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Pharmacokinetics of Cyanamide in Dog and Rat

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Abstract—A pharmacokinetic study of cyanamide, an inhibitor of aldehyde dehydrogenase (E.C. 1.2.1.3) has been made in the beagle dog and Sprague-Dawley rat. Cyanamide plasma levels were determined by a sensitive high performance liquid chromatographic assay, specific for cyanamide. In the dog, i.v. administration of cyanamide at 1, 2 and 4 mg kg^{-1}, produced a dose-dependent pharmacokinetic behaviour. Statistically significant changes were observed in plasma clearance values (12·6 to 19·7 mL kg^{-1} min^{-1}), half life values (39 to 61 min) and mean residence times (50 to 79 min). Peak plasma concentrations, after oral administration of 4 mg kg^{-1} were achieved at 30 min and oral bioavailability was about 65%. In the rat after i.v. or oral administration, cyanamide (2 mg kg^{-1}) had a half life of 30 min, a total plasma clearance of 117 mL kg^{-1} min^{-1} and a mean residence time of 26 min. Oral bioavailability was about 69%.

Cyanamide is an aldehyde dehydrogenase (E.C.1.2.1.3) inhibitor that is used as an adjunct in the treatment of chronic alcoholism. The drug blocks ethanol metabolism by inhibition of aldehyde dehydrogenase which increases both hepatic and blood acetaldehyde levels after ingestion of ethanol (Brien et al 1978).

Enhanced levels of acetaldehyde in man are responsible for some unpleasant responses (i.e. tachycardia, hypotension, facial flushing). These adverse effects appear at the time ethanol is ingested and this facilitates compliance with therapy at its onset (Peachey & Naranjo 1984).

Few studies on the pharmacokinetics of cyanamide have been reported. Some have been with high doses (Obach et al 1981, 1986), others (Brien & Loomis 1983; Loomis & Brien 1983) report a profile after the oral administration of a prodrug, calcium cyanamide, to the rat and a single pharmacokinetic parameter, a half life of 92 min.

An HPLC method for the determination of cyanamide in plasma has recently been published (Pruñonosa et al 1986). This sensitive technique allows the determination of plasma concentrations of cyanamide in human therapy.

The present study characterizes the pharmacokinetic profile of cyanamide and reports its pharmacokinetic parameters in the Sprague-Dawley rat and the beagle dog.

Materials and Methods

Chemicals and reagents

Cyanamide was obtained from Fluka (Buchs, Switzerland). Acetonitrile and methanol (HPLC grade) were purchased from Sherlau. All other chemicals and solvents used were of analytical grade.

Animals

Male Sprague-Dawley rats from our colony, obtained from Charles River (France), 200–220 g, were maintained in an air-conditioned room at 21 ± 2°C at 50 ± 10 per cent relative humidity and under a 12 h light/dark cycle. Rats were assigned to two groups of 48 animals each for intravenous and oral dosing and were housed in Makrolon cages. Tap water and standard diet (UAR A04, PANLAB S L Spain) were freely available. The absence of contamination of the diet by cyanamide was established by analysis of the chow. Animals were deprived of food for the period starting 18 h before drug administration until the last blood sample was collected. Four rats were used for each experimental time.

Five male beagle dogs (9–16 kg) were deprived of food but had water freely available for 18 h before administration of the drug. The absence of contamination of the diet was also established by analysis of the chow (IPS-P90 LETICA).

Drug administration

Intravenous route. An aqueous cyanamide solution (2 mg mL^{-1}) was administered at 2 mg kg^{-1} to the rat by the caudal vein, and at 1, 2 and 4 mg kg^{-1} to the dog through the left fore cephalic vein. Blood samples were obtained by cardiac puncture 1, 3, 10, 15, 20, 30, 45, 60, 90 and 120 min after drug administration to the rat. In the dog, blood samples of 2 mL were withdrawn from the right fore cephalic vein immediately before administration of cyanamide (blank sample) and at different times up to 6 h after drug administration. Each dog received three single different doses with an interval between administration of one week.

Oral route. An aqueous cyanamide solution (0·2 mg mL^{-1}) was administered to rats by gavage at 2 mg kg^{-1}, and an 80 mg mL^{-1} solution was administered to the dogs at 4 mg kg^{-1}, one week after intravenous administration. In the rat, blood samples were taken at 1, 5, 7, 10, 15, 20, 30, 45, 60, 90 and 120 min. In the dog, blood samples were withdrawn as described for the intravenous route.

Blood samples were centrifuged at 1000 *g* for 20 min and plasma removed and stored at −70 °C until analysis.

Drug analysis

Cyanamide plasma concentrations were determined using an HPLC technique (Pruñonosa et al 1986) based on the spectrofluorimetric detection of a dansyl derivate of cyanamide. In our hands the technique was accurate (maximum relative error was 5%) and reproducible (maximum relative

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standard deviation was 15%). Linearity was assessed between the range of 25 to 500 ng mL^{-1}.

Chromatographic analyses were on a Waters HPLC system (Waters Associates, Mildford, MA, USA) equipped with two M-510 solvent delivery systems, a M-721 solvent programmer, a WISP 710B automatic injector and a M-420 AC fluorescent detector.

Pharmacokinetic treatment

A non-compartmental approach was used to estimate the following pharmacokinetic parameters: Plasma clearance (Clp), β elimination half life ($t_{\beta\frac{1}{2}}$), area under the plasma concentration curve (AUC^{0}_{∞}), mean residence time (MRT) and volume of distribution (Vd_{ss}). After oral administration experimental peak plasma concentration (C_{max}) and time to peak (T_{max}) were also estimated.

Estimation of the above parameters was using the PKCALC program (Shumaker 1986), following the methods proposed by Perrier & Mayersohn (1982).

To check if cyanamide showed a dose-dependent pharmacokinetic behaviour in the dog, the following parameters were compared: Plasma clearance (CLp), elimination half life ($t_{\beta\frac{1}{2}}$), volume of distribution (Vd_{ss}) and area under curve AUC^{0}_{∞} normalized by the administered dose.

Statistical analysis

Comparison of pharmacokinetic parameters at different doses was by ANOVA, and previous assessment of homogeneity of variances by means of Barlett's test. Comparison between groups was by Scheffé assay.

When variances were not homogeneous, statistical comparison was assessed by the "H" Kruskal-Wallis assay and comparison between groups, by the "U" Mann-Whitney test.

The level of significance adopted in all cases was $P = 0{\cdot}05$.

Results

Studies in dogs

The mean time courses of cyanamide in plasma following the drug injection in dogs at 1, 2 and 4 mg kg^{-1} are shown in Fig. 1. All plasma levels showed a biphasic disposition profile. A non-compartmental treatment of these provided the pharmacokinetic parameters summarized in Table 1.

Plasma clearance values (13·4 and 12·6 mL kg^{-1} min^{-1}) obtained after 2 and 4 mg kg^{-1} intravenous administrations were lower than the corresponding value after 1 mg kg^{-1} (19·7 mL kg^{-1} min^{-1}) ($P < 0{\cdot}01$). The cyanamide elimination half life values increased with the doses administered. The half life value obtained after 4 mg kg^{-1} was also enhanced to 61 min, a value higher than that obtained after 1 mg kg^{-1} (39 min) ($P < 0{\cdot}01$). The half life value obtained after 2 mg kg^{-1} was 47 min.

MRT values also increased. MRT after 4 mg kg^{-1} was 79 min, a value higher than those obtained after 1 and 2 mg kg^{-1}

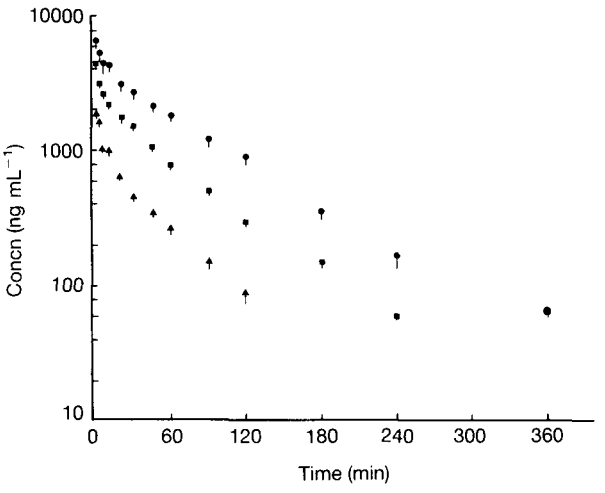

FIG. 1. Mean ± s.e.m. cyanamide plasma concentration after intravenous administration to five beagle dogs at doses of 1 (▲), 2 (■) and 4 mg kg^{-1} (●).

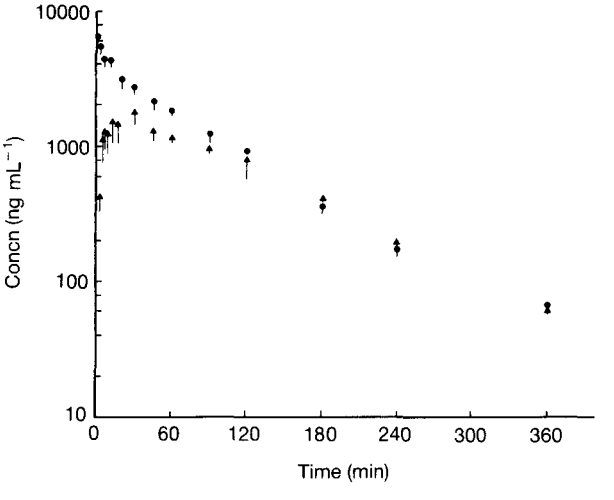

FIG. 2. Mean ± s.e.m. cyanamide plasma levels after intravenous (●) and oral (▲) administration to five beagle dogs at 4 mg kg^{-1}.

Table 1. Mean pharmacokinetic parameters of cyanamide after intravenous administration to the dog.

	Mean values (±s.e.m.) at doses:			
Parameters	1 mg kg^{-1}	2 mg kg^{-1}	4 mg kg^{-1}	Units
Total plasma clearance (CLp)	19·7 ± 0·9	13·4 ± 0·6[a]	12·6 ± 1·6	mL kg^{-1} min^{-1}
Elimination half life ($t_{\frac{1}{2}}$)	38·7 ± 3·5	47·2 ± 1·2	61·3 ± 6·1[a]	min
Mean residence time (MRT)	49·7 ± 4·1	61·5 ± 1·9	78·6 ± 4·6[a,b]	min
Volume of distribution (Vd_{ss})	963·4 ± 45·7	824·4 ± 51·4	1090·0 ± 198·4	mL kg^{-1}
Area under curve (AUC^{0}_{∞})	51 322 ± 2644	150 614 ± 6329[a]	334 352 ± 34567[a,b]	ng mL^{-1} min

[a] Significant difference with respect to 1 mg kg^{-1} dose ($P < 0{\cdot}01$ ANOVA followed by Scheffé test).
[b] Significant difference with respect to 2 mg kg^{-1} dose ($P < 0{\cdot}01$ ANOVA followed by Scheffé test).

Table 2. Mean pharmacokinetic parameters after oral administration of 4 mg kg^{-1} to the dog.

Parameters	Mean values (±s.e.m.)	Units
Area under curve (AUC_∞^0)	213 895 ± 23000	ng mL^{-1} min
Elimination half life ($t_{\frac{1}{2}}$)	62·0 ± 4·8	min
Mean residence time (MRT)	99·4 ± 9·8	min
Peak plasma concentration (C_{max})	2298 ± 583	ng mL^{-1}
Time to peak (T_{max})	32·8 ± 8·9	min
Bioavailability (F)	65·4 ± 7·2	%

Table 3. Mean pharmacokinetic parameters of cyanamide after intravenous administration of 2 mg kg^{-1} to the rat.

Parameters	Mean Values (±s.e.m.)	Units
Total plasma clearance (CLp)	117·1 ± 27·9	mL kg^{-1} min^{-1}
Elimination half life ($t_{\frac{1}{2}}$)	32·5 ± 3·6	min
Mean residence time (MRT)	26·4 ± 1·6	min
Volume of distribution (Vd_{ss})	3088·8 ± 924·5	mL kg^{-1}
Area under curve (AUC_∞^0)	17080 ± 4077	ng mL^{-1} min

(50 and 62 min, respectively) ($P < 0.01$). The distribution volume at steady state (Vd_{ss}) did not change according to the administered dose (values ranged between 824 and 1090 mL kg^{-1}). Mean cyanamide plasma levels obtained after 4 mg kg^{-1} oral and intravenous administrations are shown in Fig. 2. Cyanamide was rapidly absorbed after oral administration. The mean peak plasma concentration (2298 ng mL^{-1}) was reached at 33 min. Pharmacokinetic parameters of cyanamide obtained after oral administration at 4 mg kg^{-1} are summarized in Table 2. The bioavailability of cyanamide after oral administration at 4 mg kg^{-1} was around 65%.

Studies in rats

The mean plasma time courses of cyanamide after intravenous and oral administration to rats at 2 mg kg^{-1} are shown in Fig. 3.

After intravenous administration, plasma clearance of cyanamide in the rat was 117 mL kg^{-1} min^{-1} and elimination

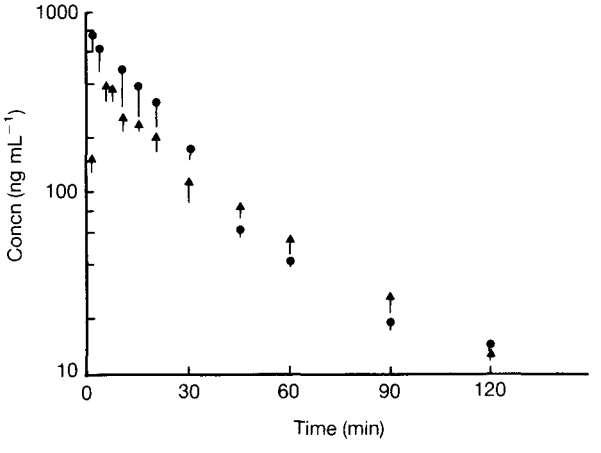

FIG. 3. Mean ± s.e.m. cyanamide plasma levels after intravenous (●) and oral (▲) administration to the rat at 2 mg kg^{-1}. Each mean value was obtained from 4 rats.

Table 4. Mean pharmacokinetic parameters of cyanamide after oral administration of 2 mg kg^{-1} to the rat.

Parameters	Mean Values (±s.e.m.)	Units
Area under curve (AUC_∞^0)	11 744 ± 2011	ng mL^{-1} min
Elimination half life ($t_{\frac{1}{2}}$)	27·2 ± 0·3	min
Mean residence time (MRT)	36·2 ± 0·5	min
Peak plasma concentration (C_{max})	388 ± 68	ng mL^{-1}
Time to peak (T_{max})	5	min
Bioavailability (F)	68·7 ± 4·6	%

half life was 33 min, the mean residence time was 26 min and Vd_{ss} 3089 mL kg^{-1} (Table 3).

Also in rat the absorption of cyanamide after oral administration was rapid. When cyanamide was orally administered at 2 mg kg^{-1} the peak plasma concentration (388 ng mL^{-1}) was achieved at 5 min. The absolute bioavailability was 68·7%. Values of main pharmacokinetic parameters of cyanamide in the rat after oral administration are shown in Table 4.

Discussion

The pharmacokinetic behaviour of cyanamide in dogs over the range of the intravenously administered doses (1–4 mg kg^{-1}) was dose-dependent. Plasma clearance decreased when the dose increased ($P < 0.01$). Also, the elimination half life of cyanamide changed with doses (from 39 min after 1 mg kg^{-1} to 61 min after 4 mg kg^{-1}). As the volume of distribution Vd_{ss} did not change with the dose administered ($P < 0.05$), the dose-dependent behaviour of cyanamide could be related to its elimination process. It is possible that the drug may not be completely acetylated to the rapidly excreted *N*-acetylcyanamide on the first pass. Unmetabolized drug could be distributed to other compartments from which it slowly passes into the blood stream. This might explain the decrease in plasma clearance and increase in half life as the dose of cyanamide increased.

Shirota et al (1984) demonstrated that, after giving 1·7 mg kg^{-1} of [^{14}C]cyanamide to dogs, between 80 and 99% of the dose was recovered in urine. The biotransformation of cyanamide was basically by *N*-acetylation. The 11% of radioactivity recovered corresponded to cyanamide and 87% to *N*-acetylcyanamide. Shirota et al have shown that while *N*-acetyltransferase in the dog does not catalyse the acetylation of aromatic amines, it does catalyse the acetylation of cyanamide. The amount and/or activity of the enzyme is probably low, since the dog is adversely affected by high doses of cyanamide. The observed dose-dependent pharmacokinetic behaviour in dogs could be related to a saturation of the cyanamide acetylation process.

The fact that AUC/D values did not remain constant at different doses agrees with a dose-dependent behaviour of the drug in the dog. The mean residence time is short (between 50 and 79 min) and is obviously longer when the dose increased ($P < 0.01$). This pharmacokinetic dose-dependent behaviour has been observed with higher doses of cyanamide in rats and rabbits (Obach et al 1979, 1981).

In the dog, oral absorption of cyanamide was fast, peak plasma concentrations being achieved 30 min after adminis-

tration. Absolute bioavailability was estimated at 65%, but taking into account the non-linear behaviour of the drug, this value has to be considered "approximate".

Studies in the rat at 2 mg kg^{-1} showed a short elimination half life of ca. 30 min, a value less than that reported by Obach et al (1986) (56 min after 35 mg kg^{-1}). The differences in t1/2 values suggested a dose-dependent behaviour towards the drug. Furthermore, the total plasma clearance at 2 mg kg^{-1} was 117 mL kg^{-1} min^{-1}, notably higher than the value observed at 35 mg kg^{-1} (20 mL kg^{-1} min^{-1}). Also, the half life we observed in rats was shorter than that (1 h) observed by Loomis & Brien (1983). Nevertheless, those authors reported the value after oral administration of 7 mg kg^{-1} of calcium carbimide. The shorter half life with free cyanamide in the rat via the oral route compared with that reported by Brien & Loomis (1983) is probably due to calcium cyanamide being less soluble than cyanamide. Cyanamide must first be liberated from this prodrug (Nagasawa et al 1986) thereby limiting its ready bioavailability, unlike free cyanamide which is readily absorbed (Obach et al 1986).

The oral absorption of cyanamide in rats was rapid. The peak plasma concentration was achieved at 5 min, which is in accordance with the value obtained after an oral dose of 35 mg kg^{-1}. Nevertheless, the time to peak observed by Loomis & Brien (1983) after oral administration of cyanamide calcium salt was higher. The fast absorption of cyanamide could be explained by taking into account its low molecular weight and its structure which allows it to diffuse through gut epithelial membranes and pores (Plá-Delfina & Moreno 1981).

The oral bioavailability in rats after 2 mg kg^{-1} was 69%, a value closer than that obtained after oral administration of 4 mg kg^{-1} to the dog (65%) and lower than those reported after oral administration of 35 mg kg^{-1} (ca 90%) (Obach et al 1986).

Deitrich et al (1976) reported that after oral administration of [^{14}C]cyanamide, almost all administered radioactivity could be recovered in urine, thus demonstrating the complete absorption of the drug. They were also the first to show that cyanamide is extensively metabolized to a metabolite of unknown structure in rodents. Shirota et al (1984) found that this urinary metabolite was *N*-acetylcyanamide and that cyanamide is *N*-acetylated in the dog and man as well as in the rat and rabbit. They also found cyanamide to be extensively metabolized and, that *N*-acetylcyanamide, the main metabolite, was present in the urine of rat, dog and rabbit. These findings assure the virtually complete absorption of the drug. For this reason, and because the bioavailability of the drug in rats and in dogs after a low dose oral administration was low, a first-pass effect could not be discounted.

Volumes of distribution Vd_{ss} in the rat (3·8 L kg^{-1}) are higher than the corresponding values in the dog (around 1 L kg^{-1}). This difference between species could be explained in part by the different affinity showed by cyanamide for erythrocytes of both species (Obach et al 1984).

The high clearance values obtained in both species and the short half lives estimated contrast with the duration of the cyanamide activity and support the idea reported by some authors (Deitrich 1976; DeMaster et al 1983, 1984) who proposed that the pharmacological activity was due to an active metabolite of unknown structure. Current evidence indicates that the active metabolite is formed via a minor metabolic pathway catalysed by the enzyme, catalase, and the active chemical species is most likely nitroxyl (Shirota et al 1987).

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