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EXTRAHEPATIC METABOLISM OF RECOMBINANT TISSUE-TYPE PLASMINOGEN ACTIVATOR (tPA) IN DOGS. K.L.L. Fong, K.E. Boyle, C.S. Crysler, M.S. Landi, H.E. Griffin, and R.K. Lynn, Departments of Drug Metabolism and Laboratory Animal Science, Smith Kline and French Laboratories, Swedeland, PA 19479. USA.

Hepatic uptake has been proposed as the major mechanism of tPA clearance from systemic circulation. However, our recent studies demonstrated that tPA was rapidly inactivated through complexation with protease inhibitors in dog plasma *in vitro*, and that tPA-inhibitor complexes were present in plasma of dogs receiving tPA. Therefore, the present work was undertaken to differentiate hepatic from extrahepatic clearance of tPA. Pharmacokinetics of tPA were determined in anesthetized beagle dogs with either intact hepatic circulation or with interrupted hepatic blood flow achieved by hepatic artery ligation and portal caval shunt. Recombinant two-chain tPA was administered as an intravenous bolus dose (80 µg/kg) and plasma active tPA concentrations were measured using a modified and validated S-2251 chromogenic assay. Following tPA administration to intact dogs, plasma active tPA concentration declined biexponentially with time with 84% of the active tPA eliminated during the α-phase. The t1/2's of the α and β-phase were 1.76 ± 0.74 and 6.23 ± 1.56 min, respectively. The systemic clearance was 25.98 ± 1.13 ml/min/kg and the volume of distribution at steady state (VDss) was 73.9 ± 15.1 ml/kg. Upon the elimination of hepatic blood flow, the systemic clearance was reduced by 54% while VDss was unaffected. The contribution of plasma inactivation of tPA to the systemic clearance was estimated from *in vitro* inactivation studies in 37°C plasma. Based on the pseudo first order inactivation rate constants of 0.184 min^{-1} and 0.095 min^{-1} at initial tPA concentrations of 25 and 250 IU/ml respectively, clearance rates from 5.02 to 9.2 ml/min/kg were calculated. These data suggest that (1) in intact dogs, 46% of the tPA clearance occurs extrahepatically and (2) inactivation of tPA in plasma accounts for a major portion of the extrahepatic clearance.

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DOSE-DEPENDENT PHARMACOKINETICS OF RECOMBINANT TISSUE-TYPE PLASMINOGEN ACTIVATOR (tPA) IN ANESTHETIZED DOGS FOLLOWING INTRAVENOUS INFUSION. K.L.L. Fong, C.S. Crysler, B.A. Mico, K.E. Boyle, G.A. Kopia and R.K. Lynn, Departments of Drug Metabolism and Pharmacology, Smith Kline and French Laboratories, Swedeland, PA 19479 U.S.A.

The pharmacokinetics of SK&F recombinant two-chain tissue-type plasminogen activator (tPA) following intravenous (i.v.) infusion were characterized in anesthetized, open chested mongrel dogs in which artificial intracoronary thrombi were formed. SK&F tPA was infused at rates of 0.5, 1, 2, 4, and 8 µg/kg/min (n=3 to 5 per dose) for 90 min and arterial blood samples were withdrawn during and after infusion for determination of functionally active tPA concentrations using a modified and validated S-2251 chromogenic assay. At all doses studied, steady state active tPA plasma concentrations were achieved 10-20 min after the onset of infusion. Upon cessation of infusion, active tPA plasma concentrations declined rapidly with a t1/2 of 2-3 min. The active tPA plasma concentration at steady state (Css) and the area under the tPA plasma concentration-time curve (AUC) increased linearly with dose in the range of 0.5-4 µg/kg/min. However, as the dose was increased 2-fold from 4 to 8 µg/kg/min, the AUC and the Css increased 2.5 fold. The systemic clearance ranged from 15-16 ml/min/kg at doses of 0.5-4 µg/kg/min but decreased to 11.7 ml/min/kg at the 8 µg/kg/min dose. With exceptions in 3 dogs, the volume of distribution at steady state approached or slightly exceeded the blood volume. Plasma tPA antigen concentrations were also determined in the dogs receiving the 2 µg/kg/min dose. At steady state, active tPA accounted for 40-60% of the total tPA antigen. The post-infusion t1/2 of the tPA antigen was considerably longer (13.46 ± 5.94 min) than that of active tPA. These results suggested that non-plasminogen activating metabolites (e.g., tPA-inhibitor complex(es)) are present in the plasma of dogs receiving tPA. It is also concluded from the present study that (1) distribution of the i.v. administered tPA molecule was limited primarily to the intravascular space, and (2) tPA may display dose-dependent pharmacokinetics within the optimum thrombolytic dose range.

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CELLULAR CATABOLISM OF RECOMBINANT TISSUE-TYPE PLASMINOGEN ACTIVATOR: IDENTIFICATION AND CHARACTERIZATION OF A NOVEL HIGH AFFINITY UPTAKE SYSTEM ON RAT HEPATOCYTES. C. Bakhit, D. Lewis, R. Billings, and B. Malfroy. Genentech, Inc., South San Francisco, CA, U.S.A.

The uptake, internalization and intracellular degradation of ^{125}I-labeled rt-PA (^{125}I-rt-PA) by isolated rat hepatocytes was investigated. Incubation at 37°C resulted in internalization of ^{125}I-rt-PA, followed by the appearance of labeled trichloroacetic acid-soluble (TCA) material in the inclubation media due to degradation of rt-PA. Degradation of rt-PA was inhibited by the presence of NH_4Cl (10mM) or chloroquine (1mM) (lysosoma tropic agents) in the incubation media. This suggests that rt-PA degradation occurs intracellularly, perhaps within the lysosomes. ^{125}I-rt-PA was taken up by rat hepatocytes through a specific, high affinity mechanism. Scatchard analysis of the data indicated that 10^6 molecules of rt-PA were taken up per cell/hour and the calculated dissociation constant was 10nM. Uptake of ^{125}I-rt-PA was not inhibited by glycopeptides isolated from rt-PA nor by several other glycoproteins known to be cleared by identified hepatic receptors. These results suggest that the uptake of rt-PA by rat hepatocytes involves a receptor specific for t-PA and is not mediated by a carbohydrate specific receptor.

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THE INTERACTION OF ONE AND TWO CHAIN TISSUE-TYPE PLASMINOGEN ACTIVATOR WITH INTACT AND DEGRADED FIBRINOGEN. Deborah L. Higgins. Dept. of Cardiovascular Research, Genentech, Inc., South San Francisco, CA 94080, USA.

Tissue-type plasminogen activator (t-PA) plays a central role in fibrinolysis in vivo. One-chain recombinant t-PA was isolated using lysine-Sepharose chromatography followed by chromatography on monoclonal antibody column which is specific for one chain t-PA. Two chain t-PA was produced by incubation of one chain t-PA with plasmin-Sepharose. Both forms of t-PA were incubated with plasminogen-free fibrinogen as well as fibrinogen that had been degraded with plasmin until it was ~50% clottable. Thrombin was added to catalyze fibrin formation and the amount of free t-PA in the clot supernatants was measured by ELISA. A quantitative analysis to determine the dissociation constant (K_d) and moles of t-PA bound per mole of fibrin monomer (n) was performed using a computer fit of a nonlinear regression analysis. The data obtained gave the following results:

	One-chain t-PA		Two-chain t-PA	
	K_d(M)	n	K_d(M)	n
Intact Fibrinogen	3.8×10^{-7}	1.06	5.2×10^{-7}	0.91
Degraded Fibrinogen				
2 site model	3.2×10^{-9}	0.06	3.6×10^{-9}	0.03
	5.5×10^{-6}	0.86	1.2×10^{-6}	1.20
3 site model	5.8×10^{-10}	0.02	2.0×10^{-9}	0.02
	2.2×10^{-8}	0.16	1.1×10^{-7}	0.11
	1.2×10^{-6}	1.08	1.2×10^{-6}	1.04
+0.1M ε-ACA acid	5.4×10^{-10}	0.01	not done	
	2.9×10^{-8}	0.06	not done	

Plasmin degradation of the fibrinogen caused the formation of a new set(s) of sites with a significantly lower K_d(s), which caused an increase in binding to degraded fibrin when compared to intact fibrin. ε-amino caprioc acid (ε-ACA) (0.1M) was found to compete for t-PA binding to the loose sites in both intact and degraded fibrin, but not the tight sites formed by degradation. Significantly (>20%) more one chain t-PA than two chain t-PA bound to both intact or degraded fibrin. Differential binding of one and two chain t-PA as well as tighter binding to degraded fibrin may play a significant role in the regulation of fibrinolysis.