Pharmacokinetics of the Novel Recombinant Streptokinase ^{125}I-mut-C42

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ABSTRACT

Streptokinase (SK) is a worldwide used thrombolytic agent known for its immunogenicity. Mut-C42 is a mutant recombinant streptokinase lacking the 42 C-terminal aminoacids and intended to be less immunogenic than native SK. We have compared the pharmacokinetic profiles of this molecule to its recombinant counterpart SK-C2 (Heberkinasa®), and describe them here under a distinct view developed under the consideration of plasmin transfer from an activator complex to plasma inhibitory proteins, particularly α2-macroglobulin with the concomitant release of an SK free fragment and the occurrence of two independent metabolic pathways. Plasma concentration profiles after a 20 000 IU/Kg iv bolus of both thrombolytic agents to female Beagle dogs, showed a shorter plasma half-life for the activator complex as opposed to that for the ^{125}I-mut-C42 free fragment ($t_{½c}$ = 0.43 h vs. $t_{½u}$ = 7.43 h) with a consequent increased clearance for the activator complex (Cl_c= 520 mL/h vs. 53.52 mL/h). The results show the existence of two distinct elimination pathways, for the activator complex and SK-free fragment. When plasma profiles of ^{125}I-SK-C2 and ^{125}I-mut-C42 were compared, a significantly longer elimination half-life with a reduced clearance rate for ^{125}I-mut-C42 was evidenced. The kinetic behaviour reported here might imply a better therapeutic effect for mut-C42 as compared to SK-C2.

Keywords: pharmacokinetics, plasmin, plasminogen, streptokinase

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RESUMEN

Farmacocinética de la nueva estreptoquinasa recombinante ^{125}I-mut-C42. La estreptoquinasa (SK) es un agente trombolítico ampliamente utilizado, bien conocido por su inmunogenicidad. La molécula mut-C42 es un mutante de la estreptoquinasa recombinante que carece de los 42 residuos aminoacídicos del extremo C-terminal, diseñada con el objetivo de disminuir su carácter inmunogénico con relación a la SK nativa. En este trabajo hemos comparado los perfiles farmacocinéticos de esta molécula con los de su contraparte SK-C2 recombinante (Heberkinasa®) y los analizamos bajo una perspectiva que toma en consideración la transferencia de la plasmina desde el complejo activador hacia proteínas plasmáticas inhibitorias, particularmente la α2-macroglobulina, con la consiguiente liberación de un fragmento libre de SK y el procesamiento por dos vías metabólicas independientes. Los perfiles de concentración plasmática luego de la administración de 20 000 UI/Kg de ambos trombolíticos en bolo i.v. a perras Beagle, mostraron tiempos de vida media plasmática menores para el complejo activador en relación con el fragmento libre de ^{125}I-mut-C42 ($t_{½c}$ = 0.43 h vs. $t_{½u}$ = 7.43 h) con un consiguiente aumento del aclaramiento del complejo activador (Cl_c= 520 mL/h vs. 53.52 mL/h). Estos resultados sugieren la existencia de dos vías distintas de eliminación para el complejo activador y el fragmento libre de SK. Cuando los perfiles plasmáticos de la ^{125}I-SK-C2 y la ^{125}I-mut-C42 fueron comparados, resultó evidente un tiempo de vida media de eliminación más prolongado y un aclaramiento menor de la ^{125}I-mut-C42. El comportamiento cinético que aquí reportamos podría implicar un mejor efecto terapéutico de la mut-C42 sobre la SK-C2.

Palabras claves: estreptoquinasa, farmacocinética, plasmina, plasminógeno

*I*ntroduction

Thrombolytic therapy for acute myocardial infarction (AMI) is intended to restore, as fast as possible, the perfusion of the territory irrigated by the occluded artery [1, 2]. Streptokinase (SK) has largely been known as an important indirect plasminogen activator. The mechanism of human plasminogen (HPlg) activation by streptokinase-type activators has been regarded as the formation of an equimolar complex with either free or fibrin-associated HPlg [3, 4]. Major streptokinase-induced adverse events such as stroke, bleeding and allergic reactions, have been previously documented, also morbidity related to delayed reactions has been recognized and attributed to immune complex disease [5].

Anti-streptokinase antibodies are found in most individuals due to common streptococcal infections. Higher levels of antibodies, as seen in previous receivers of streptokinase therapy, cause more adverse reactions and may result in a lower efficacy [6]. Earlier studies have demonstrated that SK C-terminus is significantly immunodominant [7, 8].

SK-C2 (Heberkinasa®) is a recombinant SK coded by the sk-c2 gene in *Escherichia coli* [9]. It has shown similar patterns of immune associated adverse reactions in clinical trials [10]. A SK-C2 deletion mutant (mut-C42), lacking 42 C-terminal residues has been constructed in order to lower the immunogenicity of

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this bacterial born protein, while conserving its thrombolytic potency. Initial experimental works provide evidence that the novel molecule elicits a weaker humoral response and is less neutralized by antibodies as compared to SK-C2 [11].

Since interaction with antibodies may contribute to changes in plasma kinetics as well as to *in vivo* biological activity we have compared the plasma profiles of SK-C2 and mut-C42 after a single intravenous bolus (i.v.) injection to female Beagle dogs. Here we provide evidence showing different pharmacokinetic profiles probably indicating that they are distinctly recognized and processed by catabolic proteins or by antibody-mediated clearance mechanisms.

Materials and Methods

Proteins

Recombinant streptokinases, mut-C42 and SK-C2 were obtained from *E. coli* at the Center for Genetic Engineering and Biotechnology, in Havana, Cuba. Proteins were radiolabeled with 1.2 mCi Na ^{125}I using the solid state lactoperoxidase method [12] with an 88.5% recovery. All materials used in the experiments were freshly labelled. Radioactivity was measured in a LKB Clinigamma Counter (Pharmacia). The radiolabeled proteins were purified by gel filtration on Sephadex G-25. The resulting ^{125}I-SKs migrated as a single band on SDS-PAGE (Figure 1).

Plasma concentration profiles

Female Beagle dogs (four animals per treatment group), weighing 8.9 Kg, were injected an intravenous bolus containing 20 000 IU/Kg of ^{125}I-mut-C42 or ^{125}I-SK-C2 through a cephalic vein. Blood samples were collected in heparinized Eppendorf tubes at 2, 4, 6, 8, 10, 15, 20, 25, 30, 45 min and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8 h. Specimens were incubated at 37 °C for 30 minutes and centrifuged 10 min at 6000 rpm in a Sigma 201m centrifuge. Plasma was kept at –20 °C. Radioactivity was measured in a Clinigamma 1272 Counter and expressed as percent of the injected dose per milliliter of plasma and transformed to IU/mL.

Data analysis

Individual plasma profiles were adjusted to a biexponential function by non-linear regression methods, using WinNonlin Professional 2.1, Pharsight Co. 1998, according to the general equation:

$$C = A\, e^{-a^t} + B\, e^{-b^t}$$

Pharmacokinetic parameters (α, β, $t_{1/2\alpha}$, $t_{1/2\beta}$, AUC, CL, V_{ss}, MRT, Cl/kg y V_{ss}/kg,) were estimated using the method by Gibaldi and Perrier [13]. Statistical differences were tested by the student *t* test for all parameters. The significance level was set at $p < 0.05$.

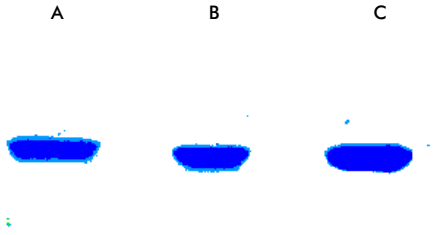

Figure 1. Composite SDS-PAGE showing migration of iodinated recombinant and non-radioactive control streptokinases. Proteins were diluted in the buffer containing 1% SDS and heated for 5 min. The samples were run on a 5% polyacrylamide gel and the individual bands (lanes A, B) were visualized by autoradiograpy; the other band (lane C) was stained with Coomassie blue. Lane A, represents ^{125}I-SK-C2 with a slower electrophoretic mobility; lanes B and C, correspond to ^{125}I-mut-C42.

Results

The mean serum concentration versus time data for mut-C42 is presented in Figure 2. The disposition profile was best described by a two-compartment model. Table 1 provides the mean pharmacokinetic characteristics of the group. The mean distribution half-life was 0.43 h, and the mean elimination half-life was 7.43 h, denoting a rapid distribution followed by a gradual elimination phase. Mean clearance value (48.54 mL/h) reveals a slow elimination of the mutant molecule from the bloodstream. Hence, it was apparent that streptokinase followed a two-compartment kinetic, but according to its known thrombolytic mechanism and its metabolism it would correspond to a different model.

Using WinNonlin professional, we have created a new pharmacokinetic model to analyse the behaviour of the molecule in which SK-plasma protein interac-

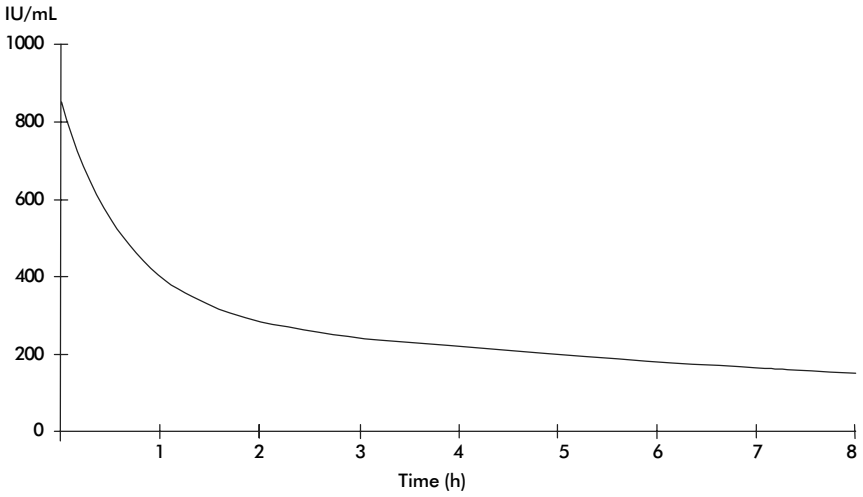

Figure 2. Mean ^{125}I- mut-C42 plasma concentration profile after a 20 000 IU/Kg i.v. bolus administration to Beagle dogs. Two-compartment modelling ($r^2 = 0.998$).

Table 1. Pharmacokinetic characterization of ^{125}I- mut-C42 in Beagle dogs. Individual and estimated mean parameters.

Parameter	Dog #1	Dog #2	Dog #3	Dog #4	Mean	%VC
AUC (IUh/mL)	3521.22	4253.56	2635.76	5345.26	3708.03	30.61
$t_{½\alpha}$ (h)	0.35	0.47	0.27	0.39	0.43	21.44
$t_{½\beta}$ (h)	5.65	8.50	7.05	13.01	7.43	49.59
K_{10} (h^{-1})	0.22	0.20	0.32	0.21	0.24	31.53
K_{21} (h^{-1})	0.79	0.74	1.58	1.16	0.83	17.03
K_{12} (h^{-1})	1.09	0.61	0.80	0.45	0.64	38.23
Vd (ml)	198.20	211.43	200.87	195.11	206.33	3.11
CL (ml/h)	44.30	41.38	64.50	40.78	48.54	30.66
MRT (h)	7.74	11.30	9.30	17.11	9.78	49.92
Vss (ml)	342.81	467.75	599.86	697.95	474.75	20.30

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tions and metabolism are taken into account. Figure 3 shows the proposed model. Based on the classic compartmental analysis, it is a one-compartment open model with two parallel first order elimination processes. It properly describes SK intravenous kinetics. The equations to determine each parameter were developed from the general equation:

$$[C = Ae^{-Kect} - Be^{-Keut}]$$

According to this model, IU/mL vs. time curves show a bi-exponential profile (Figure 4); notice the high correlation of experimentally observed and model predicted data. The initial phase of the plasma decay curve would correspond to the disappearance of the ^{125}I-SK:Pm complex and the final phase to the disposition of the ^{125}I-SK free fragment, slopes of each phase corresponding to the elimination constants of the activator complex and the released fragment, (Kec_c and Keu_u) respectively.

Pharmacokinetic parameters calculated from the model are shown in Table 2.^{125}I-mut-C42:Pm complex plasma half-life is shorter than that of free ^{125}I-mutC-42 ($t_{½c}$=0.43 h vs. $t_{½u}$ = 7.43 h). Accordingly activator complex is cleared faster than the SK fraction (Cl_c = 520 mL/h vs. 53.52 mL/h).

The slopes of the plasma concentration versus time curves for ^{125}I-mut-C42, are lower than those for ^{125}I-SK-C2 (Figures 4 and 5), indicating a delay in the elimination of the ^{125}I-mut-C42:Pm complex as opposed to the ^{125}I-SK-C2:Pm complex, along with a reduced rate of clearance for the free portion of ^{125}I-mut-C42 as compared to ^{125}I-SK-C2 (Table 2).

Statistical comparison of pharmacokinetic parameters for both molecules (Table 2) shows clear differences in clearance between ^{125}I-mut-C42:Pm and ^{125}I-SK-C2:Pm activator complexes (622.36 mL/h vs 2816.28 mL/h), also, the protracted half-life for the SK ($t_{½c}$ = 0.37 h vs. 0.067 h) mutant, is considerable.

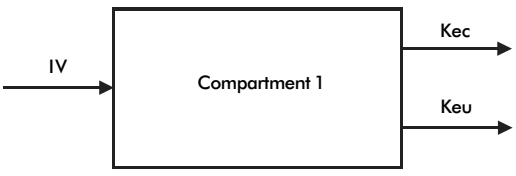

Figure 3. Proposed model representing the new one-compartment pharmacokinetic model with two parallel first order elimination processes where Kec corresponds to the elimination constant of the activated complex, ^{125}I-mut-C42-PM and Keu correspond to the elimination constant of the ^{125}I-mut-C42 fragment.

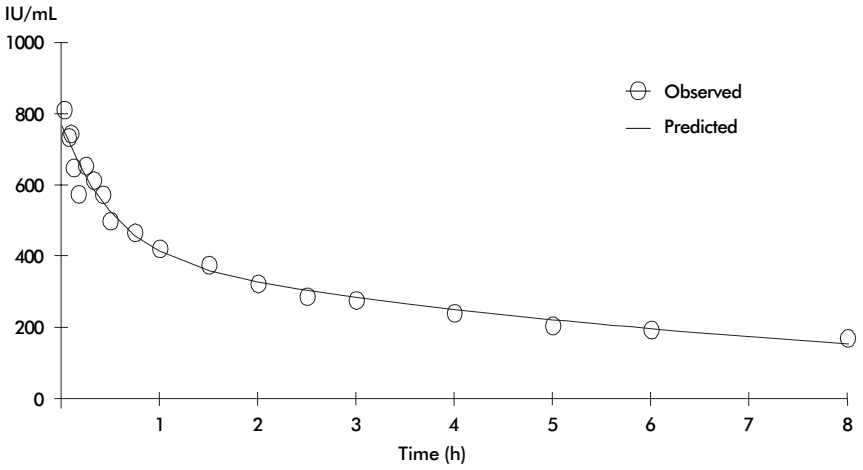

Figure 4. The mean plasma concentration profile (n=4), of recombinant streptokinase ^{125}I-mut-C42 in Beagle dogs after a 20 000 IU/Kg i.v. bolus administration. The one-compartment pharmacokinetic model with two parallel first order elimination processes (r^2 = 0.989).

Table 2. Pharmacokinetic parameters for the recombinant streptokinases ^{125}I-mut-C-42 and ^{125}I-SK-C2, administered as a 20 000 IU/Kg (150 μCi) intravenous bolus.

Parameters	^{125}I- mut-C42	^{125}I-SK-C2	P
AUC (IUh/mL)	3708.95	2374.49	P<0.05.
$t_{½\,c}$ (h)	0.43	0.067	P<0.05
$t_{½\,u}$ (h)	7.43	9.20	N.S.
Vdc (mL)	322.0	443.29	N.S.
Vdu (mL)	573.97	1337.96	N.S.
CLc (mL/h)	520.63	2816.28	P<0.05
CLu (mL/h)	53.52	97.40	P<0.05
CLc/Kg (mL/h)	0.43	257.64	P<0.05
CLu/Kg (mL/h)	7.43	5.89	N.S.

* c-Activator complex SK:Pm, ** u-Free fragment ^{125}I-SK,

*D*iscussion

Considering these data it is apparent that streptokinase follows a two-compartment kinetic pattern, but according to its known thrombolytic mechanism and metabolism its kinetics in dog plasma would correspond to a different model. There are several facts sustaining this notion: 1) Streptokinase does not have intrinsic enzymatic activity, and must therefore form a complex with plasminogen. 2) The association reaction is extremely rapid, rendering a plasminogen activator complex [14, 15]. 3) The clearance of the activator complex involves the transfer of plasmin moiety to α_2-macroglobulin (α_2-M) and the cleavage of streptokinase to a lower molecular weight fragment, leading two independent metabolic pathways for the α_2-M-plasmin complex and free streptokinase [16]. 4. SK:Pm activator complex is eliminated from the bloodstream at a faster rate than individual components of the combination.

All these facts make us consider that although SK shows a bi-exponential pharmacokinetic profile it might not be due to a two-compartment distribution but rather to the existence of two different simultaneous clearance pathways emerging from a central compartment.

Although some people consider that the association of intravenously administered SK with dog plasminogen is limited, others have found that canine plasminogen reacts avidly with SK [17]. After a 20 000 IU/Kg bolus mut-C42 might rapidly bind to plasminogen at a kinetically non-perceptible rate, making the distribution phase indistinguishable [18].

SK complex with human plasminogen is insensitive to α_2-antiplasmin (α_2-AP) [17] but α_2-M is a potent inhibitor of SK:Pm. Although Ogloblina *et al*. found that a decrease in the α_2-AP concentration in human and dog plasma markedly enhanced the lysis of fibrin clots formed from the plasma under the action of urokinase and streptokinase [19], so it seems that α_2-AP plays a role in the ability of SK to activate plasminogen as well as in its metabolism.

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The activator complex is cleared faster than the SK free fraction. This is in good agreement with results reported by Gonias *et al*. [16] who found 50% of the injected ^{125}I-SK to be cleared from the bloodstream in 15 min as compared to the 3 min required by the activator complex in a murine model.

Both the activator complex and free SK undergo hepatic uptake by not well defined but separate pathways. ^{125}I-mutC-42 plasma performance was compared to the plasmatic course of its full 414 amino acid counterpart SK-C2, administered in a similar fashion. A comparable bi-exponential disposition was detected when the one-compartment open model approach was applied (Figure 5).

It is noteworthy that the mut-C42:Pm activator complex presents a delayed elimination.

This could be attributable to the absence of the truncated C-terminal residues not allowing its recognition by plasma inhibitors in charge of its catabolism or to the development of a non-permissive conformation once bound to plasminogen/plasmin. Furthermore, evidence of C-terminal inmuno-dominance [7, 8] encourages us to propose the hypothesis of an antibody-mediated accelerated clearance of SK-C2. In this context ^{125}I-mut-C-42:Pm would not be bound nor neutralized. This second hypothesis seems to be the most likely one according to the proposed model inasmuch as the elimination of the free fragments from both molecules occurs in a non-discernible way.

Alpha, beta and gamma domains of streptokinase are involved in plasminogen recognition and activation as judged by the crystal structure [20, 21]. Considering the notion that the absence of the C-terminal 42 amino acids favours a distinct conformation pattern to be true, this could explain the lack of recognition by degrading plasma proteins as α2-M, leading to a longer persistence of the activator complex in the dog's bloodstream.

The kinetic behavior of ^{125}I-mut-C42:Pm complex would bring about an enhanced therapeutic outcome, since more plasminogen molecules would be activated, signifying a more potent clot destruction, a faster reperfusion rate and perhaps a reduction in dosage with less adverse reactions.

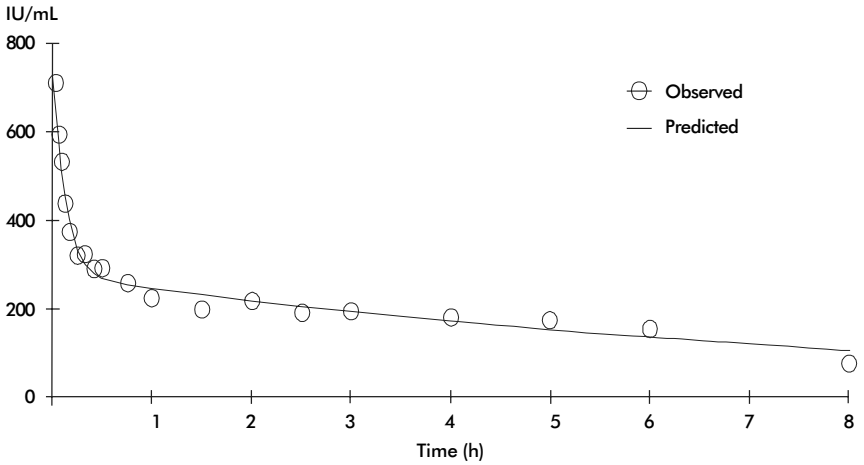

Figure 5. The kinetic course of plasma concentrations (n=4) of the recombinant streptokinase ^{125}I-SK-C2, in Beagle dogs after a 20 000 IU/Kg (150 μCi) intravenous bolus administration. The one-compartment pharmacokinetic model with two parallel first order elimination processes ($r^2 = 0.98$).

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