

# Molecular Processes in the Streptokinase Thrombolytic Therapy

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*The aim of this study is to evolve the current streptokinase thrombolytic treatment and the identification of new techniques with a higher efficiency. The biochemical processes that manage the genesis and dissolution of the thrombus represent an important topic regarding the therapy of the myocardial infarction and cerebrovascular accident.*

*Keywords: streptokinase EC 3.4.99.22, plasmin inhibitors(API), thrombolytic activity*

The aim of this research is to evaluate the current streptokinase thrombolytic treatment and to identify new techniques that will base new approaches with a higher efficiency in this area of expertise.

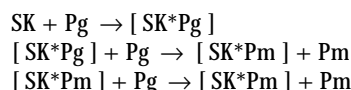
The biochemical processes that govern the genesis and especially the dissolution of the thrombus present in the blood stream, have been and continue to represent an important research topic, given the practical implications of this problem, mainly regarding the therapy of the myocardial infarction and/ or the cerebrovascular accident.

Currently, there are several options for the thrombolytic treatment, but the most utilized is the administration of streptokinase. Although the results do not always live up to the expectations, the elements concerning the costs of the treatment have imposed and are maintaining this therapy method as one of the most utilized options in the acute myocardial infarction or cerebral micro thrombosis [1].

One of the elements that set back the streptokinase therapy is that of the high heterogeneity of the response of the patients to this type of treatment. It is due to this fact that the streptokinase therapy, even if it does not have a significant number of adversaries, has come across a high degree of criticism based on clear clinical data [2]. In this

study we have tried to systematize the most positive effects and also to clarify as much as possible the limits of this treatment.

Streptokinase (STK), EC 3.4.99.22, is a bacterial protein [3], formed out of 414 amino acids with a 44kDa mass, pI 4, 7 and a 7.5 max pH activity. This protein has a specific biological activity, meaning that it can engage the plasminogen proteins (Pg) and form the STK-Pg compound that subsequently gains a proteolytic activity on other Pg proteins, thus generating plasmin (Pm) that combined with STK form the compound STK-Pm, a compound that has a high Pg and STK proteolytic activity. Through this succession of reactions, the STK interacts with the plasminogen, having as a final result plasmin and peptide segments derived from STK and Pg, [4, 5]:



The next phase identifies Pm acting with a proteolytic activity on the fibrin, that represents the frame of the blood clot, determining its degradation and the disappearance of the thrombus [6].

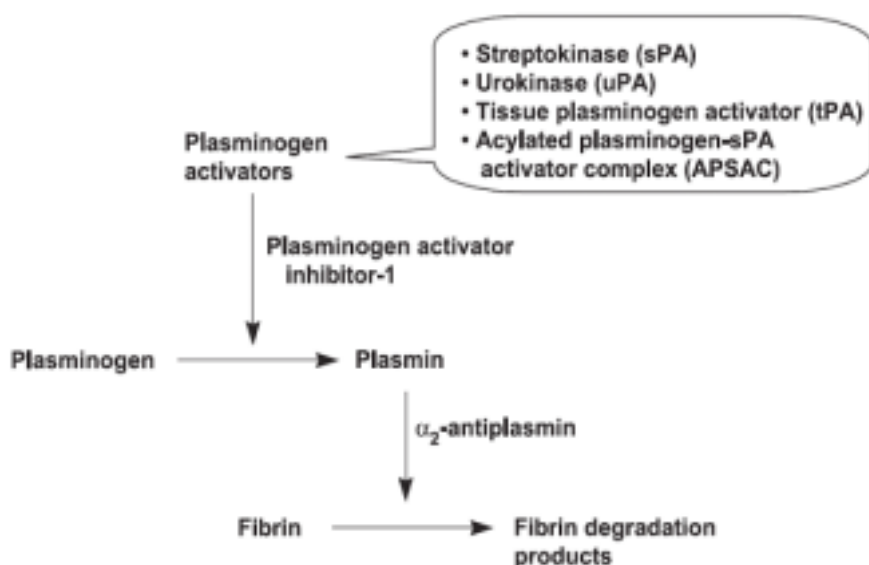


Fig.1. Schematic representation of fibrinolysis, [6]

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**Table 1**

THE PROTEOLYSIS OF THE INTEGRAL SANGUINE THROMBUS OBTAINED FROM 2mL OF BLOOD, 1 mL OF PHOSPHATE BUFFER, 0.2 mM, pH =7 AND 250 I.U. STK-NP

Crt. No.	Time of Action (min)	Percentage of clot lysis	Observations
1	5	50	Clotted blood, after 3 h.
2	10	65	Clotted blood, after 3 h.
3	15	70	Clotted blood, after 3 h.
4	30	77	Clotted blood, after 3 h.
5	60	81	Clotted blood, after 3 h.
6	90	83	Clotted blood, after 3 h.
7	30	0	NP-STK recovered from the previous reaction
8	30	<1	Clotted blood, after 3 h, cleansed with saline solution
9	30	50	Clotted blood, after 72 h.

The clinical extensive experience in the field of using STK in the thrombolytic treatment has shown three categories of aspects that, if understood accordingly, can lead to the increase of the efficiency of the streptokinase thrombolytic treatment:

-even if the producers indicate the active dosage of STK, it has been noted that, at the circulatory system level, there are important variations between the STK concentrations that prove active in every individual, a fact that leads the current physician to elevate the dose of the administrated STK, even with the risk of causing micro bleedings;

-currently, there is no possibility of a targeted administration directly into the thrombus or at least in the general area of the body where the thrombus is present, leading to the dissipation of the STK effects into the entire mass of the organism;

-given the nature and important doses utilised for the reasons above mentioned of STK, the genesis of the antigenic reply of the organism becomes a certainty, involving the anti- STK antibodies that lower upon the point of total elimination of ulterior STK therapies for a period of several weeks;

### Experimental part

Although STK does not act as an authentic enzyme, consuming itself in the process of chemical reactions that lead to the fibrin hydrolysis, most methods of evaluating the biologic activity of STK are based on the principles and reagents used for general proteolytic enzymes. The most utilised are based on synthetic substances hydrolysis that contain derivatives of benzoyl-para-nitroanilide (b-PNA) and also the accurate spectrometric measuring of the development grade of the reaction [7]. Another category of methods implies the incubation of STK with Pg (from different sources) and measuring the proteolytic activity of the Pm resulted from different substrates like fibrin or casein [8-10].

For evaluating as precisely as possible, reporting to the limitations of the STK therapy, we have imagined and elaborated an experiment concerning blood clots using magnetically vectored STK, a technique that has the advantage of conferring a high degree of handling both in a liquid environment and also in the mass of the thrombus, using an external magnetic field.

### Principle of action

The STK action on the thrombus has been evaluated by joining the blood clot with the magnetite nano-particle ( $\text{Fe}_3\text{O}_4$ ) marked STK and monitoring the thrombus lysis percentage in different moments in time.

### Reagents and materials

All of the substances used have been analytically pure.

STK, Streptase, CSL Behring 250000 I.U./ vial.

NP-STK suspension ( equivalent of 5.000 I.U./ mL) was prepared by mixing magnetite nanoparticle with STK in phosphate buffer citrate solution and NP-STK separation with external magnetic field.

Buffer solution pH 7 phosphate, 0.2M

Clotted blood 2mL/tube

Saline solution (0.9% NaCl)

Magnet, pipettes, laboratory glassware.

### Method

36 - 40 mL of blood were harvested from a healthy volunteer and were split into 2 mL fractions in graded tubes with 5-10 mL volume. A 5-15 clotting time is necessary, followed by freezer storage (3 - 7°C) until it is utilised as a reaction environment. A strict tab will have been kept on the time (hour and date) of the harvesting/storage and utilisation of the blood clot. The serum is not to be separated from the formed clot.

The NP-STK compound is cleansed with saline solution and then it is re-suspended in buffer solution so that a suspension with 500 I.U./mL activity is obtained. The reagents are placed in a thermostat for 25 min at 37°C.

250 I.U. of NP-STK suspension (0.5 mL) are placed into contact with 1 mL of phosphate buffer solution and 2 mL of clotted blood. NP-STK is vibrated through the clot using an external magnet for 5-10 s. The mixtures are then placed in a thermostat for 5-90 min and then the fraction from the blood clot that has been hydrolysed by the action of NP-STK is measured and the expressed as a percentage of the initial clot volume. The initial clot volume is calculated as a arithmetic average from three tubes, by washing the clot in the graded tubes, followed by a sieve decantation and an adding of a well determined volume, using a pipette, of distilled water until a desired gradation on the tube containing the clot is achieved. The volume of the clot is obtained by the difference between the volume indicated on the tube and the volume of distilled water added with the pipette.

### Results and discussions

The degree of the blood clot hydrolysis at several time intervals of contact with NP-STK and certain work conditions are shown in table 1.

The experimental results show more than one aspects that influence the effectiveness of the streptokinase thrombolytic treatment, from which the most important are: the high concentrations of blood antiplasmin [7, 11], the lack of an antiplasmin inactivation/elimination treatment before administrating STK and the proteolytic activity of plasmin over STK. Along the therapy act, however, these can be mixed with other processes, as shown in table 2.

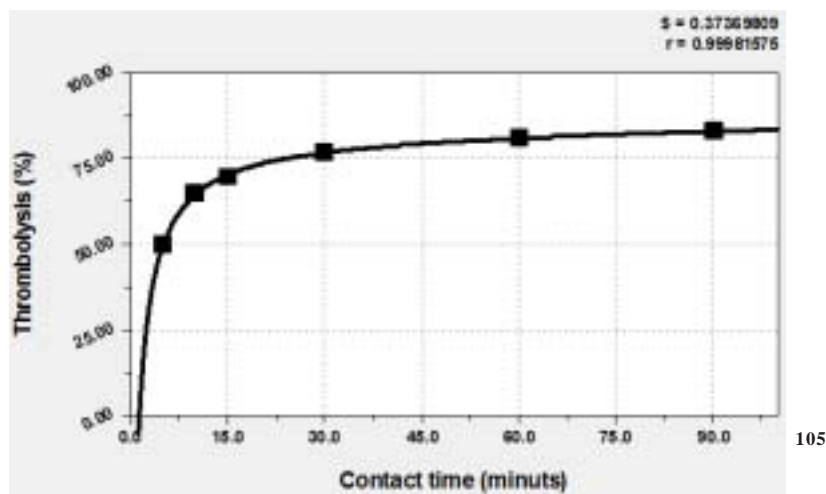


Fig. 2. The evolution of the thrombolysis degree over time

Thus, the most important process that lowers the thrombolytic activity of STK is represented by the presence of the plasmin inhibitors, mainly alfa2-antiplasmin (API), present in a concentration of aprox. 1microM. The reaction between Pm and API, being extremely quick, neutralises the plasmin and inhibits the thrombus proteolysis. But this process only occurs in a first stage, until the whole blood quantity of API is consumed. Considering the differences of the concentration between antiplasmin (0.7 – 1 microM) and plasminogen (1.5 – 2 microM), we can state that normally, given the best scenario possible, the thrombolytic activity of the STK can only achieve half of the theoretically possible activity in the lack of API. Accordingly, the physician must take into consideration that in many occasions these proportions can be modified by different pathologies like hipoplasminogenesis, sepsis, leukemia, hyperthyroidism,

hepatitis, etc. Leading to a highly lowered level of plasmin (subsequently inducing a low thrombolytic activity), regardless of the amount of administrated STK.

Another process that lowers the efficiency of the STK therapy is due to the proteolytic action of plasmin (and other reaction products between Pg and STK) over STK with an appreciable speed (the half-time of STK is aprox. 30 min).

These facts explain the allure of the curve that illustrates the advancement of the degree of thrombolysis together with the action time of the STK, presented in figure 2, as well as the ascertainment that the magnetically recovered STK in the form of NP-STK no longer produces thrombolysis (table 1, crt. no. 7).

Crt. No.	Process	Description	Solving possibility
1	High concentration of antiplasmin and quick interaction between API and Pm	API presents blood concentrations of aprox. 1 microM	Eliminating API from the blood stream.
2	The (auto)proteolytic activity of plasmin on STK	Half-time of STK of aprox. 30 minutes	Using other fibrin proteases
3	The lack of specific therapy metal-fibrin proteases	The existence of experimental models of metal-proteases (created out of venoms) that are not inhibited by API	Accelerating research in this medical domain
4	The lack of a treatment for eliminating API from the blood stream	API has a replacement rate of 2,6 days.	Administrating a consistent STK initial dose in the purpose of producing Pm that will consume from the beginning the entire quantity of API present in the blood stream. Consequently, Pg is synthesized faster than API
5	Hereditary or Pg induced deficiency	Hipo-/Displasminogenesis are hereditary. Sepsis, leukemia, hyperthyroidism, hepatitis are gained affections	Preventive medicine
6	Elevated immunogen potential of therapeutic STK	The current STK concoction is purified out of streptococci cultures and determines immune reactions in the organism	Reducing the immunogen potential of STK by genetic engineering and specific lysis.
7	The lack of a viable therapy solution for eliminating the anti-STK anti-bodies	After 7-21 days post STK administration, antibodies are created that prevent future STK efficient administrations	Elaborating a cleansing technique of the anti-STK antibodies from the blood, this technique is possible by capturing anti-STK antibodies by NP-STK

Table 2  
LIMITING FACTORS IN  
ROMBOLYTIC THERAPY

Through the non-linear regression of the degree of the thrombus hydrolysis ( $y$ , %) dependent of the contact time ( $x$ , minutes), a non-linear relationship has been highlighted,  $y = (71.95x - 89.95) / (1 + 0.86x - 0.00033x^2)$ , that can characterize the kinetics of the blood clot lysis processes under the action of STK.

Another element highlighted by the experimental data is the fact that, under the experiment conditions, even at high concentrations of STK, a complete proteolysis of the thrombus has not been achieved, leading us to the conclusion that plasmin, created as a follow-up of STK administration is not enough to completely hydrolyze the thrombus, which, in real therapy conditions, can migrate into the blood stream, leading to potential fatal consequences. For this reason, the thrombolytic therapy should hold into consideration prolonged administration of STK at least over 2-3 days, so that, as the sanguine Pg reserves are rebuilt, it can maintain a certain level of plasmin and to finally undergo the total thrombus hydrolysis, reported to the entire circulatory system.

### Conclusions

We can notice that in the first 5-10 min of the STK action, a thrombus hydrolysis of over 50% is obtained.

In the utilised concentrations, the lack of total thrombus hydrolysis is present even at 60 min of contact time. From the experimental data, in the case of a longer than 60 min contact time, the blood clot hydrolysis stops advancing.

The relationship between the contact time of the NP-STK with the thrombus and the degree of cloth hydrolysis is not linear and is based on a rational connection that can set the base for a useful model for the increase of the STK based therapy.

The NP-STK recovered using the external magnet after a 90 minutes contact time no longer presents capacity to act on a new blood clot, confirming once more that the STK has an autolytic activity through the reaction products with plasminogen.

NP-STK manifests no activity on the blood clot cleansed with saline solution 0.9%. This pleads for the hypothesis that basically, plasminogen that can be utilised as a STK substrate is only found freely in the serum and not in the structure of the thrombus. Given the results of other studies that have pinpointed the fact that 14-32% of the blood plasminogen is absorbed by the fibrin filaments through  $\alpha_2$ -API, (12), our results plead for the idea that this plasminogen can either generate plasmin that is immediately neutralized by API without being able to manifest its hydrolytic action over fibrin or, by unknown reasons, is not able to react to NP-STK. To clarify this aspect, we have set the goal to begin an extended study in the near future.

As noticed in the case of the blood clot stored in the freezer for 72 h since coagulation, the NP-STK activity is greatly decreased (about five times) by the "age" of the blood clot. This result can fully be explained if we accept the fact that while storing the blood clot, its plasminogen

levels decrease by degrading, a phenomenon consented by other research studies as well, that indicate a value of 2 days of the plasminogen half-time.

Based on the experimental data, we can appreciate that in order to increase the efficiency of the thrombolytic STK therapy, 2 directions can be followed: on the one hand, administering an initial massive STK dose so that the natural levels created by the presence of a concentration of antiplasmin in the blood that neutralizes half of the thrombolytic potential of the sanguine plasminogen are passed and on the other hand opting for a prolonged administration (3 days) of STK in order to fight the effects of incomplete hydrolysis, fragmentation and migration of the thrombus in the circulatory system.

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Manuscript received: 14.08.2015