Research Article

Pilot Investigation on Long-Term Subcutaneous Microdialysis: Proof of Principle in Humans

Franziska Simmel,¹ Claudia Kirbs,^{1,2} Zeynep Erdogan,³ Edith Lackner,³
Markus Zeitlinger,³ and Charlotte Kloft^{1,2,4}

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Abstract. Reliable drug concentration measurements at the target site are increasingly demanded and can be achieved by microdialysis. The aim of this pilot study was to demonstrate the proof of principle of long-term subcutaneous microdialysis in humans. For long-term microdialysis, a special setting implementing both concentric and linear catheters has been developed ensuring good clinical practice compliance, tolerability, and convenience for participants and personnel. As a model compound, moderately lipophilic voriconazole was selected as a well-characterized drug in *in vitro* microdialysis experiments. Multiple *in vivo* relative recovery (RR) determinations for microdialysis were performed by retrodialysis during the entire study ($n=48$ samples). Continuous microdialysis was successfully applied and well tolerated over 87 h in three adults for the first time. RR revealed low intra-individual (coefficient of variation (CV)=4.4–12.5%) and inter-individual variability (CV=4.3–12.5%) across all samples and catheters. Lower RR values were consistently determined for linear catheters. One catheter leakage was managed without an impact on the reliability of the RR values. Overall, RR values were calculated to be 73.3% (linear: CV=18.5%, $n=23$) and 84.9% (concentric: CV=5.6%, $n=23$). Long-term microdialysis application over almost 4 days was feasible by reliable multiple RR (proof of principle), well tolerated, and reduced the burden in humans avoiding several catheter insertions, thereby allowing to monitor concentration–time courses continuously. Moreover, a moderately lipophilic drug has been proven suitable for *in vivo* microdialysis, as previously suggested by *in vitro* microdialysis.

KEY WORDS: concentric/linear microdialysis catheter; long-term microdialysis; proof of principle; relative recovery; retrodialysis.

INTRODUCTION

Microdialysis is a minimally invasive catheter-based continuous sampling technique via a semipermeable membrane. As a method of choice for direct access to the interstitial space fluid (ISF) and the drug target space of agents against, e.g., extracellular domains of receptors or extracellular pathogens, it allows determining local, unbound, and, hence, pharmacodynamically active drug concentrations (1,2). Clinical drug investigations, aiming at analyzing the pharmacokinetics (PK) after single and multiple dosing, usually employ the microdialysis technique in two or more subsequent steps requiring at least two catheter insertions (3–8). Via long-term insertion, data could be obtained for several days. However, knowledge of the long-term performance of

microdialysis is limited. Prolonged microdialysis sampling over several days has been used for endogenous compounds in humans (9–23) in neonatal (10,16,17) and adult (11,12,14,18) diabetic patients, in patients admitted for breast reconstruction with transverse rectus abdominis muscle flaps after mastectomy (13), in patients with ischemic heart disease (23), and in patients for neurochemical monitoring or on the neurosurgical ICU (19–22). Some problems were reported, e.g., accidental removals of microdialysis catheters in agile infants (10,16), disturbances in perfusion flow (10), minor bleeding around the microdialysis catheter during microdialysis in one child (10), discontinuation because of obstruction of the inlet tubing of the catheter (12), or removal of a catheter because of a puncture of a small vein (16).

However, fewer investigations than for endogenous compounds have been published for xenobiotics in clinical (PK) studies (24–26). Stähle *et al.* (26) and Lindberger *et al.* (27) monitored valproic acid in epileptic patients for 3 days. The relative recovery (RR) of valproic acid was determined once on day 2. Kopacz *et al.* (25) did not assess RR at all in studying the PK of bupivacaine and dexamethasone in healthy volunteers. Recently, Konings *et al.* (24) reported a prolonged microdialysis sampling over 47 h in carboplatin-treated cancer patients. Microdialysis catheters were inserted at the tumor site and into the healthy abdominal

¹ Institute of Pharmacy, Department of Clinical Pharmacy, Martin-Luther-Universität Halle-Wittenberg, 06120 Halle, Germany.

² Institute of Pharmacy, Department of Clinical Pharmacy and Biochemistry, Freie Universität Berlin, Kelchstr. 31, 12169 Berlin, Germany.

³ Department of Clinical Pharmacology, Division of Clinical Pharmacokinetics, Medical University of Vienna, 1090 Vienna, Austria.

⁴ To whom correspondence should be addressed. (e-mail: charlotte.kloft@fu-berlin.de)

subcutaneous tissue. *In vivo* RR was performed by retrodialysis on day 1 of the study. In conclusion, the group demanded for “further research [...] to evaluate the definite role of microdialysis in subcutaneous normal tissue” (24).

Microdialysis is usually recommended for hydrophilic drugs due to the lack of interaction between the catheter membrane and the test compound. In *in vitro* microdialysis experiments, a comprehensive characterization of the permeation behavior of the moderately lipophilic antifungal model compound (voriconazole) using clinically approved concentric and linear catheters has been performed (28). Overall, the results recommended the usage of both catheter types. The recently developed linear catheters allow for simple application in scientific investigations as well as potentially in clinical routine. So far, they have rarely been deployed in pre-/clinical investigations. A systematic investigation on catheter performance comparing both types within one individual over a longer period or between individuals has never been reported before.

This work explored the applicability of long-term microdialysis in humans during a pilot part of a clinical multiple dosing trial with voriconazole, implementing concentric and linear catheters (proof of principle). Therefore, a setting and all necessary procedures should be developed meeting the following requirements: good clinical practice (GCP) compliance especially regarding tolerability and the lack of risk of infection; easy implementation particularly in the clinical routine, i.e., convenience for participants and personnel; and warrant for maximum mobility and little clinical expenses.

MATERIALS AND METHODS

Microdialysis Basic Equipment

CMA107[®] (high-precision perfusion) pumps and CMA106[®] syringes (both CMA Microdialysis AB, Solna, Sweden) were used for continuously providing the perfusate through the microdialysis catheters (concentric catheter (CMA60[®]) and linear catheter (CMA66[®]); CMA Microdialysis AB). Table I offers an overview of the technical setup.

Clinical Study

The study was approved by the Ethics Committee of the Medical University of Vienna, Austria, and the responsible competent federal authority (BASG). Registration of the

clinical trial was made at ClinicalTrials.gov (identifier: NCT01539330; <http://www.clinicaltrials.gov>) and the European Clinical Trials Database EudraCT (no. 2008-008524-32). The study was conducted at the Department of Clinical Pharmacology, Medical University of Vienna, Austria, in accordance with the Declaration of Helsinki (29), the ICH guideline for good clinical practice (30), and regulatory requirements. Written informed consent was obtained from each volunteer before study participation.

Study Design

The investigation was designed as a prospective, open-label, uncontrolled pilot trial. The pilot study was planned as the proof of principle part of a multiple dosing PK study combining long-term microdialysis and blood sampling. Two microdialysis catheters (linear CMA66[®] and concentric CMA60[®] design; see Fig. 1a) were applied in three male healthy volunteers (ID 1–3). The study individuals were young/middle-aged (median=43 years, range=34–46 years), with merely little variability in height (median=181 cm, range=176–183 cm) and weight (median=82 kg, range=77–84 kg). Microdialysis catheters resided in the tissue for 87 h to determine RR.

In Vivo Long-Term Microdialysis Study Setting

The *in vivo* microdialysis pilot study setting is illustrated in Fig. 1b (left). (1) A modified medicinal belt (Lohmann & Rauscher, Vienna, Austria) was employed allowing to cover and to fix the pump, particularly at night, directly at the human body and at the same time avoiding direct contact with the skin. (2) Moreover, the bags of the belts enabled for an easy and fast change of the syringes. (3) Both microdialysis catheters were fixed by a transparent adhesive tape. (4) Safe microdialysate sampling as well as easy and fast change of the microvials were guaranteed by outlet tubing guide channels with a precisely tailored fitting connection for the microvials (Technical Laboratory at the Institute of Pharmacy/Martin-Luther-Universitaet, Halle/Saale, Germany; see Fig. 1b, right) to guide the outlet tubing of the microdialysis catheter directly into the microvial, avoiding any loss of microdialysate. The type of connection for the microvials depended on the volume sampled and the type of the microvials used, respectively. (5) A disposable common medicinal gown protected the pumps from water when taking a shower. (6)

Table I. Microdialysis Catheters

		Concentric catheter (CMA60 [®])	Linear catheter (CMA66 [®])
Materials	Membrane	Polyamide	Polyarylethersulphone
	Inlet and outlet tubing	Polyurethane	Polyurethane
Length (mm)	Membrane	30	30
	Inlet tube	400	400
	Outlet tube	105	100
Diameter (mm)	Membrane	0.6	0.5
	Inlet tube (inner/outer)	0.15/1.0	0.4
	Outlet tube (inner/outer)	0.15/1.0	0.4
Membrane cutoff (kDa)		20	20
Membrane area (mm ²) (=length· π ·diameter)		56.6	47.1

Fig. 1. **a** Exemplary illustrations of linear and concentric catheters. *Left panel*, Linear design. *Right panel*, Concentric design. (1) Clamp; (2) Membrane; (3) Outlet tubing; (4) Luer lock connection; (5) Inlet tubing. **b** *In vivo* microdialysis setting for long-term sampling during the clinical pilot study in healthy volunteers (*left*). (6) Medicinal belt with two bags for the pumps; (7) Microdialysis pump in bag; (8) Outlet tubing guide channels with a connection for microvials; (9) Transparent adhesive tape; (10) Concentric peripheral catheter; (11) Outlet tubing guide channels with a connection for microvials; (12) Microdialysis pump; (13) Linear peripheral catheter. Outlet tubing guide channels with connection for microvials of 500 μL (*top right*) and 1,500 μL (*bottom right*). (14) Catheter outlet tubing; (15) Guide channel for outlet tubing with connection for microvial; (16) Microvial

On an optional basis, a fastened medicinal cuff was applied for additional protection during the night.

Study Procedure

Seven study visits in 87 h had to be completed (see Table II). A visit was defined as the time span between the start of a drug administration and the start of the next administration. The study drug was administered twice a day as i.v. infusion (6 mg/kg on day 1 and 4 mg/kg on day 2) and tablet (200 mg on days 3 and 4). Physical examinations before, during, and after drug administration were performed according to the study schedule. Adverse events were continuously recorded. During the study procedures, the volunteers were mostly in a resting position and were recommended to continue in this position at home.

On study day 1 (visit 1), two microdialysis catheters were inserted (concentric and linear). The skin of the periumbilical abdomen was cleaned, disinfected at the site of the microdialysis catheter insertion, and then horizontally punctured by a steel guidance cannula without anesthesia. Using this guidance cannula, the microdialysis catheters were placed into the subcutaneous tissue. The microdialysis system was connected and perfused with Ringer's solution (Mayrhofer Pharmazeutika, Linz, Austria) at a flow rate of 2.0 $\mu\text{L}/\text{min}$ provided by a microinfusion pump. After insertion of the catheter, an equilibration period of 30 min and, afterwards, a calibration were performed (see below). The volunteers stayed at the research ward only for the first night. During the nights, the flow rates were adjusted to 0.5 $\mu\text{L}/\text{min}$ until the next study visit. After the last drug administration, a final examination for safety was performed.

Calibration Procedure of Microdialysis Catheters

The *in vivo* catheter calibration procedure of the microdialysis catheters for the determination of RR was repeatedly performed covering the entire study duration by means of retrodialysis (31). During retrodialysis, the permeation of the compound from the perfusate ($C_{\text{perfusate}}$) into the surrounding tissue was taken as the relative recovery, which was calculated using Eq. 1.

$$\text{RR, \%} = 100 \cdot \left(1 - \frac{C_{\text{dialysate}}}{C_{\text{perfusate}}} \right) \quad (1)$$

where $C_{\text{dialysate}}$ is the concentration in the dialysate (in micrograms per milliliter) and $C_{\text{perfusate}}$ is the concentration in the perfusate (in micrograms per milliliter).

Retroperfusates 1 (RP1) and 2 (RP2) were prepared as follows: Voriconazole (Vfend[®], 200 mg powder for solution for infusion; Pfizer, Vienna, Austria) was added to the perfusion medium, resulting in a concentration (C_{RP}) of 20 $\mu\text{g}/\text{mL}$ (RP1) or in a C_{RP} of 200 $\mu\text{g}/\text{mL}$ (RP2). The first *in vivo* catheter calibration procedure was carried out prior to the first drug administration at study visit 1 starting with an equilibration period of 10 min with RP1, followed by retrodialysate sampling by the study personnel (two intervals of 15 min, RR1 and RR2). Afterwards, a washout period of 30 min was performed and then a baseline microdialysate sample of 15 min was taken. For microdialysis catheter calibration during model drug administration after ~25, ~59, and ~84 h (i.e., at the end of the sampling period of study visits 3, 5, and 7), the procedure started with an equilibration period of 10 min with RP2 as the perfusate, followed by retrodialysate sampling by the study personnel (two intervals of 15 min, RR1 and RR2).

Table II. Study Procedures with Time Frame

Study days						
----- 1 -----	----- 2 -----	----- 3 -----	----- 4 -----			
Study visits						
1	2	3	4	5	6	7
Time [h]						
0 - 12	12 - 24	24 - 36	36 - 48	48 - 60	60 - 72	72 - 84
Dosing schedule						
x	x	x	x	x	x	x
Insertion of microdialysis catheter (linear and concentric)						
x						
Calibration schedule						
x	-	x	-	x	-	x
Confinement to study site						
----->	----->	----->	----->	----->	----->	----->

Bioanalysis of the Samples and Explorative Statistical Analysis

Bioanalysis of the samples was performed at the Department of Clinical Pharmacy, Martin-Luther-University Halle Wittenberg, according to a previously developed robust and fully validated HPLC method for the quantification of voriconazole in microdialysate (32). In brief, for sample preparation, a simple one-step dilution procedure was sufficient: Every (retro)-microdialysate sample, initially diluted with the perfusion medium when necessary, was mixed with acetonitrile (40:60, v/v; Roth, Karlsruhe, Germany) and vortexed (laboratory shaker S 421 MLW Prüfgeräte-Werk, Medingen, Germany). The mobile phase consisting of an ammonium dihydrogen phosphate buffer (Fluka Chemie, Neu-Ulm, Germany)/ACN mixture (pH 6.0, 40 mM) was, compared to (32), modified toward a higher buffer fraction (65:35, v/v), leading to higher voriconazole t_R values (~7.5 min) accounting for a co-eluent of unidentified origin at 5 min. A volume of 20 μ L of each sample was directly injected into the HPLC system and voriconazole was detected at 254 nm. The resulting peak areas of the dialysates were used for the calculations of the concentrations and RR, respectively. In total, imprecision and inaccuracy across the entire concentration range (0.15 (LLOQ)–10 μ g/mL) were in the required limits set by the international FDA guideline for bioanalytical methods (coefficient of variation (CV)=0.5–6.1%, RE=–3.3 to +7.0%) (33). Quality assessment during the study sample measurements was realized by quality control samples covering the entire bioanalytical range (for all samples, the CV and RE values were below $\pm 15\%$ for low, medium, and high concentrations of the quality control samples).

Localization (arithmetic mean, $\bar{x}$) and dispersion (SD and CV) parameters were used to characterize the data obtained from the recovery experiments. The Wilcoxon test and Kruskal–Wallis test, respectively, were applied to compare two (multiple) (in)dependent samples. The level of significance α was set to 0.05, i.e., probability values $p < 0.05$

were considered to indicate statistically significant differences. Statistical analysis was performed using SPSS Statistics® (SPSS Inc., Chicago, IL, USA) and Microsoft® Office Excel (Microsoft Corporation, Munich, Germany).

RESULTS

Feasibility of Long-Term Application of Clinical Microdialysis

Long-Term Setting in Humans

During the study, all three healthy volunteers adhered to the study protocol demonstrated by no dropouts and replacements. Moreover, the conduction took place according to the developed protocol and procedures in terms of drug administration and (retro)microdialysate sampling.

Tolerability of the Long-Term Setting by Volunteers

The tolerability of the procedure in healthy volunteers was good over the entire study based on standard procedure according to GCP for self-reporting of any adverse event by the volunteers as well as monitoring and interviewing of the volunteers by the study personnel. The acceptance of and positive attitude toward the catheters resulted from their sufficient fixation and protection, implying only marginal restrictions for the daily life of the volunteers. The reliable fixation of all components ensured the easy and fast exchange of the pumps, syringes, and microvials and guaranteed almost complete mobility of the volunteers. The dialysate samples were obtained also during the night by the study personnel.

Functionality and Performance of the Long-Term Setting

Apart from one leakage problem due to a disjuncture within one linear catheter tubing, the microdialysis device and

membranes of the catheters kept their functionality over the entire study duration (~87 h) and (retro)microdialysates were continuously obtained after fixation (with few exceptions; see below). Since no premature displacement of the catheters was indicated in any individual, (retro)microdialysate samples could be obtained from the catheter over the entire study duration according to an extensive sampling schedule.

In total, 419 of 420 scheduled (retro)microdialysates were sampled (99.8%). Only five samples (1.2%) had less volume than expected. The reasons were found to be sourced in the position of the healthy volunteer leading to a backflow of the microdialysate when moving to an upright position and the tubing slipping out of the sample vial holder. An additional fixation of the tubing at the outlet tubing guide channel avoided reoccurrence of that event. Microdialysis pumps worked reliably, apart from battery-related interruptions resulting in a discontinuation of the perfusate flow (once, ≤ 10 h) during the night. Managing both the leakage (*via* fixation by two tubing connectors) and the battery failure (change of batteries) led to the continuation in all volunteers until the planned end of the trial. After removal at ~87 h, the microdialysis catheters were complete without any damage and the sites of insertion were normal, except for one small hematoma at the site of insertion of one catheter.

In Vivo Relative Recovery

Sample Set

The feasibility of the long-term application of a microdialysis catheter was analyzed using extensive RR investigations leading to an overall number of 46 of 48 scheduled RR values (95.8%) for the three volunteers. Due to flow problems and insufficient volume, two values were lacking (ID 2, visit 3—concentric catheter; ID 3, visit 5—linear catheter). RR and RP samples were measured *via* HPLC and the RR calculated according to the retrodialysis method (31). In all individuals, ISF_c (obtained by the concentric catheter) and ISF_l (obtained by the linear catheter) concentrations were below 7.29 $\mu\text{g/mL}$ when retrodialysis started resulting from multiple dosing of the model compound. With the chosen perfusate concentration used for the calibration after multiple dosing, it widely exceeded the ISF concentrations (>20-fold). In conclusion, the perfusate concentrations were sufficient for calibration on all study visits.

RR Results in Concentric and Linear Catheters

The RR results for each catheter (concentric: RR_c; linear: RR_l) in each individual per study day, study visit, and RR samples 1 and 2 are listed in Table III. The tables include the concentrations of the RP samples (C_{RB} with R of CV=3.2–16.9%).

Concentric Catheters

Overall, RR_c ranged from 74.6% to 92.1% for both subsequently taken samples (separate for RR1=74.6–91.2%; separate for RR2=78.4–92.1%). The mean RR_c ranged from 82.3% to 86.8% for the first RR sample and from 82.9% to 87.8% for the second one. This narrow range for each RR

sample without significant differences between the IDs ($p=0.212$) was supported by low SDs and CVs predominantly in the single-digit magnitude and in general <8.3%, demonstrating a high consistency of RR_c.

Linear Catheters

Overall, the RR_l values ranged from 51.9% to 98.8% for both subsequently taken samples (RR1=57.0–98.8%; RR2=51.9–96.4%). The mean RR_l ranged from 65.7% to 92.5% for the first RR sample and from 61.8% to 84.2% for the second one. However, since one leakage (broken inlet tubing) occurred in ID 2 on study day 1/visit 2 overnight, the results, i.e., the larger range with significant differences ($p=0.009$), shown by these linear catheters than by the concentric ones was further differentiated and then no longer statistically significant ($p=0.199$, without the RR values of visits 3, 5, and 7 for ID 2). On the subsequent study visit 3, this leakage was successfully managed using a tubing connector (Braun, Melsungen, Germany) which joined both parts together. As a result, there was only one missing sample caused by missing fluid directly after the reconnection and one subsequent sample with a larger volume than the nominal one. The latter one resulted from the additional manual flush of the catheter realized by an external syringe filled with Ringer's solution. The RR_l yielded afterwards were elevated up to 98.8% (Table III), leading to the reversed results regarding the ratio of RR_c and RR_l compared to the results in the other individuals (Fig. 2).

Overall, the results (if visits 3, 5, and 7 were evaluated separately from visit 1) showed reproducible RR even after the leakage (RR1: mean $\pm$ SD=96.5 $\pm$ 1.98%, visit 3, 5, and 7; RR2: mean $\pm$ SD=88.6 $\pm$ 7.74%, visits 3, 5, and 7).

Comparison of RR in Concentric and Linear Catheters

Statistically significantly lower RR values were predominantly determined for linear catheters (RR_l; $p=0.002$ for all IDs; $p\leq 0.018$ for IDs 1 and 3; $p=0.499$ for ID 2) represented by a ratio of RR_c and RR_l primarily higher than 1 (Fig. 2). In total, the RR_l results were comparable to the RR_c results in terms of low SDs as well as CVs, which were predominantly in the single-digit magnitude and in general <13.3%, demonstrating a high consistency in RR_l.

Comparison of Subsequently Taken RR Samples

Compared to RR1, the RR2 samples consistently showed lower or very comparable coefficients of variation, except for the RR_l for ID 2. The exceptional behavior might be explained by the leakage event in ID 2. This was the basis to correct the drug concentrations in the dialysate for the RR obtained by RR2 samples and not RR1 or a mean value from both of the samples. For this investigation, the RR1 value could be seen as a backup value for RR2, as Table IVa shows: The variability between the RR1 and RR2 samples was low. In general, a lower variability (intra-visit variability) was found for RR_c (CV $\leq$ 5.8%) than for RR_l (CV $\leq$ 20.5%).

Intra-individual Intervisit and Inter-individual RR Variability

Considering the leakage event—differentiated evaluation of visit 1 and visits 3, 5, and 7 for ID 2—the intra-individual

Table III. RR Values of Concentric (c) and Linear Catheters (l) of the *In Vivo* Calibration

Individual	Visit	RR sample	RR ^{a,b} (%)		$\bar{x}$, % RR1,RR2		SD, % RR1,RR2		CV, % RR1,RR2	
			c	l	c	l	c	l	c	l
1	1	1	90.8	60.1	86.8	65.7	4.77	8.77	5.5	13.3
1	3	1	91.0	76.2						
1	5	1	82.1	57.0						
1	7	1	83.3	69.4						
1	1	2	92.1	64.3	84.6	61.8	5.18	7.79	6.1	12.6
1	3	2	83.8	70.5						
1	5	2	82.4	60.5						
1	7	2	80.2	51.9						
2	1	1	90.7	80.3	85.8	92.5	4.50	8.26	5.2	8.9
2	3	1	88.5	95.8						
2	5	1	82.9	98.8						
2	7	1	81.2	95.0						
2	1	2	91.3	71.1	87.8	84.2	2.99	10.83	3.4	12.9
2	3	2	–	80.9						
2	5	2	85.8	96.4						
2	7	2	86.4	88.6						
3	1	1	81.1	73.3	82.3	69.0	6.83	3.79	8.3	5.5
3	3	1	82.3	66.1						
3	5	1	91.2	–						
3	7	1	74.6	67.5						
3	1	2	82.0	70.8	82.9	65.7	3.91	3.59	4.7	5.5
3	3	2	83.4	64.2						
3	5	2	87.9	65.1						
3	7	2	78.4	62.6						

$\bar{x}$ mean, SD standard deviation, CV coefficient of variation, RP retroperfusate

^a Concentric catheters: mean C_{RP} within visit 1 (CV, in percent): 15.5 $\mu\text{g/mL}$ (3.2), $n=6$. Mean C_{RP} within visits 3, 5, and 7 (CV, in percent): 155.8 $\mu\text{g/mL}$ (8.2), $n=17$

^b Linear catheters: mean C_{RP} within visit 1 (CV, in percent): 15.1 $\mu\text{g/mL}$ (4.4), $n=6$. Mean C_{RP} within visits 3, 5, and 7 (CV, in percent): 141.3 $\mu\text{g/mL}$ (16.9), $n=18$

^c Differential evaluation (only visits 3, 5, and 7) due to leakage

intervisit variability (CV=4.4–12.5%; Table IVb) and inter-individual variability (CV=4.3–12.5%; Table IVc) were low as well across all samples and catheters over the 4-day period.

Total RR for all Individuals and Visits (and Catheters)

In some cases, the RR values obtained at study visit 1 (prior to the drug administration) were higher than the RR

values obtained for the following ones (Table IVa). Finally, the calculated overall recovery value for all visits and individuals revealed a mean recovery of 84.9% with a CV of 5.6% for RR_c (Table IVd). The higher variability for RR_l (CV=18.5%) was explained by the leakage event and was lower while performing a differentiated evaluation (without visits 3, 5, and 7 in ID 2), leading to a CV of 10.7% with a mean recovery of 66.5% ($n=17$; Table IVd). The mean recovery for both catheters resulted in 79.1% and 77.1% including the leakage, respectively, with variability values in comparable range (14.7% and 14.1%, respectively; Table IVe).

DISCUSSION

The study presented here successfully implemented long-term microdialysis over ~87 h in healthy subcutaneous ISF in a pilot phase of a PK study. On the one hand, this applies to the tolerability in healthy volunteers and for reliable sampling until the last sample after multiple dosing. On the other hand, successful implementation was demonstrated by the maintenance of catheter functionality and sufficient drug diffusion across the membrane. Three aspects should be emphasized: (1) The residence period of the concentric and linear catheters is guaranteed to be 5 days (CMA Microdialysis AB, Solna, Sweden, personal communication), but knowledge of this long-term insertion is very limited and has been very scarcely employed in clinical drug investigations; (2) a clinical

Fig. 2. Ratio of relative recoveries of concentric and linear catheters over ~87 h (seven study visits)

Table IV. Means and Variability of Relative Recovery of Concentric (RR_c) and Linear Catheters (RR_l)

		RR _c					RR _l				
ID	Visit	<i>n</i> _{RRsamples/visit}	<i>n</i> _{total}	$\bar{x}$ (%)	SD (%)	CV (%)	<i>n</i> _{RRsamples/visit}	<i>n</i> _{total}	$\bar{x}$ (%)	SD (%)	CV (%)
a) Relative recovery for RR samples 1 and 2 within one visit and one individual											
1	1	2	2	91.4	0.93	1.0	2	2	62.2	2.99	4.8
1	3	2	2	87.4	5.09	5.8	2	2	73.3	4.05	5.5
1	5	2	2	82.2	0.20	0.2	2	2	58.8	2.46	4.2
1	7	2	2	81.8	2.16	2.6	2	2	60.6	12.4	20.5
2	1	2	2	91.0	0.41	0.4	2	2	75.7	6.57	8.7
2	3	1	1	88.5	—	—	2	2	88.4	10.5	11.9
2	5	2	2	84.4	2.04	2.4	2	2	97.6	1.68	1.7
2	7	2	2	83.8	3.67	4.4	2	2	91.8	4.57	5.0
3	1	2	2	81.5	0.64	0.8	2	2	72.1	1.73	2.4
3	3	2	2	82.9	0.72	0.9	2	2	65.1	1.38	2.1
3	5	2	2	89.5	2.34	2.6	1	1	65.1	—	—
3	7	2	2	76.5	2.71	3.5	2	2	65.1	3.49	5.4
b) Relative recovery for all study visits per individual during the entire study duration											
1	1, 3, 5, 7	2	8	85.7	4.75	5.5	2	8	63.7	7.96	12.5
2	1, 3, 5, 7	2 (1)	7	86.7	3.77	4.4	2	8	88.4	9.95	11.3
3	1, 3, 5, 7	2	8	82.6	5.16	6.3	2 (1)	7	67.1	3.79	5.6
— _a											
2	1	—	—	—	—	—	2	2	75.7	6.57	8.7
2	3, 5, 7	—	—	—	—	—	2	6	92.6	6.65	7.2
c) Relative recovery for all study individuals at study visits 1, 3, 5, and 7											
1, 2, 3	1	2	6	88.0	5.02	5.7	2	6	70.0	7.07	10.1
1, 2, 3	3	2 (1)	6 (5)	85.8	3.78	4.4	2 (1)	6 (5)	75.6	11.7	15.5
1, 2, 3	5	2 (1)	6 (5)	85.4	3.67	4.3	2 (1)	6 (5)	75.6	20.3	26.9
1, 2, 3	7	2	6	80.7	4.04	5.0	2	6	72.5	16.3	22.5
— _a											
1, 3	3	—	—	—	—	—	2 (1)	4	69.2	5.33	7.7
1, 3	5	—	—	—	—	—	2 (1)	3	60.9	4.08	6.7
1, 3	7	—	—	—	—	—	2	4	62.8	7.85	12.5
d) Overall relative recovery: mean RR for concentric and linear catheters for all RR samples obtained during the study											
1, 2, 3	1, 3, 5, 7	2 (1)	23	84.9	4.76	5.6	—	—	—	—	—
1, 2, 3	1, 3, 5, 7	—	—	—	—	—	2 (1)	23	73.3	13.6	18.5
— _a											
1, 3	1, 3, 5, 7	—	—	—	—	—	2 (1)	17	66.5	7.09	10.7
2	1										
e) Overall relative recovery: mean RR for both catheters for all RR samples obtained during the study											
1, 2, 3	1, 3, 5, 7	2 (1)	46	79.1	11.63	14.7					
— _a											
1, 3	1, 3, 5, 7	2 (1)	40	77.1	10.88	14.1					
2 (RR _c)	1, 3, 5, 7										
2 (RR _l)	1										

^a Differential evaluation of leakage (linear catheter, ID 2)

comparison between concentric and linear catheters has not been reported; and (3) clinical microdialysis in humans with the moderately lipophilic drug voriconazole has never been performed. So far, first experiences regarding the microdialysis of voriconazole resulted from own *in vitro* investigations and preclinical research (34) as well as from the literature (35–37). However, all these explorations had not been performed in humans.

Long-term microdialysis application over ~3.5 days was appraised as feasible (proof of principle) determined by reliable multiple RR over the whole study duration permitting transformation of the measured dialysate drug concentrations into ISF drug concentrations in the future. Intra-individual variability results for the RR determination at study visit 1 (prior to the drug administration) were in accordance with the findings of Konings *et al.* (24) in tumor tissue, but further determinations of

RR during and after the sampling time have not been performed there. In the healthy adipose tissue, RR was relatively low, over time decreasing and highly fluctuating in comparison to malignant tissue. In their work, the variability in RR within a patient in normal adipose tissue over the observation period was comparable only among three of six patients. In the remaining patients, a higher variability occurred due to leakage problems, incorrect flow settings, or an extreme decrease in recovery. In contrast to the study presented herein, those leakage problems could not be managed and led to the removal of the catheter. During the pilot phase presented here, the leakage of one linear catheter was successfully managed using a tubing connector, and the RR_l yielded afterwards by the catheter were elevated up to 98.8%, probably due to the long duration without perfusate flow within the catheter as a result of the leakage and subsequent manual flush of the catheter. If the membrane's performance

had been impaired, a more facilitated passage of voriconazole molecules expressed in the RR_1 value would have resulted. However, the results showed reproducible RR even after the leakage and, thus, were considered in the calculation of the study samples for this individual.

Based on the results, for this investigation, it was suggested to correct voriconazole concentrations in the dialysates for the RR obtained by RR2 samples and not RR1 or a mean value from both of the samples due to the lower variability in the RR2 samples. These might originate from the longer equilibrated retrodialysis for RR2. Nevertheless, the RR1 value was seen here as a well usable backup value for RR2. To overcome differences in RR1 and RR2, longer equilibration periods can be recommended for future investigations. The RR values were highly consistent over the ~87-h period.

Cases of RR values of visit 1 being lower than the RR values of the following visit might be explained with the insertion and subsequent adaptation to the environment which might have taken about one study day since the following recovery values at the subsequent study visit were more comparable. Konings *et al.* implanted the catheter the day before drug infusion in order to allow enough time for adverse tissue reactions (inflammatory effects (2)) to subside (24). Moreover, they allowed for the drug to be washed out from the peripheral medium while not starting with drug infusion until 2 h after the recovery determination by retrodialysis. In this study, microdialysate baseline samples have shown significant amounts of voriconazole after retrodialysis, indicating a washout phase of 30 min being insufficient. For the subsequent (main) part of the study, it was strongly suggested to waive the retrodialysis on study visit 1 and to invest the ~2-h period as a longer equilibration period consisting of the former equilibration period of 30 min, the *in vivo* catheter calibration procedure (40 min), the washout phase (30 min), and the baseline sampling (15 min). RR determinations on study visits 3 and 5 allowed a washout period of 10–12 h.

Regarding inter-individual variability, Konings *et al.* (24) reported much more different RR values (tumor tissue: mean $RR=44.8\text{--}75.8\%$, $n=6$; normal tissue: mean $RR=10.8\text{--}62.9\%$, $n=5$) than the present study revealed. So far, examinations using both the concentric and the linear catheters, allowing a direct comparison between them, have not been published yet. Lower RR (median = -20.2% , range = -35.4% to -9.5% , leakage considered) were predominantly determined for linear catheters (RR_1) probably due to the slightly smaller lateral surface area of the linear catheter membrane caused by a shorter diameter and/or possibly due to the different membrane materials (see Table I). However, in *in vitro* experiments, both catheters revealed similar RR values (28). The stable performance of the catheters demonstrated by consistent RR values in the present study was well in line with Bolinder *et al.* (12) and Baumeister *et al.* (10). They published unchanged recovery of glucose across the microdialysis membrane after 3, 6, and 7 days (12) and after 8 and 9 days (10).

Voriconazole as a moderately lipophilic drug has been proven suitable for *in vivo* microdialysis, as already suggested by own *in vitro* microdialysis (28). *In vivo* RR was predominantly lower than *in vitro* (*in vitro* concentric catheters: mean RR (CV) = $91.5 (1.8\%)$; linear catheters: mean RR (CV) = $90.8 (4.6\%)$ (28); *in vivo* concentric catheters: mean RR (CV) = $84.9 (5.6\%)$; linear catheters: mean RR (CV) = $66.5 (10.7\%)$). This study also confirmed that the perfusate concentrations chosen were

sufficient for calibration on all study visits. Taken together, the hypothesis that the diffusion of voriconazole was unhindered and led to unaffected accurate recovery results was accepted.

Long-term microdialysis was well tolerated without any local complication/inflammation at the site of implantation and reduced the burden in humans due to avoidance of several catheter insertions. The setting developed (Fig. 1b) for the conduction of the pilot trial aimed at and, in fact, ensured (1) convenient and easy procedures for the study personnel using low-priced additional equipment, (2) GCP during the trial, and, at the same time, (3) maximum mobility for the individual to be able to transfer the setting in the future to patients in clinical routine. The easy handling resulting from this setting, enabling, e.g., safe sample collection and fast and easy sample vial exchange, resulted in only marginal time deviations, being highly important for PK studies. Subcutaneous periumbilical adipose tissue was easily accessible for catheter implantation and disruption/alteration of the membrane by the abdominal fat (preliminary investigation, figure not shown) was prevented by a perpendicular to the body axis and horizontal application of the catheter (see Fig. 1b).

For further investigations, the following recommendation can be given: (1) For the determination of RR, a one-time/twice (instead of four times) measured value for each individual (even though the inter-individual variability was low) and each catheter ideally at the end of the study duration is sufficient to achieve an adequate conversion factor. In our setting, this reduction would lead to a 75% saving of the retrodialysate samples, perfusate samples, and the time spent for the recovery procedure. Waiving the recovery determination on study visit 1 will avoid potential drug concentrations in the baseline and subsequent samples. The question of whether it is sufficient to determine an overall recovery per individual or per catheter in our setting is drug-specific and should be assessed in future clinical trials with other model drugs displaying different properties. (2) Concentric catheters appeared to be more adequate due to (a) higher recoveries, (b) leakage problems being considered to be less possible than for the linear catheters since the disruption of the inlet tubing occurred exactly at the position where the tubing from the syringe is joined to the inlet tubing leading to the membrane, and (c) the linear catheters holding the risk of infection when the outlet tubing has to be removed.

CONCLUSION

For reproducible microdialysis *in vivo* measurements over several days in humans, a special setting for long-term microdialysis implementing both concentric and linear catheters has been developed. The application of the setting, the devices, and all necessary procedures developed were demonstrated to be feasible; easy to be implemented and low in material expenditure; and comfortable in terms of mobility and tolerability for healthy volunteers, complying with GCP requirements in a pilot study (proof of principle). Practical feasibility recognized during the study procedures was confirmed by reproducible RR values within each catheter and catheter type obtained over the entire study period of ~87 h. Finally, future long-term microdialysis PK clinical trials in healthy volunteers/critically ill patients will benefit from the recommendations regarding optimized time points for relative recovery determinations along with a drastically reduced number of RR determinations.

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