References

Calixto, J. B., Nicolau, M. (1982) Ações farmacológicas do ácido tânico. I. Efeito sobre a musculatura lisa e o sistema cardiovascular. In: Leite, D. S. B. (ed.) VII Simpósio de Plantas Medicinais do Brasil. Imprensa Universitária, B. Horizonte, Brasil, pp 479–492

Deutsch, H. F., Diniz, C. R. (1955) Some proteolytic activities of snake venoms. J. Biol. Chem. 216: 17–26

do Amaral, A. (1930) Campanhas anti-ophidicas. Mem. Inst. Butantan 5: 193–232

Feldberg, W., Kellaway, C. H. (1937) Liberation of histamine from the perfused lung by snake venoms. J. Physiol. 90: 257–279

Feldberg, W., Kellaway, C. H. (1938) Liberation of histamine and formation of lysolecithin-like substances by cobra venom. J. Physiol. 94: 187–226

Hurwitz, L., Suria, A. (1971) The link between agonist action and response in smooth muscle. Ann. Rev. Pharmacol. 11: 303–326

Kaiser, E., Michl, H. (1968) Chemistry and pharmacology of the venoms of *Bothrops* and *Lachesis*. In: Bucherl, W., Buckley, E. E., Deulofeu, V. (eds) Venomous Animals and their Venoms. Vol. II, Academic Press, New York, pp 307–318

Kellaway, C. H. (1939) Animal poisons. Ann. Rev. Biochem. 8: 541–556

Mohamed, A. H., Zaki, O. (1957) Effect of Egyptian black snake toxin on histamine of blood and its relation to blood eosinophils. Am. J. Physiol. 190: 113–116

Paton, W. D. M. (1957) A pendulum auxotonic lever. J. Physiol. 137: 35P–36P

Rocha e Silva, M., Beraldo, W. T., Rosenfeld, G. (1949) Bradykinin, a hypotensive and smooth muscle stimulating factor released from plasma globulin by snake venoms and by trypsin. Am. J. Physiol. 156: 261–273

Sarkar, N. K., Devi, A. (1968) Enzymes in snake venoms. In: Bucherl, W., Buckley, E. E., Deulofeu, V. (eds) Venomous Animals and their Venoms. Vol. I, Academic Press, New York, pp 167–216

Schild, H. O. (1954) Non competitive drug antagonism. J. Physiol. 124: 33P–34P

Weinberg, J. (1975) Perfusão de órgão isolado com volume constante—técnica apropriada para estudo de interação droga-receptor. Cienc. Cult. 27: 286–290

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The disposition of nifurtimox in the rat isolated perfused liver: effect of dose size

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Abstract—The disposition of nifurtimox was studied in the rat isolated perfused liver using a recirculating system. The drug was administered as a bolus (5·0, 15·0 or 30·0 $\mu g\ mL^{-1}$), and its disappearance was monitored by analysing perfusate samples. In all experiments perfusate disappearance was monoexponential, and no significant difference was found between the three doses for the elimination constant (0·016, 0·011 and 0·012 min^{-1}, respectively), half-life (46·6, 65·8 and 66·8 min, respectively), extraction rate (0·128, 0·091 and 0·099, respectively) and distribution volume (41·1, 47·3 and 30·7 mL g^{-1}, respectively). At 30 $\mu g\ mL^{-1}$ the hepatic clearance was lower than the other concentrations of nifurtimox (0·66, 0·51 and 0·34 mL $min^{-1}\ g^{-1}$, respectively). Relatively little parent drug was recovered from the liver at the end of the perfusions. In summary, nifurtimox is cleared slowly from the rat isolated perfused liver, is poorly extracted by hepatocyte cells and is completely metabolized from 2 to 4 h after perfusion.

Nifurtimox is a substituted nitrofuran that has been used successfully for the treatment of *Trypanosoma cruzi* infections. It appears to be effective in amastigote and epimastigote parasite forms, and the reproductive forms are more sensitive to the drug. In-vitro, the minimum inhibiting concentration is 1 μM but over 10 μM is needed to prevent the trypamastigote form from entering the vertebrate cells (Bock et al 1969; Webster 1985).

The mechanism of action of nifurtimox is not completely understood. The trypanocidal action of nifurtimox appears to be related to its ability to form chemically reactive radicals that cause the production of toxic, partially reduced products of oxygen (Docampo & Moreno 1984).

The dose of nifurtimox required to maintain a therapeutic effect, ranges from 5 to 20 mg kg^{-1}. The usual dose is 15 mg kg^{-1}. Adverse effects occur in 40–70% of patients. The central nervous system is mostly affected and effects are dose-related (Brener 1979; Laplume et al 1982).

Little attention has been given to the study of the mechanism of action, the kinetics or the metabolism of this drug, despite the wide morbidity of Chagas' disease and wide nifurtimox use in South America. The dose of this drug has been established by trial and error and the factors which may affect the kinetics and drug disposition remain to be clarified.

Pharmacokinetic studies of nifurtimox in healthy volunteers (Paulos et al 1989) and in patients with chronic renal failure (González-Martin et al 1992) have been made in our laboratories. In these studies we have reported that this drug possesses peculiar pharmacokinetic characteristics. We found low serum concentrations of nifurtimox after an oral dose (15 mg kg^{-1}) suggesting an important presystemic effect with diminished bioavailability. Furthermore, nifurtimox seems to exhibit metabolic features which indicate that the liver could play an important role in its pharmacokinetics.

The main objective of this study was to determine the hepatic disposition of nifurtimox using a rat isolated perfused liver technique.

Materials and methods

Chemicals. Nifurtimox was a gift from Bayer Laboratories (Buenos Aires, Argentina), carbamazepine was a gift from Recalcine Laboratories (Santiago, Chile). All solvents were of HPLC grade and other reagents and chemicals were purchased from Merck Química Chilena, Santiago, Chile.

Animals. Male Sprague-Dawley rats, 140–200 g (Instituto de Salud Pública, Santiago, Chile), were housed in well-ventilated

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cages and kept at a room temperature of approximately 24°C, with free access to pelleted food and tap water until 24 h before surgery. Animals were cared for in accordance with the principle of The Guide for the Care and Use of Laboratory Animals (Governmental Activities Relating to the Use of Animals in Research 1985).

Isolated perfused livers. Rats were anaesthetized with urethane (1·5 g kg^{-1}, i.p.). The abdominal cavity was opened, and the portal vein and the thoracic inferior vena cava were cannulated. The liver was cut free and placed in a humidified and thermoregulated cabinet. The perfusate was pumped (Masterflex, Cole Palmer, Chicago, IL, USA) through a filter, to a membrane lung (Silastic medical grade tubing, Dow Corning, Midland, MI, USA) where it was oxygenated with 95% O_2–5% CO_2, in a 37°C thermoregulated bath, to a bubble trap before reaching the liver. In the recirculating mode, the perfusate exited through the inferior vena cava cannula and dropped back into the glass reservoir before returning to the pump.

The perfusate consisted of 500 mL of a modified Krebs-Henseleit bicarbonate buffer containing 1 mg mL^{-1} glucose. The perfusate was divided in two flasks each containing 250 mL. Nifurtimox, dissolved in 2 mL dimethylsulphoxide, was added to one of these flasks to a final concentration of either 5, 15 or 30 μg mL^{-1}. The flow rate was adjusted to about 3 mL min^{-1} g^{-1} assuming a liver weight of 3·5% of total body weight. Constant perfusate pH of 7·4 was maintained.

The liver was perfused for 120 min with nifurtimox in the recirculating mode after an equilibrium period of 30 min in the single-pass mode with blank perfusate. For the nifurtimox assay, efflux perfusate samples of 2 mL were collected at 5, 10, 15, 20, 30, 45, 60, 90 and 120 min. At the higher concentrations (15 and 30 μg mL^{-1}) additional samples at 150, 180 and 240 min were obtained. The samples were frozen until assay.

It was established that nifurtimox did not bind to the experimental apparatus (injector, tubing, cannulas or collection apparatus) used in the perfusion studies. In this work, five livers were perfused for each dose level.

Nifurtimox assay. A 2·0 mL sample of perfusate was mixed with 100 μL internal standard solution of carbamazepine (250 μg mL^{-1}) dissolved in dimethylsulphoxide and extracted by passage through a SepPack C_{18} cartridge (Waters Associates, Inc.) fitted to a Luer-lock glass syringe. The SepPack C_{18} cartridge was prepared by flushing with 2 mL methanol. The perfusate samples were passed through the cartridge and washed with 5 mL distilled water. Unchanged nifurtimox was eluted with 2 mL methanol. The methanol fractions were evaporated to dryness under nitrogen. The residues were dissolved in 500 μL of mobile phase (methanol-phosphate buffer pH 7·0 (50:50)). Recovery was 97%. Twenty microlitres was injected in a Shimatzu LC-9A model pump. Chromatographic separation was achieved using a Merck LiChrospher 100 RP-18 (5 μm) in a LiChroCART 125–4 4·6 × 12·5 mm column. Retention times for nifurtimox and internal standard were 2·8 and 6·2 min, respectively. A Shimatzu UV-spectrophotometer set at 270 nm was used for the detection of nifurtimox and carbamazepine. The assay was specific for nifurtimox. Coefficients of variation were (within day) 5% at 15 μg mL^{-1}.

Pharmacokinetic calculations and statistical analysis. The perfusate concentration time data for nifurtimox were fitted by a one-compartment model. Areas under the concentration-time curves (AUC) in perfusate were measured using the linear trapezoidal method. A first-order rate constant, k_e, was calculated from the slope of the log-linear decay of the perfusate drug concentration, by linear regression analysis. Hence, a half-life ($t_{\frac{1}{2}}$) was calculated from $0{\cdot}639/k_e$. Clearance (CL) was calculated using the formula:

$$CL = \frac{Dose}{AUC}$$

The extraction rate was calculated as clearance/flow rate and distribution volume (Vd) as $Dose/(AUC \cdot k_e)$.

Comparison between parameters at different doses were made using one-way analysis of variance.

Results

In all of the experiments a monoexponential decay was found (Fig. 1). At the doses tested, the removal of nifurtimox was virtually complete 90–120 min after initiation of the perfusion. The pharmacokinetic parameters are shown in Table 1. The

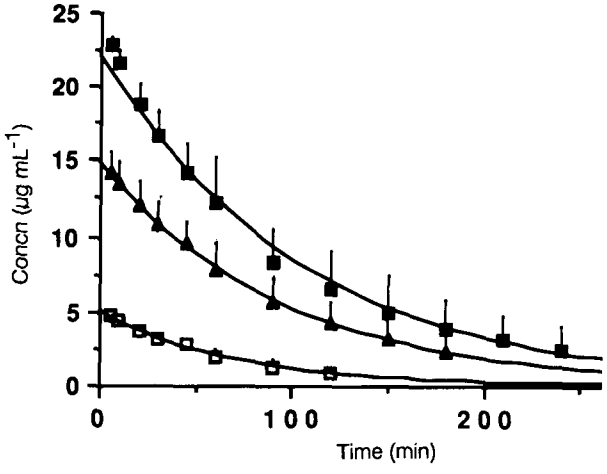

FIG. 1. Mean (±s.d.) concentrations of nifurtimox in perfusate vs time after administration of 5 (□), 15 (▲) and 30 μg mL^{-1} (■). The values represent the mean of five experiments for each concentration.

Table 1. Mean s.d. pharmacokinetics of nifurtimox in rat isolated perfused liver at different concentrations.

	Nifurtimox concn (μg mL^{-1})			
Parameters	5	15	30	*P*
k_e (min^{-1})	0·016 ± 0·004	0·011 ± 0·002	0·011 ± 0·003	NS
AUC (μg min mL^{-1} mg^{-1})	58·9 ± 13·6	95·4 ± 27·3	87·8 ± 24·1	NS
$t_{\frac{1}{2}}$ (min)	46·6 ± 12·1	65·8 ± 16·3	66·8 ± 24·6	NS
CL (mL min^{-1} g^{-1})	0·66 ± 0·19	0·51 ± 0·06	0·34 ± 0·10	< 0·05
Extraction rate	0·128 ± 0·031	0·091 ± 0·023	0·099 ± 0·028	NS
Vd (mL g^{-1})	41·1 ± 6·5	47·3 ± 8·3	30·7 ± 8·4	NS

NS = not significant.

dose-related AUC values were not statistically significant, suggesting first-order kinetics. The $t_{\frac{1}{2}}$ values were also not statistically significant. The CL values were reduced as the dose was increased. However, statistically significant differences were obtained only between 30 $\mu g\,mL^{-1}$ and the other two concentrations. The Vd values for the three doses tested did not exceed the circulating volume, indicating no hepatic drug uptake.

Discussion

In two recent studies performed in healthy volunteers and in patients with chronic renal failure (Paulos et al 1989; González-Martin et al 1992), we reported low serum concentrations of nifurtimox after a single oral dose of 15 $mg\,kg^{-1}$. Because there was not a parenteral dosage form of nifurtimox, it was not possible to know the absolute systemic availability in man. Previously, Duhm et al (1972) showed that radioactive nifurtimox is well absorbed from the gastrointestinal tract in rats. However, nifurtimox is extensively metabolized in animals, and Mendenwald et al (1972) reported that only 0·5% of a single oral dose of nifurtimox was recovered as unchanged drug in the urine of rats and dogs. These findings suggest that nifurtimox undergoes extensive first-pass metabolism in the liver. Hence, we wished to investigate in detail the pharmacokinetics of nifurtimox in the rat isolated perfused liver preparation.

In the present study, a moderate variability in the perfusate concentrations of nifurtimox of the individual preparations was found. Previous study with this compound in man also shows marked data variation. These findings suggest that metabolism of nifurtimox is under genetic control and when metabolism is a major route of elimination, interindividual variability in the pharmacokinetics of the drug can be wide (Rowland 1989). In our study, we used outbred strains of rat and this could explain the large variability found in the pharmacokinetic parameters.

We also demonstrated that the extraction rate in the rat liver was small in relation to the total liver perfusate flow. Therefore, the hepatic clearance is essentially independent of flow and it reflects drug metabolizing activity (Wilkinson & Shand 1975). As we described previously (Paulos et al 1989), the low concentration of nifurtimox found could be the result of extensive first-pass metabolism. Nevertheless, in the present study we did not find an important first-pass effect in the rat liver. On the other hand, hepatic clearance of nifurtimox (g liver)$^{-1}$ was slow but the drug was totally metabolized 3 or 4 h after perfusion. These findings indicate the presence of considerable quantities of unidentified metabolites; at least two unidentified and more polar compounds were detected by HPLC. Although there are no reported data on the pharmacokinetics of nifurtimox in laboratory animals, nitrofurazone, a nitrofuran with similar chemical structure to nifurtimox, appears to follow a similar pharmacokinetic pattern (Sorrentino et al 1987). Thus, the clearance of nitrofurazone was slow and ranged between $0{\cdot}55 \pm 0{\cdot}11$ and $0{\cdot}61 \pm 0{\cdot}07$ mL $min^{-1}\,g^{-1}$ and the extraction rate reported was $0{\cdot}18 \pm 0{\cdot}02$. These values were very similar to those found in our study.

At a nifurtimox concentration of 30 $\mu g\,mL^{-1}$ in the perfusate, the hepatic clearance of this drug was significantly decreased with respect to the other concentrations, suggesting that the removal process was operating over its V_{max} at concentrations of 30 $\mu g\,mL^{-1}$,

In conclusion, our study indicates that nifurtimox is a drug with a poor first-pass liver effect, with a slow but complete metabolism. Assuming that the results in rats can be extrapolated to man, the low serum concentrations found in the volunteers could be explained by a decreased absorption or an extensive extrahepatic metabolism. There are many examples of drugs which are extensively metabolized this way. For example, the nitroderivative compounds undergo metabolic processes in the gut by bacterial action (Ilett et al 1990). The reduction of metronidazole in the gastrointestinal tract has been shown by Goldman et al (1986). Nifurtimox with a nitro group in its structure may undergo metabolism in the gut, decreasing the bioavailability.

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References

Bock, M., Gonnert, R., Haberkorn, A. (1969) Studies with Bay 2502 in animals. Bol. Chile. Parasitol. 24: 13–16

Brener, Z. (1979) Present status of chemotherapy and chemoprophylaxis of human trypanosomiasis in the Webster hemisphere. Pharmacol. Ther. 7: 71–90

Docampo, R., Moreno, S. N. J. (1984) Free radical metabolites in the mode of action of chemotherapeutics agents and phagocytic cells on *Trypanosoma cruzi*. Rev. Infec. Dis. 6: 223–238

Duhm, B., Maul, W., Medenwald, H., Patzchke, K., Wagner, L. (1972) Investigation of nifurtimox-S 35 in the rat and the dog. Arzneim. Forsch. 22: 1617–1623

Goldman, P., Koch, R. L., Yeung, T. C., Chrystal, E. J. T., Beaulieu, B. B., McLafferty, M. A., Sudlow, G. (1986) Comparing the reduction of nitroimidazoles in bacteria and mammalian tissues and relating it to biological activity. Biochem. Pharmacol. 35: 43–51

González-Martin, G., Thambo, S., Paulos, C., Vásquez, I., Paredes, J. (1992) The pharmacokinetics of nifurtimox in chronic renal failure. Eur. J. Clin. Pharmacol. 42: 671–674

Governmental Activities Relating to the Use of Animals in Research (1985) Laboratory Animal Welfare: US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training. Ilar News, volume XXVIII, 4: 7–14

Ilett, K., Tee, L., Reeves, P., Minchin, R. (1990) Metabolism of drugs and other xenobiotics in the gut lumen and wall. Pharmacol. Ther. 46: 67–93

Laplume, H., Baroussè, A., Cabrera, H. (1982) Efectos indeseables de nifurtimox y benzmidazol. Medicina (Buenos Aires) 42: 55–58

Medenwald, H., Brandau, K., Schlossmann, K. (1972) Quantitative determination of nifurtimox in body fluids of rat, dog and man. Arzneim. Forsch. 22: 1613–1616

Paulos, C., Paredes, J., Vásquez, I., Thambo, S., González-Martin, G. (1989) Pharmacokinetics of a nitrofuran compound, nifurtimox, in healthy volunteers. Int. J. Clin. Pharmacol. Ther. Toxicol. 27: 454–457

Rowland, M. (1989) Variability. In: Rowland, M., Tozer, T. N. (eds) Clinical Pharmacokinetics: Concepts and Applications. 2nd edn, Lea and Febiger, Philadelphia, pp 197–212

Sorrentino, D., Bode, W., Hoener, B. (1987) Nitrofurazone disposition by perfused rat liver. Effect of dose size and glutathione depletion. Biochem. Pharmacol. 36: 915–918

Webster, L. T. (1985) Drugs used in the chemotherapy of protozoal infections. In: Gilman, A., Goodman, L. S., Rall, T. W., Murad, F. (eds) The Pharmacological Basis of Therapeutics. 7th edn, Macmillan, New York, pp 1060–1078

Wilkinson, G. R., Shand, D. G. (1975) A physiological approach to hepatic drug clearance. Clin. Pharmacol. Ther. 18: 377–390