EUROPEAN JOURNAL OF DRUG METABOLISM AND PHARMACOKINETICS, 1989, Vol. 14, No. 4, pp. 299 - 302

Pharmacokinetics of atracurium besylate in the pig after a single i.v. injection

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Received for publication : July 13, 1989

Keywords : Atracurium, neuromuscular relaxants, pharmacokinetic, pig

SUMMARY

A pharmacokinetic study of atracurium besylate was performed in the pig after a single i.v. bolus injection of 2 mg/kg, the dose needed to produce surgical neuromuscular blockade. The plasma concentration values were obtained by high performance liquid chromatography. Using a two-compartment pharmacokinetic model, the elimination half life was found to be 28.6 ± 6.3 min (mean ± SEM), the total volume of distribution 341 ± 56 ml/kg and the plasma clearance 8.7 ± 1.1 ml/min/kg. Although the doses required to obtain a satisfactory neuromuscular blockade as well as the plasma level, volume of distribution and plasma clearance values were higher in the pig than in man, the distribution and elimination half-lives were similar to those recently reported.

INTRODUCTION

Atracurium besylate (2,2'–(3,11–dioxo–4,10–dioxatridecylene)–bis–[6,7–dimethoxy–1–(3,4–dimethoxybenzyl)–2–methyl–1,2,3,4–tetrahydroquinolinium]) is a potent nondepolarizing neuromuscular blocking agent with an intermediate duration of action (1) which is degraded by two nonoxidative processes. It undergoes degradation in plasma by a spontaneous mechanism, the Hofmann elimination, as well as by a chemical or enzymatic ester hydrolysis due to the chemical structure of atracurium which contains an ester group. The plasma clearance of atracurium is therefore not dependent on hepatic and renal function (2). The recovery of neuromuscular function following an i.v. bolus of atracurium is thus controlled by the pharmacokinetic elimination (β-phase) half-life, rather than the redistribution (α-phase) half-life (3).

The anaesthetized pig was proposed by Muir and Marshall (4) as an additional model for new nondepolarizing neuromuscular blocking agents. This model was found to have better cardiovascular and neuromuscular stability than the cat. The time course and potency values of these agents in pig, agreed well with those reported for man and suggest that the pig may provide more reliable time course data than the anaesthetized cat (4). Recently, Motsch et al. showed that the influence of hepatic uptake and distribution on the neuromuscular effects of muscle relaxants in the pig may be used to predict the time course of the neuromuscular blockade since the cardiovascular and liver enzyme system of the two species (pig and man) are similar (5).

The aim of the present study was to establish the plasma concentrations of atracurium besylate in pig as a function of time and to determine its major pharmacokinetic parameters after a single i.v. bolus dose and to compare the values obtained with those reported for humans.

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MATERIALS AND METHODS

Animals

Five pigs weighing 23.5 to 38kg were premedicated with azaperon 4 mg/kg, ketamine 7.5 mg/kg and fentanyl 4 µg/kg i.m. and anaesthetized with isoflurane 2–3% in oxygen. The trachea was intubated without the use of a muscle relaxant and anaesthesia was maintained with isoflurane (0.5% in oxygen) and fentanyl (2 µg/kg/h). Saline solution was infused intravenously through the jugular vein at 6 ml/kg/h. Ventilation was controlled during the entire anaesthesia procedure to keep end-tidal pCO_2 at 30–40mm Hg and body temperature was maintained at 35–37°C with thermoblankets. Arterial pH was maintained within the range of 7.30–7.50 by means of an $NaHCO_3$ infusion, if necessary. Heart rate (HR), mean systemic arterial pressure (MAP) and central venous pressure (CVP) were measured using a carotid arterial cannula and a central venous catheter connected to calibrated quartz pressure transducers (1290 A Hewlett-Packard), positioned at the mid-axillary line, and recorded every 15 minutes with a chart recorder (Hewlett-Packard 78172A). In a previous study, we found that the ED_{95} value of atracurium in pigs was 2.0 ± 0.2 mg/kg (6). After a stable anaesthetic level was obtained, a single i.v. bolus injection of atracurium (2 mg/kg) was administered and blood samples for atracurium levels were drawn prior to and at 2, 5, 7, 10, 15, 30, 45 and 90 min after injection. Table I gives the animal weights and the doses of atracurium administered.

Sample preparation

Stabilisation of plasma samples

Aliquots of whole pig blood (1 ml) were collected into heparinized tubes and immediately centrifuged at 1000 *g* for 30 s, and 200 µl of plasma was transferred to an Eppendorf tube containing 800 µl of 0.015 M sulphuric acid. Each tube was quickly frozen to –70°C in a mixture of acetone and solid carbon dioxide and stored at this temperature until the contents were analyzed, usually no more than 5 days later.

Table I : Weight of animals and doses of atracurium besylate administered

No.	*Weight (kg)*	*Dose of atracurium (mg)*
1	23.5	47
2	29	58
3	25	50
4	38	76
5	28	56

Analytical procedure

Samples were spiked with 20 µl of D-tubocurarine solution (100 µg/ml) which was used as internal standard, extracted with Bond Elut Phenyl cartridge and measured by high performance liquid chromatography with fluorescence detection as previously described (7).

HPLC was carried out using a Spherisorb S 5 CN 250 x 4 mm (Knauer) column linked to a fluorescence detector at 280 nm for excitation and 230 nm for emission. The chromatographic system was used at room temperature (22.0 ± 0.5 °C) The mobile phase consisted of acetonitrile: 0.02 M sulphuric acid containing sodium sulphate 6 g/l (60:40 v/v). The flow rate was 1.5 ml/min (7). The retention times of internal standard (D-tubocurarine) and atracurium were 3.6 and 6.8 min, respectively.

Recovery

Extraction recovery of D-tubocurarine was estimated by comparing HPLC peak areas obtained with unextracted standards with standards extracted from control plasma with Bond Elut Phenyl cartridges. This recovery value was found to be 97 ± 2%.

Drugs

Atracurium was supplied by the Wellcome Foundation (Dartford, UK) and D-tubocurarine and all other chemicals and solvents were supplied by Fluka (Buchs, Switzerland) and were of analytical grade.

Pharmacokinetic analysis

The experimental data for plasma concentration was treated using a two-compartment kinetic model described by Greenblatt and Koch-Weser (8). Calculation of the values obtained for the pharmacokinetic parameter governing the proposed model was made by non-linear regression using an Enzfitter non-linear regression data analysis program for the IBM PC.

Statistical analysis

For each pharmacokinetic parameter, the mean and the standard error of the mean (SEM) were calculated. Comparison with human data was made using an

Table II : Pharmacokinetic parameters for atracurium (mean ± SEM) in human and pig

	Humans (*n* = 6)	*Pigs* (*n* = 5)	
Dose (mg/kg)	0.31	2	
$T_{1/2}\alpha$ (min)	2.27 ± 0.32	2.99 ± 0.41	ns
$T_{1/2}\beta$ (min)	19.3 ± 0.9	28.6 ± 6.3	ns
Cl (ml/min/kg)	5.5 ± 0.3	8.7 ± 1.1	*
V_1 (ml/kg)	55 ± 8	127 ± 18	*
V_{area} (ml/kg)	153 ± 13	341 ± 56	*

* Significant difference between two groups using unpaired Student's t-test $P < 0.05$

unpaired Student's t-test. For all statistical comparisons, differences were considered significant if P value < 0.05.

RESULTS

Rectal temperature was maintained in a narrow range during the entire investigation (35.8 ± 0.2 °C). Similarly arterial pH remained stable at 7.44 ± 0.01. Systemic haemodynamic values remained within 20% of baseline values. Mean values of heart rate, systemic arterial and central venous pressures were respectively 142 ± 12 beats/min, 97 ± 12 mm Hg and 4 ± 1 mm Hg.

Figure 1 shows the atracurium plasma levels after administration of a single i.v. bolus dose of 2 mg/kg (ED_{95}). The kinetic parameters could be approximated by bi-exponential equations and atracurium was eliminated with a half-life ($t_{1/2}\beta$) of 28.6 ± 6.3 min.

The pharmacokinetic parameters were determined for each animal, and the mean and standard errors of the mean were calculated. Table II shows the calculated values of the main kinetic parameters. The distribution ($T_{1/2}\alpha$) and elimination ($T_{1/2}\beta$) half-lives were 2.99 ± 0.41 min and 28.6 ± 6.3 min respectively. The total area under the curve (AUC) was 243 ± 27 µg/ml/min, the total clearance (Cl) 8.7 ± 1.1 ml/min/kg and the drug concentration at time zero (C_o) was obtained by extrapolation to the ordinate (16.7 ± 1.7 µg/ml). The total volume of distribution (V_{area}) was 341 ± 56 ml/kg.

DISCUSSION

This pharmacokinetic study of atracurium besylate in the pig was carried out under conditions (intravenous dose of 2 mg/kg) known to produce a surgical neuromuscular blockade.

The previous HPLC techniques used to measure plasma levels of atracurium required gradient elution at 60°C during the whole assay (11, 12). Since the temperature of the column has to be maintained over a very narrow range to obtain good reproducibility and the gradient elution system requires an equilibration period, we developed a simplified method than can be performed at room temperature with an isocratic solvent medium (7) that was used in the present study. The coefficient of variation obtained with this method was less than 3.5% and the recovery was at least 95% in all cases. This technique makes possible a simple and more rapid determination of atracurium in plasma samples.

As demonstrated by Merrett et al. (13), the

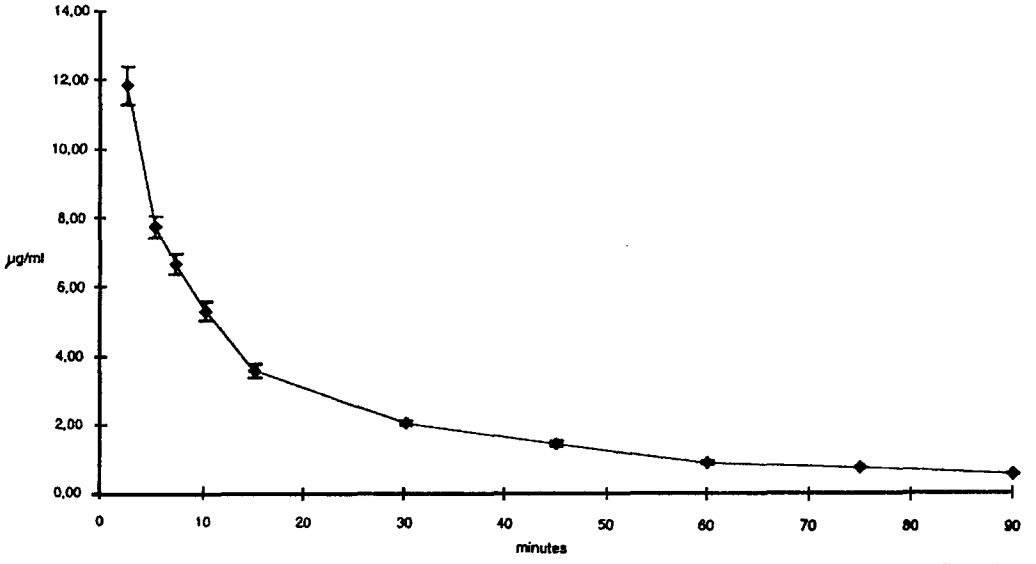

Fig. 1 : Mean atracurium plasma concentrations (µg/ml) against time after i.v. bolus doses of 2 mg/kg each to five pigs

atracurium degradation rate is influenced by the pH and temperature of the plasma. For this reason, these were maintained within a narrow range around the physiological values. The haemodynamic values remained stable during the entire anaesthesia procedure.

To calculate the pharmacokinetic parameter values, we used the two-compartment open model described by Greenblatt and Koch-Weser (8) and used by Ward and colleagues (9) for the pharmacokinetics of atracurium in humans. In this model, all elimination occurs in the central compartment. The non-enzymatic breakdown of atracurium however, occurs mainly by Hofmann elimination and this reaction is not predominantly confined to the central compartment. Atracurium pharmacokinetics are, in fact, better represented by a two-compartment open model with two microkinetic elimination rate constants from the central and the peripheral compartments. However, since with this model it is not possible to determine the microkinetic parameters, the values for clearance and total volume of distribution were derived directly from the macrokinetic parameters (A, α, B, β) using the area under the curve.

In the present study, the pharmacokinetics of atracurium was determined following a single i.v. bolus dose and the results showed an elimination half-life of 28.6 min, not significantly different to that found in man (9). The total clearance, however, of 8.71 ml/min/kg was significantly greater than in man. The large total volume of distribution (V_{area}) of 341 ml/kg implies that atracurium is extensively bound to proteins. This was confirmed by the study of Foldes and Deery (10) in which the plasma protein binding of atracurium was reported to be about 80%. The finding of a previous study (6) showed that the potency of atracurium was approximately 7 times lower in pig than in man. For this reason, correspondingly larger doses were administered to pigs in the present study. Despite this great difference in the dose, the kinetics of atracurium in pig are not very different from those reported for man. Nevertheless, the values obtained for clearance and distribution volume were found to be significantly greater in pigs than in man.

The unusual design of atracurium, which consists of a non-enzymic breakdown under physiological conditions by Hofmann elimination, confers the advantage of not depending on hepatic and renal function for elimination. Since the two parameters governing the elimination of atracurium are pH and temperature and since these physiological values are similar in man and pig, it would be expected that the elimination half-lives would be similar as well. The results of the present study showed, in fact, that the difference between the elimination half-life of atracurium in pig and in man was not significant.

The present results indicate that the anaesthetized pig described by Muir and Marshall as a good model to determine the time course and the potency of neuromuscular blocking agents like vecuronium, pancuronium, ORG 6368 (a new non-depolarizing neuromuscular blocking agent) and suxamethonium, is not valid for the determination of the potency of atracurium, but can be considered a good model for the evaluation of its time course. The finding of the present study showing that the elimination half-life which predicts the duration of action of neuromuscular relaxants, is similar in pig and in man indicate that results obtained in pig might be useful for human application of atracurium.

REFERENCES

1. Hughes R. (1986) : Atracurium: An overview. Br. J. Anaesth., 58, 2S-5S.
2. Stenlake J.B., Waigh R.D., Urwin J., Dewar G.H., Coker G.G. (1983) : Atracurium: conception and inception. Br. J. Anaesth., 55, 3S-10S.
3. Ward S., Weatherley B.C. (1986) : Pharmacokinetics of atracurium and its metabolites. Br. J. Anaesth., 58, 6S-10S.
4. Muir A.W., Marshall R.J. (1987) : Comparative neuromuscular blocking effects of vecuronium, pancuronium, Org 6368 and suxamethonium in the anaesthetized domestic pig. Br. J. Anaesth., 59, 622-629.
5. Motsch J., Hennis P.J., Zimmermann F.A., Agoston S. (1989) : A model for determining the influence of hepatic uptake of nondepolarizing muscle relaxants in the pig. Anesthesiology, 70, 128-133.
6. Pittet J.F., Tassonyi E., Schopfer C., Morel D.R., Mentha G., Fathi M., Le Coultre C., Steinig D.A., Benakis A. (1990): Plasma concentrations of laudanosine, but not of atracurium, are increased during the anhepatic phase of orthotopic liver transplantation in pigs. Anesthesiology, 72, 145-152.
7. Schopfer C., Benakis A. (1990) : Simplified method for the determination of atracurium and laudanosine in pig plasma by high performance liquid chromatography and fluorimetric detection. J. Chromatogr., 526, 223-227.
8. Greenblatt D.J., Koch-Weser J., (1975) : Clinical pharmacokinetics. N. Engl. J. Med., 293, 702-705.
9. Ward S., Neill E.A.M., Weatherley B.C., Corall I.M. (1983) : Pharmacokinetics of atracurium besylate in healthy patients (after a single i.v. bolus dose). Br. J. Anaesth., 55, 113-118.
10. Foldes F.F., Deery A. (1983) : Protein binding of atracurium and other short-acting neuromuscular blocking agents and their interaction with human cholinesterases. Br. J. Anaesth., 55, 31S-34S.
11. Simmonds R.J. (1985) : Determination of atracurium, laudanosine and related compounds in plasma by high performance liquid chromatography. J. Chromatogr., 343, 431-436.
12. Neill E.A.M., Jones C.R. (1983) : Determination of atracurium besylate in human plasma. J. Chromatogr., 274, 409-412.
13. Merrett R.A., Thompson C.W., Webb F.W. (1983) : In vitro degradation of atracurium in human plasma. Br. J. Anaesth., 55, 61-66.