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# The Pharmacokinetic Interaction of Diethyl Ether with Aminopyrine in the Rat[1] (41767)

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*Abstract.* Our studies in rats clearly demonstrate a significant depression of aminopyrine metabolism *in vivo* by ether anesthesia. The depression of aminopyrine elimination was shown both by measurements of plasma aminopyrine clearance and by depression of the [$^{14}$C]aminopyrine breath test. No apparent effect of ether was seen on aminopyrine volume of distribution. The effect of ether was prolonged, as judged by its persistence in the aminopyrine breath test for 3 hr after stopping ether anesthesia. In addition, when ether was administered in combination with a single dose of ethanol, aminopyrine clearance was inhibited significantly more than with ethanol alone. These data not only have a bearing on proper methodologic design of drug clearance studies but also may relate to the effects of some anesthetics on hepatic function.

Aminopyrine metabolism has been used extensively in the last few years as an index of hepatic mixed function oxidase activity (1). Part of this popularity has emerged from the employment of [*N-dimethyl*-$^{14}$C]aminopyrine as a noninvasive breath test for measuring hepatic function *in vivo* both in experimental animals and in man (1–9). Indeed the sequence of metabolic events involved in the $^{14}CO_2$ exhalation rate (demethylation) has been well characterized (10–12). However useful, the aminopyrine breath test provides only a measure of the drug half-life ($t_{1/2}$) and the latter depends both on the volume of distribution ($V_d$) and clearance ($C$) of the drug, i.e., $t_{1/2}(\beta) = 0.693V_d/C$ (9). Accordingly, it has been suggested that pharmacokinetic assessments based on the breath test be validated initially with conventional aminopyrine clearance measurements (9, 13). In the process of such a validation, in which the effects of ethanol on aminopyrine elimination were being investigated, we encountered a major inhibitory effect of ether anesthesia which was sufficient to confound this data. Although this effect has been previously described for a few other drugs (14–18), we believe that the common usage of aminopyrine as an index drug and ether as an anesthetic agent justify our report of the ether–aminopyrine pharmacokinetic interaction. In addition, we document this potential for confounding anesthetic interactions with ethanol–aminopyrine elimination studies.

**Materials and Methods.** Female, Sprague–Dawley rats weighing about 250 g were used. They were fasted overnight, except for *ad lib* water intake. There were three experimental aminopyrine groups—(a) intravenous aminopyrine set, (b) subcutaneous aminopyrine set, and (c) rats receiving [$^{14}$C]aminopyrine for the breath test.

*(a) Intravenous aminopyrine.* In this group the external jugular vein was cannulated under brief ether anesthesia with a PE 50 polyethylene catheter which was exteriorized on the neck via a subcutaneous tunnel. The animals were allowed to recover from the surgery with *ad lib* food and water intake for 3 days prior to the intravenous aminopyrine study. One group was then anesthetized with ether, until loss of righting reflex, and was placed on a heating blanket regulated to maintain a body temperature of 37°C, monitored by a rectal temperature probe. The other, control, group was placed in a restraining cage. Aminopyrine, 30 mg/kg, was given via the jugular catheter to each group. For blood aminopyrine analysis, 150-$\mu$l aliquots were obtained from the tail vein, spun, and 50-$\mu$l serum samples assayed by HPLC (19).

In one group of rats, the interaction of acutely administered ethanol and ether on aminopyrine elimination was assessed. One set of rats was given ethanol, 4 g/kg by gavage

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(25% v/v in water) 30 min prior to intravenous aminopyrine administration. The ethanol group was divided into two subsets. In one, the study was carried out with ether administration as described above. In the other, no ether was used, as described above for the controls.

*(b) Subcutaneous aminopyrine.* In this study, 30 mg aminopyrine/kg, was given subcutaneously in the back. One group was kept anesthetized with ether throughout the study, with temperature maintained at 37°C as outlined earlier, while the control group was kept unanesthetized in restraining cages. Blood aminopyrine assays were carried out as described above.

To assure less variation of the above data, at 2-week intervals the same rat was utilized for the ether and control studies. The order of selection was randomized.

*(c) Breath test.* For this study, [$^{14}$C]aminopyrine (Amersham Searle Corp.) labeled on both methyl groups was given intraperitoneally in saline as 0.25 ml/200 g. The aminopyrine specific activity was 73.8 mCi/mmole with 250 $\mu$Ci/140 ml saline. One group was anesthetized with ether for 2 hr prior to administration of [$^{14}$C]aminopyrine for the breath test. The ether wore off completely during the first 20–30 min of the 180-min study. Controls received no ether. Each rat was used, 2 weeks apart, for both studies. The order of experimentation was randomized. Rats in both groups were housed in individual airtight restraining cages. Exhaled $^{14}CO_2$ was drawn through concentrated sulfuric acid to remove water and then through a scintillation vial containing 10 ml of a 2:1 (v/v) methanol–ethanolamine mixture to trap all expired $CO_2$. Previous experiments with two vials placed in series demonstrated that all expired $^{14}CO_2$ was trapped in the first vial. All $CO_2$ expired was trapped in 15-min periods over 3 hr. Trapped radioactivity was determined after adding 10 ml A.C.S. (Amersham, Arlington Heights, Illinois) and counting in a liquid scintillation counter, using the automatic external standardization procedure to correct for quenching.

The elimination half-life, $t_{1/2}(\beta)$, for aminopyrine was determined from the terminal portion of the data (0.5–3.0 hr sc and 0.166–3.0 hr iv) by linear regression of the logarithm of the plasma concentration and time. The total area under the curve (AUC) was estimated by the trapezoidal rule with extrapolation to infinity using beta. Aminopyrine plasma clearance ($C$) and volume of distribution $V_d$ were calculated from the equations:

$$C = \frac{\text{Dose}}{\text{AUC}} \qquad V_{d_\beta} = \frac{C}{\beta}$$

For the intravenous aminopyrine studies, the Student's $t$ test was used to compare the ether and control groups. For the subcutaneous aminopyrine and aminopyrine breath test group, wherein each animal served as its own control, a paired $t$ test was used. Significance was accepted as $P < 0.05$, two-tailed.

**Results.** As is shown in Fig. 1, the pharmacokinetics of aminopyrine, given *intravenously,* were strikingly affected by concomitant ether anesthesia. The clearance of the drug was decreased from 10.26 ± 2.39 (mean ± SD) to 4.13 ± 0.68 ml/min/kg and the half-life was prolonged from 46.17 ± 7.89 to 111.98 ± 23.40 min ($P < 0.001$). By contrast, the volume of drug distribution was unaltered by exposure to ether.

Figure 2 indicates very similar data when the aminopyrine was administered subcutaneously to another group of five rats. In this study the control clearance was 10.80 ± 2.88 ml/min/kg and the half-life 49.92 ± 20.45 min, values which are very comparable with intravenous aminopyrine administration. As for the intravenous aminopyrine study, ether

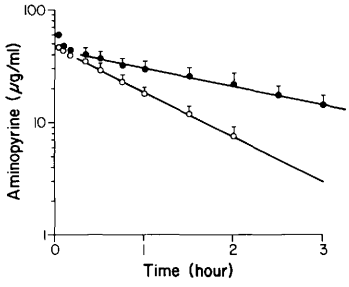


FIG. 1. Effect of ether on the pharmacokinetics of aminopyrine given intravenously. For Control, $t_{1/2}\beta$ (min) = 46.17 ± 7.89 (SD), clearance (ml/min/kg) = 10.26 ± 2.39, and $V_d$ (ml/kg) = 664.89 ± 82.47. For Ether, $t_{1/2}\beta$ (min) = 111.98 ± 23.40, clearance (ml/min/kg) = 4.13 ± 0.68, and $V_d$ (ml/kg) = 649.39 ± 41.72. $N = 7$, $P < 0.001$ for $t_{1/2}\beta$ and clearance values. ●, Ether; ○, control.

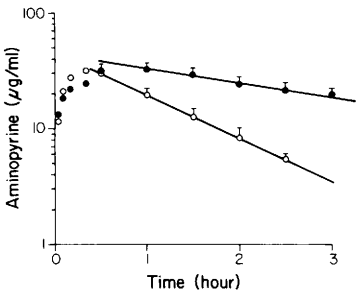


FIG. 2. Effect of ether on the pharmacokinetics of aminopyrine given subcutaneously. For Control, $t_{1/2}\beta$ (min) = 49.92 ± 20.46, clearance (ml/min/kg) = 10.80 ± 2.88, and $V_d$ (ml/kg) = 725.02 ± 124.67. For Ether, $t_{1/2}\beta$ (min) = 142.08 ± 60.93, clearance (ml/min/kg) = 4.03 ± 2.34, and $V_d$ (ml/kg) = 725.02 ± 124.7. $N = 5$. $P < 0.001$ for $t_{1/2}\beta$ and clearance. ●, Ether; ○, control.

resulted in a striking decrease in aminopyrine clearance to 4.03 ± 2.34 ml/min/kg and a prolongation of the half-life to 142.08 ± 60.93 min ($P < 0.01$). The degree of inhibition of drug metabolism was comparable to that seen with intravenous aminopyrine and ether ($P > 0.05$). The volume of distribution was unchanged. In this study, as an additional control, the same rat was used for the control and the ether study, at 2 week intervals, with the order of the study varied at random. The weight of the rats during the first study was 251.6 g and 2 weeks later 260.1 g.

Administration of [$^{14}$C]aminopyrine for measurement of $^{14}CO_2$ exhalation rate confirmed the earlier findings using unlabeled aminopyrine and measurement of the drug in plasma (Fig. 3). The decrease in exhaled $^{14}CO_2$ was evident throughout the 3-hr study, but was especially striking during the first 60 min when it averaged less than 50% of control values. The decreasing inhibitory effect of ether

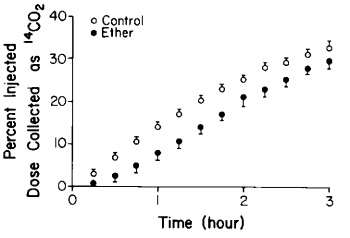


FIG. 3. Effect of ether on the rate of $^{14}CO_2$ expired during the aminopyrine breath test. $P < 0.05$ for each time point. $N = 6$. Symbols used are explained in the illustration.

with time is understandable since ether anesthesia was in effect for only the first 20–30 min of the study. It is, however, important to note that some inhibitory effect persisted for as long as 3 hr. Thus, brief use of ether and subsequent discontinuation of this anesthetic does not guarantee absence of continued depression of hepatic drug metabolism. In these studies, as in the group receiving subcutaneous aminopyrine, the same rat was utilized in random order at a 2-week interval.

As is shown in Table I, acute alcohol administration, without ether, significantly decreased the clearance and prolonged the aminopyrine half-life without affecting the volume of distribution of the drug. Administration of ethanol under ether anesthesia further strikingly depressed the clearance and enhanced the half-life of aminopyrine as compared to animals given ethanol alone ($P < 0.05$). The volume of drug distribution decreased with ethanol and ether administration as compared to control values.

**Discussion.** This study clearly shows that ether anesthesia depresses strikingly aminopyrine demethylation *in vivo* in rats (Figs. 1–3). Our data, therefore, corroborate prior observations that ether anesthesia *in vivo* impairs the oxidative metabolism of antipyrine (14), diphenylhydantoin (18), and possibly of barbiturates (17) as well as the conjugation of acetaminophen (14). The metabolism of ethanol also seems to be inhibited by ether in isolated rat liver parenchymal cells (16). Nonanesthetic exposure to ether, however, appears not to influence aminopyrine kinetics *in vivo* (20). Since aminopyrine is used extensively as a marker drug for hepatic demethylation, it is essential that the possible confounding effects of ether anesthesia in such studies be taken into consideration in any experimental design. This is especially important since, as shown in our [$^{14}$C]aminopyrine breath studies (Fig. 3), the depressive influence of ether on drug metabolism, at least in this system, persists for several hours after recovery from the anesthesia. As might be expected, however, with the passage of time, this prior effect of ether gradually decreases. These potential interaction problems are documented in the ether–ethanol studies (Table I).

Alcohol given acutely is known to inhibit hepatic microsomal oxidation of many drugs,

TABLE I. EFFECT OF ETHER ANESTHESIA AND/OR ETHANOL ON THE PHARMACOKINETICS OF AMINOPYRINE ADMINISTERED INTRAVENOUSLY

| | Half-life ($t_{1/2}$) (min) | Clearance (ml/min/kg) | Volume of distribution (ml/kg) |
|---|---|---|---|
| Control | 46.17 ± 7.89[a] | 10.26 ± 2.39 | 664.89 ± 82.47 |
| Ether | 111.98 ± 23.40[+] | 4.13 ± 0.68[+] | 649.39 ± 41.72 |
| Ethanol | 66.60 ± 14.51[+] | 6.36 ± 0.87[+] | 597.91 ± 60.84 |
| Ethanol + ether | 206.81 ± 53.39[+,*] | 1.95 ± 0.67[+,*] | 543.75 ± 51.43[+] |

[a] ±SD.
[+] $P < 0.05$ as compared to control.
[*] $P < 0.05$ as compared to ethanol alone.

although the mechanism(s) of this effect are still debatable (25). It is not surprising, therefore, that ethanol in this study decreased the clearance of aminopyrine (Table I). It is important to note, however, that ether anesthesia combined with a single dose of ethanol inhibited aminopyrine clearance much more than ethanol alone (Table I). This additive combined effect illustrates the significance of anesthesia in the design of an experimental study, in this case, the effects of ethanol on aminopyrine elimination. In addition, it confirms in an *in vivo* system, the observations of Aune *et al.* (16) carried out in isolated rat hepatocytes. Unfortunately in attempting to control for such ether effects by exposing the control group to ether alone it is difficult to predict the interaction of the two drugs, i.e., additive, synergistic. Furthermore, when two sedatives are used (i.e., ethanol and ether) and anesthesia is an end point, the amount of ether used to achieve safe anesthesia may vary, further complicating the design of the study. It follows that the use of diethyl ether and possibly other anesthetics could confound the interpretation of pharmacokinetic data and should be avoided when possible.

The mechanism(s) of this inhibitory effect of ether on hepatic drug metabolism has been studied in several ways. First, our study clearly shows that the ether influence is independent of any anesthetic-induced decrease in body temperature which was maintained at normal levels. Second, these findings probably are not explained by nonspecific ether-induced circulatory changes, as the abnormal aminopyrine demethylation persisted in animals for several hours after recovery from anesthesia (Fig. 3) and the effect of ether on other drugs has been noted *in vitro* (15, 17). This conclusion is supported by the observations that urethane anesthesia does not share the ether effect (18) and that hepatic blood flow seems to increase with exposure to ether (21). Neither are the results based on variations in the rate of absorption of aminopyrine as indicated by comparable pharmacokinetic data obtained following subcutaneous and iv administration (Figs. 1 and 2). Further, measurements of systemic blood pressure and liver blood flow *in vivo* in rats under ether anesthesia and studies utilizing the perfused rat liver are needed to verify this. Third, ether does not appear to inhibit all drug metabolizing pathways equally. Thus, in isolated rat hepatocytes, sulfanilamide acetylation was unaltered and inhibition of acetaminophen conjugation required a much higher ether concentration than impairment of antipyrine oxidation (15). Likewise, the volume of distribution of antipyrine decreased after ether anesthesia (14), but no such effect was seen by us with aminopyrine. Thus ether seems to affect the hepatic metabolism of various drugs differently. The apparent sparing of sulfanilamide acetylation (15), a cytoplasmic process, may imply that the ether effect is exerted on microsomal metabolism. However, impaired metabolism of ethanol with ether rather suggests a more generalized effect of the anesthetic. *Finally,* it appears, at least *in vitro,* that the inhibitory effects of ether on hepatic drug metabolism are dose-dependent (15). These latter studies also rule out a possible ether-induced impaired delivery of drugs to the liver as an explanation for decreased elimination of such agents. Also in these *in vitro* studies, the concentration of ether could be defined, whereas this has not been done as yet in the *in vivo* experiments.

While these data provide some insight into

ether–drug interactions, further mechanistic studies of the effects of ether on microsomal drug metabolism are clearly needed to elucidate this process. In the interim a few speculations seem warranted. It does appear, for instance, that a small amount of ether itself undergoes a cytochrome *P*-450-dependent biotransformation in liver (16, 22, 23). Thus, ether, or its metabolites, could serve as competing substrates for microsomal metabolism of other drugs. Alternately, or in addition to other effects, ether could fluidize microsomal membranes and thus may alter the microsomal enzymatic micromilieu for some drugs. The latter might be one mechanism by which diethyl ether and ethanol in combination could exert an additive impairment of microsomal drug metabolism. Finally, ether may impair hepatic oxidative energy metabolism thus interfering with oxidative drug metabolism (16, 24). Another major area for further investigation is the specificity of this effect of ether on hepatic drug metabolism. Are other anesthetic agents also inhibitory to oxidative drug metabolism? Will conjugation of therapeutic agents be affected by anesthesia and, if so, will the primary effect be on glucuronidation, sulfation, or other processes? Certainly there is already evidence that ether exposure decreases the hepatic concentration of uridine diphosphoglucuronic acid, a substrate for glucuronidation (26). These questions could have relevance for a better understanding of hepatic function in patients receiving anesthesia.

In conclusion, diethyl ether anesthesia induces a marked depression in aminopyrine metabolism *in vivo*. Also, when diethyl ether was used as the anesthetic agent in studies designed to evaluate the effects of ethanol on aminopyrine clearance, an additive combined effect was observed. These studies illustrate the potential for confounding anesthetic interactions in pharmacokinetic experiments.


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