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Clearance of levofloxacin by an *in vitro* model of continuous venovenous hemodialysis (CVVHD)

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ABSTRACT: Information about the elimination and the adequate dosing of levofloxacin during renal replacement therapy is scarce. The aim of this study was to characterize in vitro *the elimination of levofloxacin during continuous venovenous hemodialysis (CVVHD) and to investigate whether the CVVHD clearances of creatinine and urea are correlated with the levofloxacin clearance in order to facilitate dosage adjustments. An* in vitro *model of CVVHD was established using five dialyzer membranes at varying dialysate flow rates applied in the clinical setting (8, 16, 25, 33 and 41 ml/min). Plasma and dialysate samples were drawn for determination of levofloxacin, creatinine and urea concentrations to evaluate clearances by CVVHD.*

During CVVHD, the clearance of levofloxacin varied between 9.02 and 33.30 ml/min, depending on the chosen setup. Positive correlations ($p<0.001$) were received for: dialysate flow rate (QD) and creatinine/urea clearances ($R^2>0.93$); QD and levofloxacin clearance (R^2 0.59-0.71); levofloxacin and creatinine clearance (R^2 0.69-0.75); and levofloxacin and urea clearance (R^2 0.56-0.75) as well.

When dosing critically ill patients, therefore, extracorporeal as well as total clearance of levofloxacin should be considered. (Int J Artif Organs 2007; 30: 889-95)

KEY WORDS: Renal replacement therapy, Fluoroquinolones, Dialyzer membranes, Dialysate flow rate

INTRODUCTION

Levofloxacin is used extensively as an empirical or directed therapy in critically ill patients due to its broad range of bactericidal activity (1-4). Since approximately 80% is eliminated unchanged by the kidneys, dosage reduction is necessary in renal failure (5). Data regarding the effect of continuous renal replacement therapy (CRRT) on levofloxacin elimination are scarce. Dose recommendations by the marketing authorization holder do not consider elimination of levofloxacin during CRRT relevant and, therefore, do not differ between anuric patients and patients undergoing CRRT (6). Only a few studies have been published investigating the impact of continuous venovenous hemofiltration (CVVH) and continuous venovenous hemodiafiltration (CVVHDF) on the levofloxacin clearance in intensive care unit patients (7-11). All of these studies demonstrate a significant elimination of levofloxacin during CVVH or CVVHDF, but results and conclusions of the publications are controversial. As the impact of CRRT modalities was not investigated, the variability of the study results could be due to the applied different operational characteristics (e.g. dialyzer membrane type, dialysate and/or filtration rate). Indeed, Choi et al demonstrated via an *in vitro* model that the sieving coefficient of levofloxacin during CVVH depends on the filter material used or the point of dilution (12).

In addition to CVVH, continuous venovenous hemodialysis (CVVHD) is one of the most common CRRT methods used in intensive care units (13-15), but elimination of levofloxacin during CVVHD has not been investigated so far. Therefore, the purpose of this study was to identify the extent of levofloxacin transport across the dialyzer during CVVHD via an *in vitro* model, and to evaluate the influence of different dialyzer membrane types and dialysate flow rates (QD) on the levofloxacin clearance. A further aim of the study to facilitate dosage adjustment was to investigate whether levofloxacin clearance is correlated with clearance of an endogenous reference solute such as urea or creatinine.

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METHODS

An *in vitro* model of CVVHD was used to perform the experiments. The experimental setup is illustrated in Figure 1 and included a 1-liter plasma reservoir, a dialysate reservoir and a conventional dialyzer, as described in a previous publication (15, 16). Plasma circulated through the closed plasma circuit and the dialyzer with a flow rate of 150 ml/min (QB). The dialysate circuit was driven countercurrently in a single-pass design with varying flow rates depending on the experimental setup (Tab. I). Plasma and dialysate flow rates were regulated via two roller pumps. Before starting the experiment, the whole experimental setup was primed with freshly prepared SH04 solution (Braun Medizintechnik, Melsungen, Germany), representing the dialysate. The plasma reservoir was filled with human plasma augmented with urea and creatinine (urea 10 mmol/L; creatinine 150 µmol/L) (10, 11). Levofloxacin was added to reach reported peak concentrations of 8 mg/L (6). The dialysate flow rate (QD) was adjusted to the particular value before filling the plasma circuit and starting the experiment. QD was monitored throughout the duration (120 min) of the experiment via timed collection and determination of outflow.

To investigate the influence of the dialyzer and dialysate flow rate, experiments with 13 different experimental setups were performed in triplicate (see Tab. I). Five dialyzer membranes were tested at a dialysate flow rate of 25 ml/min: Hemophan® (Allwall GFS 12 Plus), polyamix (Polyflux 14L), PAN 69 (Multiflow 100, Nephral ST 300) (all Gambro Hospal GmbH, Planegg-Martinsried, Germany), and polysulfone (FX50S, Fresenius Medical Care, Bad Homburg, Germany). To investigate the influence of the dialysate flow rate, experiments were performed with the Multiflow 100 dialyzer and the FX50S dialyzer at dialysate flow rates of 8, 16, 25, 33 and 41 ml/min. Corresponding plasma and dialysate samples were taken to determine levofloxacin at the plasma inlet (Ca) and outlet (Cv) of the dialyzer and at the dialysate outflow (Cd) immediately before (0) and at 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes after starting the dialysis. To determine urea and creatinine concentration, Ca and Cd samples were drawn at the following timepoints: 0, 15, 30, 45, 60, 90 and 120 minutes.

Levofloxacin concentration in plasma and dialysate were analyzed using a validated binary gradient HPLC/fluorescence assay with moxifloxacin as internal standard (17). The stationary phase consisted of an YMC Pro C18 column (120/5 µm, 150x2 mm) and a 10x2 mm ID pre-column. The mobile phase was composed of methanol (MeOH), ammonium acetate (NH_4AC) and water (H_2O) (10:5:85 v/v/v) as eluent A and $MeOH/NH_4AC/H_2O$ (40:5:55 v/v/v) as eluent B. Fluorescence response was observed at excitation and emission wavelengths of 296 and 504 nm. The assay was found to be linear over a concentration range of 0.1 to 6 mg/L for plasma and dialysate as well. Intra- and interday precision ranged from 0.45 to 7.24%; determined intra- and interday accuracy ranged between -3.6 and 8.71%.

Determination of urea and creatinine was carried out by routine methods at the hospital clinical chemistry laboratory. Data analysis was based on the saturation coefficient (Sa), which was determined for each sampling point using the following equation: Sa = Cd/Ca (18, 19). All Sa values were used to obtain the clearances of the solutes according to the equation $Cl_{CVVHD} = Sa * QD$ (15, 18). For further evaluation, the Sa and Cl_{CVVHD} values of each single experiment were averaged and described by mean and standard deviation (SD). To evaluate whether adsorption contributes to elimination, a mass balance check was performed by comparing the elimination of levofloxacin from

TABLE I - CL_{CVVHD} (ml/min) OF LEVOFLOXACIN DEPENDING ON THE DIALYZER MEMBRANES AND DIALYSATE FLOW RATES (QD) USED FOR *IN VITRO* CVVHD (MEAN±STANDARD DEVIATION, N=3)

	QD [ml/min]				
Membrane	8	16	25	33	41
FX 50 S	9.02 ± 3.27	15.65 ± 2.16	28.05 ± 1.30	24.84 ± 6.33	32.09 ± 9.41
Nephral ST 300			25.63 ± 0.92		
Allwall GFS 12 Plus			26.91 ± 4.85		
Polyflux 14 L			27.83 ± 1.98		
Multiflow 100	10.47 ± 0.15	12.68 ± 0.19	14.27 ± 0.33	33.30 ± 2.22	31.19 ± 7.99

Membrane materials: FX 50 S (polysulfone); Nephral ST 300 (PAN = polyacrylonitrile 69 ST); Allwall GFS 12 Plus (Hemophan® = diethylaminoethyle modified cellulose); Polyflux 14 L (Polyamix= polyarylethersulphone/polyvinylpyrrolidone/polyamide); Multiflow 100 (AN 69 HF).

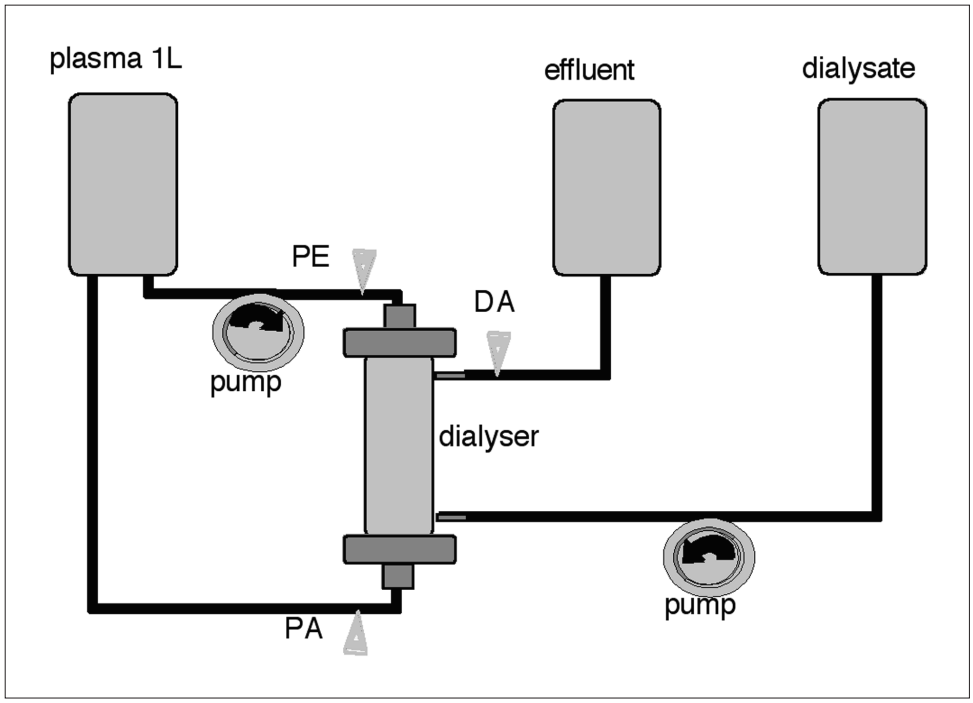

Fig. 1 - In vitro *CVVHD model. PE = plasma inlet; PA = plasma outlet; DA = dialysate outlet.*

the blood (QB*(Ca-Cv)) with the recovery in dialysate (QD*Cd). Linear regression was performed to determine the relationship between solute clearance and dialysate flow rate. For each dialyzer membrane, the clearance of levofloxacin and the clearance of creatinine or the clearance of urea were compared by paired t-test. All statistical analyses were performed with SPSS 12.0 for Windows (SPSS, Chicago, IL, USA).

RESULTS

The clearances of levofloxacin, creatinine and urea by CVVHD were investigated using five different dialyzer membranes at a dialysate flow rate of 25 ml/min (see Fig. 2) and also with two different dialyzer membranes at increasing dialysate flow rates from 8 to 41 ml/min (see Fig. 3a and b). In all experimental setups, a complete saturation of the dialysate with creatinine and urea was achieved (Sa ~1). Therefore, clearances reflected the applied dialysate flow rate ($R^2 > 0.93$; Fig. 3a/b). The elimination of levofloxacin during CVVHD was more complex and was influenced by the various dialysis conditions (Tab. I). Figure 2 illustrates the Cl_{CVVHD} of levofloxacin compared to urea and creatinine depending on the different dialyzers used at the same dialysate flow rate of 25 ml/min. Saturation coefficients close to 1, resulting in levofloxacin clearances analogous to the dialysate flow rate and the urea and creatinine clearances, were observed in four of the five tested membranes (FX50S, Nephral ST 300, Allwall GFS 12 plus, Polyflux 14 L). Only in the case of the Multi-

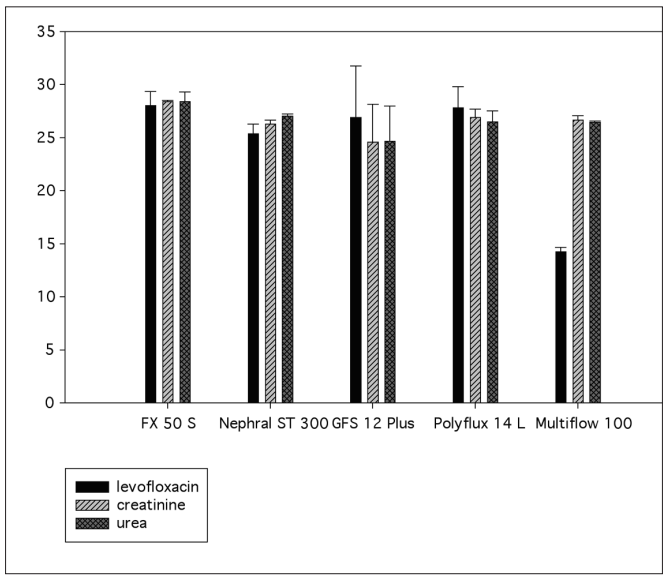

Fig. 2 - *Levofloxacin, creatinine and urea clearances with different dialyzer membranes at a dialysate flow rate of 25 ml/ min (mean ± SD).*

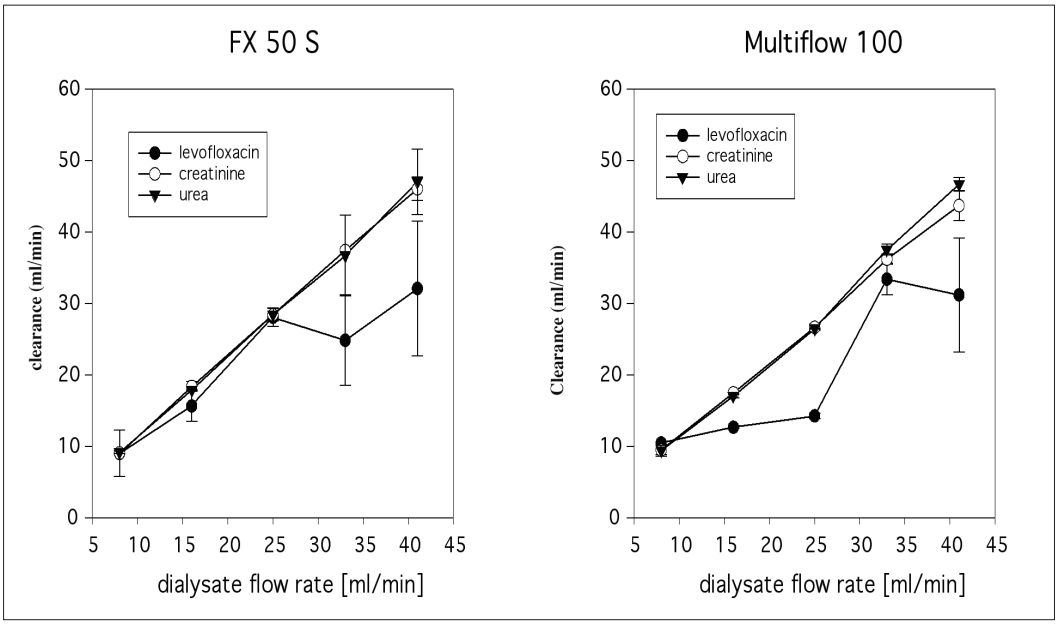

Fig. 3 - *Levofloxacin, creatinine and urea clearances with different dialysate flow rates QD (8-41ml/min) for two membranes: FX 50 S and Multiflow 100 (mean ± SD).*

flow 100 was a significantly lower Sa of 0.57 ± 0.01 observed, resulting in a significantly lower Cl_{CVVHD} value of 14.28 ± 0.39 ml/min ($p \leq 0.05$).

Analyzing the impact of the dialysate flow rate for the FX 50 S dialyzer, saturation coefficients around 1 were achieved between dialysate flow rates of 8 to 25 ml/min. Increasing the dialysate flow rate to 33 or 41 ml/min did not allow complete saturation of the dialysate with levofloxacin. Decreasing Sa (33 ml/min Sa=0.75 ± 0.19; 41 ml/min Sa=0.78 ± 0.23) yielded a Cl_{CVVHD} of 24.85 ml/min – 33.30 ml/min, which no longer directly reflected the applied flow rate. Using the Multiflow 100 dialyzer, increasing dialysate flow rates from 8 to 25 ml/min resulted in decreased saturation of the dialysate with levofloxacin. Levofloxacin clearances, therefore, increased only weakly from 10.47 ± 0.15 ml/min (at QD=8 ml/min) to 14.27 ± 0.39 ml/min (at QD=25 ml/min). When dialysate flow rates of 33 ml/min and 41 ml/min were applied, saturation of the dialysate did not continue to decrease and resulted in levofloxacin clearances of 33.38 ± 2.22 ml/min and 31.18 ± 7.99 ml/min.

Performance of a mass balance check showed that in each experimental setup and for each sampling point after equilibration, the recovery of levofloxacin in the dialysate exceeded the calculated levofloxacin elimination from the blood circuit. Via linear regression, the relationship between the solute clearance and the dialysate flow rate was determined to be significant ($p<0.001$). While creatinine and urea clearances were highly determined by dialysate flow rate ($R^2=0.99$, $R^2=0.93$ only in the case of urea clearance of FX50S), this relationship was still evident, but weaker in the case of levofloxacin clearance ($R^2=0.71$ Multiflow 100; $R^2=0.59$ FX 50S). Levofloxacin clearances were also related to creatinine clearance ($R^2=0.75$ Multiflow 100; $R^2=0.69$ FX 50 S; $p<0.001$) and urea clearances ($R^2=0.74$ Multiflow 100; $R^2=0.56$ FX 50 S; $p<0.001$).

DISCUSSION

The proposal of this study was to characterize the elimination rate of levofloxacin during various operational settings of CVVHD and to estimate a potential correlation between levofloxacin and urea or creatinine clearance.

Using an *in vitro* model of CVVHD, saturation of the dialysate with levofloxacin (0.57-1.06) as well as the clearances (9.02 - 32.10 ml/min) were related to the tested modalities of CVVHD. Linear regression performed between levofloxacin clearance and dialysate flow rate ($R^2=0.71$ Multiflow 100, $p < 0.001$; $R^2=0.59$ FX 50 S, $p<0.001$) demonstrated that the Cl_{CVVHD} of levofloxacin depends on the dialysate flow rate but is less determined by this parameter than the creatinine and urea clearance. Therefore, the dialysate flow rate has to be considered in the clinical setting. Clearances of creatinine or urea by CVVHD, which are easy to determine, may be used as sur-

rogates for the levofloxacin clearance by CVVHD. However, as presented in this study, the Cl_{CVVHD} of levofloxacin did not strictly have the same linear increase as Cl_{CVVHD} of creatinine or urea, which could result in an overestimation of the levofloxacin clearance. Especially at higher dialysate flow rates (QD=41 ml/min) the lower saturation of the dialysate with levofloxacin yielded clearances that did not directly reflect the Cl_{CVVHD} of creatinine or urea.

Despite the dialysate flow rate, the composition of the membranes, either symmetric (e.g., Multiflow 100) or asymmetric (e.g., FX50S) as well as the charge of the membrane (e.g., negative charge of Multiflow 100) are possible factors influencing the elimination of levofloxacin (19, 20). Conditioning of the membrane as a high- or low-flux membrane does not impact the elimination of levofloxacin, since the Cl_{CVVHD} was comparable for both types of membranes. Standard deviation of the clearances were comparable for all membranes based on synthetic material. Allwall GFS 12 plus, the only membrane consisting of a cellulose derivate (Hemophan®), possesses a higher variability in the clearances for all investigated solutes.

Adsorption as a reason for variability of the levofloxacin clearance seemed to be unlikely, since the concentration difference between plasma inlet and plasma outlet samples ranged around 10% for all experiments independent of the range of saturation. A comparison of the blood-side elimination and the recovery of levofloxacin in the dialysate demonstrated that levofloxacin was completely eliminated into the dialysate, independently of the experimental conditions. In contrast to the study presented here, adsorption has been reported previously (7, 12); indeed, levofloxacin plasma concentrations were extremely high (100mg/L) when adsorption was observed (12). As the adsorption of levofloxacin was found to be concentration-dependent and reversible, a relevant adsorption at clinical concentrations, which corresponds with the levofloxacin concentration in this study, is unlikely (21).

The main limitation of our study was the use of an *in vitro* model, which cannot completely reflect the situation in critically ill patients. The operational setup of the model, such as blood flow (150 ml/min), chosen dialysate flow rates (8-41 ml/min) or levofloxacin, urea and creatinine levels, corresponded well with CVVHD conditions used *in vivo*. However, there may be not predictable factors influencing the impact of clearance by CVVHD on the dosage needs of a patient (15,18, 22-25). Two major and highly variable factors in intensive care patients that have to be considered when designing optimal dosing strategies are the volume of distribution of the patient and further non-CRRT clearances (23-25). In contrast, an *in vitro* model allows for specific evaluation of the influence of the various operational setups, as demonstrated in Figures 2 and 3, thereby providing a possible guide for the dosing of patients and/or a more directed planning of pharmacokinetic studies.

Sieving coefficients of 0.79 and 0.73 reported in *in vivo* observations during CVVH or CVVHDF using the Multiflow 100 at a QD of 16 ml/min were comparable to the saturation observed in this study (11). In contrast, at a higher filtration rate (22 ml/min), Boston researchers noted a sieving coefficient of 0.96 for the same membrane in 6 CVVHDF patients (7). However, in two of these patients sieving coefficients of 0.79 and 0.8 were observed, comparable to Guenter et al. In general, a comparison between hemofiltration and hemodialysis may be limited due to the different mechanisms of solute removal. In both modalities of CRRT, the degree of the plasma protein binding of the drug can influence the extent of elimination. In the case of levofloxacin, this factor has no relevance, as the drug is only slightly protein-bound and all published *in vivo* studies agreed on a relevant elimination (demonstrated by high sieving and saturation coefficients) of levofloxacin by CRRT.

This is in accordance with an *in vitro* investigation regarding elimination during CVVH and the results of the *in vitro* study of levofloxacin removal during CVVHD presented here (12). Further *in vitro* experiments compared the elimination of various drugs during CVVHD and the albumin dialysis system MARS (indicated during liver failure) (26). In these studies, levofloxacin was completely cleared by CVVHD and MARS as well, unlike high plasma protein-bound drugs (e.g., ceftriaxone), demonstrating a significantly higher elimination during albumin dialysis.

Despite the consistent result that levofloxacin is cleared to a high extent during most modalities of CRRT, dose recommendations varied between the investigator groups. Results of this *in vitro* study indicate that the dose recommended in the product characteristics, which assumes no augmented doses in hemodialysis, but creatinine clearance dependent dosing (with increasing dosage from creatinine clearance <10 ml/min to 10-19 ml/min to 20-50 ml/min), might not be sufficient under all circumstances. Drug monitoring of levofloxacin during CVVHD would be preferable to provide sufficient plasma levels, in particular

if the varying pharmacokinetic parameters of levofloxacin in intensive care patients (especially volume of distribution and residual renal clearance) are considered (6, 23-25).

In conclusion, levofloxacin was well eliminated by CVVHD in an *in vitro* model. Therefore, in clinical practice the extracorporeal clearance of levofloxacin should be considered. Clearance values of levofloxacin were related to the conditions of CVVHD, especially the dialysate flow rate. Underexposure of the critically ill patient with levofloxacin could occur due to several reasons, such as increased volume of distribution or increased renal and extracorporal clearances. If levofloxacin concentration cannot be determined, dosage adjustments due to levofloxacin removal by CVVHD could use creatinine or urea CVVHD-clearances as surrogates. However, the *in vitro* results presented in this study show that at higher dialysate flow rates levofloxacin clearance can be overestimated when using creatinine or urea clearances by CVVHD.

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