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Review Article

# A REVIEW ON SUCCESSIVE GENERATIONS OF STREPTOKINASE BASED THROMBOLYTIC AGENTS

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**ABSTRACT**

Thrombotic disorders and their consecutive medical consequences are now well-known in both developed and developing countries. Complications associated with these disorders are emerged as the main cause of mortality in the world and is caused due to the imbalance in the regular maintenance of the homeostasis between clot formation and clot dissolution. The effectiveness of thrombolytic therapy has accelerated the attention to be paid for it, at the part of doctors, physiologists, pharmacologists, chemists, biologists, and biotechnologists. This is clearly shown by the development of both new therapeutic methods and thrombolytic preparations. The latter area has been developing in several directions, majority of them focus on the chemical modification of plasminogen activators and the development of new mutant and hybrid protein derivatives through genetic engineering. Among all other thrombolytic agents, streptokinase (SK) is widely used as a thrombolytic agent in the treatment of acute myocardial infarction (AMI) following coronary thrombosis. This review deals with the detailed description of new generation streptokinase based thrombolytic agents - their mode of action, structural modifications and current position of the drug in the clinical market.

**Keywords***:* Streptokinase; Homeostasis; Thrombolytic agents; Acute myocardial infarction.

## INTRODUCTION

A healthy homeostatic system suppresses the development of blood clots in normal circulation, but reacts extensively in the event of vascular injury to prevent blood loss. Outcomes of a failed homeostasis, leads to the formation of the blood clot (thrombus) in the circulatory system and can cause vascular blockage leading to serious consequences including stroke, pulmonary embolism, deep vein thrombosis and acute myocardial infraction [1] and even death [2]. Pathological innovation, advancement in the molecular biology and development of new tools in genetic engineering makes it possible to identify the homeostasis failures. However, to dissolve the formed blood blockages and to complement the plasminogen activators to lyse the clots, various thrombolytic molecules have been identified and developed. Out of these, most well-known agents are, Streptokinase (SK), Urokinase (UK), Recombinant tissue-type plasminogen activator (rt-PA), Acylated plasminogen streptokinase activator complex (APSAC) and single chain urokinase-type plasminogen activator (scu-PA, pro-urokinase) [3] etc. Among all the above mentioned, SK, the first generation thrombolytic agent, is the only moderately efficacious drug in practice and its intravenous administration clears approximately 50% of occluded coronary arteries within 90 minutes and reduces mortality up to 25% [3].

The molar mass of Streptokinase is 47 kDa and made up of 414 amino acid residues (aa) [4]. The crystal structure reveals that SK contains three sequential domains [5], namely α (aa 1 to 150), β (aa 151 to 287), and γ (aa 288 to 411) domains from the amino to the carboxy-termini, linked by flexible loops [6]. SK does not have any proteolytic activity as of itself, instead forms a 1:1 stochiometric complex with a plasminogen or a plasmin molecule [7], following a three step mechanism [3]. In the first step, streptokinase binds with the plasminogen to make an active complex, which undergoes a conformational change resulting in the exposure of an active site in the plasminogen moiety [8]. The C-terminal domain of streptokinase is involved in plasminogen substrate recognition and activation. Similarly, the Asp41–His48 region of streptokinase is important in binding to the substrate plasminogen [9]. In the second step, the active site catalyzes the activation of plasminogen to plasmin. In the third step, plasminogen with the streptokinase molecule is converted to plasmin streptokinase complex. Howsoever, the first 59 amino acid residues seem to have multiple functional roles in streptokinase [10, 11]. Without these N-terminal residues, streptokinase has an unstable secondary structure. Loss of residues 1–59 greatly reduces the activity of the remaining streptokinase fragment [11].

Although SK was the first FDA approved thrombolytic drug, its limited half-life, high antigenicity are becoming barriers to use this agent actively against AMI. Therefore, the ongoing thrombolytic research has focused on the development of SK based novel thrombolytic therapy. The present review has also focused the recent developments and current market status of new generation streptokinase based thrombolytic agents.

## COMPARATIVE STUDY OF STREPTOKINASE WITH OTHER PLASMINOGEN ACTIVATORS

The three major available thrombolytic agents are tPA, uPA, SK. Among of them, the microbial sourced SK is the least expensive, but immunogenic and non- fibrin specific agent. Human tPA and uPA are immunogenically benign, and trypsin-like serine proteases which activate plasminogen directly. The tPA is produced by the vascular endothelial cells and uPA is produced by the kidneys and secreted into the urine. The tPA for therapeutic use is obtained mainly from cultures of recombinant animal cells [12]. It is shown that tPA activates the clot bound plasminogen 100-fold more effectively than the circulatory plasminogen. So, tPA is said to be a good because of its clot specificity and by that it is the poor activator of circulatory plasminogen relative to the other available plasminogen activators [13]. But, the usage of t-PA in clinical applications is limited with the short half-life of the protein in *in vivo* and high cost of the treatment.

Staphylokinase (SAK), a 136 amino acid profibrinolytic agent is the other potential clot specific alternative plasminogen activator sourced from *Staphylococcus aureus*. Even though this specific clot buster finding good response in therapy, its use is limited because of its antigenicity and short half-life.

Comparative clinical trials and cost-effectiveness considerations suggest that streptokinase is the drug of choice for thrombolytic therapy, particularly in developing countries. The plasmin produced through the SK mediated activation of plasminogen, acts on the fibrin network of the thrombus. But, in addition to this clot lytic activity, it also breaks down the streptokinase in to smaller fragments that limit the *in-vivo* half-life of streptokinase to about 30 minutes [14]. Although SK survives in circulation significantly longer than does tPA (a half-life of about 3-4 min), this is still short for efficient therapy. The comparative study based on the antigenicity, half life, Fibrin Specificity (FS) of seven thrombolytic agents is summarized in Table 1.

**Table 1: Comparison of plasminogen activator**

| Thrombolytic Agent | Molecular Weight (KDa) | FS | Antigenicity | Half life (Min) | Plasminogen Activation |
|---|---|---|---|---|---|
| tPA | 72 | ++ | NO | 3-4 | Direct |
| Urokinase | 55 | - | NO | 15 | Direct |
| SAK | 15 | + | YES | 6 | Indirect |
| SK | 47 | - | YES | 25 – 30 | Indirect |
| Altreplase (rt-PA) | 70 | + | NO | 68-72 | Indirect |
| Reteplase | 40 | - | NO | 18.8-19.2 | Indirect |
| Antistreplase | 131 | - | NO | 90-112 | Indirect |

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**INITIAL CLINICAL TRIALS ON THE "WONDER DRUG"— STREPTOKINASE**

Dr. Sherry, in 1947 was the first person to report that the SK from Streptococcus could be used to treat the blood clot disorders. In 1952, Johnson and Tillett successfully used streptokinase to lyse the artificially induced intravascular clots in the marginal ear veins of rabbits. In 1955, Tillett's group performed clot lysis in patients by intravenous infusion of SK. Ruegsegger and colleagues (1959) first time successfully dissolved intracoronary clots. After successful experiments in animals, Boucek and Murphy used streptokinase in human beings. They injected streptokinase into the coronary sinus (catheterized via the right brachial artery) of patients who had occlusion of the coronary artery. In 1966, Schmutzler and co-authors in Germany published one of the largest trials of that time, involving 558 patients. They reported a mortality rate of 14.1% in the patients treated with streptokinase, compared with 21.7% in the control group [15].

These initial clinical trials with SK revealed the effectiveness on revival of ventricular function, reduction of infarct size and mortality, but the drug suffered from no fibrin specificity, short plasma half-life, hypersensitivity and need in high dosages.

In 1985, large number of trials was conducted by taking mostly small groups, in an effort to establish a standard protocol for streptokinase use in AMI. *Gruppo Italiano per la Sperimentazione della Streptochinasi nell'Infarto Miocardico* (GISSI) conducted a trial, and the initial report of this trial revolutionized the outlook of medical scientists all over the world regarding thrombolytic therapy for AMI. The protocol involved intravenous streptokinase administration in AMI patients: 11,806 patients in 176 coronary care units in different hospitals were enrolled during a period of 17 months (from February 1984 through to June 1985) for the study. After 12 months follow up period, GISSI published the mortality rate between streptokinase and non streptokinase groups which shown that the increasing effectiveness and usefulness of modified streptokinase against AMI comparing with the initial trial report.

Although t-PA has become a more popular thrombolytic agent in developed nations, SK continues to be widely used in developing nations. The introduction of SK into medical practice for acute heart attacks in the 1970s began to make its mark on the declining death rates from acute heart attack. The in-hospital death rate for acute myocardial infarction has reduced from 25% in 1970 (when early thrombolytics were becoming marginally accepted) to 10% in 1989 (when thrombolytic use had doubled and a new recombinant form of tissue plasminogen activator [t-PA] was introduced) to 8% in 2002 (after angioplasty was more widely used), for a total decline of 68% (*National Hospital Discharge Survey Annual Reports; National Heart, Lung and Blood Institute).*

A case study was conducted by Multicenter Acute Stroke Trial--Italy (MAST-I) group in 1995 with streptokinase, aspirin, and combination of both in treatment of acute ischemic stroke patients and reported that 622 patients with acute stroke when administrated with placebo or a fixed dose of SK1.5 MU *i.v* for 60 min, 27% of SK group had shown significant higher mortality compared with the placebo group (12%). However 4% of SK treated group was suffered by allergic reactions [16]. Another case study organized by Multicenter Acute Stroke Trial-Europe (MAST-E) (1996) reported that out of 310 patients with acute stroke, 34% of SK treated group shown mortality compared with control group (18.2%). This may be due to the hemorrhagic transformation of ischemic cerebral infarcts [17].

**NEED FOR STRUCTURAL MODIFICATIONS**

The importance of therapeutic proteins has grown rapidly since the emergence of the biotechnology industry more than 30 years ago and approximately 140 therapeutic proteins approved in the United States and Europe [18]. SK was the one of first FDA approved drug introduced as a therapeutic agent for acute myocardial infarction and it is included in the list of *World Health Organization Model List of Essential Medicines* [19]. As we mentioned earlier the main limitations when used in thrombolytic treatment are the immunogenicity and the relatively short half-life in circulation. Furthermore the naturally produced SK by various strains of hemolytic *streptococcus* have several other unwanted toxic products, e.g. deoxyribonucleases, streptolysin or hyaluronidase and proteases, which makes the purifying process of desired protein difficult [20]. Streptokinase not only activates the clot bound plasminogen, but also the systemic plasminogen, which can induce hyperplasminemia, depletion of the circulating fibrinogen (up to 20%) and of the V and VIII coagulation factors, with the concomitant increase of the products from the degradation of plasminogen into plasma [21].

On the other part, plasmin stimulates the conversion of kalikreinogen into kalikreine, so, the SK infusion produces a release of quinines [21]. It is partly responsible for the hypotensive effect seen in most of the patients receiving SK. Because of its bacterial origin, SK is antigenic, and therefore, it can induce allergic reactions. Four percent of the patients of the Second International Study of Infarct Survival-ISIS-2 [22] that received SK had allergic reactions, fever, shivers, urticaria or rash. The anaphylactic shock is fortunately very rare (0.1-0.5%), but the arterial hypotension needed resuscitation through fluid therapy in 7-10% patients. Based on the above mentioned resource, many structural and functional studies are underway to produce a more efficient streptokinase with extended half-life, reduced or eliminated immunogenicity, and improved plasminogen activation [23, 24]. It appears that the streptokinase domains responsible for activity, stability and immunogenicity have considerable overlaps.

Any structural change needs to be incorporated in the molecule requires a thorough structure–function analysis which in-turn leads to the subject of extensive investigation [6]. The study on streptokinase domains help to modify these molecules. The numerous preparations by recombinant DNA technology and by fusing with Polyethylene glycol have been made to enhance the half life and to reduce the antigenicity of the drug. Based on the modification and of the clinical trials on SK, it has been the classified as first generation and second generation agents, as summarized in Table 2.

**Table 2: Classification of first and second generation Streptokinase**

| First Generation | Second Generation |
|---|---|
| Natural SK | 1. Acylated plasminogen–SK activator complex (APSAC); [19]<br>2. Recombinant fusion SK; [27, 30]<br>3. Modified SK with Reduced immunogenicity; [34, 39]<br>4.Mutant SK with improved Stability; [36, 37] |

**Acylated plasminogen–streptokinase activator complex (APSAC)**

APSAC (APSAC; antistreplase) is an equimolar, non-covalent inactive complex between human plasminogen and SK**.** When it is injected, a controlled deacylation of the catalytic center occurs, activating the complex so that thrombolysis begin [25]. The main aim of constructing APSAC was to control the enzymatic activity of the plasmin (ogen)-SK complex by a specific reversible chemical, protecting its catalytic center (i.e., by titration with a p-anisoyl group). APSAC has an extended effective half-life in circulation relative to streptokinase [3] and it is immediately active in the bloodstream, combines with plasminogen to become active- and is given in a single intravenous injection. The report shown that for APSAC the plasma half-life of 70 minutes in compared with 25 minutes for the plasminogen- SK complex formed in in-vivo after the administration of SK. But patients with high SK antibody titers do not respond to APSAC. The research carried out An extensive research work is carried out to assess the relative safety and effectiveness [26] of APSAC in contrast to other thrombolytic agents.

**Recombinant streptokinase**

Considering its therapeutic applicability and clinical implications in thrombolytic therapy, attempts have been made to search for an alternative source for production of Streptokinase through recombinant routes. The production of recombinant SK (rSK), followed by different strategies, yielding a high benefit/cost or benefit/risk ratio is highly desirable. Recombinant DNA technology is used to produce high quantities of rSK avoiding the contamination risks that arise from the manipulation of pathogens such as those belonging to the streptococcus species [27]. By having sufficient information about the gene, regarding the transcriptional control [26] and its promoter [28] many attempts have been made for cloning and secure expression of the recombinant streptokinase in bacteria which are not pathogenic for humans [29].

Studies on the SK gene, isolated from various *streptococcus* species had suggested that it to be a polymorphic. In order to improve the yield of SK, particular the gene, which determines streptokinase C, A and G [30] were cloned and expressed in *E. coli* as well as in *Streptococcus sanguis*. More often the SK gene cloned from *S. equisimilis* H46A has been expressed in several gram positive and gram negative bacteria including *B. subtilis* WB600 and *E. coli* [31]. In 1990 Muller & Malke sequenced the streptokinase gene for the first time from *S. equisimilis* H46 A [32]. Production of recombinant streptokinase in the methylotrophic yeast, *Pichia pastoris*, has been attempted by Hagenson [33] and Cuban group. The insertion of a genetic construct with the gene of SK and erythromycin as a selection marker was introduced in the *S. equisimilis* H46A strain in order to select super producing clones of protein [34, 35, 36]. Plasmids designed for high-level expression of streptokinase have been successfully evaluated for producing it. The plasmid used imparted ampicillin resistance to the bacterium and ampicillin provided the selection pressure for plasmid retention. An ability to produce recombinant streptokinase greatly enhances the possibilities for beneficial structural modifications of this protein and enhanced production of the desired rSK.

The medium composition plays a vital role in microbial growth as well as enzyme production. For industrial applications any culture media should actively promote microbial growth, it should be cost effective and easy to prepare and maintain. In 1999, Wu Zhang demonstrated the production of recombinant streptokinase from *E. coli* as a host [37], 400 to 645 mg/mL SK protein had been obtained from one liter cell culture of *E. coli* using a rich and complex cell growth medium. Howsoever for higher cell biomass, yielding higher recovery of rSK from the cell extract require an inexpensive growth medium composition, which is more economical for the production of rSK. Venkat Rao (1995) demonstrated that the production of SK using a genetically engineered strain of *E. coli* PSK4 which overproduce streptokinase intracellularly by using an optimized growth medium mainly comprised of simple salts and trace-elements [29, 38].

These studies not only limited the optimization media for over production of SK, but also many attempts had been made to fuse the SK with various tags at N and C terminals to improve the stability and for easy purification of the protein from the bacterial cultures. Addition of GST tag (Glutathione S-transferase) at the N terminals of the rSK has increased the stability of the recombinant protein [15] and also helped to recover the recombinant protein in single step [34].

**Mutated streptokinase**

The drawback of SK for its clinical treatment is the presence of anti-SK antibodies (Abs) and the short life stability [39]. Patients receiving SK therapy develop high anti-SK antibody titers, which might provoke severe allergic reactions. These Abs are sufficient to neutralize a standard dose of SK up to four years after initial SK administration. It is reported that the immune-dominant epitope present in C-terminal region of native molecule and the deletion mutant lacking the 42 C-terminal residues, decrease the antigenicity when compared with the native molecule [40. One chemical modification has involved complexion of the streptokinase with PEG [41], primarily for reducing immunogenicity. In SK the cysteine residue is modified with polyethylene glycol. Polyethylene glycolated cysteine streptokinase shown the decreased antigenicity and decreased *in vivo* immunogenicity, simultaneously it has increased the proteolytic stability compared with the un-PEGylated mutant SK.

The short *in-vivo* half-life of SK limited the efficacy as an efficient blood clot dissolving agent. Therefore, the researches are mainly focusing on the increasing the half life of SK. It was found that the prevention of cleavage site at Lys59 of streptokinase prolongs the half-life of streptokinase in complex with plasmin [42]. In another study, it has shown that conversion of Lys59 to glutamine was important in extending the functional half-life of SK [43]. Another research on mutated SK claimed that either incorporating additional cysteine residues or substitute the cysteine residue for normally occurring amino acids into the non essential regions of the protein said to improve the *in vivo* half life, proteolytic activity and reduce the immune reactivity [44].

The improved thrombolytic activity resulting from resistance to degradation by human plasmin (HPlm) comprising a mutant SK in which replacing one or more amino acids segment. Reports reveal that replacement of amino acid Lys59 by Glu59, from the Pro58-Lys59-Ser60-Lys61 segment of the corresponding native SK, can help to develop the mutant SK which is more resistant to hydrolytic inactivation by human plasmin (HPlm) and more effective in activation of HPlg than the native SK [43].

**Fibrin specificity of streptokinase**

Reperfusion of the ischemic brain is the most effective therapy for acute stroke, restoring blood flow to threatened tissues. The t-PA is usually considered as the most potent fibrin specific plasminogen activator. Administration of recombinant tissue plasminogen activator for ischemic stroke patients, can improve neurological outcome within 3 h but the adverse hemorrhagic events limits its use to less than 3% of acute ischemic stroke (AIS) patients. There is a need to develop an agent which will not show any hemorrhagic complications. So the researchers are mainly focusing on to develop fibrin-specific, liquid perfluorocarbon nanoparticle that is surface modified to deliver the plasminogen activator streptokinase which can give more effectiveness and less complicated treatment for AIS compared with tPA, and this is emerging as a more efficient and cheap clot buster. It had been reported that streptokinase-loaded, fibrin-targeted perfluoro-octylbromide nanoparticles in the presence of plasminogen, induced rapid fibrinolysis (<60 min) without concurrent microbubble production and cavitation. It demonstrated that 1% surface targeting of streptokinase nanoparticles are significant for decreasing ~30% clot volumes in 1 hr [45]. Moreover ultrasound exposure had no effect on the digestion process.

The other research had shown that fibrinolytic therapy with the combination of streptokinase and the potent anticoagulant agent enoxaparin resulted in similar adjusted outcomes compared with more costly regimens utilizing a fibrin-specific lysis (FSL) [46]. In addition to the above mentioned strategies of producing SK fusions and mutated forms, the research is in progress to produce a SK fusion, tagged with a part of tPA which determines the SK as a clot specific and giving hopes to produce the molecule in prokaryotic systems at a cheaper price.

**CURRENT STATUS OF STREPTOKINASE BASED THROMBOLYTIC DRUGS**

Now-a-days many pharmaceutical companies manufacture streptokinase based thrombolytic drugs, but Hoechst Marion Roussel and Kabivitrum (Pharmacia and Upjohn) are the leading manufacturers of this product. They registered the product as Streptase® and Kabikinase®. At first Heberkinasa®, a recombinant SK commercialized by HEBER BIOTEC launched SK for acute myocardial infarction and the deep venous thrombosis. The names of generic product of SK are Propinase® (Emcure Pharmaceuticals Ltd), Ekinase® (Dabur Pharmaceuticals Ltd.), Icikinase® (ICI India Limited), Thrombosolv® (VHB Life Sciences), Fibrokinase® (Otsira Genetica), Solustrep ® (DONG KOOK PHARM Co. Ltd), Zikinase® (Indon) [19] (Table 3).

**Table 3: Trade Name and the Origin of some SK based products**

| S. No | Trade name | Distributor | Manufacturer |
|---|---|---|---|
| 1 | Heberkinase | USV Limited, India | Heber Biotec SA, Cuba |
| 2 | Streptonase | Blausiegel, Brazil | Kyung Dong Pharm Co. Ltd, Korea |
| 3 | Streptokinase TTK | TTK Pharm, India | BBT Biotech, Germany |
| 4 | Eskinase | Dabur India Ltd, India | BBT Biotech, Germany |
| 5 | Recombinant Streptokinase | China | Shanghai SIIC SMU Biotech Co. Ltd, |
| 6 | STPase | Ahmedabad, India | Cadila Pharmaceuticals Ltd, India |
| 7 | Kabikinase | Pharmacia/Upjohn, | Pharmacia Upjohn, Sweden |
| 8 | Thrombosolv | VHB Pharm, India | Korea United Pharm Inc., Korea |
| 9 | Streptase | Aventis Behring GmbH, Germany | Aventis Behring GmbH Germany |
| 10 | Treptase | Rahmah Drugstore, Jordan | Se Jong Pharm, Korea |
| 11 | SK-PDC | Aventis Behring GmbH Germany,, | Aventis Behring GmbH Germany, |
| 12 | Strek | Libra Productos | Porto Allegre, Brazil |

Varidase, a mixture of the enzymes (streptokinase and streptodornase) produced by hemolytic streptococci, is used throughout the world for the topical treatment of purulent and suppurating wounds. It is used topically on surface lesions and by instillation in closed body cavities to remove clotted blood or fibrinous or purulent accumulations. It is also used as a skin test antigen in evaluating generalized cell-mediated immunodeficiency [46].

Indikinase™ is a highly purified streptokinase produced by Recombinant technology using the specific gene of *Streptococcus equisimilis*, expressed in *E. coli*, launched by Bharat Biotech International Limited, is the first Company in India to manufacture and market Indikinase - Recombinant Streptokinase, the first line therapy for myocardial infarction/heart attack, given either by Intravenous or intra cardiac administration. It is also useful in deep vein thrombosis and pulmonary embolism, Arterial occlusions; hemodialysis shunts obstructions, intra-pleural adherences, Catheter occlusions and Prosthetic valve thrombi.

In the market of thrombolytic drugs, the rSK based medicines occupy an important place, especially in the less developed countries. According to the American Heart Association the cost of a dose of a second- or third-generation thrombolytic drug can be over $ ~2,196.00 for Alteplase or Reteplase and the $ ~2 750.00 in the case of Tecneteplase.

**CONCLUSION**

SK is a very popular and the first FDA approved thrombolytic agent sourced from a bacteria. It has gained a great clinical significance in the treatment of various blood clot related disorders particularly Myocardial infarction, Stroke and Pulmonary embolism. Though streptokinase having few side effects it is one of the most highly accepted therapeutic proteins as a clot buster because of its availability, efficiency moreover at an affordable cost.

Cardiovascular Diseases are the secondary cause of deaths in many parts of the world, although modern drugs are effective in preventing the disorders, their use is often limited because of their side effects and adverse reactions[47]. As this is said to be the era of structural biology and computer aided drug design, various trails are going on to produce a modified SK targeting its antigenicity problem, half life and more particularly to make it as a clot specific one. If SK comes out with the above said features, it stands as ever green thrombolytic molecule in the clinical market.

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