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Blood microdialysis in pharmacokinetic and drug metabolism studies

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Abstract

Microdialysis is a sampling technique allowing measurement of endogenous and exogenous substances in the extracellular fluid surrounding the probe. In vivo microdialysis sampling offers several advantages over conventional methods of studying the pharmacokinetics and metabolism of xenobiotics, both in experimental animals and humans. In the first part of this review article various practical aspects related to blood microdialysis will be discussed, such as: probe design, surgical implantation techniques, methods to determine the in vivo relative recovery of the analyte of interest by the probe, special analytical considerations related to small volume microdialysate samples, and pharmacokinetic calculations based on microdialysis data. In the second part of this review a few selected applications of in vivo microdialysis sampling to investigate pharmacokinetic processes are briefly discussed: determination of in vivo plasma protein binding in small laboratory animals, distribution of drugs across the blood–brain barrier, the use of microdialysis sampling to study biliary excretion and enterohepatic cycling, blood microdialysis sampling in man and in the mouse, and in vivo drug metabolism studies.

Keywords: Microdialysis probes; Surgical implantation; Plasma protein binding; Blood–brain barrier; Biliary excretion; Clinical applications

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1. Introduction

In recent years microdialysis sampling has become an important technique allowing the in vivo measurement of endogenous and exogenous substances in extracellular fluid [1,2]. 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 [3,4], but only during the last 10–15 years has the technique become established as an important research tool not only in the neurosciences, but more recently also in areas such as drug distribution into the CNS and other organs and tissues (e.g., tumors, eye, etc.) [5–9], transcutaneous absorption [10,11], etc.

The microdialysis sampling technique is well suited to perform pharmacokinetic studies in small laboratory animals such as mice and rats. Since microdialysis probes can be implanted in almost any organ or tissue, including blood vessels, it offers a number of important advantages over the classical approaches to carry out pharmacokinetic studies. Because there is no net fluid exchange, continuous sampling for long periods of time is possible, without interfering with the processes that govern the pharmacokinetic behavior of the drug. This results in drug microdialysate concentration–time curves with a high time resolution thus facilitating pharmacokinetic analysis. Although the unbound drug concentration in blood is undoubtedly more closely related to the activity of the drug than the total concentration, total concentrations are routinely measured in pharmacokinetic studies because the methods to determine unbound concentrations are tedious, lack accuracy and precision and are costly. Microdialysis sampling offers the unique opportunity to enable the in vivo determination of unbound drug concentrations in blood and other tissues.

In this article, the practical aspects of blood microdialysis sampling from the blood will be briefly discussed and some relevant examples highlighting the applicability of the technique to study pharmacokinetic and metabolic processes will be shown. For further information, the reader is referred to recent review articles discussing the use of microdialysis sampling in pharmacokinetic and metabolism studies [12,13].

2. Intravenous microdialysis probes

The first probes that were routinely used for implantation in the central nervous system were of the concentric type and were constructed of stainless steel needle tubing, fused silica and a semi-permeable membrane. The rigid design of these probes has the advantage that they can be secured to the skull and, therefore, the neurochemical experiments can be carried out in awake, freely moving animals. However, performing experiments in freely moving animals after implantation of such a rigid probe into a blood vessel or other peripheral tissues (subcutaneous and adipose tissue, muscle, etc.) is not ideal since the probe, especially the semi-permeable membrane, may be damaged or alternatively the steel parts may cause excessive damage to the tissue/organ. Therefore, flexible microdialysis probes were constructed in the early 1990s to allow the application of microdialysis sampling to sites other than the brain.

Flexible microdialysis probes for intravenous implantation are of two general designs: concentric or linear (Fig. 1). The shunt probe has an interesting design: a dialysis fiber is suspended within a larger tube, allowing sampling from flowing fluids such as blood or bile [14]. The easiest option is to purchase these probes from specialized companies. Alterna-

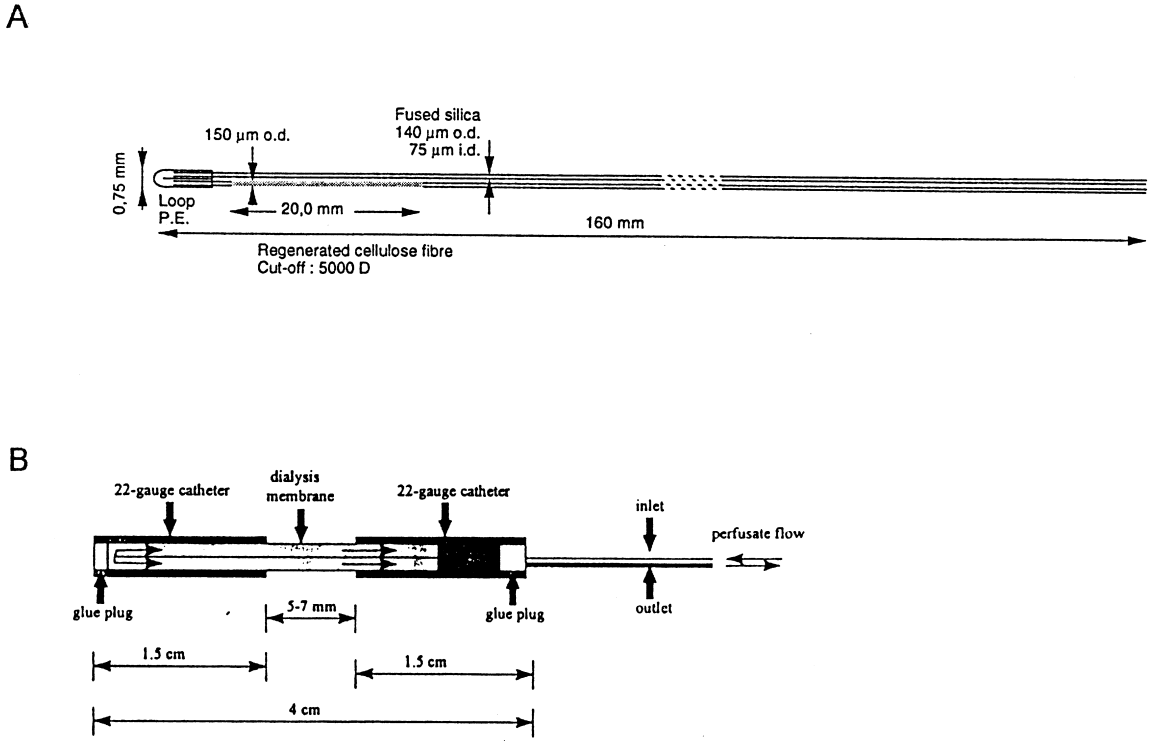

Fig. 1. Two examples of different probe design to carry out blood microdialysis sampling: (A) a simple linear design [15], and (B) a concentric design [22].

tively, it is possible to manufacture your own microdialysis probes. For instance, Evrard et al. [15] described the manufacture of a simple linear probe for implantation in a blood vessel of a small laboratory animal such as the rat or even the mouse (Fig. 1A). Páez and Hernández [16] reported a microdialysis probe for implantation in one of the superficial veins of the forearm of volunteers and used the probe to monitor blood glucose levels. Other examples of home-made microdialysis probes for blood sampling can be found in the scientific literature (e.g., Refs. [17–22]). Local construction of microdialysis probes is a viable, cost-effective option to the purchase of commercially produced probes. The advantages of home-made probes are their low cost and the versatility to construct probes for specific applications. The choice of the semi-permeable membrane material (polycarbonate-ether, cuprophan, polyacrylonitrile, etc.) and its characteristics such as molecular weight cut-off and dimensions (length, diameter) may all have an important impact on the permeability and therefore the recovery of the analyte [23]. By using home-made probes, the membrane characteristics can be chosen to optimize the in vivo recovery of any particular analyte.

The probes are generally perfused with a physiological buffer, such as Ringer's solution, or physiological saline by means of a high precision perfusion pump. Perfusate flow rates are very small and are usually around 1 µl/min. To improve the recovery by microdialysis of lipophilic substances the use of a perfusion medium containing lipids or albumin has been proposed [24,25].

2.1. Surgical implantation of the probes

For blood sampling in the rat, microdialysis probes are usually implanted into the jugular vein. Alternatively, the inferior vena cava may be used for probe implantation. There are possible advantages of implanting the probe into the inferior vena cava: (1) the blood flow is higher than in the jugular vein and

therefore recovery of the analyte may be less sensitive to fluctuations in blood flow; (2) placement of the probe in this location is less likely to interfere with blood supply to important organs; and (3) the inferior vena cava is straight over a relatively long distance (ca. 6 cm in a rat weighing 300 g) which makes it possible to use probes with longer dialysis fibers thus improving analyte recovery. Evrard et al. [15] described the surgical techniques used to place the microdialysis probe into the inferior vena cava of the rat or even the mouse. Similarly, Yang et al. [22] implanted a concentric, flexible microdialysis probe (Fig. 1B) into the inferior vena cava of rats to study the pharmacokinetics of fluconazole. Their experiments showed that using their probe/implantation technique a more stable animal preparation was obtained, compared to implantation in the jugular vein, making long-term sampling and thus repeated-measures experimental designs possible.

A major concern with intracerebral microdialysis is that implantation of the microdialysis probe may evoke inflammation and subsequent healing which may affect the results of the microdialysis experiments [26]. For intravenous implantation of the probe, blood clotting around the dialysis fiber is very likely to occur. Twenty-four hours after probe implantation into the inferior vena cava of the rat, a fibrin sheath was present around the regenerated dialysis fiber (Evrard and Verbeeck, unpublished observations). However, the presence of this fibrin sheath did not affect recovery of the analyte (flurbiprofen) by the probe up to 48 h following implantation. After 48 h of implantation, however, the in vivo recovery characteristics of the probe gradually decreased. Chen and Steger [19] also reported that membrane permeability to glucose of a microdialysis probe implanted in the right atrium of the rat rapidly decreased following implantation unless the rats were heparinized. Yang et al. [22] also found a decline in the in vivo recovery over a 10-day period of their home-made microdialysis probes following implantation into the inferior vena cava of the rat. These findings are in line with the results of others [27–29]. This reduction in membrane clearance may be attributed to fiber clotting and changes in membrane properties due to plasma protein and/or cell deposition on the surface of the membrane. A similar phenomenon has been observed in clinical hemodialysis studies and was found to be dependent on the type of dialysis membrane being used and the type of analyte studied [30]. Despite the reduction in probe recovery, Yang et al. found that when the pharmacokinetics of fluconazole where studied 2 and 10 days following implantation of the microdialysis probe into the inferior vena cava of the rat, the parameters (AUC, CL, V_{ss}, $t_{1/2}$) derived from data collected by microdialysis sampling were not significantly different from the values obtained from data collected by traditional serial blood sampling [22]. Their results show that blood microdialysis, when carefully carried out, is a feasible method to study the pharmacokinetics of fluconazole for up to 10 days post probe implantation. Consequently, the pharmacokinetics of fluconazole can be studied repeatedly over time in each rat, allowing the use of repeated-measures experimental designs.

3. Calibration of the microdialysis probes

It is very important to know the in vivo relative recovery, i.e., the concentration of the analyte in the microdialysate compared to that in the extracellular fluid surrounding the probe, because this measure allows the conversion of microdialysate concentrations into extracellular concentrations. Because in vitro recovery may significantly differ from the in vivo recovery [31], it is best to calibrate microdialysis probes in vivo. Various techniques have been proposed to determine the in vivo recovery of the analyte by the microdialysis probe: e.g., the no net flux method, the extrapolation to zero flow method, and the retrodialysis method [32–34]. The first two methods are time consuming requiring several measurements under different conditions of flow or analyte perfusate concentrations. These measurements should be carried out at analyte steady-state concentrations. In addition, the calibration is usually carried out before the start of the pharmacokinetic experiment but should ideally be repeated after completion of the study to ensure stable recovery characteristics of the probe during the entire experiment. To circumvent the major problems associated with the no net flux and extrapolation to zero flow methods, Ståhle et al. suggested the use of the reverse dialysis, or retrodialysis, method [32]. In this

case a marker with membrane diffusion characteristics close to the analyte of interest is added to the perfusate and its relative loss by retrodialysis is calculated as the difference in perfusate and dialysate concentrations divided by the perfusate concentration. If the marker is well chosen, it can be shown in vitro that loss of marker and analyte by retrodialysis is the same as their recovery by dialysis. The in vivo recovery of the analyte of interest can therefore be estimated from the loss by retrodialysis of the marker from the perfusate into the extracellular environment of the dialyzed tissue (implantation site). During the pharmacokinetic experiment, in vivo loss of marker by retrodialysis is continuously monitored and is used to convert analyte microdialysate concentrations into extracellular concentrations. The assumption then is that in vivo retrodialysis of the marker is the same as in vivo dialysis of the analyte. An advantage of this technique is that fluctuations in recovery of the probe during the experiment are taken into account by the continuous retrodialysis of the marker during the entire experiment. It is also possible to estimate absolute concentrations of analyte by using very slow perfusion rates such that equilibrium between the dialysate and extracellular fluid is approached. The drawback is that under these conditions the absolute recovery may be very low and sampling times become too long. Alternatively, microdialysis probes with long sampling windows can be used to obtain a 100% recovery of analyte in vivo. However, of all these methods the in vivo retrodialysis of a marker is the easiest and surest method to continuously monitor the in vivo recovery of the analyte.

4. Analytical considerations

One of the advantages of microdialysis sampling is the generation of samples which are generally free of proteins and other macromolecules (depending on the molecular weight cut-off of the dialysis membrane) and which therefore can be analysed on-line by techniques such as liquid chromatography or capillary electrophoresis [35,36]. Due to the low perfusion flow rates in microdialysis sampling (typically 0.2–2.0 μl/min), long sampling collection times may be necessary in order to collect sufficient quantities of analyte for a reliable quantification. The temporal resolution of a microdialysis study is determined by a combination of the perfusion rate through the microdialysis probe and the sample volume requirement (absolute recovery) of the analytical technique. Capillary liquid chromatography and capillary electrophoresis are extremely well suited for on-line analysis of microdialysis samples. For example, because of the relative purity of microdialysis samples, short capillary columns may provide fast separations without any loss in detection sensitivity due to the increased mass sensitivity of capillary columns. Furthermore, the dimensions of these columns are compatible with the minute dialysis flow rates. For example, coupling microdialysis to a microseparation method such as capillary electrophoresis in which sample volumes of a few nanoliters are sufficient can obviously greatly improve the temporal resolution of the pharmacokinetic data. Because of the small absolute amounts of analyte that are thus injected onto the column, the choice of the detector may also be critical, with electrochemical detection favored to measure low concentrations of neurotransmitters such as biogenic amines. On-line analysis of microdialysate samples by LC–MS has also been described and will become more widespread as this instrumentation becomes more readily accessible to most laboratories [37–39]. Finally, although individual microdialysate samples can be collected off-line with a fraction collector and stored for analysis at a later time, handling of these low volume samples may introduce errors due to evaporation or adsorption of the analyte to the wall of the collection tubes. More reproducible results are in general obtained by on-line analysis.

5. Pharmacokinetic calculations based on microdialysis data

A fundamental difference between serial blood sampling and microdialysis sampling is that the former is concerned with analyte concentrations measured at discrete time points while microdialysis samples are obtained during a certain time interval and therefore reflect the ‘average’ concentration of analyte during that collection interval. This should be

considered in the description of the concentration–time profiles [40–42]. A minimum requirement to represent the analyte concentrations derived from microdialysis sampling as a function of time is to use analyte concentrations at the midpoint of the sampling interval. In many cases, the midpoint concentration is a reasonable approximation, but for long sampling intervals, relative to the half-life, analyte concentrations are best calculated based on algorithms developed by Ståhle [40]. One possible drawback of microdialysis sampling to characterize the systemic pharmacokinetics of a substance is related to the fact that the first analyte concentration is obtained at the midpoint of the first collection interval. If the i.v. bolus pharmacokinetics of a substance with a rapid distribution into the peripheral tissues are studied, the first usable analyte concentration may only be obtained at 5 min (in case of a 10-min collection interval) following administration or possibly even later. This may not give a very accurate description of the early distribution phase of the substance. The key issue here is the sensitivity of the analytical technique because this will determine the actual sampling interval to collect a sufficient quantity of analyte for accurate determination. Depending on the sensitivity of the analytical technique, it may be possible to use short sampling intervals immediately following i.v. injection of the substance, because blood concentrations are highest, and to gradually increase the collection intervals as blood concentrations decrease. In general, however, the calculation of AUC and AUMC, and derived parameters such as CL and V_{ss}, is more accurate for microdialysis data than for data derived from serial blood sampling.

6. Applications

6.1. Determination of in vivo plasma protein binding parameters

Microdialysis sampling can be used to determine the plasma protein binding of drugs in vitro [42–46]. However, a much more interesting application of microdialysis sampling in this area is the determination of plasma protein binding parameters in vivo [47]. Indeed, using blood microdialysis, to determine unbound concentrations of analyte in the circulation, in combination with a classical blood sampling technique to measure total concentrations of analyte in blood, the in vivo unbound fraction of analyte can be calculated and the binding parameters B_{max} and K_d can be estimated [48]. Determination of in vivo plasma protein binding parameters has been described for flurbiprofen [48], methotrexate [49], and valproate [50]. In the case of the flurbiprofen study, a biexponential equation was fitted to the plasma and blood microdialysate flurbiprofen concentrations that were obtained simultaneously in the same rats during a 6-h period following i.v. bolus administration of flurbiprofen (20 mg/kg). The unbound fraction of flurbiprofen in blood was subsequently calculated as the ratio of the biexponential equations describing the unbound concentrations, obtained by blood microdialysis sampling, and total concentrations in plasma, obtained by serial blood sampling, of flurbiprofen as a function of time. The results showed that plasma protein binding of flurbiprofen in rats is concentration dependent. The binding parameters were estimated using a one-site binding model including a linear non-saturable binding component, and were the following (mean±standard deviation): $B_{max} = 52.5 \pm 14.3$ μg/ml; and $K_d = 0.194 \pm 0.162$ μg/ml. The same authors also determined the plasma protein binding of flurbiprofen by equilibrium dialysis in vitro and ex vivo. The in vitro results showed a systematic underestimation of the unbound fraction of flurbiprofen compared to the values obtained by in vivo microdialysis. Ex vivo equilibrium dialysis resulted in unbound fractions of flurbiprofen in plasma very close to those obtained by in vivo microdialysis. In addition, it was shown that serial blood sampling (10 samples of 250 μl over 6 h) did not significantly alter the pharmacokinetics of flurbiprofen as determined by simultaneous blood microdialysis sampling.

6.2. Distribution of drugs across the blood–brain barrier

Microdialysis sampling was initially developed to study various aspects of neurochemistry using implantation of rigid microdialysis probes into specific areas of the brain. As soon as the technique was extended to blood sampling, simultaneous implanta-

tion of probes in the brain and in a blood vessel made it possible to study the transport characteristics of drugs and other substances across the blood–brain barrier. For example, Desrayaud et al. [51] studied the effect of the P-glycoprotein inhibitor SDZ PSC 833 on the distribution of colchicine across the blood–brain barrier in the rat by using simultaneous blood and brain microdialysis. Coadministration of SDZ PSC 833 increased the brain penetration of colchicine by at least a factor of 10. This finding is consistent with the hypothesis that the P-glycoprotein pump present at the blood–brain barrier effectively limits the penetration of colchicine into the brain. However, P-glycoprotein or related multi-drug-resistant transporters are also involved in the active secretion of colchicine into bile and urine, and in vivo studies in rats have demonstrated that SDZ PSC 833 may impair the normal elimination of colchicine [52,53]. Therefore, simultaneous measurement of blood and brain concentrations of colchicine is very important for a correct interpretation of the results.

Simultaneous blood and brain microdialysis was recently also used to investigate the distribution of the highly lipophilic substance codeine across the blood–brain barrier in the rat [54]. One microdialysis probe was inserted into the right jugular vein and another one was implanted into the striatum. After a 24-h recovery period, each rat received three doses of codeine using a randomized cross-over design over 2 days: (1) a 10-min i.v. infusion of 10 mg/kg; (2) a 10-min i.v. infusion of 20 mg/kg; and (3) an exponential infusion for 2 h to rapidly reach 2500 ng/ml. The results showed that codeine was rapidly transported into the brain extracellular fluid and quickly reached distribution equilibrium with equal unbound concentrations in blood and brain. The brain transport of codeine did not show any dose dependency. The correct estimation of the relative recovery of the analyte by both probes is obviously a key issue in this type of experiments. Nalorphine was used as a retrodialysis calibrator for estimation of the in vivo recovery of codeine and results showed that in vivo retrodialysis of codeine and nalorphine were not different within and between the two study days.

Simultaneous microdialysis sampling in a blood vessel and in the brain of rats has been used to study the distribution across the blood–brain barrier of compounds such as tacrine [55], theophylline [56], bispyridinium oxime [57], and others.

6.3. Biliary excretion and enterohepatic cycling

Because of the much higher temporal resolution of the pharmacokinetic data obtained by microdialysis sampling compared to classical sampling techniques, slight irregularities in the blood drug/metabolite concentration–time profiles can be much better characterized. For example, while studying the pharmacokinetics of the flurbiprofen enantiomers in the rat using blood microdialysis sampling, Eeckhoudt et al. [58] consistently found a small secondary peak in the blood concentration–time profile of unbound *S*-flurbiprofen occurring between 2 and 4 h after i.v. administration of racemic flurbiprofen suggesting enterohepatic recycling (Fig. 2). Subsequent studies confirmed that this secondary peak was indeed due to enterohepatic recycling of *S*-flurbiprofen involving biliary excretion of *S*-flurbiprofen glucuronide with subsequent hydrolysis in the gut followed by re-absorption of the released *S*-flurbiprofen.

Scott and Lunte [14] used a specially designed flow-through microdialysis probe for sampling bile

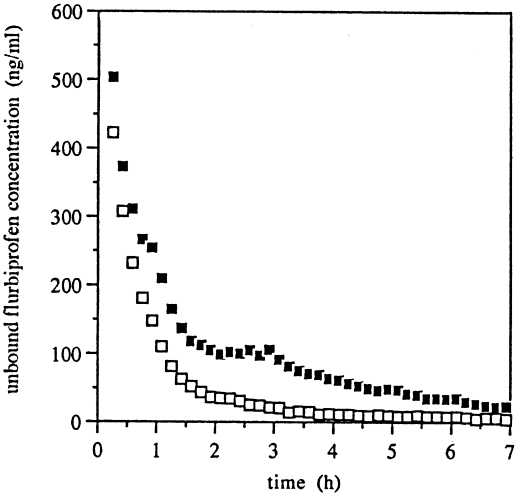

Fig. 2. Unbound concentrations of *R*-flurbiprofen (□) and *S*-flurbiprofen (■) measured by microdialysis sampling after i.v. administration of racemic flurbiprofen (20 mg/kg). The small peak in the unbound *S*-flurbiprofen concentrations in blood at approximately 3 h following drug administration is due to enterohepatic cycling [58].

in the anesthetized rat. This technique offers the advantage that the bile flow is not interrupted and that the bile salts, therefore, are recirculated in a normal manner. This allows for longer bile sampling periods without dramatically altering the animal's physiological state. This approach was used to study the disposition kinetics of phenol and its major metabolites in the rat. Microdialysis probes were implanted in the jugular vein, in the bile duct and in the median lobe of the liver. After i.v. infusion of phenol (941 μg/ml for 20 min), three metabolites could be quantified at all three sampling sites: phenyl glucuronide (the major metabolite), hydroquinone and 2-glutathionyl-hydroquinone. Similar concentration–time profiles for these metabolites were found in blood and bile, but bile concentrations were always much higher than blood concentrations indicating that the phenol metabolites were actively secreted into the bile.

6.4. Blood microdialysis sampling in humans

Microdialysis sampling in humans is mostly carried out by implanting the probe subcutaneously, intramuscularly, in adipose tissue or also intracerebrally in critical care patients and during operations [59–64]. The application of blood microdialysis in man is limited because the risk associated with implanting a probe into a blood vessel is still higher compared with the risk involved in probe implantation into subcutaneous, intramuscular or adipose tissue. However, with the recent development of flexible and robust probes that can be easily sterilized and that are introduced in arteries/veins of patients using standard clinical procedures, blood microdialysis in man has become feasible and a few reports demonstrating the use of blood microdialysis sampling in patients have recently been published. Patsalos et al. [65] have studied the pharmacokinetics of various antiepileptic drugs (carbamazepine, phenytoin, primidone and phenobarbital) in patients with intractable epilepsy. It was observed that microdialysate pharmacokinetic profiles were comparable to those obtained by direct blood sampling. In addition, plasma unbound fractions for the various antiepileptic drugs were similar to those previously reported. Patients found the microdialysis probe highly acceptable and indeed preferable to conventional blood sampling. The same group also used blood microdialysis to monitor levodopa and 3-*O*-methyldopa in patients with Parkinson's disease [66]. The flexible microdialysis probe was inserted into a forearm vein and was used to continuously collect samples over a 2–6-h period. They concluded that blood microdialysis is less invasive than frequent intermittent direct blood sampling and can readily be used to continuously monitor levodopa pharmacokinetics to optimize levodopa treatment in Parkinson's disease and to better characterize the pharmacokinetic profiles of different formulations of the drug.

Páez and Hernández [16] developed a blood microdialysis probe and used it to monitor glucose levels after administration of an oral glucose load in healthy volunteers. They showed that the procedure is easy and safe and that it would be possible to monitor blood glucose levels in an individual for several days. Their microdialysis probe is attached to an i.v. catheter and therefore it is also possible to withdraw whole blood from the catheter while blood microdialysis is taking place. Simultaneous microdialysate and whole blood sampling from the same vein area may be very interesting when studying the plasma protein binding of drugs in vivo.

6.5. Blood microdialysis in mice

Repetitive blood sampling to carry out a full pharmacokinetic study in a single mouse is not possible because of its limited total blood volume. When using the mouse for pharmacokinetic studies, therefore, a number of animals is euthanized at certain times following drug administration and thus each animal only provides one blood concentration on the concentration–time profile. Microdialysis sampling, which is not associated with fluid loss, is therefore the only practical method to carry out pharmacokinetic studies in the mouse. The most challenging aspect of carrying out blood microdialysis in the unanesthetized mouse is the implantation of the probe in a suitable blood vessel. Evrard et al. [15] described a surgical implantation technique to implant a flexible home-made probe in the inferior vena cava of the rat and subsequently applied exactly the same technique to the mouse. The applicability of the technique was shown by

measuring flurbiprofen concentrations in blood microdialysates during a 6-h period after i.v. bolus administration of flurbiprofen (20 mg/kg) to the mouse. In addition, Evrard et al. [67] also used simultaneous blood and brain microdialysis in the mouse to investigate the distribution of colchicine across the blood–brain barrier. Application of microdialysis in the mouse to carry out pharmacokinetic studies not only leads to a marked increase in the quality of the data, but also significantly reduces the numbers of animals needed to characterize the pharmacokinetic behavior (including distribution across the blood–brain barrier) of a substance in this animal species.

6.6. Drug metabolism studies

Direct implantation of microdialysis probes in the liver is possible and several reports have appeared in the literature using simultaneous microdialysis sampling in blood and the liver [68,69]. Scott et al. [12] studied the pharmacokinetics and metabolism of acetaminophen using microdialysis to monitor the concentration–time profiles of acetaminophen and its glucuronide and sulfate conjugates. This experimental approach could potentially yield more complete metabolic and pharmacokinetic data.

In another study Davies and Lunte [68] implanted microdialysis probes in three different regions of the liver of anesthetized rats. The probes were placed in the median lobe of the liver 1 cm apart in the anterior, middle and posterior position. Following intravenous administration of phenol, it was shown that concentrations of phenol and hydroquinone, one of the phenol metabolites, were similar at all three probe positions in the median lobe of the liver for a given experimental animal. For the glucuronide of phenol and for the glutathione conjugate of hydroquinone, concentrations at the anterior probe were considerably lower (100–1000 times) than at the middle and posterior positions. In another series of experiments local delivery of phenol through the probes was used to investigate whether the regional differences in metabolite concentrations in the liver observed following systemic administration of phenol were a result of differences in metabolism or differences in substrate delivery. In this case, no differences in delivery of phenol or formation of metabolites were observed at the three implantation positions. It can therefore be concluded that the much lower formation rate of the phenol glucuronide and the glutathione conjugate of hydroquinone at the anterior position of the median liver lobe is due to regional differences in the levels of enzyme activity. This study demonstrates the feasibility of in vivo microdialysis sampling at various positions in the liver to study the heterogeneous distribution of drug metabolizing enzymes in this organ.

Finally, the simultaneous implantation of probes in a blood vessel, the liver and the bile duct may yield important information concerning the processes governing the transport of drugs and/or their metabolites from the hepatocytes to the circulation or to the bile canaliculi.

7. Conclusions

The application of microdialysis sampling to pharmacokinetic studies in laboratory animals and man has seen a rapid development during the last 10 years. Important progress has been made during this short period in areas such as probe design, calibration of the probes, simultaneous use of multiple probes and evaluation of the sampling technique during long-term implantation. Especially this last issue is very important as long-term use of implanted probes would allow repeated-measures experimental designs. Further research is needed in the area of dialysis membrane materials to limit tissue damage and the effect of such tissue damage on the pharmacokinetic and metabolic behavior of the compound of interest. With the further development of very sensitive bioanalytical techniques the sensitivity problems associated with the small volumes typical of microdialysis sampling, will become less of an issue. Due to the multiple advantages of microdialysis sampling, the technique will certainly also become more popular to carry out pharmacokinetic/metabolic studies in patients or healthy volunteers. Among these advantages, perhaps the most convincing are the vastly improved quality of the data and the possibility to study processes that are difficult to investigate using the classical approaches.

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