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A REVIEW ON: POTENT PLASMINOGEN ACTIVATOR USED FOR THE TREATMENT OF THROMBOEMBOLIC DISORDERS

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ABSTRACT

Streptokinase is a well-established thrombolytic agent that has been extensively used in clinical practice for the management of thromboembolic disorders. Derived from β-hemolytic streptococci, streptokinase acts indirectly by forming a complex with plasminogen, thereby converting it into plasmin, an enzyme responsible for the degradation of fibrin clots. Owing to its fibrinolytic activity, streptokinase has played a significant role in the treatment of acute myocardial infarction, pulmonary embolism, deep vein thrombosis, and arterial thrombosis. Despite the development of newer fibrin-specific thrombolytic agents, streptokinase remains widely utilized, particularly in resource-limited settings, due to its cost effectiveness and proven therapeutic efficacy. This review highlights the mechanism of action, pharmacological properties, clinical applications, dosage regimens, and adverse effects associated with streptokinase therapy. Particular emphasis is placed on its role in restoring blood flow, reducing mortality, and improving patient outcomes in cardiovascular emergencies.

KEYWORDS: Streptokinase, thrombolytic agent, fibrinolysis, plasminogen, myocardial infarction, thromboembolic disorders.

1.INTRODUCTION

A blood clot (thrombus) forms in the blood vessels, a process that initiates a normal mechanism in the human body to heal the damaged blood vessel. Mainly treatment for myocardial infarction is percutaneous coronary intervention (PCI) and thrombolytic therapy, which is caused by the formation of unstable platelet aggregates after the rupture/erosion of atherosclerotic plaque into the arterial lumen, resulting in a significant reduction of coronary flow or acute coronary thrombosis, total occlusion of a coronary artery, which produces ST segment elevation myocardial infarction (STEMI). All hospitals do not have PCI centres in India. This makes the use of thrombolytics more common because of this unavailability of PCI. Thus, the safety and efficacy of thrombolytics in myocardial infarction patients needs to be known. This agent can cause antibodies to be formed in the body. Patients with antibodies are more likely to have an allergic reaction (including the most severe, anaphylaxis) to streptokinase. The antibodies can also reduce the action of the streptokinase. These effects make streptokinase inappropriate for more than one-time use in any one patient and the repeated use of streptokinase in a patient is not recommended. Up to 50% of the patients who present with AMI have already had streptokinase once and are thus ineligible for it. Commonly used fibrinolytic agents in the treatment of thrombosis include streptokinase (SK), urokinase (UK or Activase), and tissue type plasminogen activator (TPA or Activase).

These are called plasminogen activators because they convert the enzymatically inert plasminogen (PG) of the fibrinolytic system to an enzymatically active protease called plasmin (PN) that breaks down fibrin clots and solubilises degradation products that can be phagocytosed. This makes it easier for the blood to flow

through the blocked blood vessel. Unlike UK and TPA, which are proteases themselves, SK does not have any enzyme activity. Instead, it gets the very specific PG-activating property indirectly, as it initializes the high-affinity 1:1 stoichiometric complex of PG or PN. The resulting activator complex is a very specific enzyme, a protease, that will activate other PG molecules to become proteolytically active via a series of biochemically distinct steps. The decision of which thrombolytic to use during therapy is determined by several factors, many of which are dependent upon the relative advantages and disadvantages of the individual PG activators[4]. These include the cost of the drug, the side-effects and their severity, in vivo stability and specificity towards fibrin clots and immunological reactivity.

This allows blood to flow through the blocked blood vessel. The protease enzyme does not have any enzymatic activity as SK itself is not a protease but it is involved in the enzymatic activity of the protease enzyme. It, instead, gets the highly specific PG activating property indirectly, through the formation of a high affinity 1:1 complex of PG or PN first. The resultant activator complex is a very specific protease that catalyzes the conversion of other PGs to PN, in a series of biochemically distinct steps.

During therapy, the selection of the thrombolytic agent is based on several factors, which relies mainly on the benefits and drawbacks of each PG activator. These include the cost of the drug, the side-effects and their severity, in vivo stability and specificity towards fibrin clots and immunological reactivity [5]. Although the TPA and UK 123 10 Fibrinolysis are relatively immunologically inert when compared to SK, they have significantly shorter in vivo half-lives. However, both TPA and UK are much more costly than SK. Hence, SK is the drug of choice in the treatment of thrombolysis.

SK has become a popular thrombolytic agent among the three major agents used clinically, especially in developing countries because of its cost-effectiveness. In the treatment of myocardial infarction, and also with regard to treatment efficiency, SK is more acceptable than the other two clot dissolvers [6]. Moreover, SK is available at a low cost and is the drug of choice in most clinical situations, especially in the economically weaker countries. But in some cases, the immunogenic property of SK may cause a severe allergic reaction such as urticaria, itching, flushing, nausea etc. which limits the use of multiple doses of SK for thrombolytic therapy. Due to some of the limitations exhibited by the thrombolytic agents, including that of SK, the second generation derivatives of SK are expected to be developed.[7]

1.1 FIBRINOLYTIC SYSTEM

Extensive studies have been made over the last 25 years to understand the physiology of fibrin clot formation [8]. It has been directly demonstrated that the constituents of the fibrinolytic system are directly involved in the dissolution of the fibrin clot [9-11]

1.2 FIBRINOLYSIS

Fibrinolysis is the process in which the blood clot in the fibrin is broken down by an enzyme system found in the blood of all mammals. The fibrinolytic system includes the plasma zymogen, plasminogen, the proteolytic enzyme (activator) plasmin, activators of plasminogen, inhibitors of plasmin and of plasminogen activators as well as fibrinogen and fibrin. A limited proteolytic cleavage of plasminogen to the active proteolytic enzyme plasmin is the basic reaction of the plasma fibrinolytic system, which is mediated by different plasminogen activators [12]. Endothelial cells, other tissues synthesize and release plasminogen activators. Plasmin can break down fibrin and a number of other plasma coagulation proteins, such as fibrinogen. Plasminogen activators are regulated by inhibitors which block the activity of plasminogen activators and also block the proteolytic effect of plasmin. Plasminogen is the zymogen of a trypsin -like serine protease, two activators of plasminogen, and three inhibitors of proteases are the main components of the fibrinolytic system [13].

The two major activators that occur in the circulating blood are: the tissue TPA and the urinary type plasminogen activator (U-PA), also called urokinase.

THROMBOLYTIC THERAPY CLASSIFICATION

Generation of thrombolytic drug	Fibrin specific	Non fibrin specific
First	-	Urokinase Streptokinase
second	Recombinant tissue plasminogen activator(t-PA) alteplase	Prourokinase (scum-PA) Sk-plasminogen activating complex (APSAC)
Third	Tenecteplase (TNK-tPA) Reteplase Monteplase Lanoteplase pamiteplase	-

1.3 PLASMINOGEN

Human plasminogen (Fibrinolysin) is a single chain glycoprotein of 790 amino acids in two major forms with molecular weight of 92 and 94 kDa, respectively [14,15]. Both of these molecular forms of HPG have chemical and physical differences. Both forms have Glu at their N-terminal and Asn at the C-terminal amino acid [14, 16]. It is synthesized in the liver, but also exists in other cells and in the extravascular space of most tissues. In view of the homology between amino acid sequences of kringles in prothrombin [17] and Plasminogen, it is assumed that Plasminogen contains five 'Kringles" domains, between residues Tyr79 and Arg560. All of these kringle domains are stabilized by three disulfide bonds and play a role in the binding of HPG to fibrin as well as antiplasmin. It is 53.5 kb in length making it the largest gene in the fibrinolytic system. N-terminal position of the molecule, on which five kringle domains are located, is known as the A-chain ,and the C-terminal carries the catalytic domain, known as the B-chain. Plasmin (PN) consists of two chains (heavy and light chain) bound together by two disulfide bonds, and has a molecular mass of 85 kDa. The result of at least two peptide bond cleavages in the PG molecule results in the formation of native plasmin.

A cleavage at Arg560–Val561 is crucial for the catalytic activity to be expressed. Here, no peptides are released, as the PN molecule is stabilized by disulfide linkages between Cys557 and Lys565, and also between Cys547 and Cys665. Because of the PN formation, the autolysis of the Lys76 Lys77 residues takes place, leading to the release of the amino- terminal 76 residues in the covalent form. This PN is known as Lys77 PN. The light chain region of this PN has been demonstrated by Summaria and Robbins to be responsible for the complex formation with SK. The specificity of the active site of PN is, in general, trypsin-like and therefore it cleaves proteins and peptides at the lysyl and arginyl bonds. In the fibrinolytic system, PN is a serine protease which breaks down fibrinogen as well as fibrin [18].

It may also degrade other plasma proteins such as factors V, VIII, IX, XI and XII, insulin, growth hormones and many others. This is one of the unwanted side effects of the generalized PG activation that follows the thrombolytic therapy, and is responsible for the depletion of several plasma proteins after the administration of clot dissolver drugs. Fibrinogen consists of three chains, A-a, B-b and gamma, which are held together by disulfide bonds. The conversion of human fibrinogen to fibrin is catalyzed by the serine protease thrombin. The specificity of the active site of PN is, in general, trypsin-like and therefore it cleaves proteins and peptides at the lysyl and arginyl bonds. Fibrinogen consists of three chains, A-a, B-b and gamma, held

together by disulfide bonds. The conversion of human fibrinogen to fibrin is catalysed by the serine protease thrombin. Thrombin cleaves the A-a chain and B-b chain of the fibrin monomer to release fibrinopeptides A (16 residues) and B (14 residues), respectively, to give the a and b chains of the fibrin monomer [19]. As a result of liberation of these small peptides, the fibrin monomer polymerizes, through non covalent interactions, to yield a form of fibrin. Thrombin also catalyzes the activation of factor XIII (fibrin-stabilizing factor) which catalyzes the formation of ε- (r-glutamyl) lysine bonds between the monomers of fibrin.PN breaks down fibrinogen or fibrin first at the carboxy terminal end of the A-a chain to produce fragment X, and then breaks it down again at the amino terminal end of the molecule to produce fragments Y and D.

Further digestion by PN of fragment Y results in the formation of an additional D fragment and a fragment E. Fibrinolytic system inhibitors: Natural inhibition of the fibrinolytic system occurs both at the level of plasminogen activation and also at the level of plasmin action [3, 20, 21]. The inhibitor which acts at the level of plasminogen activator is called plasminogen activator inhibitor-1 (PAI-1). The inhibitors which act at the level of plasmin are many, the most important being alpha-2 antiplasmin and alpha-2-macroglobulin

1.4 PLASMINOGEN ACTIVATOR INHIBITOR-1

Plasminogen activator inhibitor-1 is a fast -acting inhibitor of TPA and UK [23], found at low concentrations in the blood. It is a serpin with 379 amino acids and the active sites are Arg346–Met347.

sa)ALPHA-2-ANTIPLASMIN

It is a single -chain glycoprotein of the serine protease inhibitor (serpin) superfamily, with a molecular weight of about 70 kDa [24]. It consists of 452 amino acids and has two reactive sites, Arg364–Met365 [25]. It reacts with PN to form a fast, enzymatically inactive 1:1 irreversible complex [24, 26] that inhibits PN activity. Alpha-2-antiplasmin reacts very rapidly with plasmin in two steps, first to form a reversible but inactive complex, which then slowly converts into an irreversible complex. The first step of the reaction requires the presence of free lysine binding sites and a free active centre in the plasmin molecule. A 2-antiplasmin is thought to be the most important inhibitor of PN activity. The complex of PN with SK is less reactive towards a-2-antiplasmin than PN [27].

The native molecule of a-2-macroglobulin, molecular weight 726 kDa, is a tetramer, comprised of four identical subunits of 185 kDa each [28]. Under certain conditions PN may also react with a-2-macroglobulin [29].

The in vivo studies have shown that the complex of PN with PG activators like SK, injected intravenously into mice, reacts quickly with a-2-macroglobulin and is removed from the circulation within 10 min [30].

2.PLASMINOGEN ACTIVATORS

The fibrinolytic system can be activated in two ways, viz., by PG activation through pharmacological activators and by activation of PG proactivator by active Hageman factor (a serine protease) which activates PG to PN. The most widely and commonly used (clinically approved) PG activators are SK, UK and TPA. These are also called thrombolytic agents and extensively used in the treatment of various thrombolytic disorders. Direct plasminogen activators are serine proteases that have a high specificity for plasminogen.

They break down the Arg560–Val561 peptide bond of HPG to produce the active enzyme plasmin which performs the function of fibrinolysis. Plasminogen activators can be divided into two broad categories, one related to eukaryotic origin while the second from bacterial origin. The bacterial origin plasminogen activators include SK from Streptococcus sp. and staphylokinase (SAK) from Staphylococcus sp. These, known as [31–34] activate plasminogen indirectly. Another activator, acylated plasminogen streptokinase

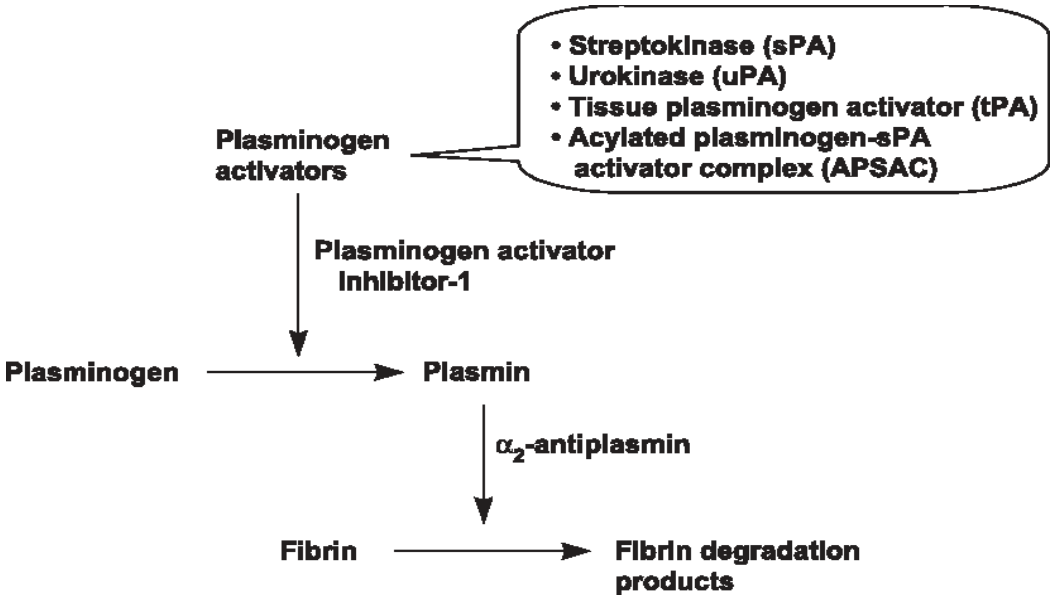
activator complex (APSAC) ,which is clinically approved, represents a complex of SK with HPG in which the active site of HPG has been acylated. It is thought that the deacylation of the active site in vivo results in a longer plasma half-life [35]. Eukaryotic PG activators include primarily TPA and UK from humans which directly activate PG. Pro-urokinase (Pro-UK) [25] is another example. Bat plasminogen activator (bat-PA), another eukaryotic PG activator, was found in the South American vampire bat's salivary glands [36]. The pro-UK is obtained from urine and is a precursor to UK, which gets activated once introduced into the bloodstream. The SAK is a product of some strains of Staphylococcus aureus. It has a mechanism of action similar to that of SK, in that it forms a binary complex with plasminogen, which then converts other PG to PN ,and a mechanism for fibrin specificity was proposed [37, 38]. To gain insight into the mechanism of plasminogen activator, Collen et al. have determined the crystal structure of recombinant SAK and identified the three non-overlapping immunodominant epitopes. The gene for SAK has recently been cloned and expressed in E. coli [39] and B. subtilis [40]. Recombinant SAK was shown to be able to induce fibrin -specific clot lysis on a human plasma milieu. Due to the known limitations of the existing plasminogen activators, efforts are being made to create better recombinant forms of these agents [41]. The greatest benefit of SK is its low cost, about $200 per dose. It is still the most widely used fibrinolytic in Australia.

3. SECOND-GENERATION PLASMINOGEN ACTIVATORS

Although several plasminogen activators are currently being used for thrombolytic therapy, it has been realized that none of them has all the desired properties in the same molecule To improve the functional characteristics of various plasminogen activators, genetically engineered mutants of those were generated. One of these mutants, comprising only the Kringle 2 (K2) and protease (P) domain of tpa was found to have a prolonged half life and was relatively fibrin -selective. Deletion of the amino acid sequence Arg179–Ser184 results in a urokinase mutant which is resistant to inhibition by PAI-1 [42].

In recent years, recombinant chimeric plasminogen activators have been developed mostly by combining various domains of TPA and single-chain urokinase type plasminogen activators (SCU-PA). The chimeric proteins might thus combine the mechanisms of fibrin-selectivity of both molecules. Chimeric molecules of TPA and U-PA were prepared. The functional properties of these recombinant TPA/U-PA chimeric plasminogen activators were comparable to those of U-PA, but were less fibrin specific than recombinant TPA (r TPA). Such chimeras did not show superior pharmacokinetic and thrombolytic properties compared to intact SCU-PA in a rabbit jugular vein thrombosis model [43].

The new generation of thrombolytic agents have not been as effective in humans as was hoped from several animal models, in terms of their thrombolytic potency and their fibrin specificity. Therefore, the quest for better thrombolytic agents still continues[44].



In this case, SK and HPG form an equimolar complex, SK.HPG. This interaction causes a conformational change in the complex to form SKHPG¢, which has an active site. This active site is located in the plasminogen part of the complex. [45-49]

After the formation of HPN, the active site of the plasminogen part of the complex catalyzes the intramolecular cleavage of the Arg560–Val561 peptide bond in the SK.HPG¢ complex to yield an altered form of SK, SK*.[50-52] This modified SK has a molecular weight of 36 kDa [53] indicating that peptides of total molecular weight of about 9 kDa have been removed. The functional properties of SK* seem to be the same as those of native SK.

Apart from its role in the activation of the PG molecule of the SK–PG complex to SK–PN, SK plays a characteristic role in modulating the substrate specificity of this 'partner' plasmin(ogen), from a general protease to a protease highly specific for the substrate molecule of plasminogen [54]. They investigated the change in substrate specificity by studying the proteolytic, esterolytic and bovine PG activation activity of PN as a function of SK concentration. They found that with an increase in the amount of SK there was a fall in general proteolytic activity, but a concomitant increase in bovine PG activation activity. Both these changes were saturated at 1:1 SK:PN. SK is able to activate plasminogen in a species-specific manner. No synthetic substrate has yet been found for SK. The only protein substrates known are plasminogens from human [55], monkey, 123 14 J Thromb Thrombolysis (2007) 23:9–23 baboon [56], chimpanzee [56], cat [55], dog [55], and rabbit [55, 57]. Sheep PG is resistant to SK activation. However, all species of plasminogen are activated equally well by the SK*human Lys77–PN complex [54, 55].

Recent evidence has shown that a protein that potentiates the activity of SK towards human plasminogen activation exists in plasma. In both cases, it was found that the same effect could be observed when fibrinogen [58, 59] or fibrinogen degradation products [60] were added to the system of interest. Kim et al. reported that the Asp41–His48 region in a SK plasminogen binary complex plays an important role in binding to a substrate plasminogen. The C-terminal domain of SK is involved in the recognition and activation of the plasminogen substrate [61]. It has been discussed that an adjacent region (residues 48-59) plays a role in plasminogen activation. The authors Wu et al. concluded that the coiled coil region of SK gam ma-domain, SK (Leu314-Ala342), is very important in the activation of HPG, as it is involved in the induction of virgin enzyme and stabilizes the activator complex. Sazonova et al. investigated the mechanism of a bacterial plasminogen activator using the plasminogen activator from S. uberis. They found that the gamma domain is not essential for SK activation of bovine plasminogen.

4.PRODUCTION OF SK

The occurrence of 4.1 in streptococci strains is described. In 1874, Billroth discovered globular microorganisms in chains in purulent exudates from erysipelas lesions and infected wounds. In scarlet fever, similar organisms were isolated from the blood, and were later named Streptococci. In 1903, Schotmuller proposed that the different varieties of Streptococcus sp. could be classified on the basis of their capacities to haemolyze erythrocytes. In addition, Brown coined the terms alpha, beta and gamma to denote the three types of haemolytic reactions seen on blood agar plates in 1919. The elaborate work of Lancefield in the early 1930's on b haemolytic Streptococci, differentiated them into a number of immunological groups designated by the letters A to O [62].

The main sources of SK are β-haemolytic Streptococci of the Lancefield groups A, C and G. The group C is preferred for SK production because they do not produce erythrogenic toxin. Christensen isolated a group C strain of Streptococcus equisimilis (H46A) (ATCC 12449) from a human source. It has been widely used as the source for the production of SK. This strain was chosen from more than 100 fibrinolytic strains due to its high level of SK activity. Furthermore, it is not as fastidious in its growth requirements as most of the group A strains, and does not produce erythrogenic toxin, so that a semi defined medium could be easily formulated for its optimal growth and SK secretion [63]. This strain has also been the main source for the SK gene, which has been expressed in E. coli, Streptococci and yeast.

4.1 SK OCCURRENCE IN RECOMBINANT STRAINS

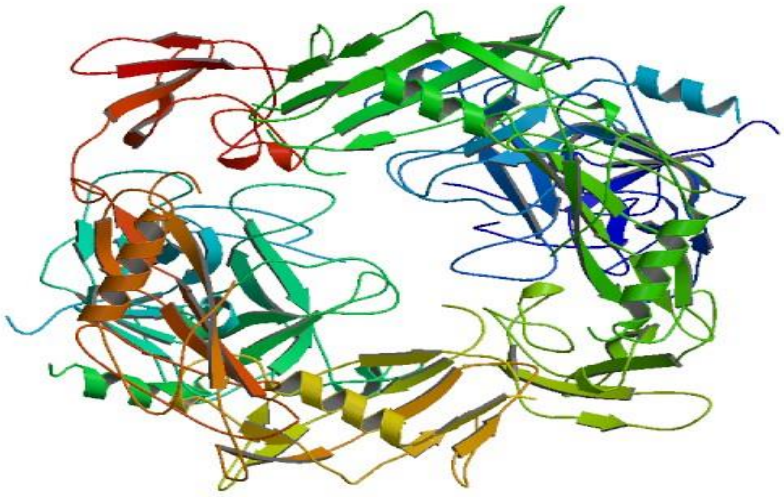
Malke and Ferretti have cloned the gene for SK into E. coli from S. equismilis H46A. Malke studied the SK gene (skc) from various sources and proposed that it was a polymorphic gene. Kapur et al. [64] found that alleles of SK in 47 isolates of S. pyogenes have a mosaic structure. The gene encoding streptokinase skc from S. equisimilis H46A was expressed in a number of heterologous Gram-positive and Gram-negative bacteria [65].

The SK gene has been over-expressed in E. coli. Muller and Malke isolated skc from an erythromycin resistant plasmid and transferred it to Streptococcus equisimilis H46A for increased SK production. They got erythromycin resistant clones producing SK at high levels. Ko et al. have developed a high level secretory expression plasmid, pSK100, for SK. It produced about 5,000 u/ml SK in LB–ampicillin medium. The SK gene was also expressed in Eucaryotic cells like Pichia pastoris.

5.SK STRUCTURE

The complete amino acid sequence of SK was first determined by Jackson and Tang [66] using automated Edman degradation of cyanogen bromide and protease fragments. Malke et al. [67] sequenced the entire nucleotide sequence of the SK gene of S. equisimilis H46A. A 2568-bp Pstl fragment of S. equisimilis H46A genome that contains the SK gene was cloned into E. coli and then sequenced.

The investigators also determined the nucleotide sequences of the adjacent regions that regulate the transcription and translation of the SK gene. Homology studies have also been performed among the SK genes as well as amino acid sequences from different groups of Streptococci to compare and contrast the regions of variability in the SK sequence. The two studies indicated that there was a high degree of variability between the different streptokinase produced by different groups of Streptococci.



6.POTENTIAL GLOBAL HEALTH CARE IMPLICATIONS OF NEW PRODUCTION

Biosafety issues are important for commercial production of SK as the protein is potentially immunogenic to process workers. Also, if SK is being manufactured with natural strains of Streptococci, care must be taken as all Streptococci that produce SK are potentially pathogenic. The various safety considerations relevant to production of biopharmaceutical proteins have been discussed by [68].

The increasing incidence of thromboembolic diseases has sustained the search for new agents able to stimulate the natural fibrinolytic system [69]. Bacterial SK and human urine UK are the first generation of antithrombotic agents. Important efforts have been made to develop, via recombinant DNA technology, agents with higher fibrin clot selectivity, like TPA and SCU-PA, that do not bind to the fibrin clot. At the same time, several laboratories are trying to develop mutants and hybrids of the plasminogen activators that have enhanced thrombolytic activity compared to the natural molecules. Diwedi et al. [70] reported that reperfusion which was the primary end point was assessed using a com bination of enzyme (Creatine Kinase) peaking and ST segment resolution was observed in 68.2% (58) in the recombinant SK and in 69.4% (34) patients in natural SK group whereas, rates reported in literature by the present criteria vary from 59 to 82% [71,72]. 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 SK, respectively. The same percentage of 70.6% was reported from previous Indian studies using natural SK [73].

Safety profile assessment, which was the secondary end point in this study, was similar in both the recombinant SK and natural SK group. These events seen were similar to those reported by other studies. Incidence of bleeding was 4.2% (4) in the recombinant SK group while it was 5.4% (3) in the natural SK group, which are similar to the rates reported in literature vary between 0.5 and 15.2% [74,75]. One anaphylactic reaction was reported in the natural SK group, but not in the recombinant SK group.

7.Chemical modification of SK

To overcome the side effects of SK, attempts have been made at the chemical modifications of SK to enhance its stability characteristics by preparing polyethylene glycol (PEG)–SK adducts [76]. The main aim of this modification was to prepare conjugated derivatives of SK, which would be less immunogenic than the native form of SK and also improve its in vivo half life. To make SK more fibrinolytic, Wu et al. made mutants in which one or more of the amino acid residues in the area of Pro58–Lys59–Ser60–Lys61 were replaced by other amino acid residues. The mutants are not susceptible to the hydrolytic inhibition of plasma clots. He showed that the preferred mutant comprises replacing the Lys59 residue with glutamic acid. Additionally, the functional properties of SK can be altered, either by deletion of a-domain or by point

mutations that affect individual domain interactions, which can lead to rearrangement of the mutant-SK plasminogen/plasmin interactions and contribution from lysine-binding site-independent and-dependent binding to complex stability [77].

This indicates that loss of function that is associated with such mutations may not be readily understood as loss of a specific function of a specific domain without taking into account the interdependency of the domain interactions with plasminogen/plasmin. The conformational properties of SK are described by structural domains. Structural domains (conformational properties) of SK are described. The majority of the structural information obtained for SK has been obtained by biophysical methods like CD, NMR, FT-IR, and various scanning calorimetry [78,79] or other relatively indirect methods, such as the determination of the physicochemical properties of various fragments of SK [80,81]. The presence of multiple domains in SK with different structural–functional properties has been shown by several investigators. The scanning calorimetric analysis of SK indicated the presence of two domains in the protein.

7.1 Fermentative production of SK

Several strains of Group A haemolytic Streptococcus tend to require a very complex, rich medium supplemented with various growth factors for optimal growth [82]. Christensen used a modified medium of that of Bernheimer et al. [83] for the production of SK by Streptococcus equisimilis H46A. This medium contained peptone, phosphate salts, glucose, an additional mixture of vitamins (biotin and riboflavin), amino acids (tryptophan and glutamine), and nucleic acids (thiamine, adenine, and uracil).

The major change was a 75% decrease in the amount of glutamine added. Also, as only certain lots of casein hydrolysate supported massive growth of the strain H46A, it was substituted with Difconeopeptone, so that massive growth could be obtained uniformly under these conditions. The low glucose concentration used first allows growth to take place and become well established without too much acid production overnight. The rest of the glucose was then added the next morning and incubation was continued to achieve high cell density. 5 N NaOH was added at regular intervals to keep the pH of the fermentation at neutral. If the pH was not controlled, growth as well as SK production was also greatly diminished. Rosenberger and Elsden [84] investigated the effect of both glucose and tryptophan limitation on the growth in continuous culture of Streptococcus faecalis.

They determined that the best yield of cells per unit energy source would be achieved if the energy source was limiting. On the other hand, for product formation, the energy source should be in excess, and some other metabolite should comprise the limiting factor. von Polnitz et al.

8. PATENT REVIEW ON STREPTOKINASE

Trace elements are usually added to the SK fermentation medium in the form of a salt mixture to furnish ions of several metals in very slight amounts. It is often convenient to prepare a salt mixture from the salts of these metals and add a small quantity of the mixture to each fermentation. Szumskireported that superior results for SK production using fermentation media comprising an enzyme-hydrolyzed casein digest, glycine, and a sulfhydryl reducing agent.

SK gene from H46A expressed in E. coli produced 10- fold higher rSK. Enhanced production of rSK was observed by Lee et al.in E.coli by removal of the N-terminal amino acid at 37o C for 12 hrs in LB medium. [85] The N-terminal domain is known to be functionally relevant in SK, but this may not include the first 13 residues.

Stuebner et al. reported the kinetic analysis and modeling of SK fermentation. Patnaik applied principal component analysis (PCA) for fed-batch fermentation for the production of SK, and he observed the suitability of PCA to formulate a minimal model for industrial-scale fermentations. Malke et al. studied the expression of the SK gene, skn, using Southern hybridization analysis. [86-88] This led to the localization of

the core promoter region of skc and identification of the active upstream region required for full promoter activity.

9.DOSE:

Safety not established in children.

ADULTS:

ACUTE MYOCARDIAL INFARCTION:

A single dose of 1.5 million IU streptokinase should be infused intravenously over one hour.

LOCAL INTRACORONARY ADMINISTRATION:

1. 2. A bolus of 20000 IU streptokinase should be followed by a maintenance infusion of 2000 IU to 4000 IU per minute over 30 to 90 minutes depending on the achievement of coronary artery patency.

10.ADMINISTRATION:

Dilute two 75,000 unit vials of streptokinase with 5ml dextrose 5% in water each, gently swirl to dissolve. Add this dose of the 1.5 million units to 150ml dextrose 5% in water.

ADVERSE EFFECTS:

- Severe systemic bleeding
- Hypotension
- Severe allergic or anaphylactic reactions
- Arrhythmias
- Other rare complications like
    1. Renal and hepatic issues
    2. Serum sickness

11.CONTRAINDICATIONS:

Contraindicated in patients with recent

• trauma or surgery

• coagulation defects

• cerebrovascular disease

- peptic ulceration
- severe liver disease
- acute pancreatitis

CONCLUSION

Streptokinase may produce more bleeding complications and less resolution of ST-segment elevation when compared to tissue-plasminogen activators such as tenecteplase. Now a-days mortality rate is very high in acute myocardial infarction. So, this can be reduced by providing tissue plasminogen activator within the time. Comparative clinical trials and cost effectiveness considerations suggest that SK is the drug of choice for thrombolytic therapy in clinical practice, but its use is not risk-free. Cloning of the SK gene in non-pathogenic microorganisms has enabled production of recombinant SK that eliminates any risk of inadvertent inoculation of patients and production personnel with potentially pathogenic Streptococci. SK can be produced inexpensively in bulk via bacterial fermentation. Various chemical modifications were

used to prepare conjugated derivatives of SK, which would be less immunogenic than the native form of SK, improving plasminogen activation and also improving its in vivo half-life in circulation.

REFERENCES

1) Md.Ramjan Ali, Mohammad Salim Hossain, Md.ArifulIslam, Md.SaifulIslam Arman, Golam Sarwar Raju, Prianka Dasgupta, and Tasnim Fariha Noshin, Aspect of Thrombolytic Therapy: A Review. The Scientific world journal, 2014, 1-8.
2) Ajay Suri, Sophia Tincey, Syed Ahsan and Pascal Meier, Novel Strategies in Ischemic Heart Disease, Intech, 2012, 123-134.
3) KD Tripathi, Essentials of MEDICAL PHARMACOLOGY.7th edition, aypee brothers medical publishers, New Delhi, 2013, 626-627.
4) Anirban Banerjee, Yusuf Chisti, U.C Banerjee, Streptokinase—a clinically useful thrombolytic agent, Biotechnology Advances, 22, 2004, 287–307.
5) Ava Mansouri, ShimaTasharoie, SaeidehJavidee, Mona Kargar, Maryam Taghizadeh-ghehi, MoloukHadjibabaie, Kheirollah Gholami, StreptokinaseAdverse Reactions: A Review of Iranian Literature, J Pharm Care, 2, 2014, 120-129.
6) A Boland, Y Dundar, A Bagust, A Haycox, R Hill, R MujicaMota, T Walley, and A Dickson. Early thrombolysis for the treatment of acute myocardial infarction: a systematic review and economic evaluation, Health Technology,7, 2003, 69-75.
7) Saira Afzal, Muhammad Arif Khan, Hafiz Muhammad, Ayesha Ashraf, Maria Afzal, Psychosocial risk factors of myocardial infarction and adverse effects of streptokinase in public sector hospitals, Pak J Medical Sciences, 31, 2015, 2015, 821-826.
8) Paoletti R, Sherry S (eds) (1977) Proceedings of the serono symposia. Thrombosis and Urokinase, vol.
9) Academic Publisher, London: p 257 9. Bick RL (1992) Disorders of thrombosis and hemostasis; Clinical and Laboratory Practice. ASCP Press, USA
10) Wu KK, Thiagarajan P (1996) Role of endothelium in thrombosis and hemostasis. Annu Rev Med 47:315–331
11) Bick RL (1982) Clinical relevance of anti-thrombin-III. Semin Thromb Hemost 8:276–287
12) Castellino FJ (1984) Biochemistry of human-plasminogen. Semin Thromb Hemost 10:18–23.
13) Francis CW, Marder VJ (1994) Physiological regulation and pathologic disorders of fibrinolysis. In: Colman RW, Hirsh J, Marder VJ, Edwin W, Salzman B (eds) Hemostasis and thrombosis. J.B. Lippincott Company, Philadelphia, pp 1076–1103
14) Summaria L, Robbins KL (1976) Isolation of human plas mid-derived functionally active light (B) chain capable of forming with streptokinase an equimolar light (B) chain streptokinase complex with plasminogen activator activity. J Biol Chem 251:5810–5813
15) Violand BN, Castellino FJ (1976) Mechanism of the uro kinase-catalyzed activation of human plasminogen. J Biol Chem 251:3906–3912
16) Wallen P, Wiman B (1972) Characterization of human plasminogen. II. Separation and partial characterization of different molecular forms of human plasminogen. Biochem Biophys Acta 257:122–134
17) Sottrup-Jensen L, Claeys H, Zajdel M, Petersen TE, Magnusson S (1972) Progress in chemical fibrinolysis and thrombolysis, vol. 3. Raven Press, New York, pp 191–209
18) Pizzo SV, Schwartz ML Hill RL, McKee PA (1972) The effect of plasmin on the subunit structure of human fibrin ogen. J Biol Chem 247:636–645
19) Blomback B, Hessel B, Iwanaga S, Reuterby J, Blomback M(1972) Primary structure of human fibrinogen and fibrin. I. Cleavage of fibrinogen with cyanogen bromide. isolation and characterization of nh2-terminal fragments of the a (“A”) chain. J Biol Chem 247:1496–1512

20) Bachmann F (1987) Fibrinolysis. In: Verstaete M, Vermylen J, Lijnen R, Arnout J (eds) Thrombosis and haemostasis, Leuven Univ. Press, Leuven, Belgium, pp 227–235
21) Collen D (1987) Molecular mechanisms of fibrinolysis and their application to fibrin-specific thrombolytic therapy. J Cell Biochem 33:77–86
22) Akoi N, Harpel PC (1984) Inhibitors of the fibrinolytic enzyme system. Semin Thromb Hemost 10:24–41
23) Kruithof EKO, Tran-Thang C, Ransijn A, Bachmann F (1984) Demonstration of a fast acting inhibitor of plasminogen activators in human plasma. Blood 64:907–913
24) Wiman B, Collen D (1978) On the kinetics of the reaction between human antiplasmin and plasmin. Eur J Biochem 84:573–578
25) Holmes WE, Pennica D, Blaber M, Rey MW, Guenzler WA, Steffens GJ, Heyneker HL (1985) Cloning and expression of the gene for pro-urokinase in Escherichia coli. Biotechnol 3:923–929
26) WimanB, Collen D (1977) Purification and characterization of human antiplasmin, the fast-acting plasmin inhibitor in plasma. Eur J Biochem 78:19–26
27) Wiman B (1980) On the reaction of plasmin or plasmin streptokinase complex with aprotinin or alpha-2-antitrypsin. Thromb Res 17:143–152
28) Harpel PC (1973) Studies on human plasma a2-macro globulin-enzyme interactions: evidence for proteolytic modification of the subunit chain structure. J Exp Med 138:508–521 29. Harpel PC (1981) Alpha2-plasmin inhibitor and alpha2 macroglobulin–plasmin complexes in plasma. Quantitation by an enzyme-linked differential antibody immunosorbent assay. J Clin Invest 68:46–55
29) Rajagopalan S, Gonias SL, Pizzo SV (1985) A non-anti genic covalent streptokinase polyethylene glycol complex with plasminogen activator function. J Clin Invest 75:413 419 Lack CH (1948) Staphylokinase: an activator of plasma protease. Nature 161:559–560
30) Collen D, DeCock F, Vanlinthout I, Declerck PJ, Lijnen HR, Stassen JM (1992) Comparative thrombolytic and immunogenic properties of staphylokinase and
streptokinase. Fibrinolysis 6:232–242
31) Schlott B, Hartmann M, Guhrs KH, Birch-Hirschfeid E, Pohl HD, Vandescheure S, Van De Werf F, Michael A, Collen D, Behnke D (1994) High yield production and purification of recombinant staphylokinase for thrombolytic therapy. Biotechnol 12:185
32) Okada K, Ueshima S, Fukao H, Matsuo O (2001) Analysis of complex formation between plasmin(ogen) and staphylokinase or streptokinase. Arch Biochem Biophys 393:339 34
33) Smith RA, Dupe RJ, English PD, Green J (1981) Fibrinolysis with acyl-enzymes: a new approach to thrombolytic therapy. Nat 290:505–508
34) Schluenring WD, Alagan A, Boidol W, Bringmann P, Petri T, Kratzschmar J, Haendler B, Langer G, Baldus B, Witt W, Donnar P (1992) Plasminogen activators from the saliva of Desmodus rotundus (common vampire bat): unique fibrin specificity. Annals NY Acad Sci 667:395–403
35) Matsuo O, Okada K, Fukao H, Tomioka Y, Ueshima S, Watanuki M, Sakai M (1990) Thrombolytic properties of staphylokinase. Blood 76:925–929
36) Lijnen HR, Vanhoef B, DeCock F, Okada K, Ueshima S, Matsuo O, Collen D (1991) On the mechanism of fibrin specific plasminogen activation by staphylokinase. J Biol Chem 266:11826–11832
37) Sako T (1985) Overproduction of staphylokinase in Escherichia coli and its characterization. Eur J Biochem 149:557–563
38) Behnke D, Gerlach D (1987) Cloning and expression in Escherichia coli, Bacillus subtilis, and Streptococcus sanguis of a gene for staphylokinase, a bacterial plasminogen activator. Mol Gen Genet 210:528–534
39) Nicolini FA, Nichols WW, Mehta JL, Saldeen TGP, Scho field R, Ross M, Player DW, Pohl GB, Mattsson C (1992) Sustained reflow in dogs with coronary thrombosis with K2P, a novel mutant of tissue plasminogen activator. J Am Coll Cardiol. 20:228–235

40) Adams DS, Griffin LA, Nachajko WR, Reddy VB, Wei CM (1991) A synthetic DNA encoding a modified human urokinase resistant to inhibition by serum plasminogen
41) activator inhibitor. J Biol Chem 266:8476–8482
42) WuXC,YeRQ,DuanYJ,WongS-L(1998)Engineering of plasmin-resistant forms of streptokinase and their production in Bacillus subtilis: streptokinase with longer functional half-life. Appl Environ Microbiol 64:824–829
43) WuXC,YeRQ,DuanYJ,WongS-L(1998)Engineering of plasmin-resistant forms of streptokinase and their production in Bacillus subtilis: streptokinase with longer functional half-life. Appl Environ Microbiol 64:824–829
44) Banerjee A, Chisti Y, Banerjee UC (2004) Streptokinase—a clinically useful thrombolytic agent. Biotechnol Adv 22:287–307
45) Christensen LR (1945) Streptococcal fibrinolysis: a proteolytic reaction due to serum enzyme activated by streptococcal fibrinolysin. J Gen Physiol 28:363–383
46) McClintock DK, Bell PH (1971) The mechanism of activation of human plasminogen by streptokinase. Biochem Biophys Res Commun 43:694–702
47) Summaria L, Arzadon L, Bernabe P, Robbins KC (1974) The interaction of streptokinase with human, cat, dog, and rabbit plasminogens. The fragmentation of streptokinase in the equimolar plasminogen–streptokinase complexes. J Biol Chem 249:4760–4769
48) Hummel BCW, Schor JM, Buck FF, Boggiano E, DeRenzo EC (1965) Quantitative enzymic assay of human plasmin ogen and plasmin with azocasein as substrate. Anal Bio chem 11:532–547
49) Reddy KNN, Markus G (1972) Mechanism of activation of human plasminogen by streptokinase presence of active center in streptokinase–plasminogen complex. J Biol Chem 247:1683–1691
50) Schick LA, Castellino FJ (1973) Interaction of streptokinase and rabbit plasminogen. Biochemistry 12:4315–4321
51) Kosow DP (1975) Kinetic mechanism of the activation of human plasminogen by streptokinase. Biochemistry 14:4459–4465
52) Bajaj AP, Castellano FJ (1977) Activation of human plasminogen by equimolar levels of streptokinase. J Biol Chem 252:492–498
53) Brockway WJ, Castellino FJ (1974) A characterization of native streptokinase and altered streptokinase isolated from a human plasminogen activator complex. Biochemistry 13:2063–2070
54) Markus G, Werkheiser WC (1964) The interaction of streptokinase with plasminogen. i. functional properties of the activated enzyme. J Biol Chem 239:2637–2643
55) Wulf RJ, Mertz ET (1969) Studies on plasminogen. Species specificity of streptokinase. Can J Biochem 47:927–931
56) McKee RJ, Hargrove CK, Smith AG, Mes H, Thompson A (1971) Precise calibration of a Ge (Li)-spectrometer using a digital to analog converter. Nuclear Instr Meth 92:421–432
57) Sodetz JM, Brockway WJ, Castellino FJ (1972) Multiplicity of rabbit plasminogen. Physical characterization. Biochemistry 11:4451–4458
58) Camiolo SM, Thorsen S, Astrup T (1971) Fibrinogenolysis and fibrinolysis with tissue plasminogen activator, urokinase, streptokinase-activated human globulin, and plasmin. Proc Soc Exp Biol Med 138:277–280
59) Takada A, Ito T, Takada Y (1980) Interaction of plasmin with tranexamic acid and alpha 2 plasmin inhibitor in the plasma and clot. Thromb Haemost 43:20–23
60) Violand BN, Byrne R, Castellino FJ (1978) The effect of alpha-, omega-amino acids on human plasminogen structure and activation. J Biol Chem 253:5395–5401
61) Kim DM,LeeSJ,KimIC, KimST,ByunSM(2000)Asp41 His48 region of streptokinase is important in binding to a substrate plasminogen. Thromb Res 99:93–98

62) 117. Feldman LJ (1974) Streptokinase manufacture. In German. German patent DE 2354019 118. Hagenson MJ, Holden KA, Parker KA, Wood PJ, Cruze JA, Fuke M (1989) Expression of streptokinase in Pichia pastoris yeast. Enzyme Microb Technol 11:650–656
63) Wong SL, Ye RQ, Nathoo S (1994) Engineering and pro duction of streptokinase in Bacillus subtilis expression secretion system. Appl Environ Microbiol 60:517–523
64) Malke H, Ferretti JJ (1984) Streptokinase: cloning, expression and excretion by Escherichia coli. Proc Natl Acad Sci 81:3557–3561
65) Kapur V, Li LL, Hamrick MR, Plikaytis BB, Shinnick TM, Telenti A, Jacobs WR, Banerjee A, Cole S, Yuen KY, Clarridge III JE, Kreiswirth BN, Musser JM (1995) Rapid mycobacterium species assignment and unambiguous identification of mutations associated with antimicrobial resistance in Mycobacterium tuberculosis by automated DNA sequencing. Arch Pathol Lab Med 119:131–138
66) Malke H, Gerlach D, Kohler W, Ferretti JJ (1984) Expression of a streptokinase gene from Streptococcus equisimilis in Streptococcus sanguis. Mol Gen Genet 196:360–363
67) Muller J, Reinert H, Malke H (1989) Streptokinase mutations relieving Escherichia coli K-12 (PRLA4) of detriments caused by the wild-type skc gene. J Bacteriol 171:2202–2208 124. Estrada MP, Hernandez L, Perez A, Rodrıguez P, Serrano R, Rubier AR (1992) High-level expression of streptokinase in Escherichia coli. Biotechnology 10:1138–1142
68) Muller J, Malke H (1990) Duplication of the streptokinase gene in the chromosome of Streptococcus equisimilis H46A. FEMS Microbiol Lett 72:75–78
69) Ko JH, Park DK, Kim IC, Lee SH, Byun SM (1995) High level expression and secretion of streptokinase in Escherichia coli. Biotechnol Lett 17:1019–1024
70) Pratap J, Rajamohan G, Dikshit KL (2000) Characteristics of glycosylated streptokinase secreted from Pichia pastoris: enhanced resistance of SK to proteolysis by glycosylation. Appl Microbiol Biotechnol 53:469–475
71) Jackson KW, Tang J (1982) Complete amino acid sequence of streptokinase and its homology with serine proteases. Biochemistry 21:6620–6625
72) Malke H, Roe B, Ferretti J (1985) Nucleotide sequence of the streptokinase gene from Streptococcus equisimilis H46A. Gene 34:357–362
73) Huang TT, Malke H, Ferretti JJ (1989) Heterogeneity of the streptokinase gene in group A Streptococci. Infect Immun 57:502–506
74) Malke H (1993) Polymorphism of the streptokinase gene—implications for the pathogenesis of post streptococcallomerulonephritis. Zentralbl Bakteriol 278:246–257
75) Chisti Y (1998) Biosafety. In: G Subramanian (ed) Bio separation and bioprocessing: a handbook, vol. 2, Wiley VCH, New York, pp 379–415
76) Pierard L, Bollen A (1990) Development of new thrombolytic agents using recombinant DNA technology. J Biotechnol 15:283–304
77) Diwedi SK, Hiremath JS, Kerkar PG, Reddy Krishna N, Manjunath CN, Ramesh SS, Prabhavati S, Dhobe M, Singh Kavita, Bhusari P, Rao Raman (2005). Indigenous re combinant streptokinase Vs natural streptokinase in acute myocardial infarction patients: Phase III multicentric randomized double blind trial. Indian J Med Sci 59:200–207
78) Goa KL, Henwood JM, Langley MS, Clissold SP (1990). Intravenous streptokinase: a reappraisal of its therapeutic use in acute myocardial infarction. Drugs 39:693–719
79) Gruppo Italiano per lo Studio della Soppravvivenza nell' Infart miocardico (GISSI-2) (1992) A factorial randomized trial of alteplase vs. streptokinase and heparin versus no heparin among 12490 patients with acute myocardial infarction. Lancet 339:753–770
80) Koide A, Suzuki S, Kobayashi S (1982) Preparation of polyethylene glycol-modified streptokinase with disappearnce of binding ability towards antiserum and retention of activity. FEBS Lett 143:73–76

81) Pautov VD, Anufrieva EV, Ananeva TD, Saveleva NV, Taratina TM, Krakovyak MG (1990) Structural dynamic and functional properties of native and modified streptokinase. Mol Biol 24:35–41
82) Bean RR, Verhamme IM, Bock PE (2005) Role of the streptokinase a-domain in the interactions of streptokinase with plasminogen and plasmin. J Biol Chem 280:7504–7510
83) Conejero-Lara K, Parrado J, Azuaya AI, Smith RAG, Ponting CP, Dobson CM (1996) Thermal stability of the three domains of streptokinase studied by circular dichroism and nuclear magnetic resonance. Protein Sci 5:2583 2591
84) Parrado J, ConejeroLara F, Smith RAG, Marshall JM, Ponting CP, Dobson C (1996) The domain organization of streptokinase: nuclear magnetic resonance, circular dichroism, and functional characterization of proteolytic fragments. Protein Sci 5:693–704
85) Beldarrain A, Lopez-Lacomba JL, Kutyshenko VP, Ser rano R, Cortijo M (2001) Multidomain structure of a recombinant streptokinase. A differential scanning calorimetry study. J Protein Chem 20:9–17
86) Markus G, Evers JL, Hobika GH (1976) Activator activities of the transient forms of the human plasminogenstreptokinase complex during its proteolytic conversion to the stable activator complex. J Biol Chem 251:6495–504
87) Siefring GE, Castellino FJ (1976) Interaction of streptokinase with plasminogen: isolation and characterization of a streptokinase degradation product. J Biol Chem 251:3913 3920
88) Rodriguez P, Fuentes D, Munoz E, Rivero D, Orta D, Alburquerque S (1994) The streptokinase domain responsible for plasminogen binding. Fibrinolysis 8:276–285
89) Reed GL, Lin LF, Parhami-Seren B, Kussie P (1995) Identification of a plasminogen binding region in streptokinase that is necessary of the creation a functional streptokinase–plasminogen activator complex. Biochemistry 34:10266–10271