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Pharmacokinetic studies using microdialysis probes in subcutaneous tissue: effects of the co-administration of ethanol and acetaminophen

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Abstract: Loop geometry microdialysis probes with membrane lengths of 40–60 mm were used to monitor the effects of acute and chronic doses of ethanol on acetaminophen pharmacokinetics in awake, freely-moving rats. Microdialysis probes used in this configuration provide very high concentration recoveries and good precision at flow rates below 2 μl min^{-1}. The ability of microdialysis to monitor pharmacokinetics in subcutaneous tissue and blood vessels is compared. Dialysates acquired simultaneously from both blood vessels and subcutaneous tissue showed corresponding disposition for acetaminophen. Acute intraperitoneal doses of ethanol (1 ml kg^{-1}) are shown to increase the relative bioavailability, measured as AUC, by 40%, elimination half-life by 24%, and changes in CL and V_d were also observed. Larger doses of ethanol, up to 2 ml kg^{-1}, had a similar incremental effect on the pharmacokinetic parameters in some animals, but apparent decreased abdominal blood flow in others caused diminished absorption and drastically altered pharmacokinetic parameters. Chronic doses of ethanol (5% in drinking water for 14 days) caused an increase in bioavailability and other pharmacokinetic parameters, but changes were not as significant as following acute doses. Acute doses of ethanol (1 ml kg^{-1}) were also observed to change the pharmacokinetics of acetaminophen at hepatotoxic levels of the drug. However, acute intraperitoneal doses of acetaminophen (10 mg kg^{-1}) were observed not to have an effect on ethanol pharmacokinetics.

Keywords: In vivo *microdialysis; subcutaneous tissue; acetaminophen–ethanol interactions; pharmacokinetics.*

Introduction

In vivo microdialysis sampling probes have been constructed in primarily three geometries; concentric [1], loop [2–4], and straight-through [5, 6]. Concentric probes are of critical importance to neuroscience applications due to their small size [7–9]. Straight-through and loop probes are more practical for use in adipose and subcutaneous tissue due to their larger size and flexible construction [4–6, 10, 11]. All three types of probes have been shown to be practical and potentially advantageous sampling devices for continuously monitoring small molecules in animals. Compared with traditional methods of blood removal using either vena puncture or catheterization, microdialysis provides a means of continuous observation without alteration of the pharmacokinetics [12, 13]. Microdialysis can be accomplished in awake freely moving animals, providing an ideal means of obtaining pharmacokinetic information. In addition, since the technique can be fully automated, for microdialysates do not contain protein or cellular matter and require no further sample preparation, microdialysis probes allow for the determination of all small ions and molecules in extracellular fluid. Probes using the loop geometry can be constructed with membrane fibres 40–60 mm long, which provide very high recoveries at flow rates below 2 μl min^{-1} [4]. Subcutaneous tissue is an attractive sampling medium for several reasons. This tissue is very uniform [14]. The extracellular fluid is in constant flux with the circulatory system [15] and a large area is available for implantation of probes.

The effects of ethanol on drug disposition and pharmacokinetics is of importance due to the frequent concurrence of ethanol and therapeutic drug intake [16]. Ethanol has been shown to effect the pharmacokinetics of a number of drugs [17, 18]. In the case of acetaminophen, ethanol has been proposed to be a potential inhibitor of acetaminophen hepatotoxicity in acute doses [19–26], and an inducer of hepatotoxicity in chronic doses [27–

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29]. Both acetaminophen and ethanol metabolism are mediated by cytochrome p-450 oxidation at high concentrations [30, 31]. Ethanol, at high levels, has been shown to inhibit the metabolism of acetaminophen by altering the ratio of NADH/NAD [18, 20, 42]. Changes in physiological conditions and possible inhibition of metabolism of both substances occur when dosed simultaneously. The pharmacokinetic parameters most likely to be affected by ethanol intake are CL, V_d, $t_{1/2}$ [17] and AUC [16].

The objectives of this study were three-fold. Our first concern was to demonstrate the effectiveness of loop geometry microdialysis probes, implanted in subcutaneous tissue, for obtaining quantitative recoveries and pharmacokinetic information. Secondly, we wanted to observe the effects of acute and chronic doses of ethanol on acetaminophen and its glucuronide and sulphate conjugate pharmacokinetics. Finally, we proposed to monitor the pharmacokinetics of acute doses of ethanol (1 ml kg^{-1}) and determine the effects of acute doses of acetaminophen, at therapeutic levels, on ethanol pharmacokinetics.

Experimental

Subjects and surgery

Hooded Long Evans rats, 6–10 weeks old, (Harlan–Sprague Dawley, Indianapolis, IN) were used. The animals were housed in a temperature controlled room under a 12 h light/dark cycle. Food and water were provided *ad libitum*, except for the group of three subjects used for chronic dosing experiments. These subjects had a solution of 5% ethanol substituted for their water 14 days prior to acetaminophen dosing. Subjects were anaesthetized intraperitonealy with a 10:1 (100 mg ml^{-1}) mixture of ketamine–xylazine (1 ml kg^{-1}). The animals were weighed to the nearest gram while anaesthetized. For implantation into subcutaneous tissue a 5 mm incision was made in the back between the shoulders. A second incision was made in the back of the animal 6 cm posterior to the neck. A thin walled 13 gauge needle was inserted through the two incisions. A loop microdialysis probe with a 60 mm long regenerated cellulose membrane (6000 MWCO, 170 μm o.d. and 150 μm i.d.) [4] was inserted into the needle. The needle was then carefully removed through the distal incision, leaving the probe in the subcutaneous tissue. The probe was sutured to the skin to secure it. The two incisions were then sutured closed. The animal was transferred to an awake animal sampling system, and connected to the liquid swivel and dialysis perfusion pump. The animals were allowed to recover from surgery for a minimum of 12 h before pharmacokinetic experiments were conducted. Implantation into the jugular vein was performed as described previously [12, 13]. The microdialysis probe had a membrane 42 mm long. All other experimental conditions were the same as above.

Microdialysis

The awake animal system used has been previously described [1, 7, 12, 13]. Briefly, the animal was attached to a counter-balanced arm through a wire tether. The arm suspended a dual channel liquid swivel, the combination of the swivel and swinging arm provides the animal with complete mobility about the cage. The microdialysis probe was connected to the swivel with 110 μm i.d. Teflon tubing (Bioanalytical Systems, West Lafayette, IN). Microdialysis was conducted with a CMA/100 microinjection pump (BAS/CMA, West Lafayette, IN). A perfusion rate of 1.0 μl min^{-1} was used for all experiments except the chronic dosing and hepatotoxic dosing; 1.5 and 2.5 μl min^{-1} were used for the chronic and hepatotoxic experiments, respectively. Samples were collected every 10 min using either a CMA 140 fraction collector (BAS/CMA, West Lafayette, IN), or a CMA/160 auto-injector coupled directly to the liquid chromatograph. For *in vitro* dialysis experiments, a solution of 3.33 μg ml^{-1} acetaminophen was constantly stirred and maintained at 30°C.

Chromatography

Liquid chromatography was carried out with a BAS 201A chromatograph (West Lafayette, IN) using a Biophase C_{18}, 5 μm, 250 by 4.6 mm column maintained at 35°C and a flow rate of 1.5 ml min^{-1}. A 5 μl injection loop was used. For acetaminophen determinations the mobile phase was acetonitrile–50 mM sodium phosphate (5:95, v/v; adjusted to pH 2.5 with phosphoric acid, 1 M). Quantitation was accomplished using UV detection at 250 nm. The separation conditions provided a baseline separation of acetaminophen and its primary metabolites, the sulphate and glucuronide con-

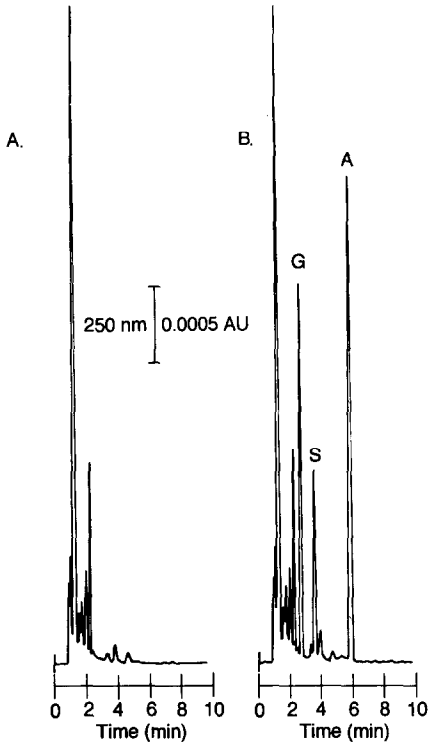

Figure 1
Separation of acetaminophen (A) acetaminophen-*O*-glucuronide (G) and acetaminophen-*O*-sulphate (S) in microdialysates by liquid chromatography (a) before and (b) 30 min after a 10 mg kg^{-1} dose of acetaminophen. Chromatography was conducted as described in the text.

jugates, and is illustrated in Fig. 1. Ethanol was determined using a prototype alcohol oxidase reactor, 3.0 × 30 mm (BAS) placed after the separation column. Detection of H_2O_2, a product of the ethanol–alcohol oxidase reaction, was accomplished using a thin-layer cell with a 3 mm platinum disk electrode maintained at a potential of +500 mV versus Ag/AgCl. The mobile phase was a 20 mM sodium phosphate buffer (pH 7.5) with 0.005% Kathon. Samples were diluted 25 or 50 times in mobile phase prior to injection.

Materials

Acetaminophen standard was purchased from Sigma (St Louis, MO) and used as received. Acetaminophen-*O*-sulphate was synthesized using the procedure of Neuberg [32], purity was confirmed using enzymatic conversion to acetaminophen with sulphatase (*Helix pomatia*, Sigma) [33] and microchemical analysis. Acetaminophen-*O*-glucuronide, obtained from human urine, was a gift from Dr C.E. Lunte (University of Kansas). Absolute ethanol was purchased from Midwest Grain Products (Parila, IL). All buffers were made from analytical grade materials purchased from Aldrich Chemical (Milwaukee, WI). Sterile Ringer's solution (consisting of 147.5 mM sodium, 2.25 mM calcium, 4 mM potassium and 156 mM chloride) and HPLC grade acetonitrile were obtained from Baxter (McGaw Park, IL). All solutions were made with double distilled deionized water and filtered through a 0.22 μm nylon filter.

In vivo *pharmacokinetic experiments*

Microdialysate samples were collected for at least 1 h prior to all experiments. Acetaminophen dosage at the therapeutic level was 10 mg kg^{-1}. A solution of acetaminophen (5 mg ml^{-1}) in Ringer's was used, and administered intraperitonealy. Hepatotoxic doses were administered intraperitonealy using a 25 mg ml^{-1} solution of acetaminophen in 10% Tween 80®/Ringer's solution [20]. Ethanol was prepared as a 50% solution and injected intraperitonealy 30 min prior to injection of acetaminophen [26]. Animals were allowed food and water throughout the experiments.

Pharmacokinetic parameters

Acetaminophen follows an open one compartment model with first order elimination and first order absorption [12, 34]. The disposition of acetaminophen can be described by the following equation.

$$C_t = A_1 e^{-\beta t} - A_2 e^{-k_a t}, \qquad (1)$$

where β is the elimination rate constant and k_a is the absorption rate constant. The half life of elimination was determined by plotting the log of the concentration against time [12, 13, 35]. Linear regression analysis provided the constant β. The first order elimination half-life ($t_{1/2}$) was determined by the equation 0.693/β. The initial concentration, $C(0)$, immediately after a single dose was determined from the *y*-intercept of the semi log plot. The area under the curve (AUC) values were determined using the trapezoidal rule, with extrapolation based on terminal half life beyond the last data point. The total body clearance (CL) was calculated as the ratio of dose/AUC and the volume of distribution (V_d) was calculated as the ratio of CL/β. Pharmacokinetic parameters were determined for individual subjects, and then the mean and standard error of the mean were computed for the group.

Ethanol follows an open one compartment model with first order absorption and zero order elimination. This allows for the elimination rate constant to be determined from the slope of a concentration vs time plot. All other pharmacokinetic parameters were determined using the methods described above.

Results

In vitro *microdialysis*

Five identical loop microdialysis probes with a membrane length of 60 mm were tested for relative recovery of acetaminophen. Five samples were obtained from each probe and assayed by liquid chromatography with alternatively assaying the standard solution. The five probes had an average recovery of 96.9 ± 0.4% (average ± SEM) for acetaminophen at a perfusion rate of 1.5 μl min^{-1} (27°C).

Pharmacokinetics of consecutive acetaminophen doses

Six consecutive 10 mg kg^{-1} doses of acetaminophen were administered to a single subject in two sets of three injections 4 h apart. Between the two sets of injections, the microdialysis probe was changed and the animal was allowed to recover from anaesthesia for 12 h. The bioavailability, measured as AUC (μg min^{-1} ml^{-1}), increased an average of 4.6% each injection. A total change of 22% over the six injections was observed. The apparent plasma clearance, CL, decreased from 22.1 to 18 ml min^{-1} over the six doses. However, the half-life of elimination (18.4 ± 0.4 min) and V_d (0.53 ± 0.01 l) showed no trend from the first to the last injection.

Comparison of in vivo *dialysates in blood vessels and subcutaneous tissue*

Microdialysis was performed in both the jugular vein (n = 3) and in subcutaneous tissue (n = 3) using 42 and 60 mm loop geometry probes, respectively. Both probes were perfused at the same rate (1.0 μl min^{-1}). The monitoring of acetaminophen pharmacokinetics in subcutaneous tissue and in blood vessels produced similar results with no statistical difference at P = 0.05. The pharmacokinetic values, AUC, CL and V_d, are dependent on probe recovery and interstitial concentration. The pharmacokinetic parameters for both methods are compared in Table 1.

Acute doses of ethanol

Pharmacokinetic parameters following an acute dose of ethanol at 1 ml kg^{-1} are summarized in Table 2. The pharmacokinetic parameters derived in the present investigation show an increase in AUC, $C(0)$, and $t_{1/2}$, while a decrease in CL and V_d. The AUC increased by 40%, while the apparent plasma clearance dropped by 44%. A 24% increase in the half-life of elimination was observed. Figure 2 illustrates these changes in three subjects.

Table 1
Comparison of pharmacokinetic parameters for acetaminophen dosed at 10 mg kg^{-1}* in the jugular vein and subcutaneous tissue

	Subcutaneous tissue	Jugular vein
$t_{1/2}$ (min)	18.3 ± 0.7	20.9 ± 0.5
AUC (μg min ml^{-1})	183 ± 25	195 ± 24
V_d (l)	0.31 ± 0.05	0.34 ± 0.03
CL (ml min^{-1})	11.2 ± 1.7	11.3 ± 1.3
$C(0)$ (μg ml^{-1})	10.0 ± 1.4	8.1 ± 0.4

*n = 3, for all parameters, mean ± SEM.

Table 2
Pharmacokinetic parameters for acetaminophen dosed at 10 mg kg^{-1}* under varying ethanol dosing conditions

	Acetaminophen only	1 ml kg^{-1} ethanol	Chronic ethanol
$t_{1/2}$ (min)	18.3 ± 0.7	23.3 ± 0.2	21.2 ± 0.4
AUC (μg min ml^{-1})	183 ± 25	257 ± 26	228 ± 22
V_d (l)	0.31 ± 0.05	0.26 ± 0.03	0.29 ± 0.04
CL (ml min^{-1})	11.2 ± 1.7	7.7 ± 0.9	9.4 ± 1.2
$C(0)$ (μg ml^{-1})	10.0 ± 1.4	11.0 ± 1.3	11.3 ± 1.3

*n = 3, for all parameters, mean ± SEM.

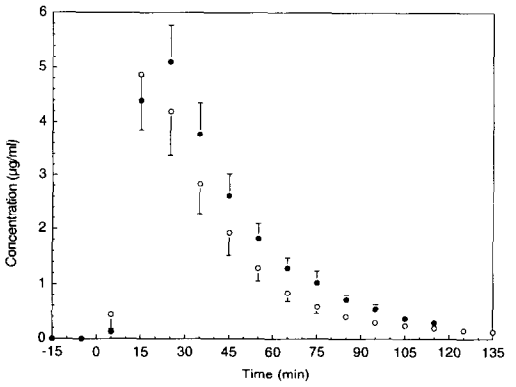

Figure 2
Comparison of subcutaneous monitoring of acetaminophen pharmacokinetics dosed at 10 mg kg^{-1} without ethanol (○) and with a 1 ml kg^{-1} dose of ethanol (●). The curves are the average of three animals with error bars representing SEM.

Furthermore, inter-subject variation increased with ethanol dose. Acute doses at 2 ml kg^{-1} caused apparent diminished abdominal blood flow in many animals decreasing acetaminophen absorption, whereas in some animals a similar, but more pronounced, effect was seen. An example of the effects of 1 and 2 ml kg^{-1} ethanol doses on acetaminophen disposition is illustrated in Fig. 3. Figure 3(a) shows a normally responding animal, whereas Fig. 3(b) shows an animal that had apparent dehydration due to the high dose of ethanol. Figure 4 shows the changes of the acetaminophen-*O*-sulphate conjugate concentration in the same animal as in Fig. 3(a) after an acute dose of 1 ml kg^{-1} of ethanol.

Chronic ethanol doses

A 5% solution of ethanol in the drinking water provided ethanol over a 14-day period. Each animal consumed an average of 31 ml day^{-1}. Increased urinary activity was pronounced in the experimental group. Delayed and erratic results were obtained from all subjects upon the first injection of acetaminophen. This could be due to dehydration and hypovolemia, reducing blood flow to the abdominal cavity. More consistent results were observed in the second dose. Decreased absorption and increased half-lives of elimination were observed. The pharmacokinetic parameters are reported in Table 2 for the second injection of acetaminophen. A 24% increase in bioavailability and a 13% increase in the elimination half-life was observed. Note that a delay in the maximum concentration was also observed in the ethanol dosed animals. The disposition of acetaminophen in control animals and in chronically dosed animals with ethanol is illustrated in Fig. 5.

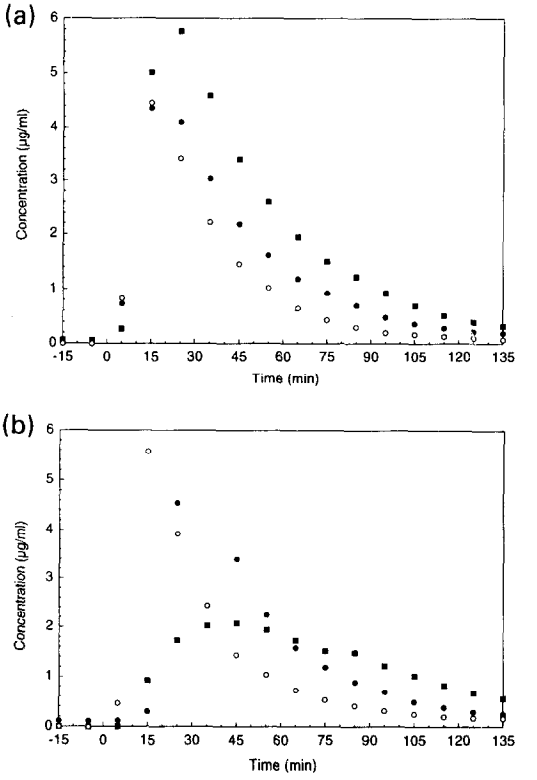

Figure 3(a) Acetaminophen monitored subcutaneously using microdialysis with no ethanol (○), 1 ml kg^{-1} ethanol (●), and with 2 ml kg^{-1} ethanol (■) in a single normal subject. (b) Acetaminophen disposition monitored using microdialysis with no ethanol (○), 1 ml kg^{-1} ethanol (●), and with 2 ml kg^{-1} ethanol (■) in another single subject that reacted negatively to the high dose of ethanol.

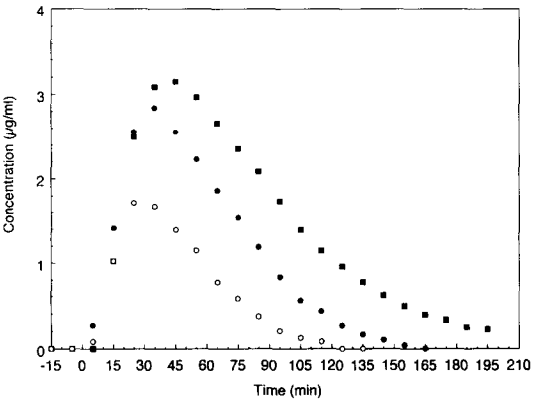

Figure 4
The concentration of acetaminophen-*O*-sulphate from the same animal in Fig. 3(a) with no ethanol (○), 1 ml kg^{-1} ethanol (●), and with 2 ml kg^{-1} ethanol (■).

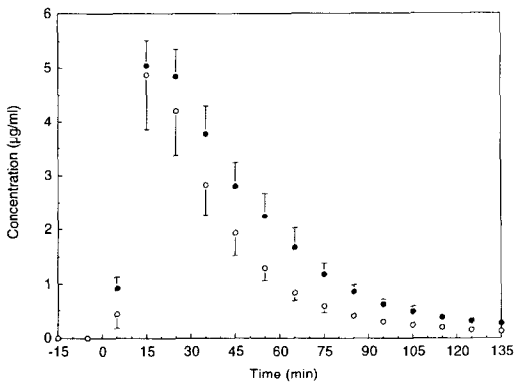

Figure 5
Comparison of acetaminophen disposition monitored in subcutaneous tissue (○) with chronically dosed with ethanol for 14 days (●). The curves are the average of three animals with error bars representing SEM.

Ethanol pharmacokinetic determinations

In a similar fashion to the experiments conducted for acetaminophen, ethanol provided 100% recoveries at flow rates <2 µl min^{-1}. Three consecutive injections of ethanol (1 ml kg^{-1}) into a single subject resulted in no statistical difference in pharmacokinetic parameters. The pharmacokinetic parameters for ethanol were determined in four subjects and the average ± SEM (n = 3) values were, 75.5 ± 6.8 (min) for $t_{1/2}$, 99 ± 14 (mg min ml^{-1}) for AUC, 1.35 ± 0.11 (mg ml^{-1}) $C(0)$, and 2563 ± 262 (ml min^{-1}) CL. Injections of 10 mg kg^{-1} acetaminophen 30 min prior to the injection of ethanol provided no statistical alteration of the pharmacokinetic parameters determined for ethanol.

Discussion

Microdialysis probes with short membranes typically have a large relative error, even when comparing probes apparently made identically [36, 37]. This is possibly due to minor differences in membrane surface area which are exaggerated due to the relatively short time it takes to completely replace the perfusate in the probe. Probes with longer membranes, like the ones used here, provide a greater period of time for the perfusate and surrounding tissue to reach equilibrium [4]. In this study the probes are operated in the plateau region of a recovery–flow rate curve. This results in probes with less physical differences and more reproducible recoveries. Five 60 mm loop microdialysis probes used in this study showed very good inter-probe reproducibility *in vitro* with an average recovery of 96.9 ± 0.4% for acetaminophen.

Many different methods for *in vivo* calibration of microdialysis probes have been proposed. These include extrapolation to zero flow [38], the zero flux method [38, 39], *in vitro* corrections [7–9], constant infusion combined with zero flux [40], and the low flow rate method [38]. However, excluding *in vitro* corrections of recoveries when blood sampling [12, 13] and the low flow rate method, none of these methods is practical when pharmacokinetic measurements are desired. They all either take a prohibitive amount of time, can not measure dynamic concentrations, or cause changes in the animals physiology and bias subsequent pharmacokinetic measurements. The approach taken in this study is to use the low flow rate method. The probes possess membranes long enough for an equilibrium condition to be obtained, between perfusate and surrounding tissue at practical flow rates between 0.5 and 2 µl min^{-1} [4, 41]. Concentrations reported are true dialysate concentrations, without the necessity for correction for mass transport limiting diffusion within the tissue.

The monitoring of acetaminophen disposition both in the jugular vein and subcutaneous tissue give similar results when both probes are used in conditions where the perfusate and sample fluid are close to equilibrium (Table 1). In the present study this was accomplished at a flow rate of 1.0 µl min^{-1} for both tissues, 42 and 60 mm probes for the jugular vein and subcutaneous tissue, respectively. The values of AUC, V_d, and CL indicate that the probes give similar recoveries and interstitial analyte concentrations. The error observed could be inter-animal related, since each probe type was used in three different animals. It has been previously shown that microdialysis monitoring in blood vessels gives identical results to traditional centrifuged ultrafiltrates of whole blood samples [12, 13].

The degradation of probe recovery over time has previously been observed, probably due to protein absorption to the membrane. In this study, three injections of acetaminophen spanning a time period of 36 h were required. In a single animal two microdialysis probes were implanted into the subcutaneous tissue and three consecutive injections made into each probe. A small increase of 4.3% in AUC was

seen after each injection of 10 mg kg^{-1} acetaminophen. The AUC increased, most likely due to physiological changes in response to the repetitive acetaminophen dosing. A decrease in recovery for the probes was not observed. There was no statistical difference between the pharmacokinetic parameters derived for the two microdialysis probes, supporting the *in vitro* recovery reproducibility. These results indicated that experiments over a 36 h time period could be conducted without large changes in the probes.

Acute doses of ethanol have been shown to inhibit the metabolism of acetaminophen in isolated liver microsomes of rats [23], hamsters [25] and mice [24, 26]. Ethanol's effects are believed to be a combination of inhibition of biliary clearance [24], cytochrome p-450 mixed function oxidase [20], and a decrease in NADPH and NADH levels. All of these studies were conducted at hepatotoxic levels of acetaminophen in liver microsomes. It has been proposed that ethanol decreases acetaminophen metabolism due to a common metabolism pathway [20, 23, 30, 31]. Likewise, acetaminophen at relatively high levels has been shown to alter ethanol metabolism and possibly pharmacokinetics [42]. Consequently, higher plasma concentrations and slower rates of elimination could be expected for acetaminophen following concurrent administration of both. Ethanol when ingested can increase the rate of drug absorption and bioavailability. Increased dissolution of the therapeutic drug in the gastric contents, increased circulation in the gastrointestinal system, and inhibition of first pass metabolism are several possible mechanisms that can explain these facts [16]. If increased enzyme levels affect the changes in pharmacokinetics due to ethanol, these differences would be seen in the chronically dosed animals. Increases in microsomal enzymes in rats occur as early as 48 h and are maximal after 2–3 weeks of ethanol administration [18].

In this study increased bioavailability, measured as AUC, and higher plasma concentrations were observed with both acute and chronic ethanol treated animals (Table 2). Higher initial concentrations of acetaminophen were also seen in all ethanol dosed animals. The total body clearance (CL) and volume of distribution (V_d) both decrease with acute and chronic doses of ethanol. More dramatic changes were observed in response to acute doses of ethanol. However, they were not statistically different at $P = 0.05$. The differences can be attributed to inhibition of acetaminophen metabolism by ethanol or increased absorption and physiological changes due to ethanol's presence. The disposition of acetaminophen in animals with acute doses of 1 and 2 ml kg^{-1} of ethanol are compared in Figs 3(a) and 3(b). At higher doses of ethanol a significant decrease in absorption and AUC was observed in some animals. Figure 3(b) illustrates an animal that reacted adversely to a 2 ml kg^{-1} acute dose of ethanol. High concentrations of ethanol can dehydrate the animal and cause decreased abdominal blood flow. This is the only known explanation for this observation at this time.

These same types of decreased absorption effects were seen for the chronically dosed animals, and the disposition of acetaminophen was quite varied after the first dose. The second doses were much more consistent, which might possibly be due to the use of Ringers solution as the carrier for acetaminophen as well as intraperitoneal injection. Significant changes in acetaminophen metabolism were not seen following the chronic ethanol treatment, which leads one to the conclusion that physiological changes, such as increased blood flow, absorption, and diminished renal clearance, are the more important factors affecting changes in the pharmacokinetics.

Acetaminophen is primarily converted to the sulphate (30%) and glucuronide (55%) conjugates *in vivo* [34]. Inhibition of the clearance of the sulphate conjugate and increased AUC was also observed, following the same trends as acetaminophen. Figure 4 illustrates the increase in bioavailability and half-life of elimination for the acetaminophen sulphate conjugate in the same animal presented in Fig. 3(a). The glucuronide conjugate, not shown, also followed the same trends, quantitation of glucuronide pharmacokinetics was not possible due to the lack of a pure standard.

The effect of ethanol on acetaminophen pharmacokinetics, at hepatotoxic levels of acetaminophen is also important due to the possible hepatotoxicity protection provided. Acetaminophen dosed at 200, 300 and 400 mg kg^{-1} showed a change in pharmacokinetics from a first order elimination kinetics to zero order kinetics. The sulphate conjugate route of metabolism was saturated at acetaminophen doses of 200 mg kg^{-1}, whereas the glucuronide

conjugate route became saturated at 400 mg kg^{-1}. The effect of ethanol on hepatotoxic doses of 200, 300 and 500 mg kg^{-1} of acetaminophen followed the same trends as therapeutic doses. Increased bioavailability and decreased clearance was seen for 1 ml kg^{-1} doses of ethanol. An example of a 200 mg kg^{-1} acetaminophen dose with and without a 1 ml kg^{-1} ethanol is illustrated in Fig. 6.

The alteration of ethanol pharmacokinetics by acute doses of acetaminophen at therapeutic levels was not observed in this study. Ethanol metabolism is dominated by alcohol dehydrogenase [18], which is apparently not inhibited by acetaminophen to any substantial degree. Figure 7 illustrates the pharmacokinetics of ethanol with and without acetaminophen administered 30 min prior in a single subject. A significant change in the liver NAD/NADH ratio due to acetaminophen likely occurs only at very high concentrations, after the two conjugate reactions are saturated.

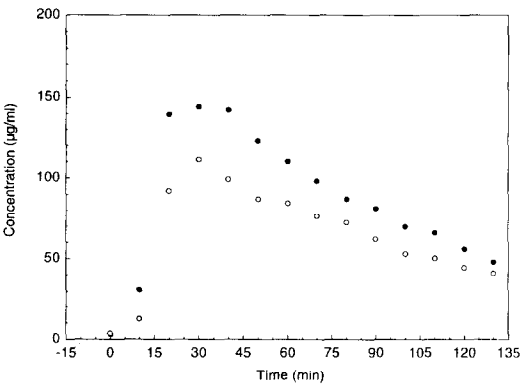

Figure 6
The effects of ethanol dosed at 1 ml kg^{-1} on a 200 mg kg^{-1} dose of acetaminophen (●) compared with acetaminophen dosed without ethanol (○).

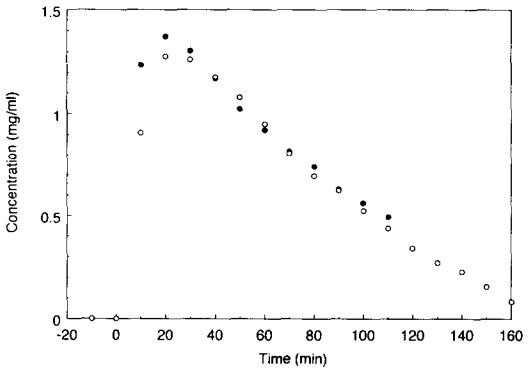

Figure 7
The effects of an acute intraperitoneal dose of acetaminophen, 10 mg kg^{-1}, on a 1 ml kg^{-1} dose of ethanol (●) compared with ethanol dosed without acetaminophen (○).

Conclusions

Microdialysis probes made with the loop geometry have been shown to be an effective practical tool for monitoring drug pharmacokinetics in awake animals. The increased membrane length of 40–60 mm provides high recoveries at flow rates below 2 µl min^{-1}. Microdialyses conducted in blood vessels and subcutaneous tissue are shown to provide similar acetaminophen disposition. Acute and chronic doses of ethanol are shown to change the pharmacokinetic parameters of acetaminophen. Increased bioavailability and elimination half-lives were observed for both acute and chronic doses of ethanol. However, the pharmacokinetics of ethanol were not affected by acute doses of acetaminophen. Thus, the changes in pharmacokinetics were due to increased absorption and other physical changes due to the ethanol and not to increased enzymatic activity. The ability to continuously monitor therapeutic drug disposition in the subcutaneous tissue of conscious animals is illustrated.

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