which resolved without medication, one patient had gastrointestinal bleeding without hemodynamic compromise and was

managed conservatively.

DISCUSSION

Reperfusion in the present study which was the primary end point was assessed using a combination of enzyme (Creatine Kinase) peaking and ST segment resolution was observed in 68.2% (58) in the r-SK and in 69.4% (34) patients in n-SK group whereas, rates reported in literature by the present criteria vary from 59 to 82%.^[1,4-6,9] A randomized comparative study was carried out in over 200 patients where a reperfusion rate of 67.1 and 70.7 was observed with recombinant and natural streptokinase respectively.^[10] A similar rate of 70.6% was reported from previous Indian studies with natural streptokinase.^[8]

Safety profile assessment which was the secondary end point in the present study was similar in both the r-SK and n-SK group. These events seen were similar to those reported by other studies. Incidence of bleeding was 4.2% (4) in the r-SK group while it was 5.4% (3) in the n-SK group, which are similar to the rates reported in literature vary between 0.5 to 15.2%.^[1,5,11,13] No anaphylactic reactions were seen in the r-SK group whereas one was reported from the n-SK group. Adverse effects of thrombolytic therapy commonly include hypotension 4.5-15%, fever 5%, rigors, nausea and vomiting 46% and bleeding (0.5-15.2%). The mortality rates were low for both the groups {3.1% (3) and 5.5% (3)}, however the present small study did not have the statistical power to show the difference in the two comparable treatment groups. Post thrombolysis, cardiogenic shock was observed

in 1 (1.2%) and 2 (3.6%) in the recombinant and natural streptokinase groups respectively whereas reinfarction was seen in 2 (2.4% and 3.6%) patients respectively in both the groups which is much less than in other studies.

The present study has its limitations as the sample size is small and the markers used for assessing reperfusion were non-invasive instead of the gold standard that is angiography, although the non-invasive methods have been validated against angiography and are proven surrogate markers of reperfusion. Since this trial was a randomised controlled trial the instigators were of the opinion that the conditions to see the efficacy and safety need some factors to be controlled including patients not more than 65 years of age and without any co-orbid conditions that might influence the outcome. A larger study with patency assessed by angiography and without influencing factors of age or others would give a precise assessment of reperfusion.

Streptokinase is the most widely used thrombolytic agent particularly in India while others like tPA are expensive. Reviews comparing other thrombolytic agents have shown that streptokinase to be as good as that of tPA or rPA in terms of both efficacy and safety.^[14,15] Recombinant streptokinase has the advantage of not containing streptolysin and streptodornase unlike streptococci-derived natural streptokinase which might make it safer, and will be cheap which is very relevant to our country's population. This technology can be further used to make more modifications in the present agents to make them more safe and efficacious.

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