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*Research Article*

# Intravenous Microdialysis in the Mouse and the Rat: Development and Pharmacokinetic Application of a New Probe

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***Purpose.*** A flexible microdialysis probe was designed for intravenous sampling in small laboratory animals.

***Methods.*** Surgical techniques were developed to implant this probe via the femoral vein in the vena cava of the mouse and the rat. The in- and outlet of the probe were exteriorized above the tail of the animal and were directly connected to the microsyringe pump for perfusate delivery and to the injection valve for on-line HPLC analysis of the microdialysate samples.

***Results.*** The in vitro recoveries of flurbiprofen and naproxen for these probes were 68.2 ± 6.9% (mean ± S.D., n = 12) and 66.5 ± 7.3%, respectively. The relative loss by in vivo retrodialysis, measured the day after the implantation of the probes, was 66.1 ± 8.8% for flurbiprofen and 60.9 ± 9.9% for naproxen. The pharmacokinetics of unbound flurbiprofen were studied following i.v. bolus administration of flurbiprofen to the mouse (n = 4) and the rat (n = 6) with on-line HPLC analysis of microdialysates every 10 minutes during 6 to 8 hours. Flurbiprofen microdialysate concentrations were converted to unbound concentrations using the in vivo loss of flurbiprofen by retrodialysis carried out just before the start of the pharmacokinetic experiment. The integrity of the probe throughout the experiment was monitored by continuous retrodialysis of naproxen.

***Conclusions.*** The developed techniques can be used to carry out routine pharmacokinetic studies in the mouse and the rat as illustrated by our experiments with flurbiprofen, a compound with very high plasma protein binding.

KEY WORDS: intravenous microdialysis sampling; flurbiprofen; pharmacokinetics; rat; mouse.

## INTRODUCTION

In recent years microdialysis sampling has become an important technique allowing the in vivo measurement of endogenous and exogenous substances in extracellular fluid (1). The principle of the technique is based on the passive diffusion of compounds down a concentration gradient across the semi-permeable membrane of a microdialysis fiber. The microdialysis fiber separates two fluid compartments: the extracellular space at the sampling site and the physiological buffer solution (the perfusate) moving through the fiber at a slow rate. Microdialysate samples can then be collected for subsequent analysis or assayed on-line by HPLC or other suitable techniques. The idea to sample substances in the extracellular environment by dialysis is not new (2,3), but only during the last 10 years has the technique become established as an important research tool in the neurosciences (4-7). It is evident that this technique also holds a lot of promise for the study of the pharmacokinetics and pharmacodynamics in laboratory animals and man (8,9). Several reports have recently been published describing the application of intravenous microdialysis sampling to the study of the pharmacokinetics of drugs in unanesthetized laboratory animals, mainly the rat (e.g. 10-16).

The objective of this work was to develop a simple flexible microdialysis probe for intravenous drug sampling in the rat and the mouse. The probe was tested in vitro and in vivo using flurbiprofen, a 2-arylpropionic acid nonsteroidal anti-inflammatory drug (NSAID) with very high plasma protein binding, as model compound. Naproxen, another 2-arylpropionic acid NSAID, was evaluated as a possible retrodialysis marker. Surgical techniques were developed to implant the microdialysis probe into the inferior vena cava of the rat and mouse. The developed methods allowed the pharmacokinetic study of a highly plasma bound drug (flurbiprofen) by intravenous microdialysis sampling in the rat and the mouse with on-line HPLC analysis of the microdialysates.

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## MATERIALS AND METHODS

### Drugs and Reagents

Flurbiprofen and naproxen were purchased from Sigma Chemical Co. (St. Louis, Missouri). Solvents were of HPLC grade and all other chemicals used were AR grade.

### Microdialysis Probe Construction

Intravenous microdialysis probes were made using polyimide-coated fused silica capillary tubing (75 μ i.d. by 140 μ o.d., Polymicro Technologies Phoenix, Arizona) and regenerated cellulose dialysis fibers (150 μ o.d., 5,000 M.W. cut-off, Spectrum Medical Industries, Los Angeles, California). To construct the tip of the probe a piece of PE-10 polyethylene tubing was stretched in a flow of hot air. An enamaled copper wire (100 μ OD) was then inserted in the stretched PE-10 tubing and after heating in a flow of hot air, an elbow was formed by compressing the PE-10 tubing between two fingers and cut to a length of 1.8 mm (i.e. a total length of 3.6 mm). A 20 cm piece of silica tubing (the inlet line) and an 8 cm piece were then inserted in each end of the elbow and glued into place with epoxy glue (Devcon, Danvers, Maine). The two silica tubing lines were then inserted into a 4 cm piece of PE-20 to ensure that the two lines were close together and parallel, and the elbow was covered with loctite 770 polyolefine primer (Loctite, Kontich, Belgium). A drop of butyl-cyanoacrylate with a viscosity of 20 centipoise (B-20 cyanoacrylate glue, 3M, Brussels, Belgium) was then applied between the legs of the elbow. Next, the 8 cm silica tubing line was cut (Capillary Cleaving Tool, Supelco, Bellefonte, Pennsylvania) at 1 mm of the elbow. A 17 cm piece of silica tubing (the outlet line) was then inserted into a 2.0 or 2.5 cm dialysis fiber and glued in place using butyl-cyanoacrylate with a viscosity of 210 centipoise (B-210 cyanoacrylate glue, 3M). The other end of the dialysis fiber was pushed over the short piece of silica tubing, glued in the elbow and fixed with butyl-cyanoacrylate B-210 glue. A mark was made with a felt pen at 9 cm of the probe tip to facilitate the exact positioning of the probe in the vena cava during surgical implantation. Special care was always taken to avoid glue penetrating too far into the polyethylene tubing or the dialysis fiber where it would then obstruct the probe. Similarly, any glue sticking to the outside of the probe was carefully avoided as this would hamper the smooth introduction of the probe into the blood vessel. Finally, the probe was perfused with filtered distilled water (Mili-Q Water Purification System, Millipore, Milford, Maine) to dilate the regenerated cellulose dialysis fiber and a drop of B-20 glue was placed between the two silica tubing lines, distal to the tip of the probe, to maintain the right tension on the dialysis fiber. The construction of the probes was carried out under a dissecting microscope. A schematic diagram of the probe is shown in figure 1.

### Implantation of the Microdialysis Probe in the Rat

The probe was implanted in male Wistar rats (Animal Breeding Facilities of the Faculty of Medicine, UCL, Brussels) weighing between 260 and 320 g. The animals were anesthetized with a mixture of 4 mg/kg droperidol and 0.08 mg/kg fentanyl (Thalamonal$^{R}$, Janssen Pharmaceutica, Beerse, Belgium) injected subcutaneously. First, the right jugular vein was cannulated. A piece of silastic tubing (0.94 mm OD, 0.51 mm ID, Dow Corning, Valbonne, France) was introduced over a distance of 3 cm into the jugular vein via the anterofacial vein. The cannula was then exteriorized at the back of the neck. Next, a 1.5 cm incision was made in the groin of the left hindleg and the femoral vein was carefully dissected and ligated with 3.0 Surgilon$^{R}$ (Davis+Geck, Cyanamid Benelux, Belgium) just above the epigastric vein. A loose ligature was then placed against the abdominal wall approximately 8 mm above the first and a clamp was placed just underneath to avoid bleeding during the incision. A small incision was then made in the wall of the femoral vein and the tip of the probe was gently introduced into the vein. The clamp was then removed, the probe was swiftly pushed over a distance of exactly 9 cm into the inferior vena cava and the ligature was tightly knot. The two ends of the probe were then exteriorized at the base of the tail via a subcutaneous tunnel. The skin incisions were closed with Surgilon 2-0. The probe inlet was connected to a small piece of teflon tubing (1/16 inch OD, 250 μ ID) which was stretched on one end in a flow of hot air. The other end of the teflon tubing was dilated and connected to the outlet of a one-way 22 G swivel (Harvard Apparatus, South Natick, MA). The inlet of the swivel was then connected to the syringe pump using PE50 polyethylene tubing. The probe outlet was connected to a 45 cm piece of silica tubing (200 μ OD, 75 μ ID) through a teflon press-fit connector. Just before the start of the pharmacokinetic experiment this piece of silica tubing was directly connected to the HPLC injector bypassing the swivel to minimize the dead volume. Both ends of the probe were protected by a metal wire cylinder (hair curler) and taped around the tail. The probe was perfused with physiologic phosphate buffer at a flow of 0.5 μl/min during the entire period preceding the pharmacokinetic experiment.

### Implantation of the Microdialysis Probe in the Mouse

For implantation of the probe in the mouse, male NMRI mice (Animal Breeding Facilities of the Faculty of Medicine, UCL, Brussels) were used weighing between 35 and 40 g. The animals were anesthetized with sodium pentobarbital i.p. (60 mg/kg). A piece of silastic tubing (0.6 mm OD, 0.3 mm ID, Dow Corning) was introduced into the right jugular

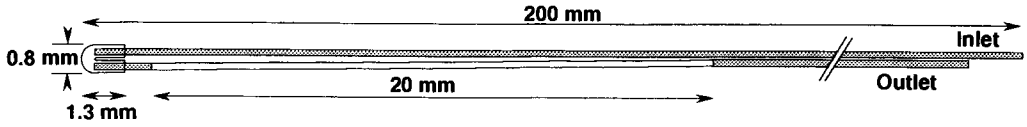


Fig. 1. Schematic diagram of the intravenous microdialysis probe. The tip of the probe including the microdialysis fiber is magnified approximately 5-fold. The real dimensions of the elbow and the length of the dialysis fiber (20 mm in this case) as well as the total length of the probe are indicated.

vein over a distance of 6 mm and exteriorized at the back of the neck between the ears. The procedure to implant the microdialysis probe was very similar to the one described above for the rat. The following adaptations were, however, necessary: (1) 7-0 silk suture was used, (2) the incision in the wall of the femoral vein was made without using a clamp, (3) the probe was pushed into the inferior vena cava over a distance of 4 cm, and (4) the skin incisions were closed with Surgilon 4-0. Transpore™ tape (3M) was wrapped around the tail and the exteriorized probe ends during the period preceding the pharmacokinetic experiment.

All surgical procedures were carried out under a dissecting microscope using microsurgical instruments (S&T Marketing AG, Rheinfall, Switzerland). During surgery and for 5 hours afterwards, the animals were kept under a 60 W lamp at a distance of 70 cm to avoid hypothermia. The animals had free access to water throughout the entire experiment, i.e. during the recovery period and the pharmacokinetic study. The described experimental procedures in animals were approved by the University Animal Experimentation Ethics Committee.

### Flurbiprofen Pharmacokinetics in the Conscious Rat and Mouse

The day after the surgical implantation of the microdialysis probe, the in vivo retrodialysis of flurbiprofen and naproxen was measured in the awake animal. In case of the rat, the animal was placed in a cage under the HPLC injection valve and its movements were unrestrained. The mouse, however, was put in a restraining tunnel in close proximity to the HPLC injection valve. The probe outlet was connected directly (mouse) or via a swivel (rat) to the injection valve. An isotonic phosphate buffer pH 7.4 solution of flurbiprofen and naproxen (250 ng/ml) was perfused through the probe at a flow rate of 2 μl/min and the microdialysate concentrations were measured by on-line HPLC analysis. These microdialysate concentrations were compared to the concentrations of both arylpropionic acid derivatives obtained by direct injection of the perfusate in the same loop of the injection valve (i.e. bypassing the probe). The perfusate was then switched to an isotonic phosphate buffer pH 7.4 containing only naproxen (250 ng/ml) which was continuously perfused through the probe during the remainder of the experiment. Flurbiprofen (20 mg/kg) was subsequently injected i.v. via the jugular cannula and the on-line analysis was simultaneously started. Microdialysate samples were automatically injected onto the HPLC column for a total duration of 6 to 8 hours following drug administration.

### On-Line Analysis of Microdialysate Samples by HPLC

The chromatographic system consisted of a high pressure liquid chromatographic pump (Model 420, Kontron Instruments, Milan, Italy), a pneumatic 10-port injection valve (Valco Instruments, Houston, Texas), a programmable fluorescence detector (Spectrasystem FL2000, Spectraphysics, San Jose, California) and a Varian model 4290 integrator (Varian Instruments, Walnut Creek, California). The injection valve was controlled by a home-made interface allowing automatic switching at regular intervals (10 minutes in our case). The microdialysis probes were used in conjunction with a syringe pump (Model 22, Harvard Apparatus, South Natick, Maine) and a Hamilton RN1001 syringe (Hamilton Co., Reno, Nevada).

The dialysate samples were collected directly into a 10 μl PEEK$^R$ injection loop (Jour, Sweden) and automatically injected every 10 minutes onto a $C_{18}$ Nucleosil column (100 × 4 mm, 5 μ, Macherey-Nagel, Düren, Germany) which was protected by a guard column packed with $C_{18}$ Corasil, 37-50 μ (Waters Associates, Milford, Maine). The mobile phase consisted of phosphate buffer 50 mM (pH 3.0) and acetonitrile (52:48, v/v) and was delivered at 1.1 ml/min. The eluent was monitored using the following excitation and emission wavelengths: 262 nm (ex) and 356 nm (em) from 0-3.25 min (naproxen), and 258 nm (ex) and 310 nm (em) from 3.25-5.7 min (flurbiprofen).

As a result of the direct on-line analysis of microdialysates which did not allow dilution of the samples prior to injection, detector response over the concentration range of interest during the in vivo pharmacokinetic studies slightly deviated from linearity. Of all the equations tested, the Hill equation best fitted the fluorescence response-microdialysate concentration data:

$$Y = \frac{A\,X^n}{B^n + X^n} \qquad [1]$$

where n represents the Hill coefficient or shape factor. Curve fitting was carried out using COMSTAT, a nonlinear least squares regression program using a steepest descent algorithm (17).

### In Vitro and in Vivo Evaluation of the Recovery Characteristics of the Probe

In vitro recoveries by dialysis were estimated as follows. The microdialysis probe was inserted through a small hole (1 mm ID) drilled through the screw cap of a 10 ml test tube containing a physiologic phosphate buffer pH 7.4 (sampling medium). The test tube was then placed in a thermostated (37 °C) organ bath and its contents were continuously stirred. To evaluate the in vitro recovery by dialysis, flurbiprofen and naproxen were added to the medium surrounding the probe to obtain a concentration of 250 ng/ml for both compounds. Naproxen, a 2-arylpropionic acid derivative like flurbiprofen, was selected as a possible retrodialysis marker to monitor probe recovery during the in vivo pharmacokinetic experiment. The probe was perfused with physiologic phosphate buffer at a flow rate of 2 μl/min. Dialysate concentrations of flurbiprofen and naproxen were analyzed on-line with HPLC. The relative recovery of a particular solute, i.e. flurbiprofen or naproxen, by dialysis ($R_{dial}$) was calculated as follows:

$$R_{dial} = \frac{C_{dial}}{C_{med}} \qquad [2]$$

where $C_{dial}$ and $C_{med}$ represent the concentrations of solute in the microdialysate and the medium surrounding the dialysis fiber, respectively.

In case of in vitro and in vivo (just before pharmacokinetic study) retrodialysis, the solutes flurbiprofen and naproxen were introduced into the perfusate (at 250 ng/ml)

and their relative loss to the medium (in vitro) or to the circulation (in vivo) during microdialysis (perfusate flow: 2 μl/min) was measured. The relative loss of solute during retrodialysis $L_{retro}$, under ideal circumstances identical to the relative recovery by dialysis, was then calculated as follows:

$$L_{retro} = R_{dial} = \frac{C_{perf} - C_{dial}}{C_{perf}} \quad [3]$$

where $C_{perf}$ is the concentration of solute in the perfusate.

Flurbiprofen microdialysate concentrations ($C_\mu$) were converted to unbound concentrations ($C_u$) as follows:

$$C_u = \frac{C_\mu}{L_{retro}} \quad [4]$$

where $L_{retro}$ is the in vivo loss of flurbiprofen by retrodialysis.

Recovery values in the text and Table II are reported as mean ± S.D.

## RESULTS

Chromatograms of microdialysate samples injected on-line contained little interfering substances. Retention times therefore could be kept short (± 2.5 min for naproxen and ± 4 min for flurbiprofen) allowing on-line injection of the microdialysate at 10 minute intervals. On-line injection of (undiluted) microdialysate samples, however, necessitated calibration over a wide concentration range. As a result these calibration curves were nonlinear (fig. 2). Using a Hill equation an excellent fit was obtained throughout the concentration range encountered during the pharmacokinetic experiment (Table I).

The in vitro recovery of flurbiprofen and naproxen was estimated for 12 probes with a dialysis fiber length of 2 cm. In vitro recoveries were very similar for flurbiprofen and naproxen and averaged 68.2 ± 6.9% and 66.5 ± 7.3%, respectively. Loss of flurbiprofen and naproxen by in vitro retrodialysis was almost identical to their in vitro recovery

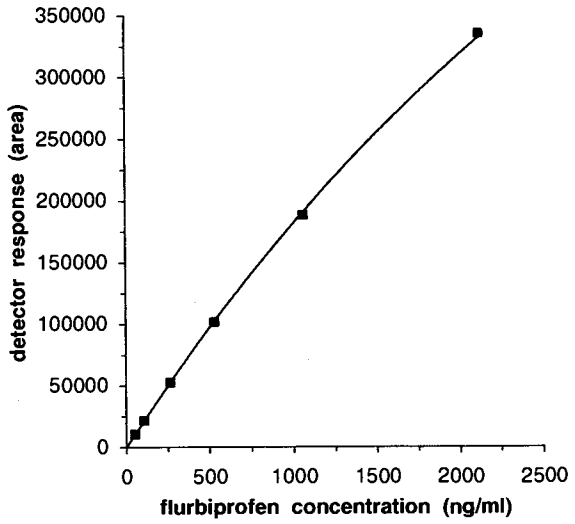


Fig. 2. Typical calibration curve for the quantification of flurbiprofen in microdialysates.

Table I. Observed and Calculated Detector Response, Based on the Nonlinear Calibration Curve for Flurbiprofen, at Different Concentrations Covering the Range Encountered During the Intravenous Microdialysis Experiments in Rats

| Flurbiprofen concentration (ng/ml) | Observed (area) | Calculated (area) | Difference (%) |
|---|---|---|---|
| 52.95 | 10,465.5 | 10,588.8 | +1.18 |
| 105.90 | 21,737.5 | 21,187.9 | −2.53 |
| 264.75 | 52,692.5 | 52,768.3 | +0.14 |
| 529.50 | 101,782.0 | 102,361.5 | +0.57 |
| 1,059.00 | 188,462.5 | 190,688.6 | +1.18 |
| 2,118.00 | 335,162.5 | 331,799.8 | −1.00 |

by microdialysis (Table II). Loss by retrodialysis determined in vivo, after implantation of these same probes in rats, was higher for flurbiprofen (66.1 ± 8.8%) as compared to naproxen (60.9 ± 9.9%), but the difference did not reach statistical significance. However, whereas the in vitro and in vivo losses of flurbiprofen by retrodialysis were not significantly different (68.3 ± 7.0% and 66.1 ± 8.8%, respectively), the in vivo loss by retrodialysis for naproxen (60.9 ± 9.9%) was significantly lower than the in vitro loss (69.6 ± 8.0%). These results are summarized in Table II.

Because no difference could be demonstrated between dialysis and retrodialysis of flurbiprofen in vitro, nor between retrodialysis of flurbiprofen in vitro and in vivo, we decided to use the in vivo retrodialysis data for flurbiprofen to convert microdialysate concentrations into unbound concentrations. The in vivo loss of naproxen during the pharmacokinetic experiment was used to monitor the integrity of the microdialysis probe as illustrated in figure 3. Pharmacokinetic experiments during which the naproxen concentrations in the microdialysate showed unusual variations (>10%) were discarded.

The applicability of the probes, implanted using the described implantation techniques in the rat and the mouse, was demonstrated by studying the pharmacokinetics of unbound flurbiprofen in both species following administration of a single i.v. dose of 20 mg flurbiprofen/kg. Fig. 4 shows the mean semi-logarithmic concentration-time profiles for unbound flurbiprofen in blood in the mouse (n=4) and the rat (n=6). These graphs clearly illustrate that by using intravenous microdialysis sampling the pharmacokinetics of unbound flurbiprofen can be studied with much detail in laboratory animals as small as the mouse.

## DISCUSSION

A flexible microdialysis probe of a simple design was

Table II. In vitro and in vivo Recovery/Loss (%) by Dialysis or Retrodialysis of Flurbiprofen and Naproxen. Values are the Mean ± SD of 12 Different Probes

| Procedure | | Flurbiprofen | Naproxen |
|---|---|---|---|
| Retrodialysis | in vitro | 68.3 ± 7.0 | 69.6 ± 8.0 |
| | in vivo | 66.1 ± 8.8 | 60.9 ± 9.9[a] |
| Dialysis | in vitro | 68.2 ± 6.9 | 66.5 ± 7.3 |

[a] Significantly different (p = 0.04) from loss by retrodialysis in vitro.

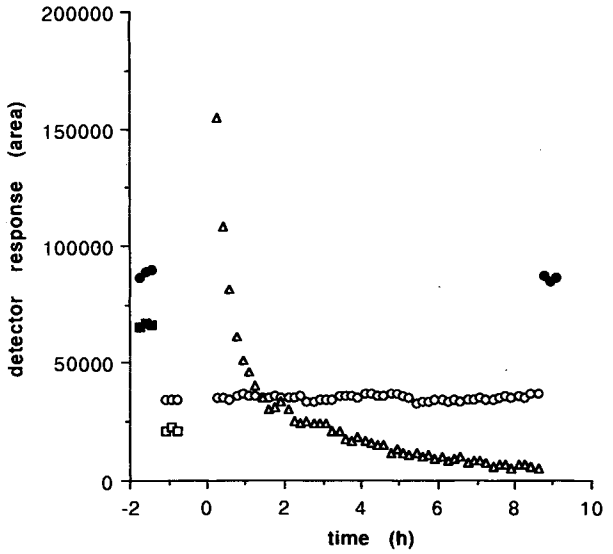


Fig. 3. Microdialysate concentrations (detector response) of flurbiprofen and naproxen obtained during the evaluation of the microdialysis probe by in vivo retrodialysis before I.V. administration of flurbiprofen at time 0 and during the actual pharmacokinetic experiment: ○, naproxen microdialysate concentrations; □, flurbiprofen microdialysate concentrations during in vivo retrodialysis; △, flurbiprofen microdialysate concentrations during the pharmacokinetic experiment. Perfusate concentrations of naproxen (●) and flurbiprofen (■) (during in vivo retrodialysis) are also shown and were used to estimate the relative loss of both compounds by in vivo retrodialysis.

developed to sample the unbound concentration of endogenous and exogenous substances in blood of the mouse and the rat. Most probes used so far for intravenous microdialysis sampling in the rat have a concentric design. Drawbacks of such design are the position of the dialysis fiber at the tip of the probe, which may result in membrane damage during the implantation procedure, and the reduction of the perfusate volume in the fiber by the central tubing. Comparing a linear and a concentric probe having the same microdialysis fiber characteristics (material, dimensions) and using the same perfusate flow, it is clear that the residence time of the perfusate in the dialysis fiber is longer for the linear probe thus leading to a higher recovery. This allowed us to use a microdialysis fiber of only 150 μ OD (2.0-2.5 cm long) and still obtain excellent recovery characteristics (approximately 68% for flurbiprofen) at a perfusate flow rate of 2 μml/min. Another distinct advantage of the developed probe is the construction of the polyethylene elbow serving as the tip of the probe. This elbow is rounded to facilitate implantation into the blood vessel without damaging its wall. The probe, as described in this report, is the result of a series of improvements made over a period of approximately 3 years in our laboratory. It is very robust due to the polyethylene tip, flexible and its dimensions are such that it can be implanted without difficulty in the vena cava of the mouse.

Several pharmacokinetic studies in the rat using intravenous microdialysis sampling have recently been published. (e.g. 10, 12, 13, 16, 18). In all these studies, the probe was implanted into the jugular vein. We feared that the blood flow around the probe implanted in the jugular vein might be small and variable thus resulting in fluctuations in the recovery. We have recently shown using an in vitro set-up that the linear velocity of medium flow around the microdialysis probe may influence the relative recovery (19). These observations prompted us to implant the microdialysis probe in the inferior vena cava where blood flow is higher than in the jugular and probe recovery may be less sensitive to fluctuations in blood flow (19). In addition, the inferior vena cava is straight over a relatively long distance (± 6 cm in a rat weighing 300 g) which makes it possible to use probes with longer microdialysis fibers to improve solute recovery. We are currently using 5 cm microdialysis fibers for our intravenous probes having a 100% recovery of flurbiprofen in the rat.

Surgical procedures were therefore developed to implant the probe in the inferior vena cava of both rat and mouse. Like the construction of the microdialysis probe, the surgical procedure has been refined over the last 2-3 years such that its actual success rate is very high (>90%) and the entire procedure, i.e. probe implantation and jugular vein cannulation, only takes approximately 40 minutes. After the surgery is finished, the animals are allowed to recover overnight before starting the in vivo evaluation of the probe followed by the pharmacokinetic experiment. This ensures recovery of the animal from the short-term effects of anesthesia and provides a sufficiently long time for the microdialysis membrane to stabilize. Exteriorization of the probe in- and outlet above the tail and the use of a one-way swivel to connect the probe to the microsyringe pump and to the injection valve allow the rat to move around freely during the entire experiment. The use of specialized equipment would also make it possible to carry out the microdialysis experiment in the freely moving mouse. However, since the only purpose of the experiments in the mouse was to show the feasibility of intravenous microdialysis sampling in this animal species, no investment was made in such specialized equipment and the animal was immobilized in a home-made restraining tunnel during the microdialysis experiment i.e.

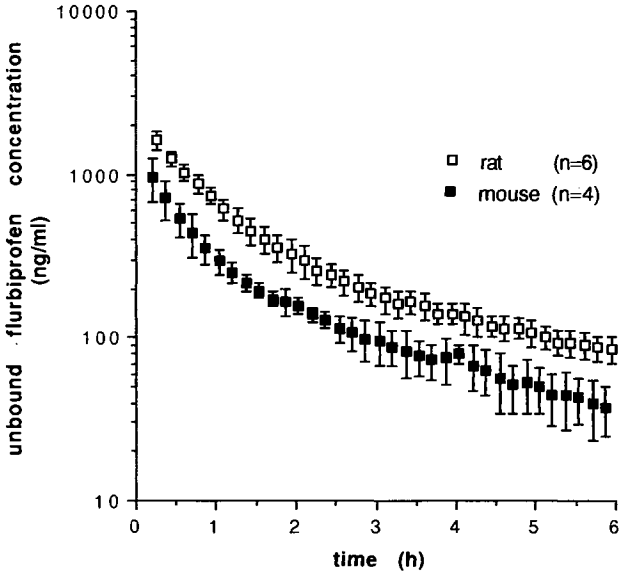


Fig. 4. Average (± S.D.) unbound concentrations of flurbiprofen as determined by intravenous microdialysis sampling following i.v. bolus injection of flurbiprofen (20 mg/kg) to 6 rats and 4 mice.

the in vivo evaluation of the probe and the pharmacokinetic study.

Intravenous microdialysis sampling in the mouse has, to our knowledge, not been described before, although brain microdialysis in this animal species has recently been reported (20). Repetitive blood sampling to carry out a pharmacokinetic study is not possible in a single mouse because of its limited total blood volume. When using the mouse for pharmacokinetic studies, a number of animals is killed at certain times following drug administration and thus each animal only provides one blood concentration on the concentration-time profile. Since no fluid is removed by microdialysis it is possible, as we have shown, to carry out pharmacokinetic studies in a single mouse using intravenous microdialysis sampling. This would make pharmacokinetic studies in the mouse more cost-effective especially because it will substantially reduce the amount of test product required to carry out disposition studies, an important consideration when dealing with expensive substances of which only small quantities are available. In addition, intravenous microdialysis sampling opens the possibility to repetitively sample unbound substances in the blood of transgenic mice, and in combination with simultaneous brain microdialysis may be a powerful approach to study the transport of substances across the blood brain barrier.

Using the approach described in this report we have investigated the i.v. pharmacokinetics of flurbiprofen in the rat. Using simultaneous microdialysis and serial blood sampling, the in vivo fraction unbound of flurbiprofen in plasma could be estimated throughout the experiment. The results showed that the plasma protein binding of flurbiprofen in the rat is concentration-dependent even following a single 20 mg/kg i.v. dose (21). In addition, a detailed analysis of the flurbiprofen plasma protein binding with estimation of $B_{max}$ and $K_d$ was possible based exclusively on the results of the i.v. microdialysis experiment. These studies clearly illustrate the enormous advantage intravenous microdialysis offers when doing pharmacokinetic studies in small laboratory animals.

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