In Vivo Assessment of Intestinal, Hepatic, and Pulmonary First Pass Metabolism of Propofol in the Rat

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Purpose. The relative contribution of the intestinal mucosa, liver and lung to the *in vivo* disposition of propofol in the rat was investigated.
Methods. Propofol (4.9–5.1 mg · kg^{-1}) was administered to groups of rats (n = 4) via the intra-arterial, intravenous, hepatic portal venous and oral routes. The AUC's of propofol were estimated and the fractions of the administered dose escaping first pass metabolism by the gut wall (f_G), liver (f_H) and lung (f_L) were calculated. In addition, transport experiments were carried out using Caco-2 cell monolayers to rule out the possibility that intestinal permeability is limiting the oral absorption of propofol.
Results. Values for f_G, f_H and f_L were the following: 0.21 ± 0.07, 0.61 ± 0.13, and 0.82 ± 0.09, respectively. The apparent permeability coefficient of propofol across Caco-2 cell monolayers was 24.2 ± 0.3 × 10^{-6} cm · sec^{-1}, which is similar to the apparent permeability coefficient obtained for propranolol (30.7 ± 1.7 × 10^{-6} cm · sec^{-1}), a compound known to easily cross the intestinal epithelial membranes. The formation of propofol glucuronide, a major metabolite of propofol, could not be demonstrated during the flux experiments across the Caco-2 cell monolayers.
Conclusions. The intestinal mucosa is the main site of first pass metabolism following oral administration of propofol in the rat. Intestinal metabolism could therefore also contribute to the systemic clearance of propofol.

KEY WORDS: propofol; in vivo pharmacokinetics; gut wall metabolism; rat Caco-2 cells

INTRODUCTION

The short-acting intravenous anesthetic agent propofol (2,6- diisopropyl-phenol) is extensively metabolized in laboratory animals and man (1,2). Propofol glucuronide is the major metabolite in man (53% of the dose) and in the bile duct cannulated rat (45%) (1,2). Systemic propofol clearance in man is very high and exceeds liver blood flow (3), indicating that extrahepatic tissues contribute to the overall metabolism of propofol. Extrahepatic metabolism of propofol has indeed been shown to occur in patients during coronary bypass surgery (4) and during the anhepatic phase of orthotopic liver transplantation (5).

In an attempt to identify extrahepatic tissues contributing to the metabolism of propofol, the relative contribution of the gut wall, the liver and the lungs to the presystemic elimination of propofol in the rat was evaluated. Propofol blood concentration-time profiles were determined following administration of the anesthetic by a number of different routes, i.e. oral, intravenous, intra-arterial and hepatic portal venous. In addition, Caco-2 cell monolayers were used to demonstrate that intestinal epithelial permeability is not the limiting factor controlling the extent of absorption of propofol following oral administration. This is an important issue to correctly interpret the blood concentration-time profiles of propofol following oral administration.

MATERIALS AND METHODS

Materials

The commercially available i.v. propofol preparation (1% w/v propofol in an aqueous emulsion of 10% w/v soya bean oil, 1.2% egg phosphatide and 2.25% w/v glycerol, Diprivan®, Zeneca) was used in the *in vivo* rat studies. For the experiments with Caco-2 cell monolayers, pure propofol (a gift of Zeneca Pharmaceuticals, Macclesfield, U.K.) was dissolved in incubation medium. HPLC grade acetonitrile was obtained from Labscan (Dublin, Ireland). The media and reagents used to culture and incubate the Caco-2 cells were purchased from Gibco (Life Technologies, Merelbeke, Belgium). All other chemicals and reagents used were of analytical grade.

In Vivo Studies in Rats

Male Wistar rats, weighing 300 to 350 grams, were used (Janssen Pharmaceutica, Beerse, Belgium). The animals were housed in an environmentally controlled room at 20–22°C with a 12-hour light/dark cycle. Food and water were provided ad libitum. All experimental procedures in rats were approved by the University Animal Experimentation Ethics Committee.

Pharmacokinetic studies were carried out in anesthetized rats. After induction of anesthesia with i.p. urethane (1.5 g · kg^{-1} using a 25% solution in normal saline), a polyethylene cannula (PE-50) was placed in the left carotid artery in a first group of 4 rats for intra-arterial (i.a.) propofol administration. A second group of 4 rats was fitted with a PE-50 cannula in the right jugular vein for intravenous (i.v.) administration of propofol. A third group of 4 rats received propofol directly into the hepatic portal vein (h.p.v.) using a 26 G infusion set (Abbott, North Chicago, IL, USA). Finally, propofol was dosed orally by direct gavage into the stomach (p.o.) of the last group of 4 rats. All rats were fitted with a PE-50 cannula in the left femoral artery for blood sampling. It was shown in preliminary studies that no appreciable loss of propofol occurred due to adsorption to the infusion cannulae when the commercial preparation of propolol was administered. Two hours after induction of anesthesia, propofol was slowly (over 6 minutes) administered via i.a., i.v. and h.p.v. routes and arterial blood samples (200 μl) were obtained at the following times: 0 (pre-dose control), 1.5, 4, 6, 10, 15, 30, 45, 60, 90, and 120 minutes. Following oral administration arterial blood was sampled at the following times: 0, 5, 10, 15, 20, 30, 45, 60, 75, 90, and 120 minutes. All blood samples were collected in heparinized tubes and stored at 4°C until analyzed (within 24 hours), The administered propofol dose varied between 4.9 and 5.1 mg · kg^{-1} for all routes of administration.

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Cell Culture

The Caco-2 cell line, originating from a human colon carcinoma, was generously provided by Dr. Y. Schneider (Laboratory of Cellular Chemistry, Université Catholique de Louvain, Belgium). Cells were used between passage 110 and 130. Caco-2 cells were cultured as described before (6,7). For transport experiments, cells were seeded into inserts (25 mm diameter, Anapore^R membrane, Nunc, Naperville, IL) and the medium was changed the day after the seeding and every other day thereafter. Apical and basolateral chamber volumes were maintained at 2 ml and the experiments were conducted 17 days after the seeding process.

The integrity of the monolayers was monitored by measuring the transepithelial electrical resistance (TEER) using an Endohm Tissue Resistance Measurement Chamber (WPI, Aston, England). Sodium fluorescein was used as a hydrophilic marker compound (leakage marker) (8).

Transepithelial Flux Experiments

The transepithelial flux across Caco-2 cells was determined for propofol and propranolol (a compound which easily crosses epithelial membranes). The polarized monolayers, after being washed extensively with Hank's balanced salt solution supplemented with 10 mM HEPES and 25 mM glucose (transport medium pH 7.4), were preincubated in the same medium for 30 min at 37°C. At the end of the preincubation, TEER was measured to check the integrity of the monolayer. Only monolayers having a resistance above 250 $\Omega \cdot cm^2$ were used in the study. The medium was removed and the experiment initiated by adding 2 ml transport medium containing 100 μM (25.9 $\mu g \cdot ml^{-1}$) propranolol or 100 μM (17.8 $\mu g \cdot ml^{-1}$) propofol to the apical side of the monolayers, and 2 ml of blank transport medium to the basolateral side. Preliminary studies showed that adsorption of propofol to the inserts, under the same conditions as those used during the transport experiments, was negligible. To maintain sink conditions during the flux experiments, the inserts were removed and placed in fresh 6-well plates containing 2 ml of transport medium after 15, 30, 45 and 60 minutes. A 1 ml sample was taken from the basolateral chamber at each of these time points, and from both the apical and basolateral chamber at the end of the experiment (i.e. at 60 min). Transport was expressed as the percentage of the concentration of the compound of interest in the basolateral compartment at the end of the experiment to the initial concentration in the donor (apical) compartment.

Analytical Methods

Propofol concentrations in blood and transport medium were determined using the HPLC-fluorometric method described by Plummer (9), having a sensitivity limit of 10 ng $\cdot ml^{-1}$. In addition, transport medium samples obtained at the end of the flux experiments were analyzed by using a direct HPLC-UV method for propofol glucuronide (10). Unlike propofol itself, propofol glucuronide does not fluoresce and the sensitivity limit of this assay is only approximately 5 $\mu g.ml^{-1}$. Propranolol concentrations in transport medium were also measured by HPLC. The system consisted of an autosampler injector (Waters 717), a pump (Waters 600 controller pump) and a UV detector (Waters Lambda-Max LC 480). Fifty microliters of the sample were injected directly on the column (Radial-Pak Cartridge Type 8NV C18, 8 mm ID, 4 μ particle size). The mobile phase (acetonitrile:0.02 M phosphate buffer pH 2.5, 50:50, v/v) was delivered through the system at a flow rate of 1 ml $\cdot min^{-1}$. The eluate was monitored at 288 nm. Under these conditions propranolol had a retention time of 4.6 minutes. Linear calibration curves were obtained between 0.6 and 20.0 μM.

Data Analysis

The area under the propofol blood concentration-time profiles (AUC) was calculated according to the linear trapezoidal rule with extrapolation from the last measured concentration to infinity. The fractions of the administered dose escaping first-pass metabolism by the gastrointestinal mucosa (f_G), liver (f_H) and lung (f_L) were calculated as follows (11):

$$f_G = \frac{AUC_{po}}{AUC_{hpv}}, \qquad f_H = \frac{AUC_{hpv}}{AUC_{iv}}, \qquad f_L = \frac{AUC_{iv}}{AUC_{ia}},$$

where AUC_{ia}, AUC_{iv}, AUC_{po} and AUC_{hpv} represent the area under the propofol blood concentration-time curve between 0 and infinity following i.a., i.v., p.o. and h.p.v. administration of propofol, respectively. In addition, following i.a. administration systemic clearance (CL), steady state distribution volume (Vss) and elimination half-life (based on the terminal linear portion of the semilog blood concentration-time profile) of propofol were calculated by noncompartmental methods.

From the results of the transepithelial flux experiments the apparent permeability coefficient (P_{app}, $cm.sec^{-1}$) was calculated according to the following equation (7):

$$P_{app} = \frac{\Delta Q}{\Delta t.60.A.C_0}$$

where $\Delta Q/\Delta t$ is the permeability rate ($\mu g \cdot min^{-1}$), A is the surface area of the membrane (4.91 cm^2) and C_0 is the initial concentration ($\mu g \cdot ml^{-1}$) of the compound in the donor chamber.

Results in the text and tables are expressed as mean ± SD. Data were analyzed using the one-way ANOVA-Bonferroni test. A P-value of 0.05 or less was considered significant.

RESULTS

After i.a. administration the following pharmacokinetic parameters were obtained for propofol: CL, 81.7 ± 5.2 ml $\cdot min^{-1} \cdot kg^{-1}$; Vss, 2.3 ± 0.8 L $\cdot kg^{-1}$; t1/2, 66.6 ± 25.4 min. The individual blood concentration-time profiles of propofol following its administration by each of the 4 routes are shown in figure 1. Propofol blood concentration-time profiles following i.v. administration were similar as those obtained following i.a. administration. Following hepatic portal venous and especially following oral administration, propofol blood concentration-time profiles were much lower as compared with i.a. administration. One-way ANOVA demonstrated a statistically significant decrease in the AUC_{po} and AUC_{hpv} as compared to AUC_{ia} (Table I). AUC_{po}, AUC_{hpv}, and AUC_{iv} represent approximately 10%, 50% and 82%, respectively, of AUC_{ia}. The fractions of the administered dose escaping first-pass metabolism by the gastrointestinal mucosa, liver and lung were as follows: 0.21 ± 0.07, 0.61 ± 0.13, and 0.82 ± 0.09, respectively.

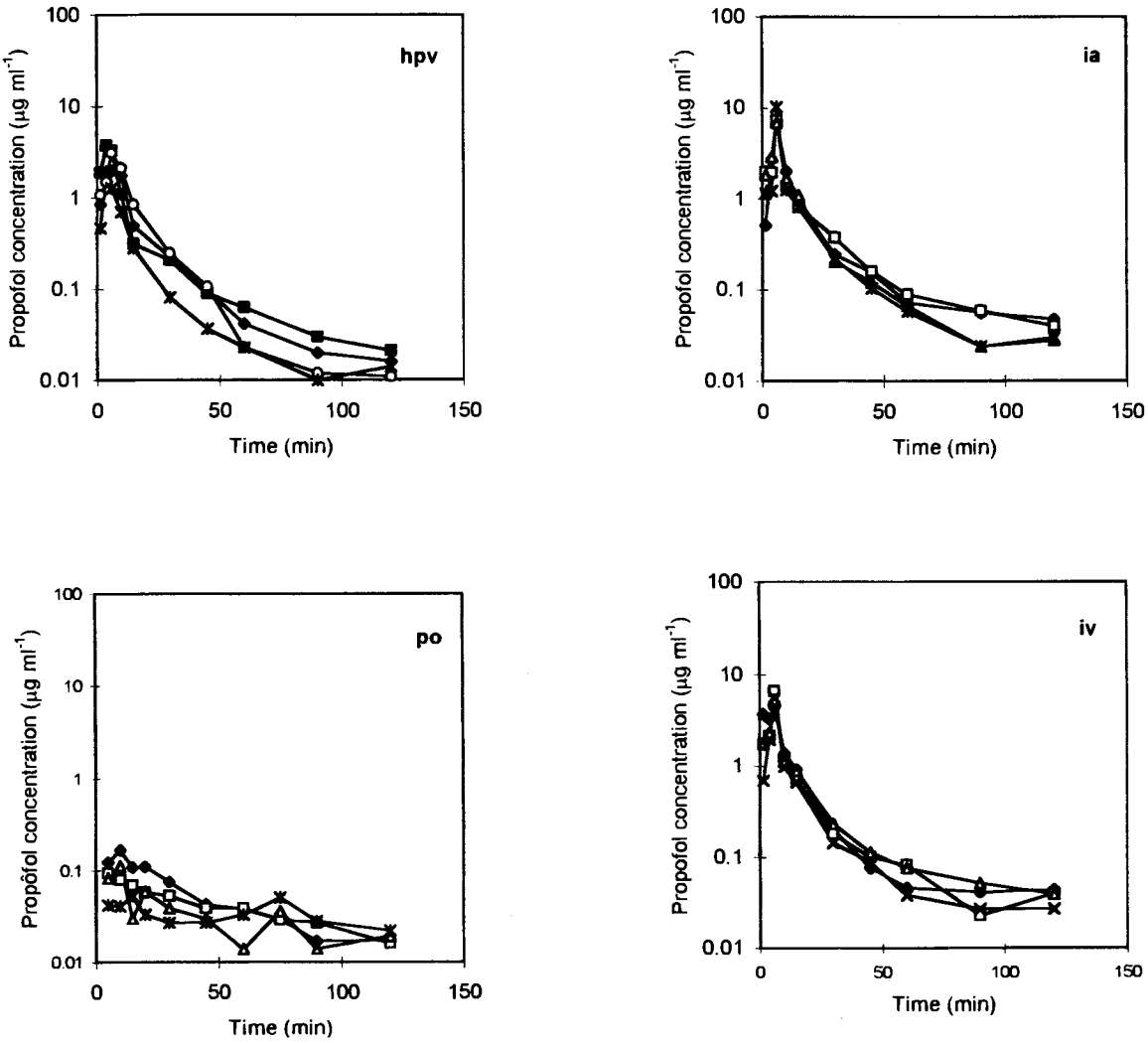

Fig. 1. Blood concentration-time profiles for propofol in individual rats following intra-arterial (i.a.), intravenous (i.v.), hepatic portal venous (h.p.v.), and oral (p.o.) administration of propofol.

The average TEER value for the Caco-2 cell monolayers was 490.3 ± 90.9 ohms · cm^2 at the start of the flux experiments (n = 8). The apical to basolateral permeation rates across the Caco-2 cell monolayers for propofol and propranolol were linear as a function of time (fig. 2). The apparent permeability coefficient of propofol was 24.2 ± 0.3 × 10^{-6} cm · sec^{-1} (n

Table I. Area Under Blood Concentration-time Curves for Propofol (AUC) Following Administration of Propofol by a Number of Different Routes

Route	AUC μg · min · ml^{-1} · kg
i.a. (n = 4)	19.90 ± 0.48[a]
i.v. (n = 4)	16.37 ± 1.25[b]
h.p.v. (n = 4)	10.06 ± 1.40[c]
p.o. (n = 4)	1.94 ± 0.19

[a] Different from h.p.v. ($p < 0.01$) and p.o. ($p < 0.001$).
[b] Different from h.p.v. ($p < 0.05$) and p.o. ($p < 0.001$).
[c] Different from p.o. ($p < 0.01$).

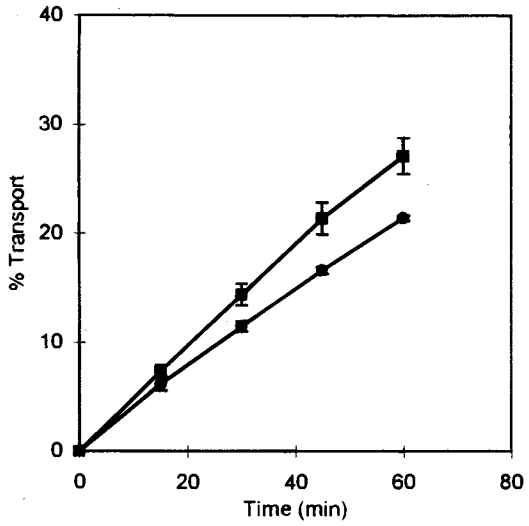

Fig. 2. Apical to basolateral transport across Caco-2 cell monolayers as a function of time for propofol (●) and propranolol (■) (n = 4).

= 4) which is 79% of the permeability coefficient obtained for propranolol ($30.7 \pm 1.7 \times 10^{-6}$ cm · sec^{-1}, n = 4). The apparent permeability coefficient of sodium fluorescein, determined at the end of each series of transepithelial flux experiments, was $0.72 \pm 0.34 \times 10^{-6}$ cm · sec^{-1} indicating that the Caco-2 cell monolayers were intact. No propofol glucuronide could be detected by HPLC analysis in the transport medium (both basolateral and apical sides) at the end of the flux experiments.

DISCUSSION

Although results of several clinical studies indicate that propofol undergoes extrahepatic metabolism in man (3–5) the organs involved have not been identified. We have recently demonstrated *in vitro* that the human intestinal mucosa and kidney possess a significant capacity to glucuronidate propofol (10). However, a simple extrapolation of these *in vitro* data to the *in vivo* situation is not possible. Factors which are not important for *in vitro* glucuronidation, such as the availability of the co-factor UDPGA, may become rate limiting *in vivo* because its supply is limited in certain extrahepatic organs (12).

Since glucuronidation is a major metabolic pathway of propofol in both man and rat, we chose to carry out a series of *in vivo* studies in the rat to evaluate the relative contribution of the intestinal mucosa, the liver and the lung to the presystemic metabolism of this anesthetic agent. However, because of the low sensitivity of the HPLC assay no propofol glucuronide could be demonstrated in the blood samples of the *in vivo* studies. It can therefore not be excluded that metabolic pathways other than glucuronidation may also have been involved in the elimination of propofol. The results demonstrate that propofol undergoes a large first pass effect when administered orally to rats: only approximately 10% of the administered dose appears unchanged in the systemic circulation. This presystemic elimination is mainly the result of propofol extraction by the intestinal mucosa. The liver and lungs also contribute but to a much lesser extent. These results are similar to the ones reported by Cassidy and Houston concerning the extrahepatic conjugative metabolism of phenol in the rat (11). They found that the hepatic enzymes only marginally contributed to the first pass effect of phenol. Intestinal and to a smaller extent pulmonary enzymes were mainly responsible for the first pass effect of phenol at the dose (1.5 mg · $kg^{-}{}_{1}$) studied.

The much smaller AUC_{po} compared to AUC_{hpv}, AUC_{iv} and AUC_{ia} could also be explained by an incomplete or slow diffusion of propofol across the intestinal epithelial wall. To exclude this possibility, *in vitro* studies were carried out using Caco-2 cell monolayers to demonstrate the ability of propofol to cross the intestinal mucosa. The results show that propofol readily crosses the Caco-2 cell monolayer with an apparent permeability coefficient similar to the one obtained for propranolol, a compound known to readily cross the epithelial membranes of the gastrointestinal tract of man and rat (13). Although the Caco-2 cell line is derived from human colon cells, good correlations have been observed for small organic molecules between the permeabilities obtained from everted rat intestinal ring uptake, in situ rat intestinal perfusion and Caco-2 cell monolayers (7,14). The good permeability of propofol across Caco-2 cell monolayers suggests that transport of this lipophilic anesthetic across the intestinal epithelium of the rat is not a limiting factor for its absorption following oral administration. It is therefore very likely that the small AUC_{po} of propofol is not due to incomplete intestinal absorption but rather to significant extraction of the anesthetic by the intestinal mucosa. Caco-2 cells have also been used to explore gut wall metabolism. Although previous reports have demonstrated that Caco-2 cells were capable of forming glucuronide conjugates of p-nitrophenol (15) and methyldopa (16), we were not able to show formation of propofol glucuronide during propofol diffusion across the Caco-2 cell monolayer, possibly because of the low sensitivity of the HPLC method used to quantify propofol glucuronide. In addition, Caco-2 is a human colon adenocarcinoma cell line and it is likely that certain enzyme systems may be less active in Caco-2 cells as compared to the enterocytes of the small intestine.

In conclusion, using *in vivo* studies we have demonstrated that the intestinal mucosa is the main site of first pass metabolism following oral administration of propofol in the rat. Whether intestinal metabolism contributes to the systemic elimination of propofol (i.e., following intravenous administration) remains to be proven.

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