REVIEW ARTICLE

Micro and Nanoparticles as Carriers for Streptokinase: A Mini-Review on Efficacy, Side Effects and Pharmacokinetics

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ABSTRACT

Streptokinase is being successfully used as a thrombolytic agent for restoring the blood flow following thromboembolism and myocardial infarction. However, high immunogenicity of the drug has limited its use in clinics. To overcome this limitation, several approaches including PEGylation, use of polymeric particles and liposomes have been suggested. Here, an overview of options for encapsulating the streptokinase has been provided. The suggested options in the literature include PEGylation, polymeric nano/micro-capsules and liposomes. In each approach, efficacy, side effect(s) and pharmacokinetic profile of streptokinase has been evaluated. The data show that while efficacy of streptokinase does not appear to change importantly, side effects and pharmacokinetics have been improved.

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INTRODUCTION

Globally, the leading cause of death is considered to be cardiovascular diseases (CVDs). In 2019, around 17.9 million deaths (~32% of all deaths) were estimated to be due to CVDs. Eighty-five percent of the CVD deaths are supposed to be from stroke/ heart attack [1]. Myocardial infarction, venous thromboembolism and ischemic stroke are major types of CVD which are caused by formation of blood clots. To restore the blood flow in a vessel (i.e. dissolve the blood clots), thrombolytic agents are commonly used in clinic [2].

A very cost-effective thrombolytic agent which is being widely used in clinic is streptokinase (SK). SK (molecular weight = 47 kDa) is produced by β-hemolytic streptococci which activates the circulatory plasminogen. However, the drug's potential is limited due to its highly immunogenic properties which makes its biological life relatively short [3]. In most cases, neutralizing antibodies are detected in the body after the first injection. The antibodies which are formed as reaction to foreign antigens [4], remain in the body for a long time, up to four years [5] and affect the SK's efficacy. Secondly, when administering SK, plasminogen is activated, not only on the surface of the thrombus, but also within the blood circulation. The activated plasma plasminogen can cause important adverse effects such as systemic bleeding [6] (see Figure 1).

To overcome the limitations, several approaches have been suggested. Mutant SKs are interesting options [7, 8], having reduced immunogenicity [9, 10]: Several antigenic sites are found on the molecule of SK. Such sites are added to the SK sequence by the Streptococci strains and when removed, the immunogenic properties decreases considerably [11]. The mutation may also be performed on SK to increase the drug's efficacy: in an in-silico study, activity of a cysteine variant of

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SK (SK319cys) was found to increase by 18 % [12]. The group, using bioinformatics tools, generated multiple mutations on SK, leading to 44 % increase in the enzyme's efficiency [13]. The mutant SKs, provided they show cysteine residues, could also be employed for addition of polyethylene glycol (PEGylation), which in turn can have enhanced activity [14].

Another approach to overcome the drawbacks of SK is encapsulating SK in different carriers. For instance, as reviewed previously, liposomes are able to prevent enzymatic degradation, increase the circulation time and target SK against desired sites [15]. The current review aims to study different carriers which have been used to improve half-life or efficacy of SK in-vivo. PEGylation, polymeric particles and liposomes have been reviewed as the most common approaches, as schematically shown in Figure 2. In each section, reports about efficacy of SK (including its clot lysis capability), side effects (arising from activation of plasminogen/fibrinogen) and pharmacokinetics (e.g. due to activation of immune system) have been summarized.

A major concern in encapsulation of SK is that the encapsulation process may modify the molecular structure of SK, thus, damage its efficacy. Therefore, the findings that have reported the efficacy of SK freshly after encapsulation have been summarized in Table 1. From the details, out of 13 reports that have made comparison of SK activity before and after encapsulation/conjugation, only 3 have mentioned more than 25% drop in efficacy, of which, 2 were for liposomes. Thus, it could be argued that the encapsulation process, especially in polymers, appears not to affect the activity of the enzyme, importantly.

PEGYLATION OF SK

PEGylation is addition of polyethylene glycol (PEG) to a substrate. Reviewing the literature, several advantages have been reported for PEGylation of peptides and proteins, including increasing their biological life. Additionally, kidney clearance and neutralizing antibodies are two main challenges for delivery of peptides/proteins, which may be hindered by PEGylation [16]. From the literature, PEGylated SK (PEG-SK) is able to improve different characteristics of SK, including:

Efficacy

Microcapsules of PEG-SK indicated faster clot lysis time both in plasma (23.8% ± 4.5 faster) and in whole blood (17.2% ± 9.2 faster), compared with free SK. Also, using confocal microscopy, the encapsulated SK was shown to have greater penetration to the clot [17]. In another study, clot-lysis activity of PEG-SK was comparable with that of free SK, i.e. both manifesting 50% lysis of the clot at 75 min [18]. Also, microcapsules of PEG-SK (particle size 5-20 μm) had more or less similar in-vivo activity in encapsulated SK and free SK, with the exception of an initial lag time (~ 15

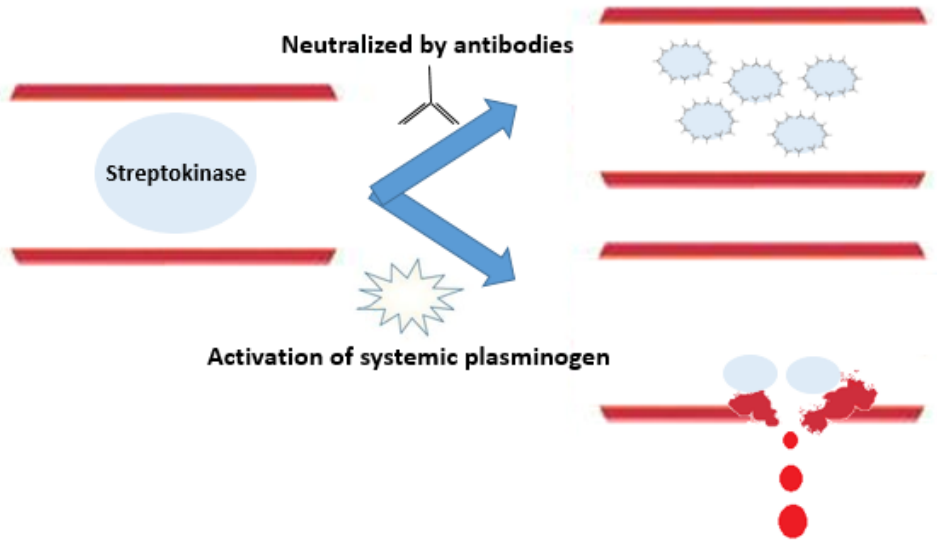

Figure 1. Drawbacks of clinical application of streptokinase. When streptokinase enters the blood circulation, it may be neutralized by antibodies, or may activate plasminogen in blood circulation, thus, lead to systemic bleeding.

min) [19]. In a report to compare reperfusion time in occlusive thrombus in rabbit, PEG-entrapped SK indicated significant reduction in reperfusion time (i.e. 7.3 ± 1.6 min, respectively), compared with free SK (i.e. 74.5 ± 16.9 min). The authors then suggested a potential fibrinolytic treatment using the encapsulated SK [20]. In our previous report, the freshly prepared PEG-grafted chitosan nanoparticles containing SK had slightly lower activity, compared with the free SK. From in-vivo studies, during the first 10 min after injection, the activity of free SK was slightly more than the SK in the nanoparticles. However, from 20 min post injection onwards, the activity of encapsulated SK was more than that of free SK [21].

Side effects

Minimum 3.5-folds lower depletion of plasminogen and fibrinogen (i.e. lower side effects) have been reported from PEG-SK in comparison

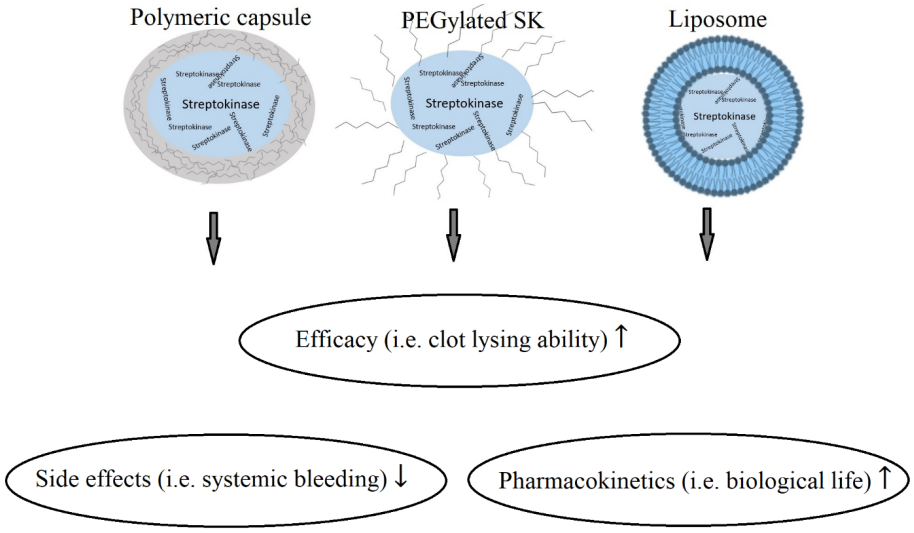

Figure 2. Schematics of the different approaches to modify efficacy, side effects and pharmacokinetics of streptokinase: encapsulation in micro/nano-polymeric particles, PEGylation and encapsulation in liposome

Table 1 The encapsulation efficiency and activity of SK after being encapsulated in different nanoparticles. To summarize the data, wherever more than one nanoparticle has been reported, only the characteristics of the nanoparticle with highest SK activity after encapsulation has been listed.

Carrier	Particle size	Encapsulation efficiency	Residual SK activity (freshly after encapsulation/conjugation process)	Reference
Liposome		Maximum 45.9 ± 34.0%	> 30%	[49]
Liposome	178 ± 40 nm	30.8 ± 4.2%	27.2 ± 4.8%	[45]
PEG-liposome	169.4 ± 5.51	32.0 ± 10.2 %	~ 100%	[46]
Dendrimer			90 ± 2	[30]
Dendrimer			79.8 ± 0.7%	[50]
PEG			98%	[22]
PEG			92%	[24]
PEG		91 ± 1%	Not significantly different	[19]
Chitosan	490 nm		98%	[33]
Liposome	178 ± 40 nm	30%	Not significantly different	[45].
Eudragit	726 ± 15 nm	90.1 ± 2.7 %	61.4 ± 3.4 %	[51]
SLN	210 nm (DLS), < 60 nm (SEM)	67.30 %	99.9%	[52]
SLN	224 nm (DLS), < 60 nm (SEM)	67.3 %	97.3 %	[53]

with the unmodified one. [22]. In an interesting publication, rabbit ear was incised. Subsequent to hemostasis, PEG-SK or free SK were infused. Microcapsules of PEG-SK (particle size ~ 0.8 µm) in comparison with the free SK, showed delayed onset of bleeding (24 ± 2 min in free SK, compared to 46 ± 7 min in PEG-SK), lesser blood loss (20 ± 5 mL in free SK, compared to 3 ± 2 mL in PEG-SK) and shorter duration of bleeding (72 ± 7 min in free SK vs. 42 ± 8 min in PEG-SK) in bleeding from ear of rabbit. The authors then suggested that the microencapsulated SK had selective thrombolysis and reduced fibrinolysis and bleeding in the microcirculation, thus, had lower adverse effects [23]. Additionally, PEG-SK microcapsules indicated less activation of plasma plasminogen. The authors then concluded a direct relation between release rate of SK and its side effects [19].

Pharmacokinetics

In two early reports, PEGylation was associated with enhanced biological stability: While the SK activity was maintained, complete loss of its antigenicity (i.e. binding ability to the antibody) was from PEG-SK [24]. PEG-SK -depending on molecular weight of PEG- also showed slightly improved biostability: After 30 min incubation with plasminogen, free SK was extensively degraded, while PEG (MW 2000)-SK was resistant cleavage by plasmin. The authors also experienced greater specific binding of SK to its antibody in free SK compared with PEG-SK (specific binding of PEG-SK was around 5% of free SK) [25]. To increase plasmin resistance and reduce immune-reactivity of SK, cysteine residues were incorporated on SK, then, the molecule was PEGylated. The biological half-life of SK increased from 15-20 min in free SK to 1-9 h in PEG-SK [18]. Furthermore, 65-67% lower immune reactivity from PEG-SK was observed, compared with free SK [18]. In a study on PEG-grafted chitosan nanoparticles, the antibody against SK was measured. Maximum level of produced antibody was 22 ng/mL for the nanoparticles, while this value was nearly double (i.e. 42 ng/mL) for the free SK, indicating the immune system hiding ability of the nanoparticles [21]. Using ^{125}I, lesser SK degradation by plasmin as well as extensive increase in clearance time of SK from blood circulation (i.e. 15 min in free SK vs. more than 200 min in PEG-SK), was shown following PEGylation. Moreover, in the same study, it was found that in mouse, the accumulation site for free SK and PEG-SK were kidney and liver/GI tract, respectively [26].

POLYMERIC NANO/MICRO-CAPSULES

Polymeric nano/micro-capsules are composed of solid polymers which encapsulate the core material and act as reservoir forms. Various natural or synthetic polymers have been successfully employed [27]. Such particles are able to modify loading, release, efficacy, side effect, bioavailability, distribution, cellular uptake or stability of the active ingredient in or out of the body [28]. When encapsulating in polymeric particles, several changes have been reported from SK, such as:

Efficacy

In a report to compare two different methods of entrapping SK in CS (i.e. bulk mixing and microfluidic), the microfluidic method showed uniform morphology (spherical shape for the particles) with particle size of 67 ± 13 nm and a sustained release pattern. In-vivo tests also indicated higher amidolytic activity for SK during 120 min study period in comparison with free SK and SK which was entrapped in CS using bulk mixing [29]. Polyamidoamine (PAMAM) dendrimers with three different generations (i.e. generations 1.5 (SK-G1.5), 2.5 (SK-G2.5) and 3.5 (SK–G3.5)) were conjugated to SK at protein–polymer molar ratios of 1:1 to 1:10. Compared with the free SK, the SK-dendrimer had clot-lysing activity of 48% to 90%, depending on the SK-dendrimer ratio and dendrimer generation [30]. Elctrospray, as a cost-effective method to load sensitive therapeutics [31] was employed to prepared PLGA-SK nanoparticles. However, the activity of SK was noticeably low (i.e. 19.2%), probably due to the spray process or use of organic solvents [32]. The activity of SK encapsulated in chitosan particles was reported to be similar (3474 IU/mL in encapsulated SK vs. 3558 in free SK) [33] and slightly reduced [34] in two different reports from our group. Compared with free SK, the plasma activity of SK was lower in mPEG-PLGA nanoparticles during the first hour of injection [35].

Side effects

The aforementioned SK-dendrimers made less activation of plasma fibrinolytic system (i.e. plasminogen, α2-antiplasmin, and fibrinogen), compared with the free SK. SK–G1.5 (molar ratio of 1:10) which had minimum clot-lysing

activity (48 ± 2%) caused almost no exhaustion in plasma plasminogen, α2-antiplasmin, and fibrinogen within 2 h [30]. In an artificial neural networks modelling study, toxicity of chitosan-SK nanoparticles was investigated. The generated model illustrated a reverse relation between size of nanoparticles and their in-vitro toxicity [36]. Also, mPEG-PLGA loading SK, nanoparticles had satisfactory blood compatibility and indicated no cytotoxicity [35].

Pharmacokinetics

The above-mentioned SK-dendrimer particles indicated more plasma stability than the free SK. In particular, SK–G3.5 particles (1:10) were the most stable conjugates after 2h incubation in human plasma at 37°C (i.e. residual activity of 76% in SK-dendrimer vs 59% in free SK) [30]. The in-vivo studies on SK-loaded chitosans showed that 120 minutes after administration, free SK lost ~50% of its initial activity, while this value was ~36% for encapsulated SK, indicating the effect of chitosan capsules in hiding SK from immune system [33]. The bioactivity data also showed that in-vivo activity of free SK was more than mPEG-PLGA encapsulating SK in the early minutes of study, while after the first hour, the encapsulated SK had better activity. The authors then suggested that while the encapsulated SK managed to maintain its activity, the free SK was deactivated during the study period [35].

LIPOSOMES

Liposomes are bilayer vesicles made from cell membrane materials (i.e. phospholipids). They can be formulated to act as pH-sensitive carriers or behave as immunological adjuvants. Liposomes are able to complex with different molecules (having positive or negative charges) and can be used to carry DNA or other nucleic acids. Additionally, they can be targeted against a special site of action [37]. Liposomes have been reported to influence the following features of SK:

Efficacy

To increase efficacy of the active ingredient and decrease its adverse effects, targeting the carrier is nowadays a common approach. A selective target-sensitive liposome (modified with RGD) containing SK, with higher accumulation in the thrombus area of murine model, managed to dissolve the thrombus significantly faster than that of free SK (i.e. thrombolytic activity 28.27% ± 1.56 vs. 17.18% ± 1.23, respectively, after 30 min of treatment) [38]. Long circulatory liposomes, targeted against platelets using RGD peptide, were employed for thrombolysis studies in wistar rats using human clots. After 1 h, the RGD-liposomes containing SK and free SK dissolved 34% and 22% of the initial clot, respectively. Interestingly, the thrombolytic activity of free SK after 30 min, was higher than that of liposomal preparations. The authors then ascribed this finding to the fact that the SK must be released from liposome in liposomal preparation, while in free SK, the SK starts to act immediately. Moreover, it was observed that the nanoliposomes penetrate the clots deeper than the free SK [39]. Using RGD, target sensitive liposomes encapsulating SK were prepared (particle size 100-120 nm). To compare the activity of the liposomal SK with that of free SK, % clot lysis as well as time for 50 % clot lysis (T_{50}) were determined. After 30 min treatment, RGD-liposome containing SK indicated 50 % clot lysis, while 30% lysis was documented with free SK. Additionally T_{50} was 30 and 55 min in free and RGD-liposomal SK, respectively [40]. Animal investigation of immunoliposomes containing SK, targeted against fibrin, showed considerable fibrinolysis with immuno- and normal liposome which had been loaded with SK. Furthermore, the ultrasound study indicated 32% reduction in residual stenosis in 3 h post injection. From the morphological assessment of the artery samples, 71.3% enhancement in degree of free vascular lumen was reported for the immunoliposome having the SK, compared with 47.7% in free SK [41].

The reperfusion time in a liposome-encapsulated SK system was significantly lower than the free SK (i.e. 19.3 ± 4.6 min vs. 74.5 ± 16.9 min, respectively), but higher than the PEG-encapsulated SK (i.e. 7.3 ± 1.6 min) [20]. Decrease in the reperfusion time from 78 ± 43 min (free SK) to 32 ± 28 min (liposomal SK) has also been reported. The remaining thrombus weight was 28.6 ± 31.5 mg and 3.4 ± 4.3 mg in free SK and liposome-encaspulated SK groups, respectively [42]. The clot dissolving time of liposomal SK has been reported as 19.3 ± 12.1 min, while it was 74.3 ± 41.4 min for free SK. The authors suggested that the improved dissolving rate was due to prevention of premature inactivation of SK [43]. The percent clot lysis in saline control (22.4 ± 3.3%) was reported to be lower than the liposomal SK (47.4

± 1.4%). Interestingly, empty liposomes (no SK) experienced some degrees of thrombolysis (32.4 ± 2.8%), close to that of free SK (36.3 ± 3.4%) and empty liposomes plus free SK (38.0 ± 2.0%). The authors contributed the effect of empty liposomes to transient redistribution of circulating platelets. In the same paper, in immunized animals, liposomal SK has shown enhanced thrombolytic activity compared to the saline group (i.e., 33.8 ± 1.5% vs. 22.4 ± 3.3%) [44].

Pharmacokinetics

Liposomes, encapsulating SK molecules, have shown preserving ability for SK. To investigate inactivation of SK during the encapsulation process, free SK and liposome-encapsulated SK were incubated for 15 min with platelet-poor plasma. The clot dissolving time of SK was compared with that of not-incubated preparations. Mean clot-dissolving time of the free SK increased from 10.7 min (not-incubated) to 15.5 min (incubated), while in encapsulated SK, the clot-dissolving time increased from 12.4 min (not-incubated) to 13.3 min (incubated). The authors suggested that the liposome managed to limit exposure of SK to the plasma, thus, minimize its deactivation [45]. Liposomes have also shown ability to increase biological life of SK. In a report, biological half-time of free SK was reported as 0.3 h while this value was 3.5 h in a PEGylated liposome encapsulating SK. The authors also reported significant increase in area under the curve (AUC_{∞}) and mean residence time of the liposomal SK, compared with the free one (i.e. 1317 ± 675 vs 152 ± 45 IU h/mL and 5 ± 1 vs 0.4 ± 0.1 h, respectively) [46].

An immunoliposome containing SK, which used monoclonal antibody FnI-3C against fibrin, (particle size ~ 140 nm, entrapment efficiency 14.1%), selectively accumulated at sites containing fibrin. Furthermore, the immunoliposome managed to increase the half-life of SK from 1.8 min to 24.1 min and T_{max} from 15 to 30 min. Also, the elimination constant reduced ~ 13 times [47]. Three vesicular systems (liposome, sphingosome and niosome) containing SK were compared in terms of their biodistribution and thrombolytic activity. After 1 and 4 h, the liposomes (particle size= 150 nm, entrapment efficiency= 11 %) accumulated mainly in blood and spleen, respectively. While niosomes (particle size= 190 nm, entrapment efficiency= 13 %) and sphingosomes (particle size= 200 nm, entrapment efficiency= 10 %) were mostly detected in spleen at both time points. Interestingly, free SK was largely detectable in kidney at both time points. Furthermore, the activity of SK in the vein with thrombosis was compared with that of vein without thrombosis. After 4 h from injection, higher activity in thrombotic vein was obtained for the vesicular preparations, showing the targeting ability of the preparations [48].

CONCLUSION

The different encapsulation/conjugation approaches, reviewed here, indicated improved efficacy, reduced adverse effects and modified pharmacokinetics for SK. In some cases, the efficacy of SK decreased transiently. However, the efficacy increased after some minutes, due to inactivation of the free SK in-vivo. Remarkably, we could not find clinical reports on the use of encapsulated SKs. Evidently, there is still a way ahead to see pharmaceutical products in market for human use

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