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Streptokinase: A Potential Therapeutic Agent

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Abstract

Enzymes have been widely used as a drug for therapy of specific medical problems and streptokinase produced by *Streptococci* is useful in the lysis of *in-vivo* blood clots. Streptokinase works by activating the fibrinolytic pro-enzyme plasminogen which is present in plasma normally. The active form of plasminogen is plasmin, which has serine protease-like activity. It attaches fibrin and cleaves it into several soluble components. Streptokinase is general a fibrinolytic remedy, integrated into the world Health Organization (WHO) model catalog of vital medicines produced by beta-hemolytic *Streptococci* as their extra-cellular enzyme. Streptokinase is useful and effective for the treatment of acute myocardial infarction as tpA or other drug but its production from a bacterial source need modifications and optimization to reduce its cost and adverse effects.

Keywords: Streptococci, Streptokinase, Fibrinolytic enzyme, Therapeutic agent, Myocardial infarction

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1. Introduction

Many fibrinolytic enzymes have been identified and discovered in various organisms including snakes, earthworm and bacteria (Babashamsi *et al.* 2009). Among bacterial strains, *Streptococcus pyogenes*, *Streptococcus equisimillis* and *Bacillus amyloquefacens* are the best bacterial strains, whereas *Fusarium oxysporum*; *Mucor* sp, *Armillaria mellea* are best fungal sources (Assiri, 2014). Fibrinolytic enzymes can be discovered in various foods such as Japanese Natto, Tofyou, Korean chugkook Jay soy sauce and edible honey mushroom. These fibrinolytic enzymes are purified from these sources and characterized for the study of their physiochemical properties (Zia *et al.* 2013). These novel fibrinolytic enzymes are very effective for thrombolytic therapy. Large quantities of such enzymes can be proficiently formed from these sources to manage heart diseases. Such enzymes are being used for food enrichment and nutraceutical applications to prevent from cardiovascular diseases (Bhardwaj & Angayarkanni, 2015).

Cardiovascular diseases are the prominent cause of death all over the world and in an imbalanced hemostasis, fibrin clots are not lysed so clot dissolving agents including urokinase, streptokinase, and tissue plasminogen activator (tpA) have been widely used as thrombolytic agent (Abdelghani *et al.* 2005). But these enzymes are often expensive, thermolabile and can also produce undesirable side effects (Chitte & Dey, 2000). Streptokinase is an extracellular enzyme extracted from various strains of beta-hemolytic *Streptococcus*.

The use of streptokinase-streptodornase (SK-SD) is frequently efficient, and the curative outcome differs from patient to patient. These two enzymes produced by hemolytic *Streptococci* are different from each other in all respects. Their production and their mode of accomplishment are totally different from each other even

despite the fact that they are formed at the same time by the same microorganisms. SK works on fibrin while SD on DNA-protein and both belongs to the collection of substances known as fibrous unsolvable proteins (Aila *et al.* 2010). Medical treatment for heart diseases is very costly that is not affordable for the poor people of Pakistan. So, there is a great need to work on the large-scale production of SK to meet the challenges of our country.

Mode of Action of Streptokinase

It is a non-protease plasminogen activator that functions by activating plasminogen to plasmin (Fig. 1), so being used as a drug for thrombolytic therapy. Streptokinase indirectly activates the fibrinolytic system by forming a composite with plasminogen which then undergoes an alteration and plasminogen is changed to plasmin that degrades the fibrin clot (Hernández-Bernal *et al.* 2014). The most interesting characteristic about streptokinase is that it has no catalytic activity of its own until it binds to plasminogen molecule and activates it to plasmin (Vijayaraghavan *et al.* 2016). Tissue plasminogen activator shows more affinity with a clot, but a conformational change occurs due to binding of plasminogen and streptokinase. This newly formed complex is known as streptokinase human plasminogen abbreviated as SK-HPG (Kumar, 2011).

This transition complex is readily converted into streptokinase human plasmin (KS-HPN), where a peptide bond present between Agr560-Val 561 is cleaved for the formation of new complex. After plasmin formation, streptokinase is separated from the complex and its molecular weight changed from 47 kDa to 36 kDa. In overall reaction, streptokinase loses 9 kDa but this new streptokinase with reduced molecular weight shows some properties as that of native ((Babu & Devi, 2015).

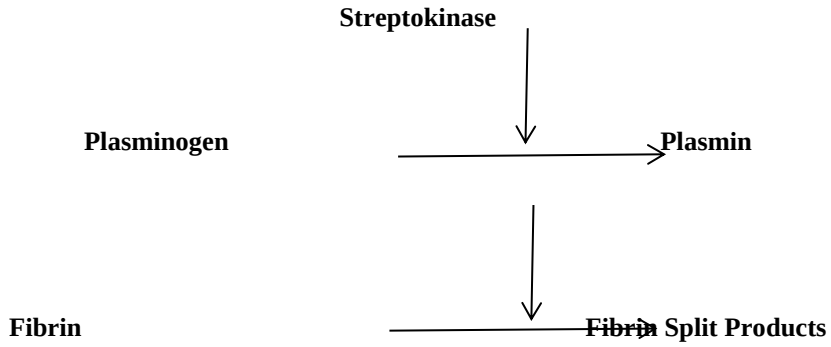

Fig. 1. Mode of action of streptokinase and its products

Physiology & Thrombolysis

The liquefying properties of hemolytic *Streptococci* facilitate the microbes to spread throughout the body and discover wider areas to grow so in this way they cause diseases and may be lethal for the host. Group A beta-hemolytic *Streptococci* possess the fibrinolytic property, so they are most common human pathogens. According to bacterial enzyme classification, the fibrinolytic property belongs to the collection named as constitutive enzymes, which do not need the existence of substrate for their production. On the other hand, adaptive enzymes are produced in the existence of their particular substrate. Streptokinase is a powerful therapeutic agent produced by beta-hemolytic *Streptococci*. It is a metalloenzyme which is being used as an economical but effective clot dissolving agent in a myocardial infraction and pulmonary embolism (Murray & Lopez, 1997).

Streptokinase is clinically in practice for the last three decades and is being used successfully for the treatment of acute myocardial infarction especially in developing countries. Regardless the use of various thrombolytic drugs including tissue plasminogen activator, SK is more effective in first or initial three to four hours of myocardial infarction to avoid any major complication (Sikri and Bardia, 2007). In some clinical practices, recombinant streptokinase is also used and represented adequate preliminary information. This recombinant streptokinase may become a novel drug for the treatment of hemorrhoid emergency (Quintero *et al*., 2010).

It is the primary aim of medical professionals to avoid lethal pulmonary embolism in small time. For this purpose, a quick return of the venous system to the ordinary condition is essential and this can be obtained by removal of thrombus before the destruction of valves of the vein. The only two possible ways for this removal include either by remedial thrombolytic or surgical thrombectomy. The conventional anticoagulant therapy can prevent further thrombosis and can also reduce the risk of pulmonary embolism, but this therapy cannot recanalize the blocked vein. As thrombolytic agents influence all thrombi nearby in the venous system so now this thrombolytic has to turn out to be the cure of preference. By using these venous thrombi of the inferior limb and pulmonary artery can be treated at the same time (Faran *et al.* 2015).

No doubt this thrombolysis also has demerits and restrictions including risks, so it is a challenge for clinicians to identify such patients who can get benefit from thrombolysis despite developing serious hemorrhage. In thrombolysis, the administration of a fibrinolytic medicine activates the endogenous fibrinolytic system. All the presently accessible fibrinolytic agents potentially increase the plasmin action on fibrin which is present in the clot. The size of the thrombus gets reduced as the fibrin is degraded. On the other hand, use of such fibrinolytic agents like streptokinase results in degradation of plasma fibrinogen which is lytic state of plasma and this is the main side effect of such therapeutic agents (Banerjee *et al*. 2004).

Anticoagulants and Fibrinolytic Agents

Acute myocardial infarction (AMI) and angina have a relationship with coronary blockage and the concept was further supported by William Osler who described that a common cause of angina and death is the clotting coronary artery. Aspirin was useful in early 1950's but useless in acute phase for MI (Craven, 1950). For the relieve of coronary spasm, use of atropine and papaverine followed by sublingual nitroglycerin were also routinely used. Also in addition, anticoagulants support the therapeutic cocktail to avoid coronary blockage, and thrombosis.

In the 1940s, an anticoagulant (dicumarol) obtained from spoiled sweet clover was used orally for the treatment of MI (Wright *et al*. 1948). Later on, warfarin became superior and replaced the dicumarol eventually, but up to the 1960s, there was no significant decline in death rates in patients treated with anticoagulants after acute MI. Then, heparin was proved to me effective as compared to other anticoagulants (Hilden *et al.* 1961).

All fibrinolytic agents are classified into (1) Non-specific fibrinolytic including streptokinase, urokinase and antistreplase and (2) Fibrin specific fibrinolytic including recombinant tissue type plasmin activator (rt-PA), reteplase (r-PA), tenecteplase (TNK-tPA) and lanoteplase (n-PA). But most commonly and frequently used thrombolytic agents are streptokinase, urokinase and recombinant tissue plasminogen activator (Kunamneni *et al.* 2007).

Another way to classify thrombolytic agents is known as a generational classification that is based on the time when the drug is administered clinically, where urokinase and streptokinase are included in first generation thrombolytic agents. The second generation consists of tissue plasminogen activator (t-pA), SK-Plasminogen activating complex (APSAC) and pro-urokinase (scum-PA). Reteplase (r-PA) TNK fall in the third generation of thrombolytic agents (Hernández-Bernal *et al.* 2014).

History of Development of Streptokinase

A powerful fibrinolytic agent, first discovered by Tillett in 1933 when *Streptococci* were found to produce a fibrinolytic substance, which can lyse fibrin (Tillett & Garner 1933). Tillett observed the agglutination of

Streptococci due to fibrinogen and concluded that fibrinogen is adsorbed on the exterior of *Streptococci*. It was further concluded that a fibrinolytic substance was present in sterile, cell-free filtrates of bacteria. This substance was named as fibrinolysin which later became streptokinase (Tillett & Garner, 1933). It was isolated worked as an enzyme which showed high specificity for human fibrin (Tillett *et al.* 1934). It was further demonstrated that fibrinolysin is a unique secretion of *Streptococci* and this cannot be associated with any other bacterial species.

Streptokinase was named in 1945, and commercial production was initiated from *Streptococcus equsimilis* because it produces fewer toxins and can be easily grown on synthetic media (Christensen, 1945). Streptokinase faced a severe downfall in clinical practice because the signs and symptoms before and after SK therapy were ambiguous. Clinical trials in various parts of the world like in Germany, Australia, and the UK. were carried out during 1970-80 but could not launch a well-defined drug treatment until SK was injected directly on thrombus in the coronary artery in 1984 (Laffel & Braunwald, 1984).

Administration of streptokinase is not risk-free and may cause allergy, hypersensitivity, bleeding and other complications which cannot be neglected (Verheugt *et al.* 1985). To overcome the issue related to SK therapy, GISSI (Gruppo Italiano per la Sperimentazione della streptochinasinell infarto Miocardico) prepared a framework to improved survival rate with decreased adverse effects (Vermeer *et al.* 1986). Currently, tissue plasminogen activator is in clinical use for treating AMI, but due to reasonable cost, SK is still a drug of choice in third world countries (Sikri & Bardia, 2007).

Risks Factors Related to SK

Streptokinase used for the treatment of blood clot resulted into death of cardiac muscles when used for the first time (Ruegsegger *et al.* 1959). From more than 50 years, streptokinase was being used to lyse the blood clots but still today it is controversial and likely to remain of very restricted medical value (Johnson & McCarty, 1959).

Risks associated with streptokinase administration are classified as cardiac and extra-cardiac factors. The extra-cardiac risk factors include hemorrhage in various body parts including brain (Marx & Levin, 1986). Besides the advantages of SK, it also has various side effects including sublingual hematoma, bleeding, reperfusion arrhythmia and hypersensitivity, especially in the case of elderly age patients and allergic response is also a key factor being an antigenic nature of SK (Klugmann *et al.* 1983; Verheugt *et al.* 1985). Such adverse effects were minimized after the development of recombinant SK that can exert nausea, hypotension and mild antigenic reactions (Babu & Devi, 2015).

Future Perspectives

Thrombolytic treatment include, ignoring the risks and cost of this therapeutic agent (Menon *et al.* 2004). In patients having the initial phase of AMI (less than 12 hours), any of the fibrinolytic agents including streptokinase is recommended, but on the other hand, fibrinolytic therapy may be fatal in patients having acute posterior AMI (Verheugt *et al.* 1985).

In the modern nations including the US, UK or EU, recombinant tissue plasminogen activator is widely accepted over other thrombolytic agents mainly due to low side effects. But in developing nations including Pakistan, SK is being used as a preferred drug. This is because of the differences in health-care systems and health-care policies. So, in developing countries, SK is the most economical but effective available fibrinolytic agent for the treatment of AMI (Diwedi *et al.* 2005).

Streptokinase was introduced as a powerful therapeutic agent for decades, and is a part of World Health Organization (WHO) model catalog of vital medicines. In the current era, medical history has been changed by all thrombolytic agents including streptokinase (SK), urokinase (UK) and tissue plasminogen activator. Treating cardiac disorders has become more effective due to the development of hybrid proteins having the properties of fibrin specificity and delayed thrombolysis (Babu & Devi, 2015) but immunogenic reactions of

SK needs more attention (Banerjee *et al*. 2004). A strong research is required for the allergen-free production, purification, and marketing of this (SK) fibrinolytic agent.

Amylases are among the most important enzymes and have great significance in many fields especially present day biotechnology (Sundarram and Murthy, 2014). These can be produced from several sources such as plants, animals and micro-organisms like bacteria and fungi (Pandey *et al*., 2000).

Fungal sources are not only confined to terrestrial isolates but also on marine isolates mostly to *Aspergillus* and *Penicillium* species (Gouda and Elbahloul, 2008). The growth of microorganisms on humid solid substrates with minor free water is known as Solid state fermentation (Pandey *et al*., 2001; Raul *et al*., 2014). For cost effective production of α-amylases agro-industrial wastes have been reported as very good substrates (Kumar and Duhan, 2011; Kumar *et al*., 2011).

Those have been studied extensively due to an important group of hydrolytic enzymes and their latent applications in the biotechnological based products like pharmaceutical industries, food, paper, and detergent (Maarel *et al*., 2002; Krishnan *et al*., 2006). Purpose of this paper is to optimize different parameters of fermentation for maximum production of α-amylase and to found an association in *A. sydowii* and culture medium of waste bread (Ellaiah *et al*., 2002; Maryam *et al*., 2010).

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