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Pitfalls of the application of microdialysis in clinical oncology: Controversial findings with docetaxel

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Abstract

Microdialysis is a novel and minimally invasive sampling technique, based on the diffusion of analytes from the interstitial compartment through a semi-permeable membrane, and enables direct assessment of tissue disposition and penetration of drugs. Variable antitumor responses may be associated with differences in tumor vascularity, capillary permeability or tumor interstitial pressure resulting in variable delivery of anticancer agents. In preparation of pharmacokinetic studies, aimed at measuring docetaxel concentrations in healthy and malignant tissues *in vivo*, in preclinical as well as clinical studies, *in vitro* recovery experiments were performed. In contrast to published data, the recovery experiments suggest that docetaxel has a very low recovery as a result of non-specific binding to currently available microdialysis catheters. Here we discuss our findings with docetaxel in a historical perspective and we report on our experience using polysorbate 80 to eliminate the non-specific binding and its effects on the recovery of docetaxel.

Keywords: Microdialysis; Non-specific binding; Polyurethane; Docetaxel

1. Introduction

It is currently unclear why within a patient with solid tumors there can be a reduction in the size of some tumors while tumors at other sites in the patient's body can progress during or after treatment, even though the genetic composition of the tumors is identical [1]. Such variable antitumor responses within a single patient may be associated with inherent differences in tumor vascularity, capillary permeability, and/or tumor interstitial pressure that result in variable delivery of anticancer agents to different tumor sites [2,3]. However, studies evaluating the intratumoral concentration of anticancer agents and factors affecting tumor exposure in preclinical models and patients are scarce [3–5]. In addition, preclinical models evaluating tumor exposure of anticancer agents and factors affecting tumor exposure may not reflect the disposition of chemotherapeutic agents in patients with solid tumors due to differences in vascularity and lymphatic drainage [2,3]. It is logistically difficult to perform the laborious studies required to evaluate the tumor disposition of anticancer agents and factors that determine the disposition in patients with solid tumors, especially in sites that are not easily accessible. Thus, there is need to develop and implement techniques and methodologies to evaluate the disposition and exposure of anticancer agents within the tumor matrix.

Microdialysis is a relatively novel and minimally invasive sampling method/technique based on the diffusion of analytes from the interstitial compartment (i.e., extracellular fluid, ECF) through a semi-permeable membrane (Fig. 1). Microdialysis was originally developed for the research of endogenous cerebral neurotransmitters. It is also used to determine the non-protein bound fractions of exogenous compounds such as small molecules and drugs in other tissues, tumors, or body fluids [6–12]. In oncology, the technique enables direct assessment of

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tumor disposition and penetration, which in turn can contribute to the optimization of anticancer therapy and the development of tumor-targeted drugs [13]. Since sensitive analytical methods such as LC–MS/MS, which allows one to quantitatively determine low drug concentrations in small volumes, are becoming the standard equipment of many pharmacological laboratories, microdialysis is finding its place in pharmacokinetic oriented studies of small molecules, including anticancer agents.

The catheter (Fig. 1) of the microdialysis system consists of an inlet through which a fluid, ideally with equal composition and osmolarity to the extracellular fluid, will be infused, i.e., the perfusate. The perfusate flows along a semipermeable membrane over which diffusion of small molecules from the extracellular fluid into the perfusate will take place. The dialysate (i.e., the solution which exits the probe) is then collected for analysis. As microdialysis experiments are not performed under equilibrium conditions, the concentration of small molecules in the dialysate is lower than the concentration in the extracellular fluid. One of the challenges concerning microdialysis is the estimation of the relative recovery (i.e., the ratio between the concentration of the analyte under investigation in the dialysate versus its concentration in the extracellular fluid).

The anticancer drug docetaxel (Taxotere®) is approved for the treatment of patients with locally advanced or metastatic breast cancer, non small-cell lung cancer (NSCLC), hormone refractory prostate cancer and the treatment of patients with advanced gastric cancer (see www.taxotere.com). The plasma pharmacokinetic profile of docetaxel is characterized by large interpatient variability in drug clearance, indeed typical values for variability range from 30 to 45%. Accordingly, this wide interpatient pharmacokinetic variability may explain, in part, why inadequate therapeutic effects (i.e., undertreatment) are seen in some patients and unacceptable severe toxicities (i.e., overtreatment) in others. Indeed, Bruno et al. [14] demonstrated that docetaxel systemic exposure was a significant predictor of time to tumor progression in NSCLC patients. However, intra-tumoral pharmacokinetic variability following administration of docexaxel may be more predictive of tumor response. The pharmacokinetic variability in tumors is significantly greater than the plasma pharmacokinetic variability [15]. Thus, plasma exposure of docetaxel is not a reliable measure of tumor exposure. Consequently, the application of microdialysis, allowing one to continuously monitor unbound docetaxel concentrations in the extracellular fluid of malignant and healthy tissues of cancer patients treated with docetaxel, may assist to evaluate the relationship between intra-tumoral pharmacokinetics and treatment efficacy.

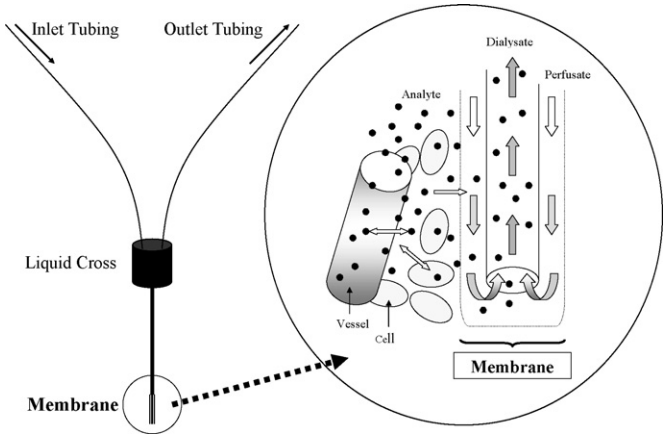

Fig. 1. Schematic presentation of microdialysis catheter and principle of dialysis.

Recently, it was reported that docetaxel is a candidate for microdialysis, with high relative recoveries up to 60% observed [16]. In preparation of planned pharmacokinetic studies, aimed at measuring docetaxel penetration and disposition in healthy and malignant tissues *in vivo*, in pre-clinical as well as clinical studies, we performed several *in vitro* microdialysis experiments.

Here we report on our experience with the non-specific binding of docetaxel to the microdialysis tubing and membrane, the use of polysorbate 80 to eliminate this non-specific binding and its effects on the recovery of docetaxel.

2. Experimental

2.1. Materials

CMA 70 microdialysis brain catheters (CMA, Stockholm, Sweden) with a membrane length of 10 mm and a molecular cut-off of 20 kDa were used throughout the initial experiments. The membrane of the CMA 70 catheter is made of polyamide, whereas the shaft (length, 6 cm), the inlet tubing (length, 60 cm) and the outlet tubing (length, 22 cm) are composed of polyurethane. Experiments were conducted either with the complete catheter or with 55 cm of the inlet tubing. In subsequent experiments 50 cm fluorinated ethylene propylene tubing (FEP, Teflon ™) was used alone and in combination with a 4 mm CMA12 Elite microdialysis probe made of polyarylethersulfone with a molecular cut-off of 20 kDa. Catheters and tubings were connected to CMA 107 pumps, with adjustable flow settings from 0.1 to 5.0 μL/min.

Docetaxel reference compound was a gift of Sanofi-Aventis (Anthony Cedex, France), while polysorbate 80 was purchased from Buva BV (Uitgeest, The Netherlands). Ringer's solution was obtained from Baxter (Utrecht, The Netherlands) and consisted of 147 mmol/L sodium (Na^+), 2.25 mmol/L calcium (Ca^{++}), 4.0 mmol/L potassium (K^+) and 156 mmol/L chloride (Cl^-). Paclitaxel, used as internal standard in the analytical method, was purchased from Sigma (St. Louis, MO, USA). After approval, fresh lithium heparinized plasma was obtained from a healthy volunteer. All chemicals were of analytical grade or higher.

As docetaxel was shown not to bind to the surface of 15 mL polypropylene centrifuge tubes (Techno Plastic Products, Trasadingen, Switzerland; data not shown), these tubes were used for the solutions and experiments in the absence of polysorbate 80.

2.2. LC–MS/MS system

Docetaxel was quantified using reversed-phase liquid chromatography coupled to tandem mass-spectrometric detection (LC–MS/MS). Samples were injected onto an Alltima HP C18 HL 3 μm column (50 mm × 2.1 mm internal diameter, Alltech Applied Science, Breda, The Netherlands) by a model 2795 XC chromatograph (Waters, Mildford, MA). The mobile phase was composed of acetonitrile and water containing formic acid (0.1% v/v) and delivered using different linear gradient settings, depending on the matrix analyzed, at a flow rate of 0.2 mL/min. Detection was performed with a MicroMass Quatro Micro triple-quadropole mass spectrometer (Waters) in the positive ion mode. The electrospray ionization was set at 3.8 kV and the cone voltage at 18 V. The collision energy for the fragmentation of docetaxel (*m/z* 808.3) and paclitaxel (*m/z* 854.3) was set at 9 and 20 eV, respectively. Argon was used as collision gas at a pressure of 0.004 mbar. The daughter ions of docetaxel (*m/z* 527.2) and paclitaxel (*m/z* 286.2) were monitored through the third quadropole and used for the quantitation. The dwell time per channel for data collection was set at 0.15 s.

2.3. Binding to tubing

With the flow-rate set at 5.0 μL/min (i.e., the maximum flow-rate), Ringer's solutions spiked with 100 ng/mL docetaxel (i.e., a concentration assumed to be clinically relevant) and different polysorbate 80 concentrations were perfused through 55 cm of the polyurethane inlet tubing (0.10–0.25–0.50–1.0% polysorbate 80) of the CMA 70 catheter and 50 cm of FEP-tubing (0.01–0.05–0.10% polysorbate 80). Three consecutive 15 min samples (i.e., 75 μL) from the dialysate were collected, which were then analyzed for docetaxel. By using the maximum flow rate, experiments could be performed within a reasonable time frame.

2.4. In vitro recovery with the CMA 70 microdialysis brain catheters

The recovery of docetaxel in aqueous solution was determined by dialysis of Ringer's solution containing 1.0% polysorbate 80 against Ringer's solution spiked with 100 ng/mL docetaxel at a flow rate of 5.0 μL/min (i.e., extraction efficiency method; Eq. (1); Fig. 2A):

$$\text{recovery}\,(\%) = \frac{\text{conc}_{\text{dialysate}}}{\text{conc}_{\text{solution}}} \times 100 \quad (1)$$

Subsequently the recovery of docetaxel in aqueous solution was determined, at the same flow rate, by dialysis of Ringer's solution containing 1.0% polysorbate 80 spiked with 100 ng/mL docetaxel against blank Ringer's (i.e., retrodialysis method; Eq. (2); Fig. 2B):

$$\text{recovery}\,(\%) = \frac{(\text{conc}_{\text{perfusate}} - \text{conc}_{\text{dialysate}})}{\text{conc}_{\text{perfusate}}} \times 100 \quad (2)$$

Next, docetaxel spiked at a concentration of 100 ng/mL in Ringer's solution containing 1.0% polysorbate 80, was dialyzed at the same flow rate, against docetaxel spiked at a concentra-

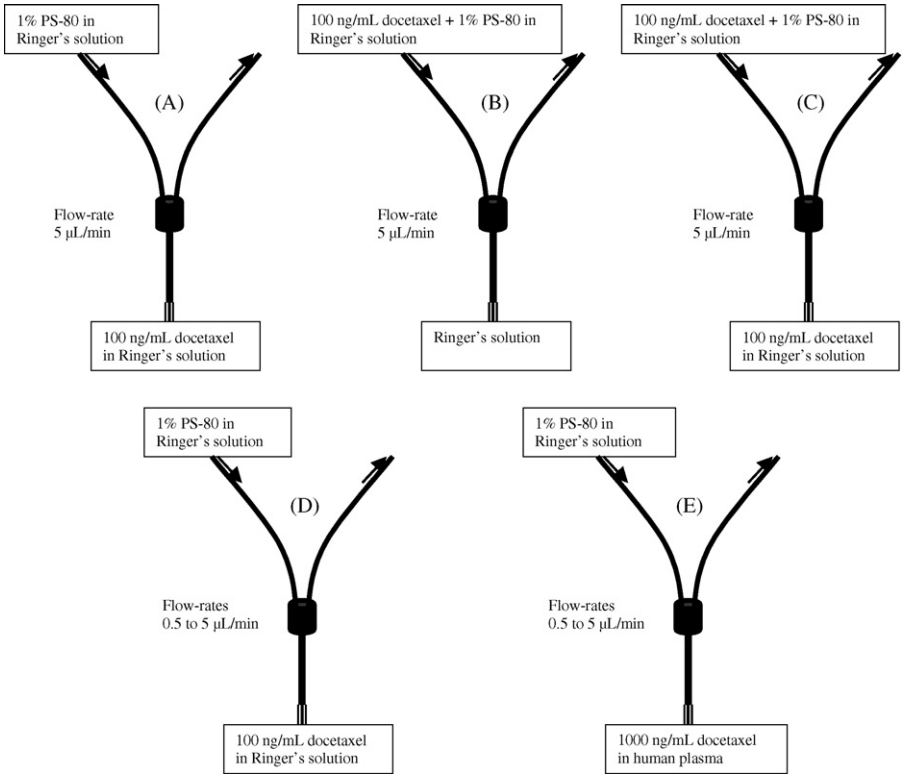

Fig. 2. Different experimental conditions used during the *in vitro* recovery experiments with the CMA 70 microdialysis brain catheters. See the text for the description of the experiments.

tion of 100 ng/mL in Ringer's solution. In quadruplicate, 15 min samples (i.e., 75 μL) were collected, after a pre-perfusion of 15 min, which were then analyzed for docetaxel (Fig. 2C).

In a second set of experiments, Ringer's solution containing 1.0% polysorbate 80 was dialyzed at flow rates in the range of 0.5 to 5.0 μL/min against Ringer's solution spiked with 100 ng/mL (Fig. 2D) or freshly obtained human heparinized plasma spiked with 1000 ng/mL docetaxel (Fig. 2E). Samples were collected in triplicate and analyzed for docetaxel. The sample time depended on the flow rate used.

Experiments were conducted in 15 mL polypropylene centrifuge tubes in a shaking waterbath with the temperature set at 37 °C.

2.5. *In vitro recovery with FEP-tubing connected to the CMA 12 Elite probe*

To the inlet as well as the outlet of the CMA12 Elite probe, 50 cm of FEP-tubing was connected. The recovery of docetaxel (100 ng/mL) in Ringer's solution containing 0.10% polysorbate 80 was determined by the extraction efficiency method and the retrodialysis method, both at the flow rate set at 1.0 μL/min. In triplicate, 45 min samples (i.e., 45 μL) were collected, after a pre-perfusion of 30 min, which were then analyzed for docetaxel. Experiments were conducted in 15 mL polypropylene centrifuge tubes in a shaking waterbath with the temperature set at 37 °C.

3. Results

As shown in Table 1, the non-specific binding of docetaxel to the polyurethane inlet tubing of the CMA70 catheter depends on the concentration of polysorbate 80 in Ringer's solution, with minimal or no binding in the presence of 1.0 % polysorbate 80 (of note high variability observed). At lower polysorbate 80 concentrations, a substantial amount of docetaxel binds to the tubing. In contrast, even at low polysorbate 80 concentrations as low as 0.01%, docetaxel did not bind to the FEP-tubing (Table 1).

Using the CMA-70 catheter, the apparent recovery of docetaxel in aqueous solutions at a flow rate of 5.0 μL/min as determined by the efficiency method (33.3 %) was approximately 3-fold higher compared to the recovery as determined by the retrodialysis method (12.2 %). In accordance, docetaxel has a higher affinity for Ringer's solution containing 1.0% polysorbate 80 compared to Ringer's solution without polysorbate 80, as shown by the increased concentration of docetaxel in the dialysate when docetaxel spiked in Ringer's solution containing 1.0% polysorbate 80 was dialyzed against docetaxel in Ringer's solution without polysorbate 80 (Table 2). Polysorbate 80 did not diffuse across the probe membrane from the perfusate to the aqueous solution and thus has no influence on the constitution of the solution (data not shown).

By lowering the flow-rate, the apparent recovery of docetaxel increased, with recovery values exceeding 100% when Ringer's solution containing 1.0% polysorbate 80 was dialyzed at a flow-rate of 1.0 μL/min against docetaxel spiked in Ringer's solution without polysorbate 80 (Fig. 3A). Apparent recoveries of docetaxel spiked in human heparinized plasma markedly increased upon lowering the flow-rate, with apparent recoveries of 30% at a flow rate of 0.5 μL/min (Fig. 3B).

As shown in Table 3, using the CMA 12 Elite probe connected to the FEP-tubing, the recovery as determined by the extraction recovery method as well as by the retrodialysis method decreases over time, with lower recoveries at later time points.

Table 1
Binding of docetaxel to polyurethane and FEP-tubing (data are presented as mean ± S.D. of three measurements)

Perfusate spiked with 100 ng/mL docetaxel	Polyurethane (%)[a]	FEP (%)[a]
Ringer's solution plus 0.01% (v/v) PS80	ND	1.9 ± 3.6
Ringer's solution plus 0.05% (v/v) PS80	ND	−1.6 ± 5.5
Ringer's solution plus 0.10% (v/v) PS80	43.6 ± 5.7	1.6 ± 5.1
Ringer's solution plus 0.25% (v/v) PS80	34.3 ± 5.4	ND
Ringer's solution plus 0.50% (v/v) PS80	11.1 ± 3.4	ND
Ringer's solution plus 1.0% (v/v) PS80	5.2 ± 5.6	ND

Abbreviations: FEP, fluorinated ethylene propylene; PS80, Polysorbate 80; ND, not determined.

[a] Percentage binding calculated as: $1 - (\text{conc}_{\text{dialysate}}/\text{conc}_{\text{perfusate}}) \times 100$.

Table 2
Recovery determination of docetaxel with the CMA-70 catheter (data are presented as mean ± S.D. of four measurements)

Experiment	Dialysate (ng/mL)	Recovery (%)
Extraction efficiency method	33.9 ± 3.79	33.3
Retrodialysis method	92.3 ± 3.53	12.2
Docetaxel spiked at both sites	120 ± 2.90	NA

Abbreviation: NA, not applicable.

4. Discussion

Several methodologies for recovery determination for microdialysis experiments exists [9,17,18]. As part of *in vitro* studies, the relative recovery can easily be determined using the extraction efficiency method (Eq. (1)). The catheter is placed in a solution containing the analyte of interest and perfused with blank solution. *In vitro* calibration alone is insufficient and not feasible for *in vivo* studies, but may be useful to investigate potential adsorption of small molecules to the microdialysis probe. The recovery of drug as part of *in vivo* microdialysis studies is highly variable in different sites with the same tissue or tumor and across studies. Thus, the recovery of drug must be

Table 3
Recovery determination of docetaxel with the CMA-12 Elite probe and FEP-tubing (data are presented as individual data)

Experiment	Dialysate (number)	Recovery (%)
Extraction efficiency method	First 45 min	15.2
	Second 45 min	13.9
	Third 45 min	12.2
Retrodialysis method	First 45 min	24.0
	Second 45 min	18.5
	Third 45 min	10.5

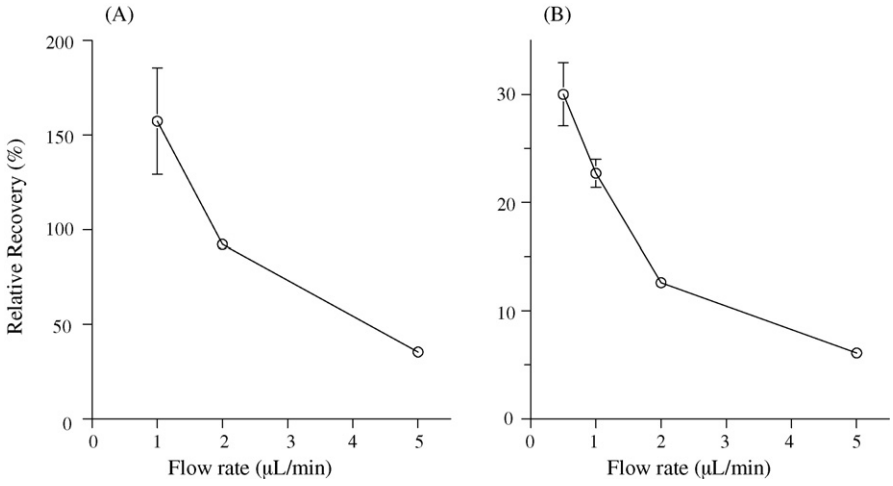

Fig. 3. Apparent relative recovery of docetaxel from (A) Ringer's solution spiked with 100 ng/mL docetaxel and (B) fresh human lithium heparinized plasma spiked with 1000 ng/mL docetaxel, both dialyzed against 1.0% polysorbate 80 in Ringer's solution. Data are presented as mean ± S.D. of three measurements (two measurements for flow rate set at 1.0 μL/min in (A) and four measurements for flow-rate set at 5.0 μL/min in (B)).

performed in each probe in each study [15]. Since the diffusion rates of most small molecules differ between aqueous solutions and the extracellular fluid in tissue, validation of the *in vivo* recovery is essential for the quantitative determination of extracellular fluid concentrations. A commonly applied technique to determine the *in vivo* recovery is the so-called retrodialysis method, in which the analyte of interest is spiked in the perfusate (Eq. (2)). Since diffusion processes are considered to be equal in both directions over the membrane, the loss of analyte in the perfusate is equal to the *in vivo* recovery. Since the drug of interest is added to the perfusate solution for the standard retrodialysis method this procedure cannot be used to evaluate the disposition of drug in tissue or tumor ECF after the drug has been administered. To overcome this problem, retrodialysis calibration using a tracer agent can be used [15]. This methodology allows the estimation of the recovery using the tracer agent at the same time as sampling of the drug.

Previous studies have evaluated the plasma, tumor, and tissue disposition of docetaxel [19–21]. Zamboni et al. were the first to report extended tumor exposure of docetaxel as compared to plasma and normal tissue. High docetaxel concentrations were maintained at late time points as compared to plasma and other tissues with the retention of docetaxel at 24 h being 132-fold and 15-fold higher in tumor as compared with plasma and liver, respectively [19]. This prolonged retention of docetaxel in tumors has not been reported for other anticancer agents except for geldanamycin-analogues and carrier-mediated anticancer agents (e.g., liposomes, nanoparticles, and conjugates) [22–24]. The factors associated with the prolonged tumor retention of docetaxel are unclear. Microdialysis may allow to evaluate whether docetaxel is primarily located in the tumor ECF or bound to tumor matrix associated proteins or plasma related proteins.

In preparation of planned pharmacokinetic studies, aimed at measuring docetaxel penetration and disposition in healthy and malignant tissues *in vivo*, in pre-clinical as well as clinical studies, we conducted a set of *in vitro* microdialysis experiments. During initial *in vitro* recovery experiments, performed with docetaxel in Ringer's solution and CMA 70 catheters (CMA, Stockholm, Sweden), we observed irreproducible results and low apparent recoveries of docetaxel. In addition, we noted that docetaxel dissolved in Ringer's solution substantially binds to several laboratory tubes. Addition of human serum albumin, a substance known to saturate the surface of several plastics including polyurethane [25,26], thereby potentially reducing the non-specific binding of compounds, did not resolve the non-specific binding of docetaxel. Though, after the addition of the non-ionic surfactant polysorbate 80 (also used to solubilize docetaxel in the commercial drug formulation, Taxotere®) to Ringer's solution at a concentration of 0.10% (v/v), docetaxel no longer bound to laboratory tubes and microdalysis collection vials (data not shown).

The (relative) recovery of any drug/analyte evaluated using the microdialysis technique depends on several determinants, including perfusate flow rate, physicochemical properties of the drug and the microdialysis materials (i.e., dialysis membrane, internal tubing and outlet tubing). Regarding the analyte the most important determinants are the degree of binding of the analyte to the membrane or (inlet/outlet) tubing and its physicochemical properties. Indeed, a prerequisite for reliable estimations of unbound drug concentration based on the application of microdialysis is that the drug does not bind to the microdialysis equipment, or that if binding does occur, that the binding variability is acceptably low and predictable. Docetaxel is a highly lipophilic drug (water solubility, 3 μg/mL) and exhibits a high degree of protein binding, notably to albumin [27]. Previously, recovery problems of other agents due to adsorption/binding to the dialysis membrane and the plastic tubing material have been attributed, in part, to the lipophilicity of the compound of interest [28]. Investigated solutions include the addition of albumin [29] or of beta-cyclodextrin [30] in order to saturate the binding sites on the microdialysis equipment. However, in our experiments, in the presence of albumin (up to 4%), glucose or dextran we still observed substantial binding (up to 80–90%) of docetaxel when

evaluated at clinically relevant concentrations (data not shown). Moreover, by addition of albumin to the perfusate, a third additional compartment with a different composition compared to the interstitial fluid will be introduced. It is unknown what effect this will have on the diffusion over the semi-permeable membrane; for instance this may potentially result in the formation of a gradient of the drug in the tissue, as the affinity of the drug for the compartments might be different.

It has been hypothesized that the degree of protein binding could predict the degree of binding to the outlet tubing, however, this does not appear to be universally the case [28]. Indeed, although both protein binding and lipophilicity seem to be involved to a certain degree in drug adsorption to microdialysis equipment, specific interactions with, for instance polyurethane most likely also play an important role. Improvement of the (*in vitro*) recovery has also been achieved by the addition of organic solvents (e.g., ethanol and 1-propanolol 33% v/v) [26]. However, addition of such solvents to the perfusate solutions for *in vivo* use clearly has disadvantages.

Recently it was reported that docetaxel is a candidate for microdialysis [16]. High relative recoveries of docetaxel up to 60% were observed. In contrast to the studies presented here with the CMA-70 catheter, very long equilibration periods, up to 3 h, were required before the relative recoveries were determined. It thus may well be that during this equilibration time; the tubing and membrane were saturated with docetaxel. As very high, clinically irrelevant concentrations were used, saturation of the microdialysis catheter might occur within this equilibration period. Moreover, the equilibration period in the no-net-flux method, in which Ringer's solution was dialyzed against plasma spiked with docetaxel was longer than the equilibration period when dialysis was performed against docetaxel in Ringer's solution. As docetaxel is highly protein bound and only the unbound fraction is able to cross and bind to the membrane, the exposure of docetaxel in the presence of plasma proteins to the microdialysis catheter is lower and saturation of the system, as a consequence, takes longer. In addition, as in the study by Schuck et al. [16] the recovery observed by retrodialysis method was slightly higher than those observed by the extraction efficiency method binding to the system seems logical. As also discussed by the authors, in the case of retrodialysis docetaxel needs to pass the inlet tubing, the membrane and the outlet tubing, while in the case of the extraction efficiency method, docetaxel is only exposed to the membrane and outlet tubing.

At present, clinically available microdialysis catheters all consist of a polyurethane tubing. For preclinical use also fluorinated ethylene propylene tubing (FEP, Teflon^{TM}) is available. It has been suggested that FEP-tubing may be a more suitable material for substances, which show considerable adsorption to the polyurethane tubing. As shown in Table 1, in contrast to the polyurethane tubing no binding of docetaxel was observed to the FEP-coated tubing. However, the recovery of docetaxel, using the 4 mm CMA 12 probe connected to FEP-coated tubing was shown not to be stable over time. A long equilibration time is thus needed, which is most likely due, as discussed above, to non-specific binding of docetaxel to the dialysis membrane.

In conclusion, it would seem that other drug specific mechanisms of binding play a role and that non-specific binding to microdialysis catheters should be evaluated *in vitro* in each individual case at clinical relevant concentrations before *in vivo* application of microdialysis can be contemplated. In the case of docetaxel, the addition of polysorbate 80 to avoid binding to the microdialysis probes will introduce an additional compartment, resulting in an extra convection of docetaxel into the microdialysate. The distribution of docetaxel in the tissue directly surrounding the membrane of the microdialysis probes will thus be disturbed and docetaxel concentrations in the perfusate will be altered and not represent true tissue concentrations. Docetaxel is thus not a candidate for microdialysis in clinical as well as in pre-clinical studies.

Conflict of interest

The authors declare no conflict of interest.

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