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The use of microdialysis for the determination of plasma protein binding of drugs*

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Abstract: Microdialysis sampling was used for the determination of the protein binding and the free therapeutic drug concentration of drugs in plasma *in vitro*. Several drugs with varying extent of protein binding and for which the plasma monitoring is important were studied. To mimic the *in vivo* situation, an artificial blood vessel was constructed and filled with spiked plasma circulating at the flow rate of human blood at 37°C. The microdialysis probe (16 mm membrane length, 20000 MW cut off) was placed in the vessel and perfused with 0.9% NaCl at 5 μl min^{-1}. Dialysates were collected every 10 min and were analysed by reversed-phase LC with UV detection. The free concentration of the drug was calculated by correcting the concentration in the dialysate for the recovery of the probe, which was also determined in the artificial blood vessel after the experiment. The data confirm that microdialysis is a valid alternative technique for the determination of protein binding or free therapeutic plasma concentration of drugs on a comparative basis. Reference to literature values indicates that the results of the proposed method correspond reasonably well with accepted values.

Keywords: *Protein binding; microdialysis; LC–UV; plasma; artificial blood vessel.*

Introduction

The determination of concentrations of drugs in different body compartments such as blood, urine, brain, liver, etc. is necessary for studying absorption, distribution, biotransformation and excretion. In this respect, plasma protein binding is an important factor in establishing pharmacokinetic and pharmacodynamic properties of a drug, as only the free fraction of the drug is pharmacologically active. Equilibrium dialysis and ultrafiltration are two techniques for determining protein binding. However, neither are applicable for *in vivo* pharmacokinetic studies of *free* drug concentrations.

Microdialysis is a bioanalytical sampling technique for drugs and/or endogenous substances after implantation of a dialysis membrane into the tissue of interest. The technique is developing very fast and apart from numerous studies on laboratory animals, a few studies have also been done in man. The value of the technique has already been proven in brain, liver, muscle tissue, eye and adipose tissue [1–5].

Validation of microdialysis sampling for the determination of protein binding has been established recently by Herrera *et al.* [6] and Ekblom *et al.* [7] by comparing this technique with ultrafiltration and equilibrium dialysis, respectively.

This paper describes the use of microdialysis sampling for the determination of plasma protein binding of a series of drugs with varying extent of protein binding and for which the monitoring of plasma concentrations is of clinical importance. An LC–UV system was developed and validated for the drugs under study. As opposed to the former studies, the protein binding experiments were carried out in a self constructed artificial blood vessel, filled with spiked plasma. The free fraction of the drugs was then determined by microdialysis sampling. The protein binding was calculated, taking into account the known amount of drug spiked to the plasma and the obtained free drug plasma concentration.

Experimental

Chemicals

Paracetamol, theophylline and sodium phenobarbital were obtained from Bios (Brussels, Belgium). Carbamazepine was a gift from Ciba-Geigy (Basle, Switzerland) and procainamide HCl was purchased from Janssen

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Chimica (Beerse, Belgium). Caffeine came from Fluka (Buchs, Switzerland), lidocaine HCl from Ludeco (Brussels, Belgium) and sodium phenytoin from Parke Davis (Brussels, Belgium). Human plasma was obtained from the blood transfusion centre of the university hospital. All other chemicals were analytical reagent grade or better and purchased from Merck (Darmstadt, Germany).

Stock solutions of the drugs were prepared in a 0.9% NaCl solution. For solubility and stability reasons some stock solutions were made using different methods. Paracetamol was dissolved in 0.001 N NaOH in 0.9% NaCl solution. Carbamazepine was dissolved in propylene glycol–0.9% NaCl solution (50:50, v/v) and sodium phenytoin was dissolved in the same solution adjusted to pH 11.5 with NaOH 1 N. Further dilutions were made in 0.9% NaCl solution (adjusted to pH 11.5 for sodium phenytoin).

Chromatographic conditions

The concentrations of the drugs in the microdialysates were determined by LC with UV detection. The chromatographic system consisted of a Gilson 305 piston pump (Villiers le Bel, France) with a manometric module 805 as a pulse dampner, connected to a Kontron 433 UV detector (Milan, Italy). Separation occurred on an Ultrasphere^{R} ODS column, 5 μm particle size, 25 × 0.46 cm i.d. (Beckman, San Ramon, CA, USA). The guard column (30 × 4 mm i.d.) was filled with μBondapak C18/Corasil, particle size 37–50 μm (Waters Chromatography Division, Milford, MA, USA). The Rheodyne injection loop (Cotati, CA, USA) of 20 μl was connected to a Gilson Aspec system in the autoinjecting mode. Integration was carried out on a Merck D2500 chromato-integrator (Darmstadt, Germany). All analyses were carried out isocratically at room temperature at a flow rate of 1 ml min^{-1}.

The mobile phase consisted of filtered (0.2 μm filter) 0.05 M KH_2PO_4 adjusted to pH 2.5 with concentrated H_3PO_4. Acetonitrile (ACN) was added as an organic modifier in different percentages according to the drug under study. To minimize injection errors, an external standard (ES) was added. Separation parameters, detection parameters and choice of external standard are listed in Table 1.

Microdialysis

The microdialysis probe (CMA, Stockholm, Sweden) was the same used in previous experiments [2]. Briefly, the length of the dialysis membrane was 16 mm and its outer diameter was 0.52 mm. The MW cut off point was 20000. The probe was connected to a microinfusion pump (CMA 100, Stockholm, Sweden) and perfused with 0.9% NaCl at a flow rate of 5 μl min^{-1}. The sampling period was set at 10 min yielding dialysates of 50 μl to which 20 μl ES solution was added. A 20 μl sample was injected into the LC system.

Artificial blood vessel

To mimic the *in vivo* situation, an artificial blood vessel was constructed (Fig. 1). The

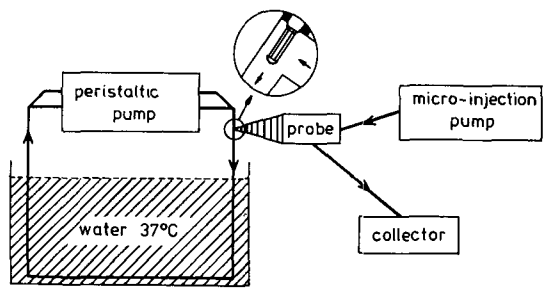

Figure 1
Schematic drawing of the experimental set-up for the determination of plasma protein binding of drugs by microdialysis using an artificial blood vessel.

Table 1
Chromatographic conditions, capacity factors (k'), detection limits (LOD) expressed as ng injected and repeatability expressed as %RSD for a standard solution of 10% of the FTPC of the drugs

	%ACN	λ	Conc. ES (μg ml^{-1})		k'	LOD	Repeatability
Paracetamol	3	220	Procainamide HCl	3	5.8	1.3	3.8
Procainamide HCl	3	220	Paracetamol	3.2	2.5	1.3	4.8
Caffeine	9	270	Theophylline	1.2	6.7	1.0	3.6
Theophylline	9	270	Caffeine	1.8	2.8	0.7	5.1
Lidocaine HCl	23	240	Na-phenobarbital	8	1.9	3.2	4.1
Carbamazepine	30	240	Lidocaine HCl	2.0	7.6	0.4	4.5
Na-phenobarbital	30	240	Carbamazepine	1	3.5	0.8	3.9
Na-phenytoin	33	240	Carbamazepine	1	5.1	1.6	3.5

tubing was made of SMA flow rate pump tubes (Technicon Instruments Corporation, New York, USA) with a total volume of 13 ml, and was connected to a peristaltic pump (Gilson, Villiers le Bel, France). The vessel could easily be filled and emptied and an opening was foreseen for entrance of an IV guide (CMA, Stockholm, Sweden) in which the microdialysis probe fitted perfectly. The dialysis membrane was positioned in the direction of the flow of the plasma or saline solution. The flow rate of the peristaltic pump was adjusted to the flow rate of human blood (15 ml min^{-1}) and the vessel was placed in a water bath (37°C).

Protein binding experiments

Blank plasma was spiked with the drug under investigation in the known total therapeutic plasma concentration (TTPC) (see Table 2) and vortexed for 5 min. The samples were then allowed to equilibrate overnight at 4°C. The next day the blood vessel was filled with the spiked plasma. The probe was placed in the vessel and was perfused at 5 μl min^{-1} during a 30 min equilibration period. Then 10 dialysates of 50 μl were collected. After the experiment the blood vessel was emptied and refilled with 0.9% NaCl solution containing the same drug in the known free therapeutic plasma concentration (FTPC) (see Table 2). Dialysates (n = 10) were again collected for determination of the relative recovery of the probe (RR%) for each substance. This was calculated by comparing the concentration of the drug found in the dialysate with the concentration of the drug in the solution from which it was dialysed:

$$RR\% = \frac{\text{conc. of the drug in dialysate}}{\text{conc. of the drug in artificial blood vessel}} \times 100.$$

Table 2
Theoretical total therapeutic plasma concentration (TTPC) and free therapeutic plasma concentration (FTPC) expressed as μg m^{-1}

	TTPC	FTPC
Paracetamol	10	8
Procainamide HCl	10	8
Caffeine	4	2.6
Theophylline	15	9
Lidocaine HCl	5	2
Carbamazepine	10	2
Na-phenobarbital	20	10
Na-phenytoin	20	2

The solution with which the plasma was spiked, was diluted and injected to determine the TTPC. The FTPC was obtained from the microdialysis data. The concentration found in the dialysates from plasma were corrected for the recovery of the probe:

$$FTPC = \frac{\text{conc. of the drug in dialysate}}{RR\%} \times 100\%.$$

The plasma protein binding (PB) was calculated using the following equation:

$$PB\% = \frac{TTPC - FTPC}{TTPC} \times 100.$$

Results and Discussion

The drugs for these experiments were chosen because of the clinical importance of their plasma monitoring (anti-epileptic drugs, theophylline) and because of their varying extent of plasma protein binding. The LC–UV method was developed so as little changes as possible were necessary for the different analyses to enable autoinjection. Table 1 shows capacity factors, detection limits and repeatability (n = 6) for a standard solution of 10% of the FTPC for the different drugs. The detection limits were taken as the amount corresponding to a S/N ratio of 3. Linearity was confirmed by injecting standard solutions in a concentration range corresponding to 10–100% of the FTPC of each drug. Specificity of the method was affirmed by injecting dialysates obtained from blank plasma at the different detection wavelengths used in the method. A major advantage of microdialysis is that it is a selective sampling technique in the sense that no plasma proteins (and therefore no protein-bound drug) or other compounds with large MW (>20000 Dalton) enter the perfusion fluid. Particularly interesting for pharmacokinetic studies *in vivo* is that no body fluids are removed during monitoring. Direct analysis without sample clean-up is possible. No internal standard is needed as it is a one-step procedure. However, it was found necessary to use an external standard to reduce injection errors of small volumes.

Another advantage of using microdialysis is that no enzymes enter the perfusion fluid enhancing the stability of some substances in the dialysates that are prone to enzymatic degradation.

Table 3
Percentage of plasma protein binding (%PB) determined by microdialysis

	RR%	Mean % PB	%RSD	%PB literature [6, 7]
Paracetamol	57.8	31.0	7.3	15–20
Procainamide HCl	48.4	18.4	5.7	15
Caffeine	65.8	46.3	7.7	±35
Theophylline	62.0	52.7	6.8	40–65
Lidocaine HCl	50.0	48.0	6.1	±50
Carbamazepine	64.2	77.2	2.2	±75
Na-phenobarbital	61.8	46.0	7.3	±50
Na-phenytoin	73.8	90.6	0.9	±90

n = 10; 10 dialysates collected.

The data of the protein binding experiments are listed in Table 3. The results are largely in agreement with those obtained from other methods such as equilibrium dialysis and ultrafiltration [8, 9]. For paracetamol and caffeine a higher extent of protein binding was observed. However, data in the literature for these drugs seem contradictory. The results described in this report are also similar to the data obtained by Herrera *et al.* [6] who also studied paracetamol, phenytoin and theophylline, and Ekblom *et al.* [7] who studied phenytoin. The precision in our experiments is better in the sense that the %RSD never exceeds 8%. The authors used a shaker bath at 50 rpm or worked under constant stirring to achieve dynamic conditions.

In the artificial blood vessel the dialysis membrane was continuously renewed with plasma. In some preliminary experiments to this study the relative recovery of a solution of paracetamol in saline was compared when (a) placed a beaker (static system) with (b) placed in the artificial blood vessel (dynamic system), in the same conditions as described above. In the static system the RR% was 51.9 ± 4.5% and in the vessel 63.4 ± 2.9% (n = 10). These results show that renewing the dialysis membrane continuously enhances relative recovery and its precision. It is important to determine the relative recovery after the experiment. Calibration is essential because several factors such as air bubbles, aging and tears of the membrane can result in changes in recovery for a certain substance. Generally, acidic compounds are known to show higher recoveries compared with basic compounds [10, 11]. However, the charge and the total lipophilic character of the molecule also determine recovery. The higher precision of our measurements in this dynamic system also shows that while the drug is being dialysed, the drug binding equilibrium is being reinstalled quickly.

The results confirm that microdialysis is valid over a wide concentration range and a wide range of degrees of drug protein binding. The only limitation for using microdialysis is that for low free drug plasma concentrations there is not always an analytical method available with sufficient sensitivity. However, this is also the case for the other methods used for studying protein binding.

Conclusions

These data confirm that microdialysis is valid for the determination of plasma protein binding of drugs on a relative basis. Results can be obtained quite quickly with good precision using the proposed dynamic system (artificial blood vessel) in which the dialysis is carried out. This, together with the fact that no volume changes occur to the sample results in no disturbance of the binding equilibrium. Good correspondence between the data produced and literature values is observed. Analytical methods with good sensitivity and appropriate calibration of the microdialysis probe are two conditions for the success of using this technique for the determination of protein binding of drugs *in vitro*.

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References

[1] S. Sarre, P. Herregodts, D. Deleu, N. De Klippel, G. Ebinger and Y. Michotte, *Naunyn Schmiedeberg's Arch. Pharmacol.* **346**, 277–285 (1992).
[2] D. Deleu, S. Sarre, P. Herregodts, G. Ebinger and Y. Michotte, *J. Pharm. Biomed. Anal.* **9**, 159–165 (1991).

[3] D. Deleu, S. Sarre, G. Ebinger and Y. Michotte, *Naunyn Schmiedeberg's Arch. Pharmacol.* **344**, 514–519 (1991).
[4] J. Ben-Nun, R.L. Cooper, S.J. Cringle and I.J. Constable, *Arch. Ophthalmol.* **103**, 254–259 (1988).
[5] P. Arner, E. Kriegholm and P. Engfeld, *J. Pharmacol. Exp. Ther.* **254**, 284–288 (1990).
[6] A.M. Herrera, D.O. Scott and C.E. Lunte, *Pharm. Res.* **7**, 1077–1081 (1990).
[7] M. Ekblom, M. Hammarlund-Udenaes, T. Lundqvist and P. Sjöberg, *Pharm. Res.* **9**, 155–158 (1992).
[8] L.Z. Benet and L.B. Sheiner, in *The Pharmacological Basis of Therapeutics*, Appendix II (A.G. Gilman, L.S. Goodman and A. Gilman, Eds), pp. 1675–1737. Macmillan, New York (1980).
[9] B. Clarke and D.A. Smith, in *Clarke's Isolation and Identification of Drugs, Part II* (B. Clarke and D.A. Smith, Eds), pp. 309–1069. The Pharmaceutical Press, London (1986).
[10] H. Benveniste and P.C. Hüttemeier, *Prog. Neurobiol.* **35**, 195–215 (1990).
[11] S. Sarre, P. Herregodts, D. Deleu, N. De Klippel, G. Ebinger and Y. Michotte, *J. Chromatogr.* **575**, 207–212 (1992).

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