

Comparative study of the reactivity of natural and mutated streptokinase with total antistreptokinase antibodies in human sera

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Streptokinase is widely used as an anticoagulant drug for the treatment of heart attacks. Because of antibody production against injected drug in individuals consuming streptokinase and causing allergic reactions, streptokinase treatment effects become neutral. Recombinant mutant type of streptokinase was prepared by removing of 42 amino acids from the C terminal region. ELISA plates were coated by natural and mutant streptokinase as antigen. Ninety-six normal serum samples as well as 27 streptokinase consumer serum samples (patients with acute myocardial infarction) were analyzed. The results showed that serum antibodies against natural streptokinase were three times more than those against the mutated streptokinase. In case of preserving thrombolytic activity, mutated streptokinase can be used as an alternative of the natural form. *Blood Coagul Fibrinolysis*

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Introduction

Currently the use of digestive blood clot drugs as standard in the treatment of patients with acute myocardial infarction has been approved [1–3] because more than 85% of coronary artery obstructions are due to blood clotting; digestive clot drugs restore the blood flow [4,5]. The tissue plasminogen activator and streptokinase are most common digestive clot drugs, but because of the economic efficiency, the medical centers of the world use streptokinase as the drug of choice [6–11].

Streptokinase is a secretory single-chain polypeptide produced by various species of β -hemolytic streptococci. This protein digests the clot through plasminogen activation. The three structural domains of streptokinase – α , β and γ – have been established through a multiple domain that reacts with plasminogen. Streptokinase γ domain is essential for activation of plasminogen. The α , and β domains of streptokinase form the plasminogen–streptokinase complex, which results in activation of plasminogen [12].

Torrens *et al.* [13] believe that the 42 amino acids from the C terminal of streptokinase play a role in antibody production. Torrens *et al.* [13,14] studied the interaction of serum antibodies with C terminal 42 amino acids of streptokinase; they believed that this part of the molecule

is most important in streptokinase–serum interaction [14,15].

The most important issue in streptokinase drug consumption is the risk of neutralizing antibodies that are found in populations that suffer from streptococcal infection [16–19]. Studies show that in populations with streptococcal infection [20–22], the level of these antibodies is more than that in the population with healthy conditions [23–32]. This phenomenon results in reduced efficiency and increased risk of drug hypersensitivity reactions in these populations [26–28]. However, there are some controversies in this field [15,32]. In addition, consumption of streptokinase in patients with myocardial infarction increased the level of neutralizing antibodies in their body [33,34].

Studies showed that if the 42-amino-acid C-terminal region of the streptokinase molecule is deleted, there will be no enzyme activity; however, less neutralizing antibodies will be produced [13–15,35,36]. It has been shown that the use of mutated streptokinase is better than the natural (wild-type) in patients with acute myocardial infarction in the populations where streptococcal infections are common, and also in patients who experienced myocardial infarction within the previous year. The aim of this study was to compare the level of

antibodies in the normal population and streptokinase receivers (with myocardial infarction) against natural (heberkinase) and mutated streptokinase.

Materials and methods

Recombinant mutant streptokinase was prepared as described previously [37,38]. Recombinant protein (mutated streptokinase) was purified by affinity chromatography using N-terminal T7 Tag [39] and used for coating of ELISA plate wells [40]. Polystyrene microtiter plates were coated with purified recombinant protein and native streptokinase (heberkinase), as previously described [41]. Briefly, to remove traces of urea and exchange chromatography elution buffer, the purified recombinant protein was dialyzed at 4°C for 2 h against carbonate/bicarbonate sodium buffer (0.1 mol/l, pH 9.5) with ELISA coating buffer. The antigens (recombinant and heberkinase) were diluted up to 1 µg per 300 µl for each well.

ELISA pates were washed three times with phosphate-buffered saline-Tween 20, and then 100-fold diluted serum samples were added to each well and incubated on the coated antigen for 1 h at 37°C. Plates were washed and antihuman IgG conjugate (γ-chain; 1:3000) was added; it was incubated for 1 h at 37°C and then washed six times; the substrate (OPD + H₂O₂) H₂O₂) was added; the reaction mixture was incubated for 10 min and then stopped by the addition of 0.5 mol/l H₂SO₄. The OD of the reaction mixture was measured at 492 nm with a 620 nm reference filter using a Tekan ELISA reader (Tekan LTD, Moscow, Russia).

Ninety-six normal sera and 27 received streptokinase serum samples were evaluated by the ELISA method. The OD values were converted to antibody concentration (molar concentration) using the formula at MyCalcTool website (<http://www.calctool.org/CALC/FAV>). The molar value of antibodies was compared against mutant streptokinase and heberkinase for normal and received streptokinase sera.

Statistical analysis

The ELISA results were analyzed by SPSS 16 software (SPSS Inc., Chicago, Illinois, USA).

Results

Comparison of concentrations of 96 normal serum samples with mutated streptokinase and natural streptokinase (heberkinase) by the ELISA method showed that concentrations of normal sera with mutant streptokinase were very low compared with those with natural streptokinase, with Fig. 1 indicating that the Iranian normal population have shown a lower immune response to mutant streptokinase.

Protein concentration and serum dilution for both antigens (natural and mutated streptokinase) were 10 µg/ml and '20th', respectively.

Comparison of ELISA assay results of 96 normal samples analyzed by SPSS16 software with paired samples t-test showed significant differences ($P_v < 0.0001$) and SD 66.607, SE 6.8.

The 27 serum samples of received streptokinase (people with acute myocardial infraction) were evaluated by

Fig. 1

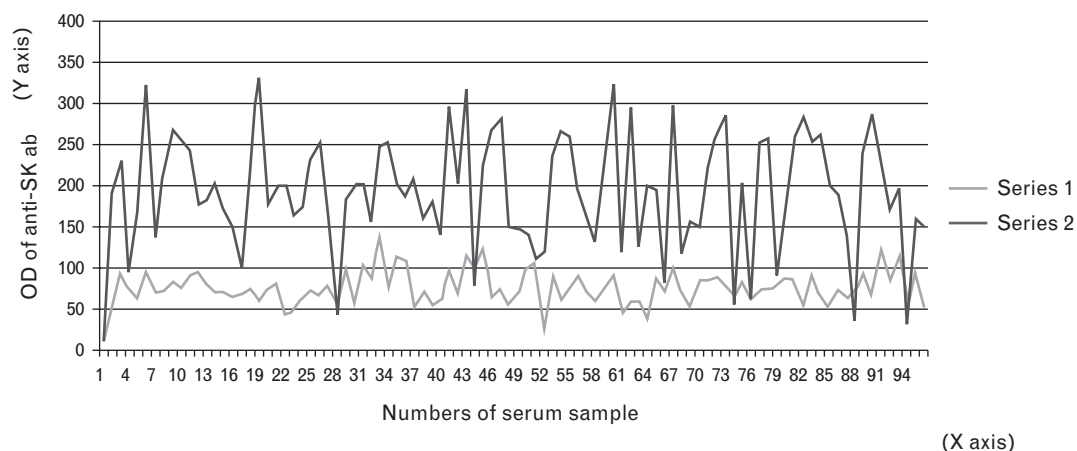


Diagram of molar concentration of antistreptokinase antibodies (anti-SK ab) in 96 normal serum samples. Series 1, molar concentration against recombinant streptokinase (lower OD); and series 2, molar concentration against heberkinase [higher optical density (OD)]. The X-axis represents numbers of sample used in the test, and the Y-axis represents the OD of antibody for each sample. As shown in the figure, the amount of antibody produced against recombinant antigen (mutated streptokinase) is less than that produced against heberkinase (as natural streptokinase). However, a comparison of results of 27 sera who had received heberkinase showed a significant difference between the two groups [probability value (P_v) < 0.0001], with SD 185.19 and SE 35.64.

Fig. 2

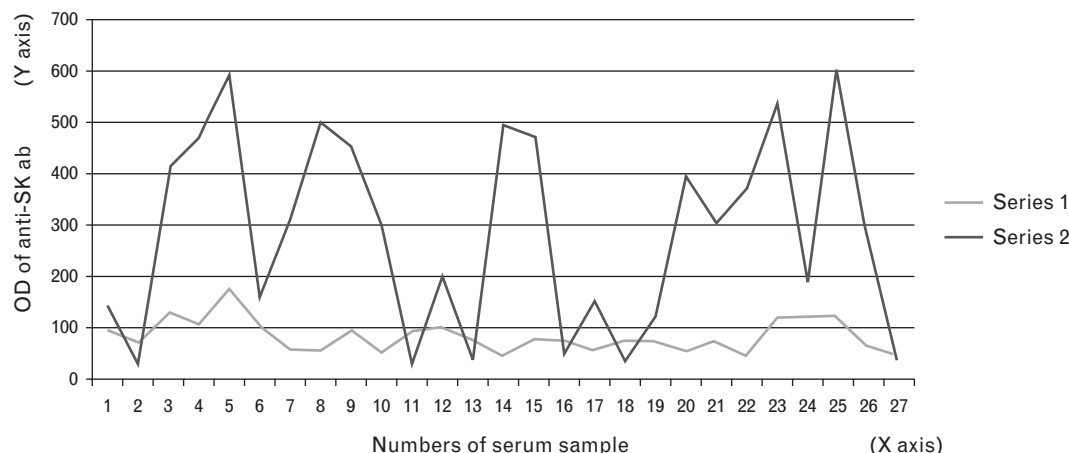


Diagram of molar concentration of antistreptokinase antibodies (anti-SK ab) in 27 heberkinase serum samples. Series 1, molar concentration against heberkinase (higher OD); and series 2, molar concentration against recombinant antigen (mutated streptokinase) (lower OD). The X-axis represents numbers of sample used in the test, and the Y-axis represents OD of antibody for each sample. As shown in the figure, the amount of antibodies produced against recombinant antigen (mutated streptokinase) is less than that produced against heberkinase (as natural streptokinase).

ELISA against two antigens (mutated streptokinase and heberkinase). The results showed that antibody production against mutated type was much less than against natural streptokinase (Fig. 2). Protein concentration and serum dilution for both antigens (natural and mutated streptokinase) were 10 µg/ml and '20th', respectively.

Discussion

Currently the use of fibrinolytic drugs for clot digestion in patients with acute myocardial infarction has been approved [1–3]. The main factor behind more than 85% coronary artery obstruction is blood clotting; thrombolytic drugs help to re-establish blood flow [3–5]. The most common drugs used for thrombolytic agents are tissue plasminogen activator and streptokinase, but economically, medical centers use streptokinase as the drug of choice [6–8,42].

Streptokinase is used for clot digestion, often accompanied by allergic reactions, due to antibodies against streptokinase. High-titer antibodies neutralize streptokinase activity, which can prevent the fibrinolysis activation by the fibrin lysis system, so the efficiency of thrombolytic therapy is reduced [8,19,22,24].

Based on research performed in Tehran Heart Center in 2010 on 677 people, 59.2% of controversial disordered drug reaction cases in the cardiovascular system are related to consumption of streptokinase [43]. Saadat *et al.* [44] believe that due to streptococcal infections, the antistreptokinase antibody level in the Iranian population is higher compared to other endemic areas. The level of antibodies against streptokinase in Pakistani people who had consumed streptokinase and normal individuals were measured and it was determined that

1.5 million units are appropriate for the initial treatment [45].

Researchers have made a lot of efforts in order to reduce the immunological effects of streptokinase [13–15,35,36]. In the present study, by genetic manipulation, recombinant mutated streptokinase was prepared and used for assay of antistreptokinase antibodies. This study showed that molar concentration of antibodies by natural antigen in people who have received streptokinase as choice drug was three times higher than measuring antibodies against mutated antigens. Namely, the deletion of 42 amino acids from the C terminal region of the streptokinase protein reduced its immunogenicity, and the antibody has not been identified. Similar findings have been reported by Torrens *et al.* [13,14].

Normal serum assessment showed that there are low statistical differences between serum reaction with normal streptokinase and mutated streptokinase because of no previous contact with antigen. Generally little response to antigens has been shown. However, serum assessment of received streptokinase which has had contact with antigen (heberkinase consumption) showed some reactions with mutated and natural streptokinase. Serum consumer streptokinase will react to natural antigen on further exposure, but the reaction of mutated streptokinase with serum consumer streptokinase is weaker, and there is less produced antibody against the mutated streptokinase.

There was some controversy about the interaction between streptokinase and plasminogen for streptokinase–plasminogen complex formation. Mundada *et al.* [46] believe that the first 59 amino acids are less involved

in activating plasminogen; other amino acids are more active in this case. Kim *et al.* [47] reported that amino acids His48 and Asp 41 play an important role in binding of streptokinase to plasminogen, but Tharp *et al.* [48] replaced basic (Arg253, Lys256, and Lys257) and acidic residues (Glu249, Glu262, and Glu263) of the 250-loop of the SK β -domain with Ala. They concluded that plasminogen is identified through arginine 253, lysine 256 and lysine 257 of streptokinase, and streptokinase–plasminogen complex is formed.

The mutated streptokinase which was produced in this study contained β -domain amino acids Arg253, Lys256 and Lys257 and theoretically could affect plasminogen–streptokinase complex and subsequently plasmin formation and blood clot digestion, but more research is necessary to determine the fibrinolytic effect of mutated streptokinase in digesting blood clots. If mutated streptokinase was able to digest the blood clots, it can be used in place of normal streptokinase that is immunogenic in people who suffer from a heart attack (without risk of being immunogenic).

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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