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BIOAVAILABILITY OF CYANAMIDE IN FASTED AND UNFASTED RATS

R. OBACH*†, C. VALENTI†, J. VALLES, J. M. VALLES* AND J. DOMENECH†‡

Research Department of S.A. LASA Laboratorios, †Practical Pharmacy Department, Division of Biopharmaceutics, Faculty of Pharmacy, University of Barcelona, Avda. Diagonal 643, 08028 Barcelona, Spain

ABSTRACT

The pharmacokinetics of cyanamide after 35 mg kg^{-1} intravenous and oral administration to fasted and unfasted rats have been investigated. The plasma level are fitted well by a two-compartment open model. The half-life ($t_{1/2}\beta$) of cyanamide in the rat was 1 hour and the plasma clearance (Cl_p) was 0·02 (l kg^{-1}) min^{-1}. Food does not appear to modify the absolute bioavailability of cyanamide ($F = 93·3$ per cent fasted, $F = 85·5$ per cent unfasted), although it does retard drug absorption.

KEY WORDS Cyanamide Pharmacokinetics Oral bioavailability Food effect Rat studies

INTRODUCTION

Cyanamide is an alcohol-deterrent drug; it is used as an adjunct in the treatment of chronic alcoholism. It interferes with the metabolism of alcohol through non-competitive inhibition of aldehyde dehydrogenase;[1] increased hepatic and blood acetaldehyde levels result when ethanol has also been ingested.[2] In man the relatively high blood levels of acetaldehyde induce an aversive situation, which appears synchronously with the ingestion of alcohol, it being the alcohol itself which is manifested as a series of unpleasant organic responses e.g. tachycardia, hypotension, facial flushing, that is usually sufficient to deter drinking.[3,4]

Although the pharmacological activity of cyanamide is well known, there are few references in the literature to its disposition and pharmacokinetics.[5,6,7] Recently, a pharmacokinetic profile of cyanamide in rat after oral administration of calcium cyanamide was reported by Loomis and Brien,[8] who showed that the apparent elimination half-life of cyanamide is 92·4 min and that the maximal plasma cyanamide level is achieved 1 hour after oral administration.

‡Addressee for correspondence.

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There are no literature references to the absolute bioavailability of this drug, a biopharmaceutical parameter that would enable the determination of the most appropriate route of administration and drug formulation.

This study was undertaken to determine the bioavailability of an orally administered, aqueous cyanamide solution (COLME®) in the Sprague-Dawley rat. The absolute absorption efficiency of cyanamide (percentage of absorbed dose) and the absorption rate characteristics following gastric intubation were studied.

The influence of food on the oral bioavailability of cyanamide was also examined.

MATERIALS AND METHODS

Experimental animals

Male Sprague-Dawley rats from our colony, derived from Charles River France, and weighing 300–400 g, were used. The rats were assigned at random to three groups of 5 animals each and housed in makrolon cages containing 5 rats per cage. Animals were acclimatized for 1 week before starting the study.

Tap water and U.A.R/AO4 rodent laboratory chow were provided *ad libitum*. The absence of contamination of the diet by cyanamide was established by analysis of the chow.

The animals were maintained in an air-conditioned room at $20 \pm 2°$, at 50 ± 10 per cent relative humidity and under a 12 h light/dark cycle.

Animals were deprived of food for the 18-h period immediately before the start of the study and maintained with water *ad libitum*.

Drugs

Cyanamide was purchased from Fluka (Buchs, Switzerland) and cyanamide aqueous solution was prepared from COLME® (6 per cent oral cyanamide solution batch number S-13, S.A. LASA Laboratorios, Barelona, Spain) by dilution with distilled water to a concentration of 1·75 per cent p/v. Stability of the cyanamide aqueous solution was established. The purity of the cyanamide solution was determined on TLC aluminium sheets, cellulose (Merck Darmstadt, GFR) of 0·2 mm thickness and with a solvent system of 1-butanol–ethanol–water (4/1/1, v/v/v). Spots were detected by spraying the plate with a reagent solution that contained equal volumes of a 5 per cent aqueous solution of potassium ferricyanide (Merck), a 5 per cent aqueous solution of sodium nitroprussiate (Merck), and a 5 per cent aqueous solution of sodium hydroxide (Merck). The chromatogram contained a single violet spot of $R_f = 0{\cdot}63$; possible dicyandiamide and urea contamination would be detected by this procedure as pink spots with $R_f = 0{\cdot}38$ and $R_f = 0{\cdot}27$, respectively.

Method

All the rats were anaesthetized with sodium pentobarbital (50 mg kg^{-1} i.p.) and the right carotid artery was cannulated for blood sampling (0·4 ml per sample). The jugular veins of 5 fasted rats were also cannulated for i.v. administration of 35 mg of cyanamide aqueous solution. A second group of 5 fasted rats received 35 mg kg^{-1} of cyanamide aqueous solution by gastric gavage. In all cases, blood was sampled from the carotid artery at intervals over the 0 to 120 min post-administration period.

Anaesthesia and loss of blood volume during sampling induced a slight modification of the haemodynamics, i.e. a decrease of 5·6 per cent in mean blood pressure and an increase of 10·8 per cent in heart rate.

Plasma cyanamide levels were determined using the spectrophotometric technique described by Buyske *et al.*,[9] conveniently adapted for 0·15 ml plasma samples, which allowed the determination of a plasma level vs time curve for each animal.

The assay procedure involved the formation of cyanamide and sodium pentacyanoammineferrate coloured complex and it was sufficiently accurate and precise for the concentration range of 3 to 80 μg ml^{-1}. The mean relative error and the mean coefficient of variation were 1·2 and 5·3 per cent, respectively.

Pharmacokinetic treatment

The plasma levels obtained after 35 mg kg^{-1} i.v. cyanamide administration were fitted to the biexponential equation representative of the two-compartment open model

$$C = A_o.e^{\alpha t} + B_o.e^{-\beta t} \quad (1)$$

Where C is the plasma concentration of cyanamide at any time (t), A_o and B_o are coefficients, and α and β are the rate constants for the distribution and elimination phases, respectively.

The plasma levels obtained after 35 mg kg^{-1} oral cyanamide administration were fitted to the following biexponential and/or triexponential equations:

$$C = B_o.e^{-k_{el}\,t} - A_o.e^{-k_a t} \quad (2)$$

$$C = A_o.e^{-\alpha t} + B_o.e^{-\beta t} - P_o.e^{-k_{01} t} \quad (3)$$

Where P_o is coefficient, k_a and k_{01} are the absorption rate constants for the one- and two-compartment open models, respectively, and k_{el} is the elimination rate constant.

Selection of the most probable equation, i.e. (2) or (3) was performed using the MAICE method (minimum value of AIC).[10]

The experimental data were fitted to theoretical equations in a TEKTRONIX 4051 computer with a non-linear, least squares regression program using the Marquardt algorithm.[11]

The values of the areas under the plasma level curves between zero time and infinite time (AUC_0^∞) were calculated from equations for the theoretical curve to which the experimental data fit best.

The extent of absorption (F) was estimated from the ratio of the areas under the plasma level curves obtained after oral and i.v. administration by the relation:

$$F = \frac{AUC_0^\infty \text{ oral}}{AUC_0^\infty \text{i.v.}} \tag{4}$$

The absorption characteristics of cyanamide following gastric intubation were assessed by establishing the pharmacokinetic parameters: absorption rate constant (k_{01}), time to peak (t_{max}), and peak concentration (C_{max}). The estimation of the absorption rate constant was performed by least squares regression analysis of data from the best-fit fitting the plasma level curves; t_{max} and C_{max} were obtained from the theoretical equation that best fitted the experimental data.

The lag time (t_o) values were calculated iteratively from (3) as the time value that provides a cyanamide plasma concentration C equal to zero.

Data analysis

Statistical comparison of AUC_o^∞ and t_{max} values obtained after oral administration to fasted and unfasted animals was conducted by an unpaired t-test. As variances of fasted and unfasted groups were not homogeneous according to the F-test, the parameters C_{max} and k_{01} were compared by Mann–Whitney's U test.

RESULTS

Intravenous kinetics

The pharmacokinetic parameters summarized in Table 1 were calculated by fitting the cyanamide plasma levels, obtained after i.v. administration of 35 mg kg^{-1} in rats, to an open bicompartmental model.

The mean plasma cyanamide level-time curve is plotted in Figure 1.

The two-compartment open model fitted the experimental data with precision (range of r for individual fittings was 0·987–0·999).

The half-life ($t_{½}\beta$) of cyanamide in the anaesthetized rat is about 1 hour, its plasma clearance (Cl_p) is 0·02 l($1\,kg^{-1}$) min^{-1} and its volume of distribution in the central compartment (V_c) is 0·52 $1\,kg^{-1}$. The return to the central compartment is not a limiting factor of its elimination in accordance with the ratios $k_{21}/k_{13} = 1{\cdot}94$.

From the physicochemical properties of cyanamide and the volume of distribution value in the central compartment, it appears that the drug is

Table 1. Mean pharmacokinetic parameters of cyanamide after i.v. administration of 35 mg kg^{-1} in rat

Parameters*	Mean values	± S.E.M.	Units
Disposition constant of α phase	0·229	0·035	min^{-1}
Disposition constant of β phase	0·013	0·001	min^{-1}
Half-life ($t_{½}\beta$)	56·6	5·3	min
Distribution constant (k_{12})	0·128	0·022	min^{-1}
Return constant (k_{21})	0·075	0·012	min^{-1}
Elimination constant (k_{13})	0·039	0·004	min^{-1}
Volume of distribution of central compartment (V_c)	0·52	0·04	$l\,kg^{-1}$
Total plasma clearance (Cl_p)	0·02	0·005	$(l\,kg^{-1})\ min^{-1}$
Area under curve (AUC_0^∞)	1807·2	112·7	$(\mu g\,ml^{-1})\ min^{-1}$

*Values are expressed as mean ± S.E.M. from 5 animals.

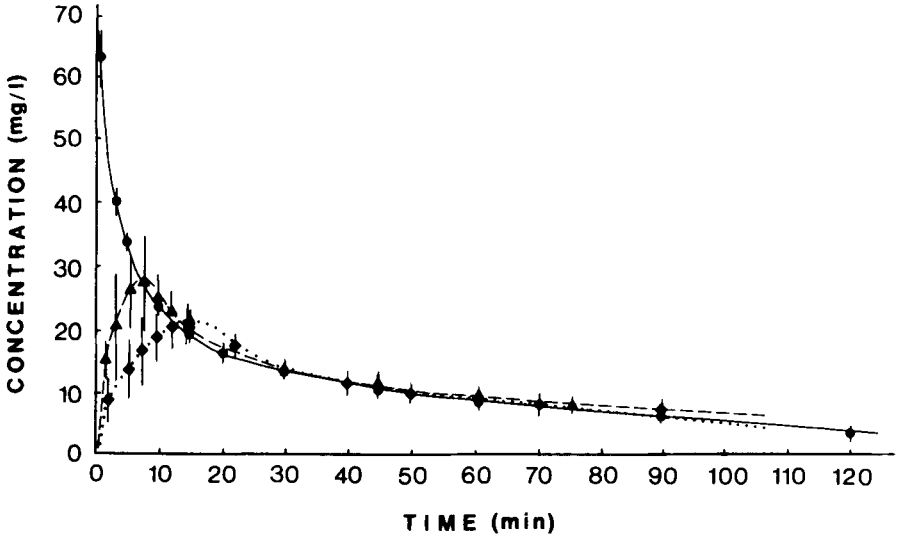

Figure 1. Mean cyanamide plasma levels (± SEM) after 35 mg kg^{-1} intravenous administration (●) and after oral administration of the same dose to fasted (▲) and unfasted (◆) rats ($n = 5$)

Table 2. Mean pharmacokinetic parameters of cyanamide after oral administration of 35 mg kg^{-1} to fasted and unfasted rats

Parameters*	Fasted	Unfasted	Units
Absorption constant (k_{01})	0·608 ± 0·151	0·197 ± 0·045	min^{-1}
Lag time (t_0)	0·36 ± 0·27	1·4 ± 0·7	min
Peak concentration (C_{max})	34·5 ± 9·9	19·8 ± 3·8	$\mu g\ ml^{-1}$
Time to peak (t_{max})	6·3 ± 2·2	14·7 ± 2·6	min
Area under curve (AUC_0^∞)	1686·8 ± 279·3	1545·9 ± 222·8	$(\mu g\ ml^{-1})$.min
Bioavailability (%F)	93·3	85·9	

*Values are expressed as mean ± S.E.M. from 5 animals.

distributed throughout the whole body water. (The volume of distribution in steady state (V_{dss}) was 1·39 ± 0·061 kg^{-1}.)

Oral kinetics

Fasted rats. The pharmacokinetic treatment allowed the determination of the individual and mean parameters. The latter are summarized in Table 2.

The mean plasma cyanamide levels and corresponding standard errors are shown in Figure 1.

Cyanamide is quickly absorbed in fasted animals. The absorption half-life is about 1 min and the value of t_{max} is just 6 min. The absorption rate constant was 0·608 ± 0·151 min^{-1}. The relative bioavailability (93 per cent) of cyanamide in fasted rats indicates that practically all the administered dose is absorbed.

Unfasted rats. The pharmacokinetic treatment carried out provided the individual and mean parameters (Table 2). The mean plasma cyanamide levels and corresponding standard errors are shown in Figure 1. Cyanamide is also quickly absorbed in unfasted animals; its absorption half-life is about 4 min and t_{max} is about 15 min. The absorption rate constant is 0·197 ± 0·045 min^{-1}. The bioavailability of cyanamide in unfasted animals is also high: 86 per cent.

Comparison between fasted and unfasted rats. No statistically significant differences ($p > 0·05$) were observed between AUC_0^∞ values from fasted and unfasted animals.

The t_{max} for the unfasted animals (14·7 min) was significantly greater ($p < 0·05$) than that for the fasted animals (6·3 min).

The absorption rate constant obtained with fasted animals (0·608 min^{-1}) was significantly greater ($p < 0·05$) than that with unfasted animals (0·197 min^{-1}).

The C_{max} obtained with fasted animals (34·5 μg ml^{-1}) was higher than that observed in unfasted animals (19·6 μg ml^{-1}). However, the difference was not statistically significant ($0{\cdot}1 > p > 0{\cdot}05$).

Lag time values (t_o) were negligible in both cases.

DISCUSSION OF THE RESULTS

The pharmacokinetic behaviour of cyanamide after i.v. administration of 35 mg kg^{-1} to rats can be accounted for by an open two-compartment model and a relatively short half-life of 1 h. This value is shorter than that reported by Loomis and Brien[8] after an oral administration of 7 mg kg^{-1} of calcium carbimide (calcium salt of cyanamide) to the conscious rat. The difference might be explained by the fact that Loomis and Brien measured plasma levels of cyanamide for a longer period of time and/or because the concentrations used to estimate the half-life of cyanamide after oral administration of calcium cyanamide may include a residual absorption component.

Comparison of t_{max} values obtained by Loomis and Brien,[8] and in the present study, indicate that, in both fasted and unfasted, anaesthetized rats, cyanamide absorption is faster from orally administered aqueous solutions of cyanamide than calcium carbimide.

This difference may be due to the different forms of cyanamide used in the two studies, i.e. free cyanamide in our study and calcium cyanamide in Loomis and Brien's study; cyanamide may not be released rapidly from its calcium salt under the conditions used by Loomis and Brien.[8]

The absorption of cyanamide is almost complete after oral administration of 35 mg kg^{-1} of cyanamide solution to unfasted or fasted rats, the relative bioavailabilities being 86 and 93 per cent, respectively. The presence of food does not modify the extent of absorption of cyanamide as evidenced by the statistical comparison of AUC_0^∞ values for the fasted and unfasted animals. However, the presence of food in the gastrointestinal tract appears to delay the absorption of cyanamide. This is indicated by the lower absorption rate constant, the higher t_{max} and the slower C_{max} values observed in the unfasted rats.

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