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Animal Pharmacokinetics of Inogatran, a Low-Molecular-Weight Thrombin Inhibitor with Potential Use as an Antithrombotic Drug

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ABSTRACT: Pharmacokinetics, excretion, and metabolism of inogatran, a low-molecular-weight thrombin inhibitor, were studied in the rat, dog, and cynomolgus monkey. After intravenous administration the half-life was short in all three animal species, due to a small volume of distribution and a relatively high clearance. At doses of 0.1–5 μmol kg^{-1}, the mean residence time was about 10 min in the rat, 35 min in the dog, and 20 min in the cynomolgus monkey. The oral bioavailability of inogatran was incomplete, presumably due to a low membrane permeability, and dose dependent. The bioavailability was 4.8% at 20 μmol kg^{-1} and 32–51% at 500 μmol kg^{-1} in rats, 14% at 10 μmol kg^{-1} and 34–44% at 150 μmol kg^{-1} in dogs, and 2.1% at 1 μmol kg^{-1} in cynomolgus monkeys. The radioactivity excreted in urine and faeces was predominantly unchanged inogatran. After intravenous administration the percentage of the radioactivity recovered in faeces was about equal to or higher than the urinary recovery, which indicates biliary excretion of inogatran. After oral dosing, most of the dose was excreted in faeces, as expected from the estimates of oral bioavailability. The plasma protein binding of inogatran in rat, dog, and human plasma, was 20–28%. The blood–plasma concentration ratio was 0.39–0.56, indicating limited distribution into red blood cells. © 1998 John Wiley & Sons, Ltd.

Key words: inogatran; animal pharmacokinetics; thrombin inhibitor

Introduction

Inogatran (Figure 1) is a synthetic low-molecular-weight thrombin inhibitor, under evaluation for prophylaxis and treatment of arterial and venous thrombosis, that binds to the active site of thrombin with a K_i of 0.015 μmol L^{-1} [1]. The binding is rapid, reversible, and competitive. High selectivity of inogatran for thrombin compared to other serine proteases has been shown [1]. Thrombin has a central role in haemostasis and thrombosis and is a primary target for antithrombotic drugs [2]. The activation of platelets, which leads to platelet aggregation, is partially mediated by thrombin and the cleavage of fibrinogen to fibrin is catalysed by thrombin. Thrombin also activates factor XIII, which stabilises the fibrin, and thrombin interacts with several enzymes in the coagulation cascade, which results in both positive and negative feed-back depending on the level of thrombin. Inhibition of thrombin thus causes both inhibition of platelet aggregation and plasma coagulation. The antithrombotic effect of inogatran has been demonstrated *in vivo* at plasma concentrations of about 0.5 μmol L^{-1} in models of arterial and venous thrombosis in rats [3].

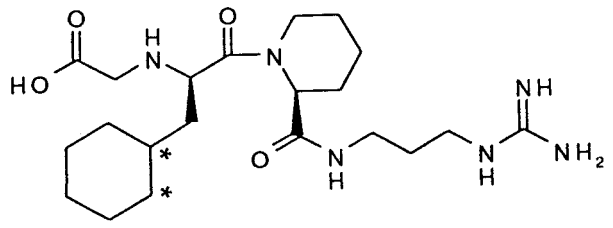

Figure 1. The chemical structure of inogatran (MW, 438.6 Da). The asterisks show the positions radiolabelled with tritium

Anticoagulants currently used in antithrombotic therapy are unfractionated heparin [4], low-molecular-weight heparins [5], and coumarins [6], which all have important limitations. Heparin can only be used parenterally and relies on the interaction with antithrombin III for its thrombin inhibition. Clot-

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bound thrombin is poorly inhibited by heparin. The coumarins, warfarin and dicumarol, can be given orally but the pharmacokinetics are highly variable due to drug and food interactions. In addition, the coumarins inhibit the formation of thrombin, which gives a delay in the anticoagulant effect of several days. Individualised therapy with frequent controls of the anticoagulant effect is necessary because of a narrow therapeutic index with risk of bleeding complications. Consequently, there is a medical need for improved treatments of thrombo-embolic diseases. Directly acting thrombin inhibitors are regarded as a new class of antithrombotic agents that has been shown to be a promising alternative [7]. Hirudin, a polypeptide derived from medicinal leeches, was the first compound in this class to be developed for parenteral use [8]. Synthetic low-molecular-weight compounds, such as inogatran, with the potential of being orally available are under evaluation [9,10].

In the present studies the pharmacokinetics and metabolism of inogatran were investigated in the rat, the dog, and the cynomolgus monkey. The coagulation time in plasma after addition of thrombin, which is prolonged by the thrombin inhibitor, was used to determine plasma concentrations of inogatran in pharmacokinetic experiments in the rat and dog. Tritium-labelled inogatran was synthesised to investigate excretion, metabolism, and pharmacokinetics of inogatran in rats, dogs, and cynomolgus monkeys. The blood–plasma concentration ratio and the plasma protein binding of inogatran were determined *in vitro* in rat, dog, and human plasma.

Materials and Methods

Chemicals

Inogatran (glycine, N - (2 - (2 - (((3 - ((aminoiminom - ethyl)amino)propyl)amino)carbonyl)-1-piperidinyl)-1-(cyclohexylmethyl-2-oxyethyl)-, (2R-(2S)))) and tritium-labelled inogatran, with two tritium atoms in the cyclohexyl ring, were synthesised by Astra Hässle AB, Mölndal, Sweden. The chemical structure of inogatran is shown in Figure 1. Aqueous solutions of inogatran, prepared as a dihydrochloride salt, a dihydrobromide salt, or the free base, in isotonic saline, were used for dosing in the animal experiments.

Animals and Experimental Design

The pharmacokinetics of inogatran was evaluated in rats and dogs after administration via different routes. Measurement of thrombin time in plasma was used to determine the plasma concentration of inogatran in samples collected after dosing.

Table 1. Pharmacokinetic parameters (mean (S.D.)) of inogatran after intravenous administration of unlabelled compound

	Rat ($n = 5$)	Rat ($n = 6$)	Dog ($n = 1$)
Dose (μmol kg^{-1})	2	5	2
Cl (mL min^{-1} kg^{-1})	83 (13)	90 (23)	9.0
V_{ss} (L kg^{-1})	0.77 (0.09)	1.06 (0.34)	0.31
$t_{1/2}$ (min)	10 (3)	29 (13)	40
MRT (min)	10 (2)	12 (4)	35

Rat. One day prior to dosing, male Sprague Dawley rats weighing 250–400 g were anaesthetized with a mixture of 2 mL kg^{-1} Ketalar and 0.25 mL kg^{-1} Rompun and a cannula was placed in the carotid artery for collection of blood samples. For dosing intravenously, into the duodenum, or into the colon a cannula was also inserted in the jugular vein, the duodenum, or the colon, respectively. The catheters were exteriorized at the nape of the neck and sealed. After surgery, the rats had free access to water but were starved until the end of the experiment. Single doses of inogatran were given via six different routes to groups of three to eight rats. Each rat was only given one dose. Intravenous (IV), subcutaneous (SC), or intraperitoneal (IP) bolus injections (1–2 mL kg^{-1}) were given. Oral (PO) doses were given by gavage and doses into the duodenum (ID) or the colon (IC) were given via a surgically inserted catheter (5–10 mL kg^{-1}). The doses of inogatran given are shown in Tables 1 and 2. Blood samples (300 μL) were collected, before and at selected times after dosing, in tubes containing 30 μL of an aqueous solution of 0.13 mol L^{-1} trisodium citrate

Dog. A female beagle dog weighing 11 kg was given an oral dose and then an IV dose of inogatran on two different occasions separated by a wash-out period of 1 week. Freshly prepared solutions of inogatran in isotonic saline were given PO (1.5 mL kg^{-1}) by gavage and IV (0.3 mL kg^{-1}) in a peripheral leg vein. The doses of inogatran are shown in Tables 1 and 2. The dog was starved overnight before dosing and until the last blood sample was collected but had free access to drinking water. Blood samples (2 mL) were collected, before and at selected times after dosing, in tubes containing trisodium citrate.

Tritium-labelled inogatran was given to rats, dogs, and cynomolgus monkeys to investigate the mass balance, metabolic fate, and pharmacokinetics of inogatran. The solutions of inogatran prepared for dosing had a chemical purity of 98% and a radiochemical purity of 96% or higher.

Rat. Groups of four female and four male Sprague Dawley rats were used in the experiments. The rats were 3 months old, females weighing 215–250 g and males weighing 340–375 g. Three days

Table 2. Pharmacokinetic parameters (mean (S.D.)) of inogatran after extravascular administration of unlabelled compound

	Rat ($n = 3$)	Rat ($n = 3$)	Rat ($n = 4$)	Rat ($n = 4$)	Rat ($n = 8$)	Rat ($n = 3$)	Dog ($n = 1$)
Route	SC	SC	IP	PO	ID	IC	PO
Dose (μmol kg^{-1})	2	5	13	20	20	20	10
AUC (μmol min L^{-1})	30.5 (2.1)	57.6 (5.1)	124 (25)	11.6 (4.6)	8.4 (2.9)	4.1 (3.2)	150
C_{max} (μmol L^{-1})	1.44 (0.20)	2.43 (0.27)	5.69 (1.17)	0.087 (0.040)	0.099 (0.054)	0.045 (0.027)	1.17
t_{max} (min)	5 (0)	5 (0)	5 (0)	34 (14)	31 (12)	12 (6)	90
$t_{1/2}$ (min)	15 (3)	18 (1)	nc[a]	nc	nc	nc	66
MRT (min)	20 (4)	26 (2)	27 (4)	93 (13)	91 (15)	76 (46)	124
F (%)	127	96	79	4.8	3.5	1.7	14

[a] Not calculated.

prior to dosing, arterial and venous catheters, if needed, were surgically inserted under anaesthesia as described above. The rats were always starved overnight before and until 6 h after dosing (the total starving period was about 20 h) but had free access to drinking water. The IV and PO doses of inogatran, given as solutions in isotonic saline in a volume of 1 and 5 mL kg^{-1}, respectively, are listed in Tables 3 and 4.

The mass balance and metabolic patterns of urine and faeces were investigated in two groups of rats. Single IV bolus doses of tritium-labelled inogatran (5 MBq kg^{-1}) were given to rats in one group and single PO doses (10 MBq kg^{-1}) were given by gavage to the other group of rats. The rats were placed in metabolic cages for separate collection of urine and faeces in 24 h intervals for 7 days after dosing.

The pharmacokinetics of inogatran was investigated in two groups of rats. Single doses of tritium-labelled inogatran were given IV (73 MBq kg^{-1}) as a bolus injection, or PO (159 MBq kg^{-1}) by gavage. Blood samples were collected in heparinized tubes via the arterial catheter prior to dosing and at selected times up to 48 h after dosing.

Dog. Four male and four female beagle dogs, 3–6 years old and weighing 11–17 kg, were used in the experiments. The dogs were starved overnight prior to dosing and until 6 h after dosing but had free access to drinking water and were fed twice daily for the remaining time. Each dog received first an IV dose and then, on a later occasion, an PO dose of inogatran (Tables 3 and 4). Solutions of inogatran in isotonic saline were infused into a leg vein over a period of approximately 1 min, in a volume of 0.5 mL kg^{-1}, and given orally by gavage in a volume of 1 mL kg^{-1}. The amounts of radioactivity were 2 and 6 MBq kg^{-1} for the IV and PO doses, respectively. After dosing the dogs were individually placed in metabolic cages permitting separate collection of urine and faeces. Excreta were collected at intervals for 5 and 7 days after oral and intravenous dosing, respectively. Blood samples were collected from a peripheral leg vein, in heparinized tubes, and centrifuged and the plasma separated.

Cynomolgus monkey. Two male and two female cynomolgus monkeys, weighing 2.6–3.5 kg, were each given first an PO dose and, after a wash-out period of 1 month, an IV dose of inogatran. The doses of inogatran are listed in Tables 3 and 4. The oral dose was given by gavage in a volume of 1 mL kg^{-1}. The IV dose, infused into a saphenous vein over a period of approximately 30 s, was given in a volume of 0.5 mL kg^{-1}. The amounts of radioactivity were 11 and 14 MBq kg^{-1} for the PO and IV doses, respectively. The monkeys were starved overnight prior to dosing and for the first 4 h post-dose. Water was available *ad libitum*. Each animal was housed singly in metabolic cages and excreta were collected for 5 days. Venous blood samples were collected in heparinized tubes, centrifuged and the plasma separated.

Plasma protein binding and blood–plasma concentration ratio of inogatran were determined *in vitro* in freshly collected blood and plasma from Sprague Dawley rats, beagle dogs, and humans. Tritium-labelled inogatran was added at concentrations of 0.1, 10, and 100 μmol L^{-1} in rat and dog blood–plasma and at concentrations of 0.1 and 10 μmol L^{-1} in human blood–plasma. The radiochemical purity of the tritium-labelled inogatran was 96% or higher. Duplicate determinations were made for three or four blood and plasma samples. Plasma was separated from blood by centrifugation at $2000 \times g$ for 10 min at room temperature. Plasma protein binding was determined as the ratio of inogatran concentration in ultrafiltrate and plasma. Ultrafiltrate of plasma was prepared using Amicon micro-partition system devices with a YMT ultrafiltration membrane. After addition of plasma (2 mL) the device was centrifuged at $2000 \times g$ for 10 min at 37°C. No adsorption of inogatran to the membrane or the ultrafiltration device was observed.

Sample Analyses

In experiments where unlabelled inogatran was given the plasma samples were analysed by measurements of thrombin time. After administration of tritium-labelled inogatran, the analyses of urine, faeces, and plasma samples were performed using

Table 3. Pharmacokinetic parameters (mean (S.D.)) of inogatran after intravenous administration of tritium-labelled compound

	Rat ($n = 4$)	Rat ($n = 4$)	Dog ($n = 4$)	Dog ($n = 4$)	Cyno ($n = 4$)
Sex	Male	Female	Male	Female	Both
Dose (μmol kg^{-1})	50	50	5	5	0.1
Cl (mL min^{-1} kg^{-1})	28 (8)	26 (4)	11 (2)	10 (2)	26 (6)
V_{ss} (L kg^{-1})	0.73 (0.13)	0.68 (0.14)	0.35 (0.01)	0.34 (0.05)	0.53 (0.10)
$t_{1/2}$ (h)	1.6 (0.8)	2.2 (0.4)	1.0 (0.1)	1.2 (0.1)	2.4 (0.9)
MRT (h)	0.47 (0.12)	0.44 (0.08)	0.56 (0.09)	0.59 (0.03)	0.35 (0.08)
Cl (renal)[a] (mL min^{-1} kg^{-1})	15.7	12.2	3.4	2.9	7.0
GFR[b] (mL min^{-1} kg^{-1})	5.2	5.2	6.1	6.1	2.1

[a] Renal clearance of inogatran was calculated as the fraction of the radioactive dose excreted in the first 24 h multiplied by the mean value of clearance.
[b] Values of glomerular filtration rate in animals taken from [11].

radioactivity for detection. All biological samples were stored at −20°C until analysis except the plasma samples for thrombin time measurements. Inogatran was shown to be stable at the conditions used.

Thrombin Time Measurements. Blood samples were mixed with a 0.13 mol L^{-1} trisodium citrate solution and centrifuged and plasma was separated and kept on ice until analysis which was performed on the same day. Equal volumes (100 μL) of plasma, saline, and human thrombin solution were mixed and the clotting time was measured at 37°C with a coagulameter (model KC10A, Amelung). This instrument measures the coagulation time mechanically by detection of the change in viscosity in the sample. The concentration of thrombin (Sigma) in the solutions used, prepared in Tris buffer (0.05 M, pH 7.4) with 0.1% bovine serum albumin, was about 9 and 5 NIH units mL^{-1} for rat and dog plasma, respectively. The thrombin time in rat and dog plasma samples collected before dosing was adjusted to about 20 s by the concentration of thrombin solution added. Solutions of inogatran in rat and dog plasma were prepared and analysed as the plasma samples to prepare standard curves. The concentrations were estimated by linear interpolation between points on the standard curve. Plasma samples with high concentrations of inogatran ($> 4–5$ μmol L^{-1}) were diluted with blank plasma.

Determination of Total Radioactivity. The total radioactivity in urine, faeces, blood, plasma and ultrafiltrate was determined by liquid scintillation counting (Beckman 3800). Urine, plasma, and ultrafiltrate samples (0.1–1 mL) were mixed with liquid scintillation fluid (ReadySafe, Beckman) and analysed. Blood samples (0.1 mL) were incubated at 40°C with a mixture of BTS-450–isopropanol (1:2) for 2 h and then with 30% hydrogen peroxide at 80°C for 1 h before addition of acetic acid and ReadySafe. Faeces and cage debris were homogenized in water and an aliquot of the homogenate was diluted in water and a 1 mL volume of this solution was added to ReadySafe. For the experiments in monkeys, faeces and cage debris were homogenized in a 1% aqueous carboxymethylcellulose solution and an aliquot of the homogenate was combusted using a Packard Tri-Carb 306 sample oxidizer. The tritiated water formed as a result of the combustion was collected by absorption in Monophase scintillation fluid. The fraction of volatile components in urine collected from monkeys (0–24 and 96–120 h) was determined by comparison of the radioactivity in urine before and after drying.

Determination of Metabolic Patterns in Urine and Faeces. The metabolic patterns of key samples of urine and faeces, containing more than 5% of the given dose, were determined by reversed-phase liquid chromatography with on-line radioactivity detection. A volume (150 μL) of untreated urine or supernatant of faeces homogenate was injected by an ASPEC or a Spectra Physics 8880 autoinjector onto a Brownlee Newguard precolumn (cyano of RP-2). The separation column was a Vydac C-18 (250 mm × 4.6 mm). The mobile phases A and B consisted of 10 and 60% acetonitrile in 0.1 mol L^{-1} phosphate buffer (pH 2.2). After injection of the sample, the mobile phase B was increased linearly from 0 to 75% over a period of 17 min. Before injection of the next sample the system was equilibrated with phase A for at least 5 min. The radioactivity in the eluate was recorded by an on-line radioactivity detector (Berthold LB 506) using homogenous counting with RiaLuma. The retention time of inogatran was about 9 min.

Determination of the Plasma Concentration of Unchanged Inogatran

The plasma concentration of unchanged inogatran was determined by solid-phase extraction, using an advanced automated sample processor (AASP, Varian) for on-line injection to the LC system identical to that used for determination of metabolic patterns. A volume of the sample (300–1000 μL),

Table 4. Pharmacokinetic parameters (mean (S.D.)) of inogatran after oral administration of tritium-labelled compound

	Rat ($n = 4$)	Rat ($n = 4$)	Dog ($n = 4$)	Dog ($n = 4$)	Cyno ($n = 4$)
Sex	Male	Female	Male	Female	Both
Dose (μmol kg^{-1})	500	500	150	150	1
AUC (μmol h L^{-1})	97.1 (25)	163 (59)	105 (20)	86.5 (27)	0.014 (0.01)
C_{max} (μmol L^{-1})	31.4 (12.6)	65.2 (21.8)	53.3 (7.6)	31.7 (18.7)	0.0076 (0.0056)
t_{max} (h)	0.7 (0.9)	1.0 (0.7)	0.6 (0.1)	1.2 (0.7)	0.9 (0.3)
$t_{1/2}$ (h)	2.1 (0.7)	1.9 (0.4)	1.6 (0.7)	2.5 (0.3)	nc[a]
F (%)	32	51	44 (5)	34 (13)	2.1 (2.0)

[a] Not calculated.

200 μL phosphate buffer (0.1 mol L^{-1}, pH 8) and 50 μL of an aqueous inogatran solution (about 0.3 mmol L^{-1}) was added to an AASP C-8 cartridge. The mixture was left for 5 min before it was passed through the cartridge. The cartridge was then placed in a AASP autosampler and the sample was injected. The eluate from the LC column, collected in 1 mL fractions, was mixed with ReadySafe and analysed by liquid scintillation counting.

Pharmacokinetic Analysis

Pharmacokinetic parameters of inogatran were estimated using non-compartmental methods. The area under the plasma concentration versus time curve (AUC) was calculated using the log–linear trapezoidal rule for IV, SC, and IP experiments and the linear trapezoidal rule for extravascular experiments. AUC was extrapolated to infinity by adding C_{last}/k, where C_{last} is the predicted concentration at the last sampling time (t_{last}) with a measurable plasma concentration and k is the elimination rate constant. C_{last} and k were estimated from linear least-squares regression of log plasma concentration versus time in the terminal phase of the decline. The area under the first moment curve (AUMC) was calculated using the linear trapezoidal rule, and extrapolated to infinity by adding $C_{last}t_{last}/k + C_{last}/k^2$. The half-life was calculated as $0.693/k$. The half-life was not estimated for some of the experiments where inogatran was given via extravascular routes because the plasma levels were low and variable. For these experiments the AUC and AUMC were not extrapolated past the last measurable plasma concentration. Mean residence time (MRT) was calculated as AUMC/AUC. For the intravenous experiments clearance (Cl) was calculated as dose/AUC, and volume of distribution at steady state (V_{SS}) was calculated as MRT Cl. The absolute bioavailability (F) for the extravascular experiments was calculated as $(AUC_{EV}/AUC_{IV})(dose_{IV}/dose_{EV})100$. For the experiments in rats the mean values of AUC were used to estimate bioavailability, while in the dogs and monkeys receiving both an intravenous and an oral dose F was calculated using the AUC values for each individual animal.

Results

Pharmacokinetics of Inogatran

Thrombin time is a sensitive and simple coagulation assay where thrombin inhibition results in a prolongation of the coagulation time. An approximately linear prolongation of the thrombin time with increasing plasma concentrations of inogatran was observed in both rat and dog plasma (Figure 2). A twofold prolongation of the thrombin time was observed at a plasma concentration of about 0.1 μmol L^{-1} (final concentration 0.033 μmol L^{-1} in the diluted sample). The lowest prolongation of the thrombin time that could be measured with adequate accuracy was 5 s, observed at inogatran concentrations of 0.02 and 0.04 μmol L^{-1} in rat and dog plasma, respectively.

The pharmacokinetics of inogatran was evaluated after IV and extravascular administration to rats and a dog using the thrombin time measurements to estimate the plasma concentration of inogatran. It was then assumed that the prolongation of thrombin time was caused entirely by inogatran and not due to any metabolites with thrombin inhibitory activity. Representative curves of the plasma concentration of inogatran versus time after dosing to rats and a dog are shown in Figure 3. Pharmacokinetic parameters of inogatran are summarised in Tables 1 and 2.

After IV and PO administration of tritium-labelled inogatran, the plasma concentrations of total

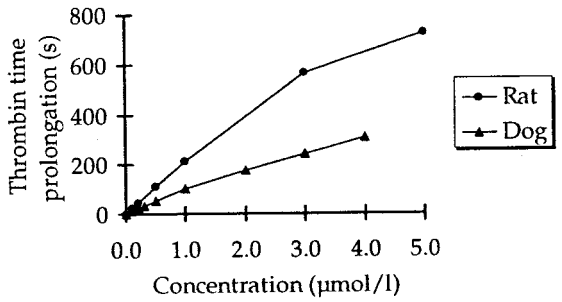

Figure 2. Thrombin time prolongation versus concentration of inogatran in rat and dog plasma

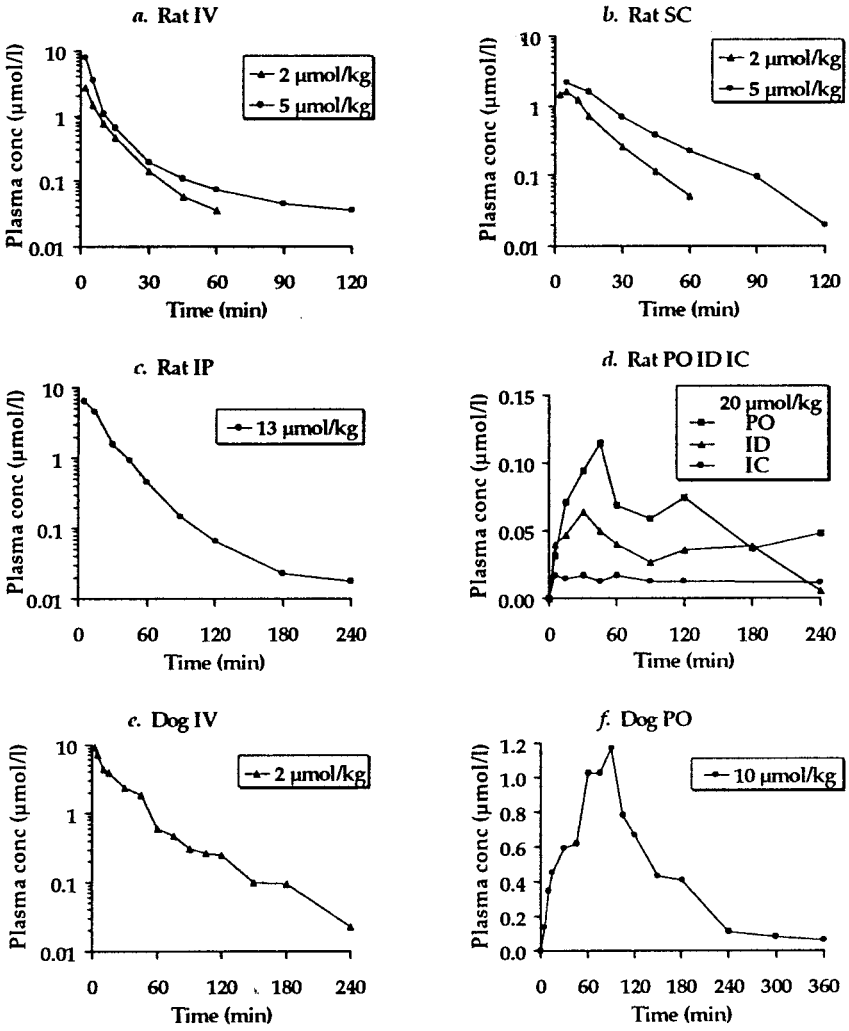

Figure 3. Plasma concentrations of inogatran after intravenous and extravascular administration to rats and dogs. Representative results for one animal are shown. The doses and routes of administration are shown in each graph

radioactivity and unchanged compound were similar in rats and dogs. For cynomolgus monkeys the plasma concentration of total radioactivity was higher than unchanged inogatran after oral dosing and at later sampling times after intravenous dosing. Representative curves of the plasma concentrations of total radioactivity and unchanged inogatran versus time after dosing are shown in Figure 4. Pharmacokinetic parameters of inogatran, calculated using the plasma levels of unchanged inogatran, are summarized in Tables 3 and 4.

Mass Balance and Metabolic Fate of Radiolabelled Inogatran

The cumulative amounts of total radioactivity excreted in urine and faeces after IV and PO administration of tritium labelled inogatran, expressed as a percentage of the given dose, are shown in Figure 5. A large percentage of the radioactivity was excreted in faeces after IV administration, indicating biliary excretion. Following PO dosing, most of the radioactivity was excreted in faeces, which is consistent with an incomplete absorption. The fraction of radioactivity absorbed, calculated as the ratio of the percentage of the dose excreted in urine after PO and IV dosing, was 30–35% for rats, 35–40% for dogs, and 1–3% for cynomolgus monkeys. Irrespective of the route of administration, most of the radioactivity excreted in urine was collected during the first 24 h after dose and the excretion in faeces was essentially completed at 48 h.

For all three animal species, most of the radioactivity in urine and faeces eluted from the LC system with the same retention time as inogatran and was therefore assumed to be unchanged inogatran. The fraction of the radioactivity excreted as unchanged inogatran was estimated to be 80–90% of the dose. Only minor peaks with other retention times than that of unchanged inogatran were observed in the chromatograms of urine and faeces. Most of these minor peaks of radiolabelled material eluted earlier than inogatran and may be metabolites that are more polar than inogatran.

The amount of volatile radioactive components in urine collected from cynomolgus monkey accounted

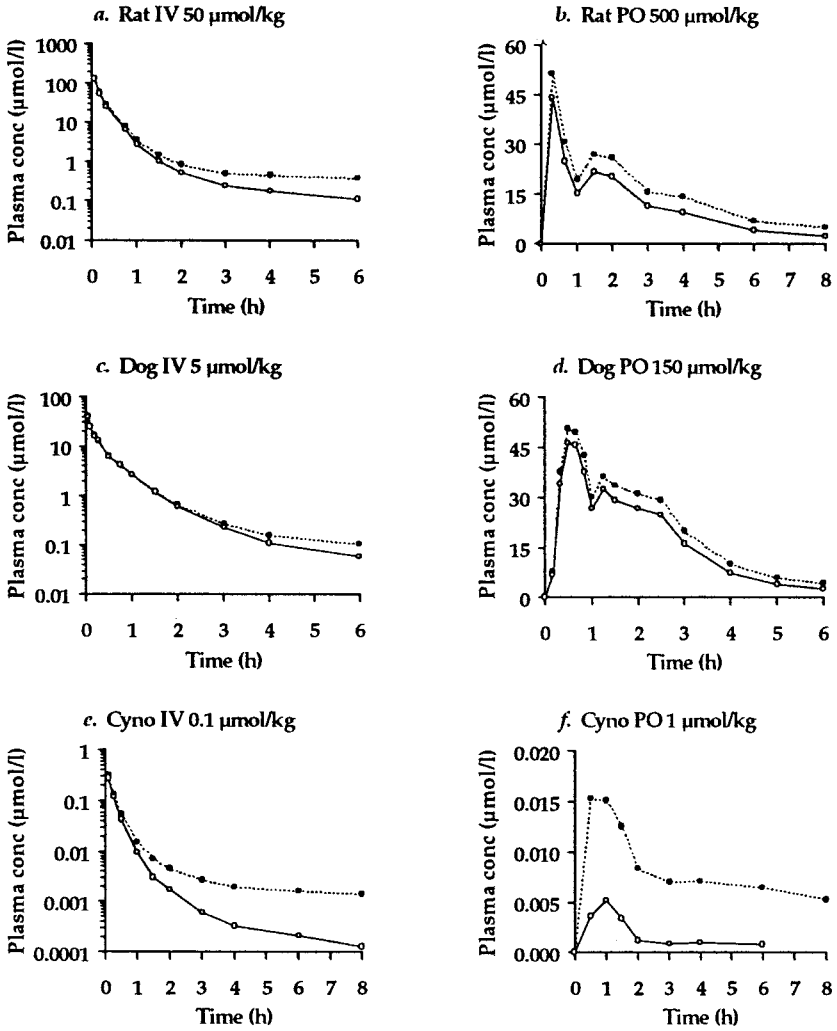

Figure 4. Plasma concentrations of total radioactivity (filled circles) and unchanged inogatran (open circles) after IV and PO dosing to rat, dog, and cynomolgus monkey. Representative results for one animal are shown. The doses and routes of administration are shown in each graph

for less than 1% of the dose. However, in samples collected 96–120 h after IV and PO dosing the amounts of volatile radioactivity were 21–37 and 66–91% of the amount of radioactivity in the samples, respectively. This may be due to formation of tritiated water as a result of metabolic conversion of inogatran and/or presence of tritiated water in the dose given.

Plasma Protein Binding and Blood–Plasma Concentration Ratio

The protein binding of inogatran in rat, dog, and human plasma was low and independent of the concentration. Mean values of the percentage of inogatran bound to plasma proteins were 20–28% (S.D. < 4%). Blood–plasma concentration ratios of inogatran were 0.39–0.56. No difference was observed between rat, dog, and human blood and the ratio was independent of the concentrations studied. The low blood–plasma concentration ratio of inogatran suggests that the distribution into red blood cells was limited.

Discussion

The pharmacokinetics and metabolism of inogatran was investigated in the rat, dog, and cynomolgus monkey after single intravenous and extravascular doses. In all three animal species, the plasma concentrations of inogatran declined with a short half-life after intravenous administration. The terminal half-life was 1–2 h but most of the AUC was accounted for during the first hour after dosing when the half-life was shorter. Mean residence time was therefore a better estimate of the effective half-life. At doses of 2–5 μmol kg^{-1} the mean residence time was about 10 min in the rat and 35 min in the dog. In the cynomolgus monkey, given an IV dose of 0.1 μmol kg^{-1}, the mean residence time was 20 min. The rapid elimination of inogatran was accounted for by a small volume of distribution and a relatively high clearance. Clearance of inogatran, expressed relative to body weight, was highest in the rat and lowest in the dog. In the rat, the clearance of inogatran was lower at the higher dose of

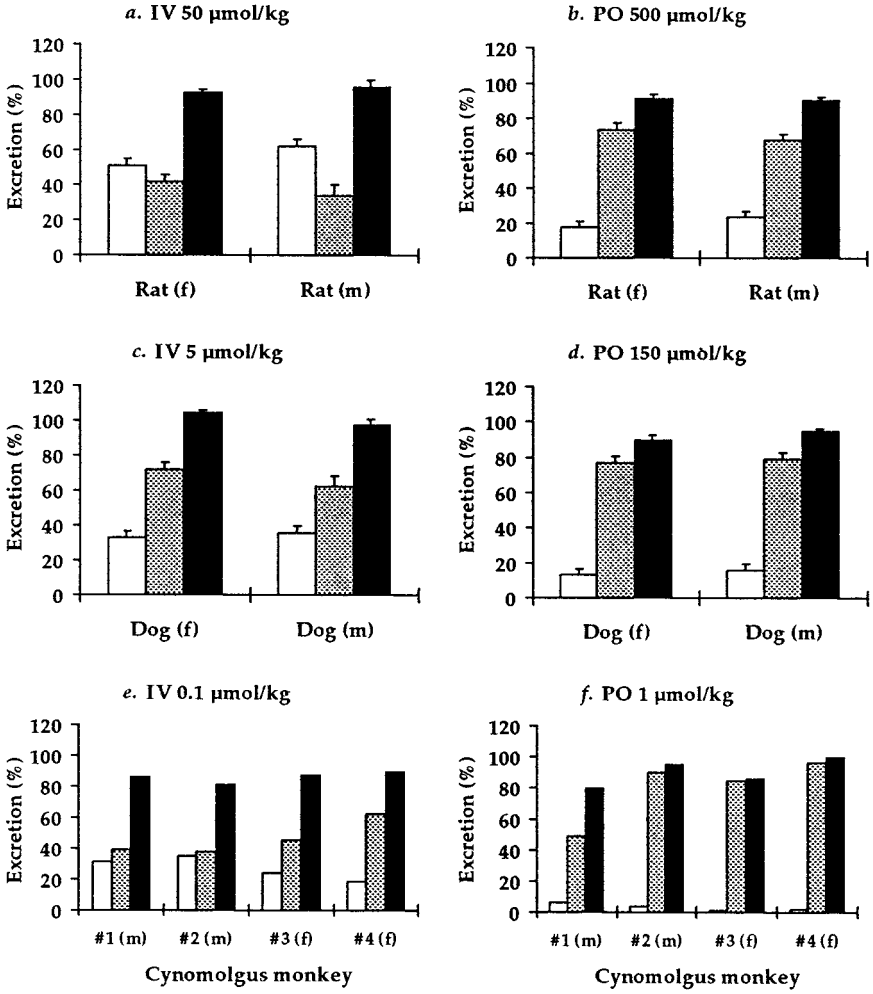

Figure 5. Radioactivity in excreta collected over 5–7 days after IV and PO dosing to rats, dogs, and cynomolgus monkeys. The amounts in urine (white) and faeces (grey) and the total amount recovered including cage wash (black) are expressed as a percentage of the given dose. Male (m) and female (f) animals are shown separately. For the rat and dog, mean values (S.D.) of four animals are shown while individual results are shown for cynomolgus monkeys

50 µmol kg^{-1}, indicating a capacity-limited elimination.

Renal clearance of inogatran was estimated as the total clearance multiplied by the fraction of radioactivity excreted in urine in the first 24 h after dosing, when most of the radioactivity was recovered as unchanged inogatran. The estimated renal clearances were higher than the glomerular filtration rate (GFR) for rats and monkeys [11]. The rate of filtration of inogatran is expected to be approximately equal to GFR because the plasma protein binding of inogatran is low. Thus, the observed differences between renal clearance and GFR indicate net renal secretion for rats and monkeys. Inogatran is a polar molecule and it is therefore expected to be excreted unchanged in urine. Based on interspecies allometric scaling [12] of the clearance values estimated for the lower IV doses in the rat, dog, and cynomolgus monkey, the predicted clearance in humans with a body weight of 70 kg was estimated to be about 300 mL min^{-1}. Assuming a volume of distribution of about 0.3 L kg^{-1}, as in the dog, the predicted half-life in humans would be about 50 min. These predictions agree well with the observations in healthy male subjects given inogatran intravenously [13]. Short half-lifes and small volumes of distribution have also been reported for the thrombin inhibitors hirudin and argatroban. Hirudin, a polypeptide derived from medicinal leeches (MW, 7000 Da), is cleared by the kidneys [8]. Argatroban, a low-molecular-weight thrombin inhibitor, was predominantly excreted in faeces after IV administration to dogs and rabbits, indicating significantly biliary excretion of drug and/or metabolites [9].

After oral dosing the bioavailability of inogatran was low, especially in rats and monkeys. This was supported by the high fraction of the dose excreted in faeces after PO doses. At the higher oral doses given to rats and dogs the bioavailability was increased. Maximum plasma concentrations were observed early after dosing but a second lower peak was observed in many animals. Administration into the duodenum of the rat resulted in a bioavailability similar to that after oral dosing while the

bioavailability after infusion into the colon was lower. Oral bioavailability was not limited by metabolism in the gastro-intestinal tract or in the liver because faeces, urine, and plasma contained mostly unchanged inogatran even after the low doses given to cynomolgus monkeys. First-pass elimination in the liver via secretion into bile is not supported by the estimated values of nonrenal clearance. Low membrane permeability is the most likely reason for the low oral bioavailability because inogatran is a polar molecule with three ionised groups at physiological pH, the negatively charged carboxylic acid (pK_a 1.3) and two positively charged groups, the secondary amine (pK_a 7.6) and the guanidine ($pK_a > 12$). Saturable binding of inogatran to constituents in the gastro-intestinal tract or increased membrane permeability at higher doses are two possible explanations for the dose dependent bioavailability.

After IP dosing to the rat, maximum plasma concentrations were observed 5 min after dose and the estimated bioavailability was close to 100%, suggesting that the absorption was rapid and complete and there was no first-pass extraction in the liver. Complete and rapid absorption was also observed after SC doses to the rat. The half-life and the mean residence time after subcutaneous dosing were longer than after IV dosing and the decline was approximately monoexponential.

Radiolabelled material was excreted, predominantly as unchanged inogatran, in both urine and faeces after IV dosing. The total amount recovered in urine and faeces was about 90% or more of the given dose and the excretion of radioactivity was essentially completed in the first 2 days after dosing. The high percentage of the dose recovered in faeces, which was about the same as, or higher than, the urinary recovery, indicates excretion of radioactivity via the bile. Inogatran, which is a polar, cationic molecule with a molecular weight of 439 Da, is expected to be excreted in the bile [14]. Rapid and extensive excretion in the bile has also been reported for other thrombin inhibitors [15,16] with chemical structure similar to inogatran.

The plasma concentrations of unchanged inogatran and total radioactivity were similar indicating that no metabolites were present in plasma, which was supported by the low amounts of metabolites in urine and faeces. Only small differences between the concentrations of total radioactivity and unchanged inogatran were observed at later times after intravenous dosing and after oral doses to rats and dogs. In the cynomolgus monkey the difference was larger which may be due to the high specific radioactivity of the dose given. Even a low percentage of tritiated water, present in the dose solution or formed *in vivo*, would give an observable contribution to the plasma concentration of total radioactivity in the terminal phase of the concentration–time curve after IV administration and after PO dosing. Tritiated water has a relatively long biological half-life (about 12 d) compared to inogatran and the oral absorption of tritiated water is complete. Volatile radioactivity, which accounted for less than 1% of the given dose, was found in urine and faeces collected from cynomolgus monkeys. In summary, a small volume of distribution and relatively high clearance resulted in a rapid elimination of inogatran after intravenous doses. Active transport systems appear to be involved in the elimination processes. Inogatran was absorbed when given orally but the bioavailability was low. At high oral doses given to rats and dogs a dose dependent increase of the bioavailability was observed. Inogatran was excreted unchanged both in urine and in faeces, presumably via the bile.

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