Effect of Isoflurane Anesthesia on Antipyrine Pharmacokinetics in the Rat

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INTRODUCTION

The selection of a suitable anesthetic is a subject of importance to researchers engaged in performing surgical procedures on laboratory animals. In pharmacokinetic studies, the optimal anesthetic must not only provide a rapid onset and desired duration of anesthesia, but also have a minimal effect on the normal physiology and the metabolic and excretory functions of the organism. In a recent report in this journal, Gumbleton and Benet (1) examined the influence of several laboratory anesthetic protocols upon the disposition of antipyrine, which was used as an indicator of intrinsic hepatic drug metabolism, in the rat. These authors found that antipyrine clearance in rats anesthetized with pentobarbital, urethane, and ketamine/xylazine was significantly reduced compared with that in a conscious group. As these tested compounds are all metabolized by the microsomal mixed-function oxidase system, it was suggested that they may interact with the membranes of the hepatic endoplasmic reticulum and influence the metabolism of other drugs.

Not discussed in the above paper is the administration of anesthetics via the inhalation route. Isoflurane, 1-chloro-2,2,2-trifluoroethyl difluoromethyl ether, is an agent in this class which has been commonly applied to a broad spectrum of laboratory species. Because of its low solubility in blood (2), isoflurane is not widely distributed to tissues. It undergoes virtually no metabolism (3) and is largely (95% of dose) expired unchanged (4), thus allowing a rapid recovery from anesthesia. Myocardial function is well maintained with isoflurane (5), and cardiac output is unaffected (6,7). All of these characteristics suggest a minimal influence of isoflurane on the metabolism and disposition of other drugs.

The objective of the present investigation is to compare the pharmacokinetics of antipyrine in isoflurane-anesthetized rats with that in conscious animals. The anesthetic regimen of ketamine/xylazine, which showed the greatest influence in the study by Gumbleton and Benet (1), was also included as a positive control. Further, the potential effect of the duration of anesthesia on drug metabolism was also examined.

MATERIALS AND METHODS

Male Sprague–Dawley rats (Charles River, CD) weighing (mean ± SD) 270 ± 18 g were used. The rats (n = 6 for each experimental group) were housed in standard rodent cages and had access to food (Purina Rodent Chow) and water. The dose of antipyrine (20 mg/kg) was prepared as a 20 mg/ml solution in 0.9% sodium chloride (Kendall McGaw Laboratories, Irvine, CA) and injected into the jugular vein exposed surgically under anesthesia (Groups 1–4). The skin incision was closed with suture after dosing. Throughout the study, anesthetized rats were kept warm using a heat lamp in order to avoid hypothermia. For rats implanted with a jugular vein catheter (Group 5), the dose was injected via the catheter.

The following anesthetic regimens were used in the five groups.

1. Isoflurane anesthetized. Anesthesia was induced with 5% vaporized isoflurane (Forane, Anaquest, Madison, WI) in oxygen (v/v) and maintained using 1.5% isoflurane in oxygen (v/v) throughout a 4-hr study period. The procedure was performed using a Vetroson nonrebreathing anesthesia machine (Summit Hill Laboratories, Navesink, NJ).
2. Ketamine/xylazine anesthetized. Ketamine (Ketaset, Aveco Co., Fort Dodge, IA), 80 mg/kg i.p., was given in combination with xylazine (Rompun, Mobay Corp. Animal Health Division, Shawnee, KS), 13 mg/kg i.m., for induction of anesthesia. Subsequent doses of ketamine (15 mg/kg i.p.) were administered every 50 min to maintain anesthesia throughout the 4-hr study period.
3. Transient isoflurane. Rats were anesthetized with vaporized isoflurane (5% in oxygen) but were allowed to regain consciousness after antipyrine dosing.
4. Transient ketamine/xylazine. Rats were anesthetized using ketamine (80 mg/kg i.p.) and xylazine (13 mg/kg i.m.) but were allowed to regain consciousness after antipyrine administration. No maintenance dose of ketamine was given.
5. Chronically catheterized conscious animals. To enable implantation of a jugular vein catheter, rats were anesthetized with vaporized isoflurane (1.5% in oxygen) for a period not exceeding 15 min. The pharmacokinetic study began 24 hr following completion of the surgical catheterization.

After antipyrine administration, serial blood samples (250 μl) were collected in heparinized micropipettes (Microcaps, Drummond Scientific Co., Broomall, PA) from the tail vein of each rat over a 4-hr period. Plasma was separated immediately by centrifugation and stored frozen until analysis. Plasma antipyrine was determined using a previously published (8) HPLC method, with slight modifications. To each plasma sample (100 μl) was added 20 μg of benzoic acid (in 20 μl of water) as internal standard and 0.4 ml of methanol. After mixing on a vortex mixer for 15 sec, protein was

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precipitated by centrifuging at 1000*g* for 20 min. The supernatant was filtered through a 0.45-μm filter unit (Millex-HV13, Millipore Filter Co., Bedford, MA), and 50 μl of the filtrate was injected onto the chromatograph. The HPLC system consisted of two Beckman 110A pumps, a Beckman 210A sample injection valve, a Beckman 164 variable wavelength ultraviolet detector, a 5-μm C_{18} Adsorbosphere guard column (Alltech Assoc., Inc., Deerfield, IL), a 300 × 3.9-mm-I.D., 10-μm μ-Bondapak C_{18} column (Phenomonex, Ranchos Palos Verdes, CA), and a Spectra-Physics SP4270 integrator (Spectra-Physics, San Jose, CA). The mobile phase was acetonitrile:1% acetic acid in water (35:65, v/v) at a flow rate of 1 ml/min. Ultraviolet absorption was measured at 254 nm. Under these conditions, no interfering peaks resulted from the metabolites of antipyrine or the anesthetic agents used. The reproducibility over a 40-day test period was indicated by coefficients of variation of 5.4% at 3 μg/ml and 0.4% at 50 μg/ml (n = 16). Accuracy was to within 10 and 0.7% at 3 and 50 μg/ml, respectively. The limit of quantification, 3 μg/ml, was considerably lower than the minimum concentration observed in this study.

Pharmacokinetic and Statistical Analysis. The log-transformed, antipyrine plasma concentration–time data were fitted to a one-compartment open model by linear regression. The predicted rate constant (k) was used in the calculation of elimination half-life ($t_{1/2} = 0.693/k$). The area under the plasma concentration–time curve (AUC) was calculated using the trapezoidal rule and extrapolated to infinity, the residual area after the last measurable concentration (C^t) being C^t/k. Total-body clearance (CL = $\text{Dose}_{i.v.}$/AUC) and volume of distribution (V_{area} = CL/k) were also calculated.

Statistical analysis was performed using one-way analysis of variance and Duncan's multiple-range test (9). Results are statistically different at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

The pharmacokinetic parameters of antipyrine in the five groups of rats are summarized in Table I. Rats that were under anesthesia with either ketamine/xylazine or isoflurane throughout the 4-hr study period showed the longest half-life and lowest clearance of antipyrine. Conscious rats given transient exposure to isoflurane were the only group which showed no significant differences from the chronically catheterized, conscious animals in antipyrine clearance or half-life. Antipyrine clearance in the catheterized, conscious group is in excellent agreement with previously reported values for rats undergoing similar treatment (1). The volume of distribution of antipyrine appeared to be larger in the ketamine/xylazine-anesthetized and catheterized, conscious rats than in the remaining groups, although the physiologic significance of this trend is unclear.

The plasma clearance of antipyrine has been shown to reflect the cumulative rate of oxidative formation, conjugation, and renal excretion of antipyrine metabolites as well as the renal excretion of unchanged antipyrine (10). Since antipyrine exhibits little plasma protein binding (11) and a low hepatic extraction ratio (12), changes in antipyrine clearance can be interpreted as changes in intrinsic clearance and not protein binding or hepatic blood flow. The present results suggest that acute anesthetization using isoflurane, for the duration of simple surgical procedures (<15 min), in the rat has little effect on the plasma clearance of antipyrine. The data compare favorably to earlier work conducted using ether as the anesthetic agent (13). Furthermore, it has been reported that exposure for 15 min to isoflurane, halothane, enflurane, or sevoflurane had little effect on the basal bile flow rate in the rat (14), thus minimizing the potential effect of the anesthetic on the hepatobiliary elimination of cholephilic xenobiotics. Among the commonly available inhalation anesthetics, isoflurane is considered the agent of choice due to its high controllability and relative lack of toxicity (15).

An interesting finding in the present study is the influence of the duration of anesthesia on the antipyrine elimination rate. While an acute treatment with isoflurane and ketamine/xylazine resulted in significantly different effects on antipyrine clearance and half-life, the advantage of isoflurane diminished upon prolonged exposure. In rats in which anesthesia was maintained for 4 hr, the two anesthetics caused an equally marked increase in the half-life and decrease in the clearance of antipyrine compared to catheterized, conscious animals. Although the observed effect could be due to anesthesia per se, it was also likely the result of a direct or indirect interaction between antipyrine and the anesthetic agent. Previous investigators showed that after exposure to 1.3% isoflurane for 2 hr, there was a slight but significant increase in aminopyrine half-life in the rat 2 hr after anesthesia, but this effect had disappeared by 24 hr (16).

In summary, this study provides support for the use of isoflurane as an anesthetic for performing short surgical pro-

Table I. Antipyrine Pharmacokinetic Parameters in Conscious and Anesthetized Rats[a]

	(1) Isoflurane anesthetized	(2) Ketamine/xylazine anesthetized	(3) Isoflurane conscious	(4) Ketamine/xylazine conscious	(5) Catheterized conscious	Statistical comparisons[b]
$t_{1/2}$ (min)	289 ± 45	313 ± 137	89 ± 15	194 ± 21	95 ± 7	2 1 4 5 3
CL (ml/min/kg)	1.89 ± 0.26	2.45 ± 1.17	5.77 ± 0.81	2.82 ± 0.35	6.44 ± 0.70	5 3 4 2 1
V_{area} (ml/kg)	773 ± 42	936 ± 97	725 ± 67	780 ± 42	876 ± 74	2 5 4 1 3

[a] Results presented as mean ± SD; n = 6 for each experimental group.

[b] Groups are arranged in the order of descending magnitude for each pharmacokinetic parameter. Groups jointly underlined are not significantly different ($P > 0.05$) from each other. Mean square error (MSE) in Duncan's multiple-range test: $t_{1/2}$, 4330; CL, 0.542; V_{area}, 4581.

cedures associated with pharmacokinetic studies in the rat. The results also demonstrate the importance of conducting pharmacokinetic studies in conscious animals. The metabolism of xenobiotics could be significantly hampered under the influence of anesthesia, regardless of the specific anesthetic agent used in the present study.

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