# Comparative Studies of Dialyzability of Levofloxacin and Amlodipine Using *in Vitro* Dialysis System with Three Different Types of Dialyzers

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**In the pharmacotherapy of patients receiving hemodialysis, the possible exclusion of an administered drug by hemodialysis should be recognized. The present study was undertaken to predict dialyzability of a drug during hemodialysis using an *in vitro* dialysis system with three different dialyzers made from high performance membrane. Levofloxacin having relatively low binding-affinity to plasma protein was removed ideally during *in vitro* dialysis. Amlodipine was also eliminated from the blood, although it is generally believed that such drugs with high binding affinity to plasma protein are removed less efficiently. Examination of the adsorption rate and clearance of the drug revealed that disappearance of amlodipine was due to its adsorption to dialysis membrane and its circuit. Thus, drugs with high protein binding-affinity may possibly be removed by dialyzers with high performance membrane.**

**Key words** —— hemodialysis, protein binding, levofloxacin, amlodipine, high performance membrane

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## INTRODUCTION

When urinary excretion type drugs are administered to patients with chronic renal failure, attention must be given to the tendency for such drugs to accumulate in the body as a result of renal dysfunction. The possible exclusion of an administered drug by hemodialysis(HD) should also be considered. Factors affecting the dialyzability of drugs are the molecular weight, water solubility, protein binding, volume of distribution, route of excretion, excretion rate, type of dialysis membrane, blood flow, dialysate flow and dehydration volume. Among these, we have focused on the properties of dialysis membrane in the present study.

A regenerated cellulose membrane has long been used in dialysis to remove substances with a molecular weight (MW) of 3000 or lower, especially small molecules with MW of 500 or less. The importance of removing uremic toxins of intermediate MW (middle MW hypothesis)[1] and $\beta$2-microglobulin (MW 11800) ($\beta$2-MG) was also recognized.[2] Accordingly, dialysis membranes called high performance membranes that remove substances with a MW of approximately 10000, but do not remove albumin (MW 67000) came into use. Today, dialysis membranes removing larger MW substances with slight albumin permeability are also used. These newly developed membranes have significantly improved dialyzability efficiency; for instance, the elimination of vancomycin and other substances by HD with a high performance membrane became more effective than with previously used membranes.[3,4] Therefore, having a high protein binding property or a high MW can not longer be a reason for non- or less-dialyzability on HD.

Generally, patients with specific organ disorders are not included in pre-marketing clinical trials. This makes it very difficult to obtain information of dialyzability of a drug at launch. Furthermore, most information on post-marketing surveillance is collected as case reports, but such information cannot be considered sufficient to determine an optimal administration regimen. Therefore, to predict dialyzability of a new drug without exposing patients, basic research on the drug's dialyzability *in vitro* using a dialysis system circulating bovine blood was planned in an attempt to develop clinical application of the information

gained. In the present study, three types of dialyzers made of different membrane materials were compared in an *in vitro* dialysis system for their drug dialyzability. Two drugs with different protein binding properties were chosen: the first was levofloxacin, a newquinolone antimicrobial drug with relatively low binding-affinity to protein (42%),[5] and the second was amlodipine, a calcium antagonist with a relatively high protein binding-affinity of 97.1%.[6] MW of these two drugs was comparable: 370.38 and 567.06, respectively.

## MATERIALS AND METHODS

**Drug** —— Bulk powder of levofloxacin (LVFX) Lot No.401 and amlodipine besilate(AML) Lot No.N-092 was provided by Daiichi Pharmaceutical Co., Ltd. and Sumitomo Pharmaceuticals Co., Ltd., respectively.

**Dialyzers** —— Three types of dialyzer provided by each manufacturer were used:

(1) TFW12: cellulose triacetate membrane (manufacturer: Teijin Ltd., distributor: Fuso Pharmaceutical Ind.)

(2) BK-1.0F: polymethylmethacrylate membrane (manufacturer: Toray Industries, Inc., distributor: Toray Medical industries, Inc.)

(3) PS-1.0UW: polysulfone membrane (import manufacturer: Kawasumi Chemical Industry, distributor: Kuraray Co., Ltd.)

Characteristics of the three types of dialysis membranes are shown in Table 1. These membranes are classified by their difference in $\beta$2-MG clearance into type I ($\beta$2-MG clearance: 0 ml/min) and type II ($\beta$2-MG clearance: 10 ml/min). Type II are superior in removing $\beta$2-MG and substances with 10000—20000 MW. Membranes used in the present study are all synthetic polymer membranes and classified as type II.

**Table 1.** Characteristics of Dialyzers

| | TFW12 | BK-1.0F | PS-1.0UW |
|---|---|---|---|
| Material | CTA | PMMA | PS |
| Diameter ($\mu$m) | 200 | 200 | 200 |
| Thickness ($\mu$m) | 16 | 30 | 40 |
| Length (cm) | 21 | 19.5 | |
| Area ($m^2$) | 1.2 | 1.0 | 0.9 |
| Strength for pressure (mmHg) | 500 | 500 | 500 |
| Volume (ml) | 80 | 58 | 54 |
| UFR (ml/hr · mmHg) | 18 | 13 | 35 |
| SC for albumin | 0.007 | 0.03 | below 0.01 |

UFR: ultrafiltration rate, SC: sieving coefficient, CTA: cellulose triacetate, PMMA: polymethylmethacrylate, PS: polysulfone.

**Blood for HD** —— Bovine blood for HD was obtained in the morning of experiment days from Tokyo Shibaura Zoki Co. at the meat market of Tokyo Central Wholesale Market. At blood collection, 200 ml of blood preservative solution (Teruflex, Terumo) was added per 1l of bovine blood to prevent blood coagulation, and the blood was used in the experiments approximately two hours later.

To prepare drug-containing bovine blood, bulk powder of LVFX or AML was dissolved in physiological saline at a concentration of 2.0 mg/ml and 1.0 mg/ml, respectively, then added to the blood at a final concentration of 4.5 $\mu$g/ml or 30 ng/ml. Those concentrations were assumed to be approximately three-fold and ten-fold of the maximum blood concentration after a single oral administration of 100 mg LVFX and 5 mg AML, respectively.

**Miscellaneous Reagents and Apparatus** —— To prevent coagulation in the hemodialysis circuit, heparin calcium (Caprocin Injection, Sankyo Co., Ltd.) was used, and for dialysate, Kindaly AF-No.2 (Fuso Pharmaceutical Ind.).

Also used were: artificial kidney NK-Y820P (Nikkiso Co., Ltd.) as the hemodialysis circuit, Model DBB-22 (Nikkiso Co., Ltd.) as dialysis apparatus, DKB-21 (Nikkiso Co., Ltd.) as blood pump and IP-11 (Nikkiso Co., Ltd.) as infusion pump. An ascites pooling bag, FCB-3, was used as a blood bag.

**HD Experiments** —— The HD circuit is shown in Fig. 1. The circuit and dialyzer were primed with 1 l of physiological saline,[7] then placed in the dialysis apparatus. At this point, the circuit and dialyzer were filled with approximately 200 ml of physiological saline. Heparin calcium, 5000 U, was added to 2 l of bovine blood, which was supplemented with blood preservative solution and filtered though double gauze; then the blood was placed in a blood bag. Following the addition and mixing of each drug solution, the blood was kept in a water bath at 37℃ and subjected to dialysis.

The flow rates of dialysate and blood were set at 500 ml/min and 200 ml/min, respectively. The dialysis period was four hours and the dehydration volume was set at 0 ml. To prevent blood coagulation during the experiment, 2000 U of heparin calcium was injected once into the circuit (at the arterial site), and then heparin calcium was further infused at 2000 U/h.

**Adsorption Experiments** —— The flow rate of the dialysate was set at 0 ml/min. Other conditions were the same as those in the HD experiment.

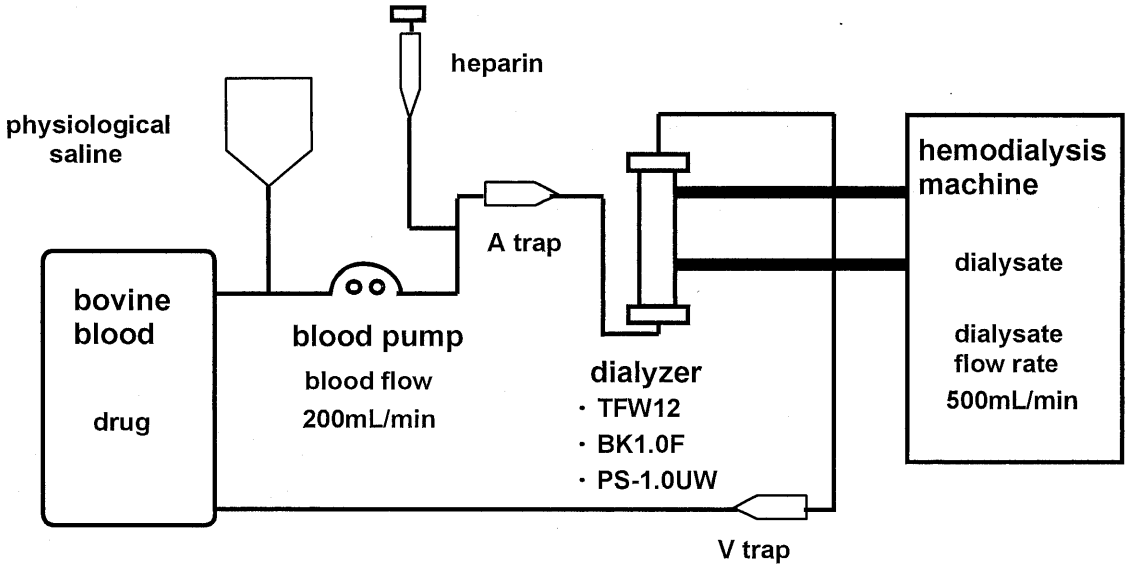


**Fig. 1.** Hemodialysis Circuit

**Blood Sampling and Plasma Separation** —— In HD experiments, blood samples were simultaneously collected from arterial (A trap) and venous (V trap) sides of the circuit immediately prior to HD (0 min) and 30, 60 and 240 min after initiation of HD. In adsorption experiments, blood samples were collected from A trap at 0 min and 240 min. After centrifugation (2000 rpm, 15 min), the supernatant (plasma) was collected and stored at 20℃ until measurement.

**Measurement Method of Plasma Drug Concentration** —— The plasma LVFX concentration was measured by the high performance liquid chromatography method[8] at SRL Co.. The limit of detection was 0.01 $\mu$g/ml. The plasma AML concentration was measured by the liquid chromatography/atmospheric pressure chemical ionization tandem mass-spectrometry method[9] at Sumitomo Pharmaceuticals Co. The limit of detection was 0.14 ng/ml.

**Calculation of Reduction Rate and Clearance** ——

(1) Reduction rate

Reduction rate was calculated by the following formula:

$$\text{Reduction rate} = \{(C\text{Bb} - C\text{Ba})/C\text{Bb}\}100\ (\%)$$

$C$Bb: plasma drug concentration at the initiation of dialysis (A trap)

$C$Ba: plasma drug concentrations at each sampling time (A trap)

(2) Clearance

Clearance was calculated by the following formula:

$$\text{CL} = \{(C\text{Bi} - C\text{Bo})/C\text{Bi}\}Q\text{B}\ (\text{ml/min})$$

$C$Bi: plasma drug concentration at inflow side of dialyzer (A trap)

$C$Bo: plasma drug concentration at outflow side of dialyzer (V trap)

$Q$B: blood flow

**Statistics** —— $n=4$ in all experiments, and each measured value was principally presented as mean standard deviation (mean±S.D.).

## RESULTS AND DISCUSSION

In the *in vitro* dialysis experiment using three different dialyzers circulating bovine blood, the plasma LVFX concentration rapidly decreased until 60 min after HD initiation, then gradually decreased thereafter in all dialyzers (Table 2). The reduction rate of LVFX after 30 min, 60 min, and 240 min was calculated to range 71—89.4%, 88.2—95.4%, and more than 99.0%, respectively (Table 3). The reduction rates at 30 min and 60 min increased in the order of BK-1. 0F<PS-1.0UW<TFW12, showing the maximal difference of approximately 20% among the dialyzers. Since the clinical dialysis period is generally 240 min, differences in membrane properties may not affect clinical cases in LVFX. However, the variations in the dialysis membranes should be considered when a drug is administered during dialysis.

Elimination of AML from bovine blood using the *in vitro* dialysis system was also examined. Plasma AML concentration decreased in a time dependent manner (Table 2) and the reduction rate of AML (Table 3) was reasonably high.

**Table 2.** Plasma Concentration of Levofloxacin and Amlodipine on *in Vitro* Hemodialysis System Using Three Different Dialyzer

| Dialyzer | Time (min) | Plasma concentration (Mean±S.D.) Levofloxacin (μg/ml) | Amlodipine (ng/ml) |
|---|---|---|---|
| TFW12 | 0 | 4.56±0.08 | 32.97±4.26 |
| | 30 | 0.49±0.23 | ND |
| | 60 | 0.21±0.09 | 4.54± 1.63 |
| | 240 | 0.02±0.02 | 0.94± 0.16 |
| BK-1.0F | 0 | 4.39±0.12 | 33.23±17.25 |
| | 30 | 1.27±0.10 | ND |
| | 60 | 0.51±0.13 | 9.38± 3.48 |
| | 240 | 0.04±0.01 | 3.84± 1.39 |
| PS-1.0UW | 0 | 4.35±0.41 | 27.93±15.58 |
| | 30 | 0.77±0.17 | ND |
| | 60 | 0.23±0.16 | 5.93± 3.63 |
| | 240 | 0.02±0.02 | 1.88± 1.22 |

**Table 3.** Reduction Rate of Levofloxacin and Amlodipine on *in Vitro* Hemodialysis System Using Three Different Dialyzers

| Dialyzer | Time (min) | Reduction Rate (Mean±S.D.) (%) Levofloxacin | Amlodipine |
|---|---|---|---|
| TFW12 | 0— 30 | 89.4±4.9 | ND |
| | 0— 60 | 95.4±2.0 | 86.5±3.1 |
| | 0—240 | 99.5±0.3 | 97.2±0.3 |
| BK-1.0F | 0— 30 | 71.0±2.0 | ND |
| | 0— 60 | 88.2±3.1 | 70.4±4.1 |
| | 0—240 | 99.1±0.2 | 87.8±2.6 |
| PS-1.0UW | 0— 30 | 82.4±3.2 | ND |
| | 0— 60 | 94.7±3.6 | 79.8±4.3 |
| | 0—240 | 99.6±0.3 | 93.0±1.7 |

Thus, like LVFX, AML was efficiently removed by this model dialysis system, though it should be noted that the extent of disappearance of AML seemed somewhat lower than that of LVFX. This difference might be explained by their different binding-affinities to plasma protein. As LVFX has a low and reversible protein binding property, it might easily dissociate from plasma protein in bovine blood[10] and permeate into the dialysate.

There was a great difference between LVFX and AML in the adsorption rate to dialysis circuit (Table 4). The adsorption rates of LVFX after 240 min were 11—21% in all three dialysis membranes, while those of AML were remarkably higher at 49—66%. High protein binding-affinity might be related to high adsorption rate.

**Table 4.** Adsorption Rate of Levofloxacin and Amlodipine in Hemoadsorption Test Using Three Different Dialyzers

| Dialyzer | Time (min) | Reduction rate (Mean±S.D.) (%) Levofloxacin | Amlodipine |
|---|---|---|---|
| TFW12 | 0—240 | 11.5± 2.5 | 48.8±16.8 |
| BK-1.0F | 0—240 | 21.1±11.7 | 65.9± 4.1 |
| PS-1.0UW | 0—240 | 16.2± 4.4 | 51.9±14.1 |

**Table 5.** Clearance of Levofloxacin and Amlodipine on *in Vitro* Hemodialysis on in Vitro Hemodialysis System Using Three Different Dialyzers System Using Three Different Dialyzers

| Dialyzer | Time (min) | Clearance (Mean±S.D.)(ml/min) Levofloxacin | Amlodipine |
|---|---|---|---|
| TFW12 | 60 | 98.1±10.0 | 1.4± 9.7 |
| BK-1.0F | 60 | 54.5± 2.1 | 1.0±28.5 |
| PS-1.0UW | 60 | 88.7± 6.0 | 2.0± 1.8 |

When clearance (CL) was calculated (Table 5), much greater difference was observed between LVFX and AML. The CLs in AML were extremely low as compared with LVFX and varied with large S.D. in all dialyzers. This indicated that little AML was eliminated from blood to dialysate in 60 min. In spite of this fact, the AML concentration continuously decreased even after 60 min (Table 2). These results suggest that the adsorption of drugs with high protein binding-affinity such as AML to dialyzer membranes and other materials of dialysis circuits probably contributes to the drug removal. Our result contradicts the general view that drugs with high affinity to plasma protein are not eliminated during HD. Thus, drugs with high protein binding-affinity are possibly removed by the newly developed dialyzers with high performance membrane. In contrast to AML, CLs in LVFX by TFW12 and PS-1.0UW were reasonably high (Table 5) and are close to those seen in clinical studies.[11] Considering the high CL and low adsorption rate of LVFX, it is concluded that drugs with low protein binding-affinity such as LVFX are probably eliminated by permeation through pores of the high performance dialysis membrane. It should be noted that CL in LVFX by BK-1.0F was significantly lower than the

other two membranes (Table 5) indicating that the BK-1.0F membrane has partly lost its capability to eliminate drugs with low MW after a short period of dialysis. The molecular basis for the relatively low dialyzability of BK-1.0F is not yet clearly understood.

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