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Research Paper

# Pharmacokinetics of clomipramine, an antidepressant, in poloxamer 407-induced hyperlipidaemic model rats

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## Abstract

**Objective** This study was undertaken to investigate the effects of hyperlipidaemia on the pharmacokinetics of clomipramine, an antidepressant, particularly addressing the change of clomipramine distribution to plasma components in poloxamer 407-induced hyperlipidaemia model rats.

**Methods** Clomipramine pharmacokinetic studies in hyperlipidaemic rats were performed with clomipramine continuous infusion. Furthermore, clomipramine protein binding and distribution to the brain and plasma components such as lipoproteins were investigated.

**Key findings** Mean plasma concentration of clomipramine at steady state during continuous infusion (17.5 μg/min/kg) in hyperlipidaemic rats (0.45 ± 0.01 μg/ml) was significantly higher than that in the control rats (0.30 ± 0.02 μg/ml). However, the amount of clomipramine in the brain in hyperlipidaemic rats (0.31 ± 0.06 μg/g) was dramatically lower than in the control rats (1.89 ± 0.13 μg/g). However, the plasma unbound fraction in hyperlipidaemic rats (0.98 ± 0.05%) was significantly lower than that of the control rats (6.51 ± 0.62%).

**Conclusions** Lower distribution to the brain and lower plasma clearance of clomipramine in hyperlipidaemic rats resulted from lower plasma unbound fraction because of higher lipid-rich protein contents in blood. Results of this study provide useful information for dosage adjustment of clomipramine in hyperlipidaemia.

**Keywords** antidepressant; clomipramine; hyperlipidaemia; lipoprotein; pharmacokinetics

## Introduction

Clomipramine is a potent and selective serotonin reuptake inhibitor of the tertiary amines from the tricyclic antidepressant family.[1] It has been used for the treatment of anxiety, fear, phobias, severe depression, and obsessive–compulsive behaviour.[2,3] However, severe side effects make clomipramine medication extremely difficult.[4] Therefore, administration planning for each patient using therapeutic drug monitoring is desired.

Clomipramine has high protein-binding characteristics (approximately 96% in plasma) and so a change of the protein binding ratio might strongly affect the pharmacokinetics of the drug, such as its tissue distribution and clearance.[4] In fact, clomipramine, a basic drug, is well bound to $\alpha$1-acid glycoprotein (AAG).[4] Additionally, it is anticipated that clomipramine binds to lipoproteins for its high lipophilicity (log $P$; approximately 5.2).[5] However, previous reports have described that the lipoprotein binding of high lipophilic drugs increases when lipoprotein increases in hyperlipidaemia.[6–8] The human immunodeficiency virus protease inhibitor nelfinavir and atazanavir, which are high lipophilic and basic drugs, bind well to AAG and lipoproteins. The distribution volume and clearance of nelfinavir and atazanavir in hyperlipidaemic rats were decreased because of the decreasing plasma unbound fraction caused by the increase of lipoproteins.[9,10] Furthermore, the blood clearance of ciclosporin, a highly lipophilic drug, decreased because of the increase of lipoprotein, which is the major complexing constituent for ciclosporin.[11] Similar results were obtained in our clinical study of ciclosporin in renal transplant recipients.[12] Therefore, investigation of the relation between the change of binding characteristics of drugs, which have high affinity to lipoprotein, and the pharmacokinetic behaviour in hyperlipidaemia is

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important. As described above, clomipramine is a highly lipophilic and basic drug that is expected to bind to lipoproteins. Therefore, the possibility exists that hyperlipidaemia changes the pharmacokinetics of clomipramine as well as the other drugs, suggesting that therapeutic drug monitoring data of clomipramine in patients with hyperlipidaemia must be evaluated carefully. Nevertheless, little is known about the pharmacokinetics of antidepressants in hyperlipidaemia. In this study, to investigate the effect of hyperlipidaemia on protein binding characteristics, distribution in plasma components and pharmacokinetic properties of clomipramine (especially distribution to the brain), we quantified the protein binding ratio and clomipramine distribution to the plasma lipoproteins. We performed pharmacokinetic studies of clomipramine in poloxamer 407 (P-407)-induced hyperlipidaemic model rats.[13,14] To date, as far as we know, there has been nothing published regarding the pharmacokinetics of clomipramine in the presence of hyperlipidaemia. The intent of this study was to shed more light on this issue using clomipramine and hyperlipidaemic rats.

## Materials and Methods

### Materials

Evans blue was purchased from Nacalai Tesque Inc. (Kyoto, Japan). Clomipramine and Pluronic F-127 (poloxamer 407, P-407) were obtained from Sigma–Aldrich Co. (Steinhein, Germany). Fluvoxamine, used as an internal standard for analysis using the liquid chromatography-mass spectrometry (LC-MS) method as described below, was purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). All other reagents were of analytical grade and were used without further purification.

### Animal preparation

All animal experiments were performed in accordance with Guidelines for Animal Experimentation of Kyoto Pharmaceutical University. Male Wistar rats (10-weeks-old, 319 ± 10 g) were obtained from Nippon SLC Co., Ltd (SLC, Hamamatsu, Japan) and had free access to food and water. The rats were maintained in a temperature-controlled facility with a 12 h light/dark cycle for at least five days before use. The hyperlipidaemic rats were prepared by intraperitoneal administration of P-407 solution (0.1 g/ml in saline) at a dose of 1 g/kg. Control rats received the same volume of vehicle without P-407. The pharmacokinetic studies of clomipramine in each group were performed 36 h after P-407 administration. Rat plasma used for the protein binding study, distribution study to lipoprotein fractions and determination of the biochemical parameters as described below, was obtained at 36 h after P-407 administration also. No differences were found in body weight between hyperlipidaemic and control rats immediately before the experiments.

Determinations of biochemical parameters such as high-density lipoprotein ratio (HDL%), low-density lipoprotein ratio (LDL%), very low-density lipoprotein level (VLDL%), HDL-cholesterol level (HDL-ch), and LDL-cholesterol level (LDL-ch) were performed by a commercial laboratory: Kyoto BIKEN (Kyoto, Japan).

### Evaluation of the blood–brain barrier function in hyperlipidaemic rats

For the evaluation of blood–brain barrier (BBB) function, Evans blue, which is bound completely to albumin in blood, was used as described by Song Y *et al*.[15] Briefly, Evans blue in 0.3 ml saline (50 mg/kg), was injected intravenously into the jugular vein in hyperlipidaemic rats. At 30 min after the administration of Evans blue, the rats were killed by cervical dislocation. Their brains were perfused with phosphate-buffered saline (PBS) using an infusion pump (Fusion 200; ISIS Co., Ltd, Osaka, Japan) to remove blood. After the brain was removed and blotted using filter paper, it was weighed and homogenized in 5 ml formalin using a glass homogenizer. The homogenate was incubated at 60°C for 24 h. The supernatant fractions obtained after the removal of cell debris by centrifugation at 1000*g* for 5 min were used to determine the Evans blue concentration using a spectrophotometer (UV-1600; Shimadzu Corp., Kyoto, Japan; $\lambda = 609.5$ nm).

### In-vitro protein binding study

The erythrocyte vs buffer or plasma partitioning method was used to determine the unbound clomipramine concentration in rat plasma.[16] Briefly, hyperlipidaemic and control rats were anaesthetized and blood was collected into centrifuge tubes by cardiac puncture. The blood was divided equally into two tubes. Plasma was separated from blood cells by centrifugation of whole blood at 2500*g* for 10 min at 25°C. After removal of the plasma and buffy-coat layers, blood cells were washed in an equal volume of PBS containing 25 mM glucose (PBS-Glu, pH 7.4), with subsequent centrifugation at 2500*g* for 10 min at 25°C. This washing procedure was repeated three times. After the third wash, the volume of total erythrocytes was noted in each tube. Then either PBS-Glu or 10-times diluted plasma by PBS-Glu was added to make a haematocrit of 0.3. Clomipramine solution in methanol was then added to erythrocyte-buffer and erythrocyte-diluted plasma suspension to produce final concentrations of 0.5, 1 and 5 μg/ml for both control and hyperlipidaemic rat groups ($n = 5$, respectively). This concentration range corresponded to the approximate value observed in plasma in the previous pharmacokinetic study. The total methanol concentration was 0.4%. Previous reports had described that a methanol concentration of up to 4% in plasma was permissible for binding studies.[17] Erythrocyte-buffer and erythrocyte-diluted plasma samples were incubated for 1 h at 37°C. After centrifugation at 9000*g* for 10 min, the concentration of clomipramine in the supernatant was determined using the LC-MS method, as described below.

The calculation of unbound fraction ($f_u$) is described as follows. The erythrocyte concentration of clomipramine in the erythrocyte-diluted plasma sample ($C_E$) was determined using the following equation:

$$C_E = \frac{C_B - C_P \cdot (1 - HCT)}{HCT} \quad (1)$$

where $C_B$ is the total concentration of clomipramine in the blood cell suspension and $C_P$ is the concentration of clomipramine in the plasma, and where $HCT$ is a haematocrit; the value of $HCT$ is 0.3 in this study, as described above.

Similarly, to estimate the erythrocyte concentration of clomipramine in the erythrocyte-buffer sample ($C_E$*), the total concentrations of clomipramine in erythrocyte-buffer samples were substituted for $C_B$, and the concentration of clomipramine in buffer was substituted for $C_P$. The $f_u$ values were determined as follows:

$$f_u' = \frac{P_P}{P_b} \tag{2}$$

$$f_u(\%) = 100 \times \frac{d \cdot f_u'}{1 - f_u' \cdot (1 - d)} \tag{3}$$

where $f_u'$ is the free fraction in dilution plasma, $d$ signifies the dilution factor (e.g. $d = 0.1$ in the case of a tenfold dilution of the plasma), and partition coefficients for erythrocyte-diluted plasma or buffer are represented, respectively, by the terms $P_P$ ($C_E/C_P$) and $P_b$ ($C_E$*/$C_B$).

## In-vitro distribution study in rat blood

The clomipramine methanolic solution was added to drug-free blood samples obtained from both control and hyperlipidaemic rats at total clomipramine concentrations of 0.5, 1 and 5 μg/ml, and total methanol concentration of 0.4%, as described above. After incubation at 37°C for 1 h, clomipramine concentrations in the mixtures were measured as the whole blood concentration. The remaining mixtures were centrifuged at 2500*g*, at 25°C for 10 min to obtain the plasma fraction, and clomipramine concentrations were measured as the plasma concentration. The plasma–whole blood concentration ratio (P–B ratio) was calculated by dividing the plasma concentration by the whole blood concentration.

## Pharmacokinetic study of clomipramine in hyperlipidaemic rats

The hyperlipidaemic and control rats were fasted overnight before the administration of clomipramine with free access to water. Under anaesthesia by an intraperitoneal injection of 50 mg/kg sodium pentobarbital, rats were placed supine on a heating pad under a surgical lamp to maintain body temperature during the experiment. For the intravenous study, clomipramine solution (5 mg/ml in saline) at a dose of 5 mg/kg was administrated into the external left jugular vein in control and hyperlipidaemic rats ($n = 5$). Blood samples from the external right jugular vein (250 μl) were withdrawn into the heparinized microcentrifuge tubes at 5, 15, 30, 60, 90, 120, 180, 240 and 360 min after drug administration. The blood samples were centrifuged at 9000*g* for 10 min to collect plasma samples. These samples were stored at −80°C until analysis of clomipramine using the LC-MS method, as described below.

To investigate the distribution of clomipramine to the brain and liver at the steady state, the femoral vein of hyperlipidaemic and control rats ($n = 5$) was catheterized with polyethylene tubes (SV-45; Natsume Corp., Tokyo, Japan). Clomipramine was then administered as a continuous intravenous infusion, starting with a bolus loading dose to reach the steady state quickly. The dosage solutions were prepared at the appropriate concentration for each rat so that the same total volume was infused into hyperlipidaemic and control rats. Clomipramine (6.3 mg/kg) dissolved in 3 ml saline solution was then infused into the femoral vein via polyethylene tubes at a rate of 17.5 μg/min/kg, starting with a bolus loading dose of 5 mg/kg (dissolved in 0.3 ml saline) administration into the external left jugular vein. Blood samples from the external right jugular vein (250 μl) were withdrawn into the heparinized microcentrifuge tubes at 30, 60, 90, 120, 150, 180, 240 and 360 min after the start of infusion. The blood samples were centrifuged at 9000*g* for 10 min to collect plasma samples. These samples were stored at −80°C until analysis of clomipramine using the LC-MS method, as described below.

The amount of clomipramine in each tissue sample was measured to investigate the distribution of clomipramine to the brain and liver. After collecting final blood samples (at 360 min after clomipramine infusion), the control and hyperlipidaemic rats were killed by cervical dislocation. Their brains and livers were perfused with PBS to remove blood using an infusion pump. After the brain and liver were removed and blotted using filter paper, they were weighed and homogenized in PBS (9-fold and 3-fold volume of each sample weight, respectively) using a glass homogenizer. The supernatant fractions obtained after the removal of cell debris by centrifugation at 1000*g* for 5 min were stored at −80°C until clomipramine analysis.

## Pharmacokinetic analysis

Noncompartmental pharmacokinetic analysis was applied to the plasma concentration-time data using a computer program, WinHARMONY.[18] The terminal elimination rate constant ($\lambda_z$) was determined by the linear regression of at least three data points from the terminal portion of the plasma concentration–time plots. The area under the plasma concentration–time curve ($AUC$) was calculated using the linear trapezoidal rule up to the last measured plasma concentration ($C_{p(last)}$) and extrapolated to infinity using a correction term, namely $C_{p(last)}/\lambda_z$. The area under the first-moment curve to the last measured plasma concentration ($AUMC$) was calculated using the linear trapezoidal rule and the addition of the concentration term after the last measured point ($t_{(last)}$) to infinity, namely, $t_{(last)}C_{p(last)}/\lambda_z + C_{p(last)}/\lambda_z^2$. The mean residence time ($MRT$) was calculated by dividing $AUMC$ by $AUC$. Total body clearance ($CL_{tot}$) was calculated by $D/AUC$, where $D$ represents the dose administered. The terminal elimination half-life ($t^l/_2$) was determined by dividing ln2 by $\lambda_z$. The steady state volume of distribution ($Vd_{ss}$) was calculated by multiplying $CL_{tot}$ by $MRT$. The concentration of clomipramine in a steady state ($C_{ss}$) was calculated as the average clomipramine concentration after time reached the steady state during infusion. The total body clearance at steady state ($CL_{tot,ss}$) was calculated by dividing the infusion rate (17.5 μg/min/kg) by $C_{ss}$.

## Plasma lipoprotein separation

Lipoproteins were isolated from clomipramine-spiked plasma in control and hyperlipidaemic rats (final concentration 5 μg/ml) based on their hydrated density with a single-step

procedure using ultracentrifugation on a potassium bromide (KBr) gradient, as described previously by Terpstra *et al*.[19] The main lipoprotein fractions were isolated using density ranges of < 0.95, 0.95–1.006, 1.006–1.063, 1.063–1.210 and > 1.210 g/ml for chylomicron, VLDL, LDL, HDL and lipoprotein-deficient fractions (LPDF), respectively. The collected fractions were frozen at −80°C until clomipramine analyses.

### Assay procedure

The clomipramine in plasma and the other samples in this study was assayed using LC-MS. The methods previously reported by Tournel *et al*.[20] and Shinozuka *et al*.[21] were used as references. Briefly, 10 µl fluvoxamine (internal standard: 50 µg/ml in methanol) and 150 µl 2% $ZnSO_4$ in 50% methanol solution were added to portions of a 100 µl plasma sample in a 1.5-ml microcentrifuge tube and vortexed vigorously for 15 s. Diethyl ether (1 ml) and 100 µl 1 M sodium hydroxide were then added to the tube, vortexed for 30 s, and centrifuged at 12 000*g* for 5 min. The aqueous phase in the test tube was frozen in a cold bath at −10°C. The ether phase was transferred to HPLC sample vials. The organic phase was evaporated to dryness at 70°C in a water bath under the flow of $N_2$ gas. The residues were reconstituted with 100 µl mobile phase and then 30 µl was injected into the LC-MS system (Shimadzu Corp., Kyoto, Japan), which included the following components: a SIL-10A system controller, LC-10ADvp pump, SPD-10A UV detector, SIL-10ADvp automatic injector, CTO-10A column oven and an LC-MS-QP8000a mass spectrometer equipped with a CLASS-8000 work station. The analytical column for the separation of clomipramine was a Quicksorb ODS (2.1 mm i.d. × 150 mm, 5 µm size; Chemco Scientific Co. Ltd, Osaka, Japan) and column temperature was maintained at 60°C for all separations. Elution was conducted isocratically at a flow rate of 0.2 ml/min with 90% methanol containing 1% acetic acid. Mass spectrometry was performed using atmospheric pressure chemical ionization (APCI) in the negative mode. The voltages of the APCI probe and the curved desolvation line (CDL) were set respectively to 5 kV and −30 V. The flow rate of the nebulizing gas ($N_2$) was set at 2.5 l/min. The temperatures of the APCI probe and CDL were set to 400 and 250°C, respectively. The voltage of deflectors was set at −80 V. The peaks of fluvoxamine and clomipramine were detected as deprotonated ions at 319 and 315 m/z, respectively. Clomipramine was quantified by calculating the peak area ratio of clomipramine against fluvoxamine. The detection limit of this assay method was 0.01 µg/ml from 100 µl sample.

### Statistical analysis

All values are expressed as the mean ± SE Differences of the means were inferred as statistically significant when $P < 0.05$ by Student's unpaired *t*-test.

## Results

The biochemical parameters of control and hyperlipidaemic rats are presented in Table 1. The HDL-ch and LDL-ch levels in rats with hyperlipidaemia were fivefold those in control rats. The HDL% in hyperlipidaemic rats was remarkably lower compared with control rats. In contrast, LDL% and VLDL% were higher than in the control rats. In contrast, Evans blue, which is completely bound to albumin in blood, was not detected in the brain after intravenous injection in either control or hyperlipidaemic rat, suggesting that the BBB function was not impaired in rats with hyperlipidaemia.

Table 2 presents the plasma unbound fraction and the plasma–whole blood concentration ratio (P–B ratio) in control and hyperlipidaemic rats. We performed the protein binding study based on the erythrocyte vs buffer or plasma partitioning method. Schuhmacher *et al*.[16] investigated the protein binding ratio of various drugs and the precision of this method, concluding that it depended not only on the true $f_u$ value; it was more significantly determined by the $P_P$ ($C_E/C_P$) value. Therefore, it was recommended that in the case of highly protein bound drugs, protein binding should be determined in diluted plasma, where $f_u$ was increased and where $P_P$ was > 0.5. Accordingly, we tested a series of diluted plasmas (1-, 2-, 4- and 10-times) to obtain the optimal $P_P$ value. The $P_P$ value of 10-times diluted plasma showed 11.9 and 2.5 for control and hyperlipidaemic rats, respectively. Therefore, this value was used for this study. The plasma unbound fractions

**Table 1** Biochemical parameters in hyperlipidaemic rats

| Biochemical parameter | Control rats | Hyperlipidaemic rats |
|---|---|---|
| HDL-ch (mg/dl | 53.0 ± 3.8 | 253.5 ± 5.2** |
| LDL-ch (mg/dl) | 9.8 ± 0.9 | 51.5 ± 2.1** |
| HDL (%) | 43.5 ± 1.2 | 3.8 ± 0.6** |
| LDL (%) | 20.0 ± 0.4 | 41.8 ± 3.3** |
| VLDL (%) | 36.5 ± 1.1 | 54.5 ± 3.4** |

Each value represents the mean ± SE of four rats. **$P < 0.01$ statically significant difference against control. HDL-ch, high-density lipoprotein cholesterol level; LDL-ch, low-density lipoprotein cholesterol level-;HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

**Table 2** Plasma unbound fraction and the plasma-whole blood concentration ratio of clomipramine in hyperlipidaemic rats

| Clomipramine concentration (µg/ml) | Plasma unbound fraction (%) | | Plasma–whole blood concentration ratio | |
|---|---|---|---|---|
| | Control rats | Hyperlipidaemic rats | Control rats | Hyperlipidaemic rats |
| 0.5 | 7.78 ± 0.71 | 0.98 ± 0.08** | 1.07 ± 0.09 | 1.06 ± 0.08 |
| 1 | 6.51 ± 0.62 | 0.98 ± 0.05** | 1.00 ± 0.06 | 0.98 ± 0.05 |
| 5 | 7.79 ± 0.43 | 0.88 ± 0.04** | 0.72 ± 0.02 | 0.93 ± 0.03** |

Blood used in both studies was collected 36 h after poloxamer 407 (1 g/kg) intraperitoneal administration (hyperlipidaemic rats) or the same volume of vehicle without poloxamer 407 (control rats). Each value represents the mean ± SE of five rats. **$P < 0.01$ compared with control rats.

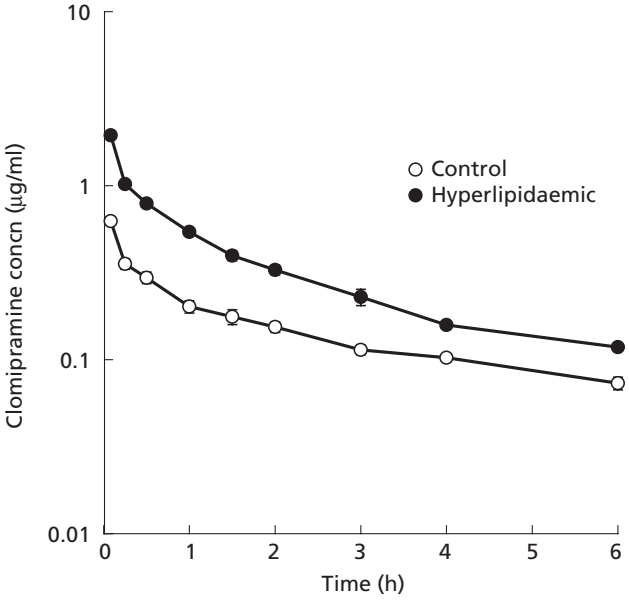


**Figure 1** Time mean plasma concentration profiles of clomipramine after its intravenous administration to hyperlipidaemic rats
Clomipramine dose: 5 mg/kg. Experiments were performed at 36 h after P-407 (1 g/kg) intraperitoneal administration (hyperlipidaemic rats) or with the same volume of vehicle without P-407 (control rats). Each symbol with a bar represents the mean ± SE of five rats.

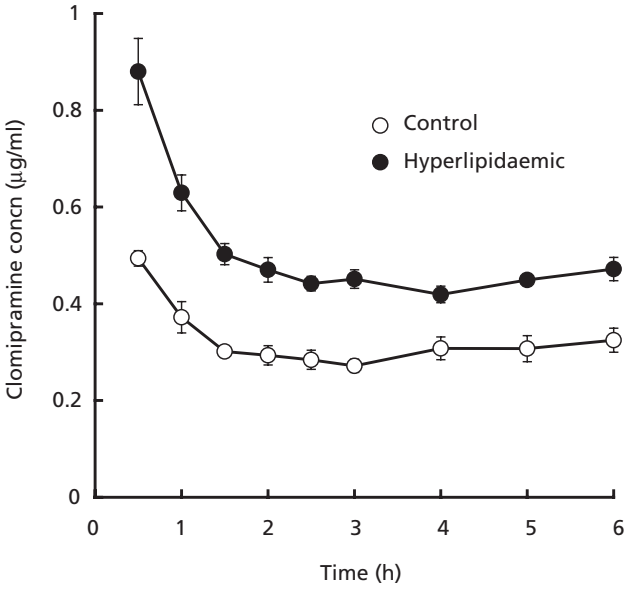


**Figure 2** Time course of plasma clomipramine level after bolus injection of 5 mg/kg clomipramine and continuous infusion of 17.5 mg/min/kg clomipramine to hyperlipidaemic rats
Experiments were performed at 36 h after poloxamer 407 (1 g/kg) intraperitoneal administration (hyperlipidaemic rats) or with the same volume of vehicle without poloxamer 407 (control rats). Each symbol with a bar represents the mean ± SE of five rats.

**Table 3** Pharmacokinetic parameters after intravenous administration of clomipramine (5 mg/kg) to control and hyperlipidaemic rats

| Pharmacokinetic parameter | Control rats | Hyperlipidaemic rats |
|---|---|---|
| $t^{l}/_{2}$ (h) | 4.05 ± 0.32 | 3.29 ± 0.30 |
| $AUC_{0\text{-}\infty}$ (μg·h/ml) | 1.31 ± 0.02 | 2.67 ± 0.08** |
| $CL_{tot}$ (l/h/kg) | 3.93 ± 0.15 | 1.88 ± 0.06** |
| $Vd_{ss}$ (l/kg) | 19.91 ± 1.30 | 6.66 ± 0.52** |

Each value represents the mean ± SE of five rats. $^{**}P < 0.01$, $^{*}P < 0.05$ compared with control rats. $t^{l}/_{2}$, half life; *AUC*, area under the plasma concentration–time curve; $CL_{tot}$, total body clearance; $Vd_{ss}$, volume of distribution at steady state.

**Table 4** Pharmacokinetic parameters of clomipramine at the steady state in hyperlipidaemic rats

| Pharmacokinetic parameters | Control rats | Hyperlipidaemic rats |
|---|---|---|
| $C_{ss}$ (μg/ml) | 0.30 ± 0.02 | 0.45 ± 0.01** |
| $CL_{tot,ss}$ (l/h/kg) | 3.56 ± 0.22 | 2.35 ± 0.07** |

Each value represents the mean ± SE of five rats. $^{**}P < 0.01$ compared with control rats. $C_{ss}$, concentration of clomipramine in a steady state; $CL_{tot.ss}$, total body clearance at the steady state.

of each sample (0.5, 1 and 5 μg/ml of clomipramine concentration) in hyperlipidaemic rats were significantly lower than each corresponding sample obtained from control rats. Furthermore, no difference was found between the plasma unbound fractions of 0.5, 1 and 5 μg/ml samples in either control or hyperlipidaemic rats. No difference was found between the P–B ratios of 0.5 and 1 μg/ml blood samples in control and hyperlipidaemic rats. Nevertheless, in the blood sample of 5 μg/ml, the P–B ratio of hyperlipidaemic rats was slightly higher than that in control rats.

Mean plasma clomipramine concentration vs time curves after intravenous bolus injection of clomipramine solution at a dose of 5 mg/kg to control and hyperlipidaemic rats are shown in Figure 1. Pharmacokinetic parameters of clomipramine for each group are shown in Table 3. After intravenous bolus injection of clomipramine solution, $CL_{tot}$ and $Vd_{ss}$ in hyperlipidaemic rats were significantly lower than those in control rats. In particular, $Vd_{ss}$ in hyperlipidaemic rats was approximately one-third of that in control rats. On the other hand, the plasma concentrations of clomipramine in hyperlipidaemic rats were higher than those of control rats and the $AUC_{0\text{-}\infty}$ of hyperlipidaemic rats showed approximately twofold increases in comparison with control rats. No difference was found for $t^{l}/_{2}$ between hyperlipidaemic and control rats.

Figure 2 shows the clomipramine plasma concentration vs time curves during continuous infusion at a rate of 17.5 μg/min/kg with a loading dose of 5 mg/kg. In addition, the pharmacokinetic parameter values are presented in Table 4. During continuous infusion of clomipramine, the plasma clomipramine concentrations in control and hyperlipidaemic rats reached steady state at 90 and 150 min, respectively, after the start of infusion. Actually, $C_{ss}$ in hyperlipidaemic rats was significantly higher – 1.5-times higher – compared with control rats. The level of $CL_{tot,ss}$ in hyperlipidaemic rats was 34.2% lower than that in control rats.

**Table 5** Clomipramine distribution in the brain and liver, ratio to plasma concentration and to plasma unbound concentration at steady state

| | Control rats | Hyperlipidaemic rats |
|---|---|---|
| $W_{brain}$ (g) | 2.27 ± 0.03 | 2.23 ± 0.05 |
| $X_{brain}$ (μg/g) | 1.89 ± 0.13 | 0.31 ± 0.06** |
| $X_{brain}/C_{ss}$ | 6.29 ± 0.44 | 0.69 ± 0.12** |
| $X_{brain}/C_{ss,u}$ | 85.4 ± 6.02 | 73.0 ± 12.6 |
| $W_{liver}$ (g) | 14.84 ± 0.62 | 14.00 ± 0.37 |
| $X_{liver}$ (μg/g) | 0.81 ± 0.08 | 0.26 ± 0.03** |
| $X_{liver}/C_{ss}$ | 2.71 ± 0.26 | 0.58 ± 0.07** |
| $X_{liver}/C_{ss,u}$ | 36.8 ± 3.54 | 61.3 ± 7.12* |

Each value represents the mean ± SE of five rats. $^{**}P < 0.01$, $^{*}P < 0.05$ compared with control rats. $W_{brain}$, weight of the brain; $X_{brain}$, amount of clomipramine in the brain; $X_{brain}/C_{ss}$, brain to plasma concentration ratio; $X_{brain}/C_{ss,u}$, brain to plasma unbound concentration ratio; $W_{liver}$, weight of the liver; $X_{liver}$, amount of clomipramine in the liver; $X_{liver}/C_{ss}$, liver to plasma concentration ratio; $X_{liver}/C_{ss,u}$, liver to plasma unbound concentration ratio.

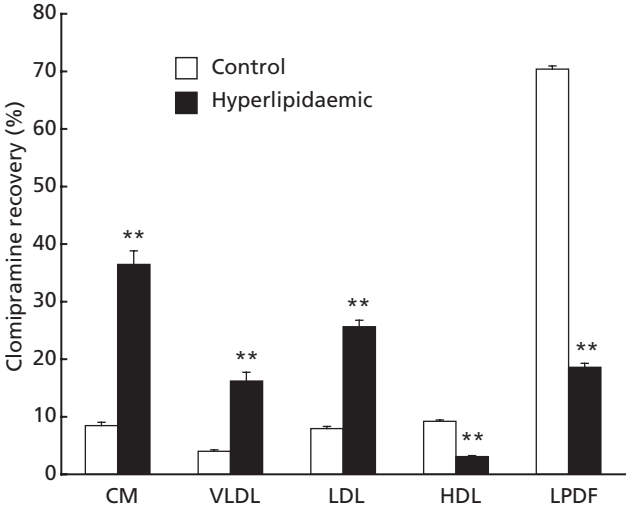


**Figure 3** Distribution of clomipramine in rat plasma lipoproteins
Plasma from hyperlipidaemic and control rats was spiked with clomipramine (5 μg/ml). Blood used for this study was collected at 36 h after poloxamer 407 (1 g/kg) was intraperitoneal administration (hyperlipidaemic rats) or the same volume of vehicle without poloxamer 407 (control rats). CM, chylomicron; VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; LPDF, lipoprotein-deficient fractions. Values are the means ± SE of five experiments, $^{**}P < 0.01$ compared with control rats.

The amount of clomipramine in the brain and the liver ($X_{brain}$, $X_{liver}$), their respective ratios to plasma concentration ($X_{brain}/C_{ss}$, $X_{liver}/C_{ss}$) and those to plasma unbound concentration at steady state, estimated using the plasma unbound fraction data ($X_{brain}/C_{ss,u}$, $X_{liver}/C_{ss,u}$), are presented in Table 5. The former, $X_{brain}$ and $X_{liver}$ in hyperlipidaemic rats were respectively 83.6 and 67.9% lower compared with control rats. Also, $X_{brain}/C_{ss}$ and $X_{liver}/C_{ss}$ at steady state in hyperlipidaemic rats (brain 0.69; liver 0.58) were significantly lower than those in control rats (brain 6.29; liver 2.71). However, no difference was found in the $X_{brain}/C_{ss,u}$ between control and hyperlipidaemic rats, although $X_{liver}/C_{ss,u}$ in hyperlipidaemic rats was significantly higher than that in control rats.

Figure 3 portrays the distribution of clomipramine to plasma lipoprotein fractions in the control and hyperlipidaemic rats. In hyperlipidaemic rats, higher clomipramine recovery was observed in the chylomicron, VLDL, and LDL fractions (8.4–36.5%, 4.0–16.2% and 7.9–25.6%, respectively). In contrast, clomipramine recovery in HDL and LPDF fractions was significantly lower (9.2–3.1% and 70.4–18.6%, respectively).

## Discussion

A nonionic surface active agent, P-407, which is nontoxic to cellular membranes, has been shown to cause a significant increase in circulating lipoproteins by decreasing lipoprotein hepatic lipase and increasing lecithin cholesterol acyl transferase and cholesteryl ester transfer protein activity.[13,14] The P-407-induced hyperlipidaemia rat model has been used for several pharmacokinetic studies of drugs having high binding characteristics to serum lipoproteins such as ciclosporin, amiodarone or nifedipine, because of its convenience, reproducibility and lack of undesirable underlying pathological conditions.[22–26] Previously, we reported that the cholesterol and triglyceride levels in rats with hyperlipidaemia induced with P-407 were significantly higher than those in control rats.[9] Moreover, in the preliminary experiment, we checked the hepatic and renal functions in P-407-induced hyperlipidaemic rats and obtained a result showing that no differences existed in plasma creatinine, aspartate aminotransferases (AST), or alanine aminotransferases (ALT) levels between hyperlipidaemic and control rats. Therefore, the use of the P-407-induced hyperlipidaemic rat model was suitable for the purpose of this study. The increase in lipoprotein levels in hyperlipidaemic rats were relatively intense. Therefore, it was quite likely that the biochemical parameters observed in hyperlipidaemic rats were similar to those observed in patients with severe hyperlipidaemia.

Although the plasma concentration of lipoprotein is low, lipoprotein plays an important role as a drug binding protein affecting the disposition of some drugs. For example, probucol, an antihyperlipidaemic agent, can account for as much as 95% of total drug binding in plasma.[27] Neutral and basic lipophilic drugs commonly bind to lipoproteins, and liposolubilization is probably the major mechanism for drug association with lipoproteins. Reportedly, the octanol–water partition coefficient (log $P$) of clomipramine was approximately 5.2.[5] Gershkovich and Hoffman[28] reported that drug distribution to lipid-rich lipoproteins correlated with log $P$ of the drug. Therefore, it is anticipated that clomipramine binds to lipoproteins for its high lipophilicity, as described in the Introduction. Shayeganpou *et al*.[29] investigated the influence of lipoprotein on the distribution of amiodarone, which is used for the management of life-threatening ventricular arrhythmias and has high binding characteristics to serum lipoproteins such as clomipramine in humans and P-407-induced hyperlipidaemic rat model plasma. They reported that a similar trend of amiodarone in normolipoproteinaemic plasma was noted for rat and human and the presence of hyperlipoproteinaemia caused the increase of amiodarone in

low-density lipoprotein.[29] In addition, Motoya *et al*.[30] reported that the free fraction of nelfinavir, which is a human immunodeficiency virus protease inhibitor and has high binding characteristics to serum lipoproteins such as clomipramine and amiodarone, was not affected by drugs that bind extensively to AAG or albumin. Although the well-known binding proteins of nelfinavir are AAG and albumin, it is anticipated that nelfinavir binds to lipoproteins for its high lipophilicity.[10] Although there is no information about the contribution of lipoprotein to the total binding of clomipramine in patients, there is a possibility that similar trends of clomipramine in plasma are observed with amiodarone and nelfinavir, because both amiodarone and nelfinavir are basic drugs and have high lipophilicity, such as clomipramine.

On the other hand, it has been reported that clomipramine is metabolized to desmethylclomipramine in the liver of rats. However, the distribution level of desmethylclomipramine to the brain is significantly lower than that of clomipramine, because the lipophilicity of desmethylclomipramine is lower than that of clomipramine.[31] Moreover, there is a possibility that serum lipids would affect the distribution to the brain less because of the lower lipophilicity of desmethylclomipramine. Therefore, we investigated and discussed the effect of serum lipids on the pharmacokinetics of clomipramine only.

The nonlinearity of clomipramine distribution to the red blood cell in control rat blood may be observed slightly at 5 μg/ml of blood concentration (Table 2). The plasma concentration of 5 μg/ml was at least 10-times higher than a therapeutic one. However, in our pharmacokinetic studies, the plasma concentrations were always less than 5 μg/ml. Therefore, saturation of the clomipramine distribution in blood need not be considered. No significant difference was found in the P–B ratio at blood concentrations of 0.5 or 1 μg/ml between hyperlipidaemic and control rats, suggesting that there was no effect of hyperlipidaemia on the P–B ratio under 1 μg/ml of blood concentration.

In plasma protein binding studies of drugs, equilibrium dialysis, ultrafiltration, and ultracentrifugation methods are generally used to determine the unbound drug concentration.[32,33] A preliminary experiment revealed that the degree of adsorption of clomipramine on to the membranes used in ultrafiltration and equilibrium dialysis devices was very high. In addition, the ultracentrifugation method may be impractical because of lipoprotein contamination of the plasma water supernatant.[34] These methods are unsuitable for highly lipophilic drugs such as clomipramine in hyperlipidaemic plasma; the erythrocyte vs buffer or plasma partitioning method was used to determine the unbound clomipramine concentration in rat plasma.[16] In this study, the plasma unbound fraction in control rats obtained using this method was approximately 7%, which was close to that reported in humans.[4] In contrast, the plasma unbound fraction in hyperlipidaemic rats was significantly lower than that in control rats (approximately 1%). In addition, clomipramine recovery in plasma lipoprotein of hyperlipidaemic rats was significantly higher in chylomicron, VLDL, and LDL fractions. However, in the LPDF fraction, clomipramine recovery in hyperlipidaemic rats was significantly lower (Figure 3). In addition, LDL% and VLDL% in hyperlipidaemic rats were higher than in control rats (Table 1). These results suggested that the increased low-density lipoprotein level might have decreased the plasma unbound fraction of clomipramine. In our previous report of nelfinavir and atazanavir, which are high-lipophilic and basic drugs, we obtained similar results.[9,10]

As shown in Table 3, $Vd_{ss}$ after intravenous bolus injection in hyperlipidaemic rats was approximately one-third of that in control rats. In addition, the plasma concentration of clomipramine at 5 min after intravenous bolus injection in hyperlipidaemic rats (1.95 ± 0.09 μg/ml) was approximately 3-times higher than that in control rats (0.63 ± 0.03 μg/ml). Moreover, after the start of the clomipramine infusion with the bolus injection, the clomipramine concentration in hyperlipidaemic rats at 30 min (0.88 ± 0.07 μg/ml) was significantly higher compared with control rats (0.49 ± 0.02 μg/ml) (Figure 2). These findings clearly showed that clomipramine distribution from the blood to peripheral tissues was limited, caused by the large increase in the low-density lipoprotein level resulting in a decrease of the unbound fraction in plasma. The marked decrease of $Vd_{ss}$ was the primary reason for the significantly higher values for $AUC_{0\text{-}\infty}$ and $C_{ss}$ of clomipramine in hyperlipidaemic rats, resulting in the decrease of $CL_{tot}$ and $CL_{tot,ss}$ in hyperlipidaemic rats (Tables 3 and 4).

On the other hand, Balant-Gorgia *et al*.[4] described that the plasma concentrations of clomipramine lower than 0.15 μg/ml were usually associated with nonresponse, although those greater than 0.45 μg/ml seldom engendered an improvement in the efficacy of antidepressant therapy in humans. Therefore, it is necessary that the plasma concentrations of clomipramine be maintained in a fairly narrow therapeutic range. Moreover, in clinics, the time to reach steady state for clomipramine is, in general, at approximately three weeks, because of the long apparent elimination half-life of clomipramine (approximately 24 h) in humans.[4] In this study, clomipramine was administrated as a continuous infusion following bolus injection (17.5 μg/min/kg with a bolus loading dose of 5 mg/kg) instead of being given orally as for human use in clinics, so as to reach the target steady state concentration range of 0.15–0.45 μg/ml quickly. Table 3 shows that our dosing regimen of continuous infusion was able to achieve the target steady state concentration range in both hyperlipidaemic and control rats.

Moreover, we investigated the distribution of clomipramine to the brain and liver because the brain is a target of clomipramine, an antidepressant, and clomipramine is almost completely metabolized in the liver.[4,35] The value of $X_{brain}/C_{ss}$ in hyperlipidaemic rats was significantly lower when compared with control rats (Table 5). The plasma unbound concentration at steady state estimated by the plasma unbound fraction data (Table 2) in hyperlipidaemic rats (0.0045 ± 0.0001 μg/ml) was lower than that in control rats (0.021 ± 0.001 μg/ml), although $C_{ss}$ in hyperlipidaemic rats was approximately 1.5-times higher than that in control rats (Table 4). In addition, the value for $Vd_{ss}$ of clomipramine in hyperlipidaemic rats was lower than that in control rats, as described previously. Therefore, it was suggested that the distribution of clomipramine to the brain was limited, whereas $C_{ss}$ was increased in hyperlipidaemic rats. Moreover, we estimated the values of $X_{brain}/C_{ss,u}$ and $X_{liver}/C_{ss,u}$, as presented in Table 5, because only protein-unbound drugs penetrate into tissues. The use of those estimates was valid

assuming no saturation of plasma protein binding. If the plasma unbound concentration was a limiting factor for drug access to tissues, then it would be expected that the values of both $X_{brain}/C_{ss,u}$ and $X_{liver}/C_{ss,u}$ would be the same in both control and hyperlipidaemic rats. In this study, no difference was found in $X_{brain}/C_{ss,u}$ between control and hyperlipidaemic rats. Therefore, the decrease of the plasma unbound fraction of clomipramine in hyperlipidaemic rats might show that the distribution of clomipramine to the brain was limited, although $C_{ss}$ was higher. Patel *et al*.[36] obtained a similar result for the tissue distribution of halofantrine, which is used for the treatment of malaria, in P-407-induced hyperlipidaemic rats, supporting our consideration. In addition, $CL_{tot,ss}$ of clomipramine in hyperlipidaemic rats was lower than that in control rats (Table 4) and the time to reach steady state in hyperlipidaemic rats after the start of infusion was longer than that in control rats (Figure 1; control 90 min, hyperlipidaemic 150 min). Moreover, $X_{liver}/C_{ss}$ (the distribution of clomipramine to the liver) in hyperlipidaemic rats was significantly lower when compared with control rats (Table 5). These findings showed that the metabolism of clomipramine in the liver at steady state was lower than that in hyperlipidaemic rats because of the lowered distribution from blood to the liver and to the brain. However, it was noted that despite the decrease of $X_{liver}/C_{ss}$, $X_{liver}/C_{ss,u}$ in hyperlipidaemic rats was significantly higher than that in control rats. Reportedly, a higher lipophilic drug load in the low-density lipoprotein fraction in hyperlipidaemic rats enhanced hepatic uptake of such drugs, mediated via lipoprotein receptors.[22,24,36] In addition, previous reports showed that the lipoprotein receptor density in various tissues affected the biodistribution of lipoproteins and lipoprotein-associated drugs.[37–41] Therefore, higher hepatic uptake than that in the control rats was a reason for the higher $X_{liver}/C_{ss,u}$ in hyperlipidaemic rats. Previously we reported similar observations from studies of nelfinavir and atazanavir in hyperlipidaemic rats.[9,10] We suggest that despite the increase of hepatic uptake of clomipramine in hyperlipidaemic rats, the amount of clomipramine distribution to the liver in hyperlipidaemic rats was lower than that in control rats, resulting in a decrease of metabolism of clomipramine in the liver.

## Conclusions

The distribution of clomipramine to the brain in hyperlipidaemic rats was dramatically lower compared with control rats in spite of the higher clomipramine plasma concentration, because of the lower plasma unbound fraction of clomipramine. These results suggested that the higher plasma clomipramine concentration in the hyperlipidaemic condition did not induce a greater pharmacological effect. Results suggested that the higher lipoprotein level might have caused a decrease in the plasma unbound fraction and that triglyceride-rich lipoproteins might have been an important mediator of the disposition of lipophilic drugs such as clomipramine. This suggested that monitoring of triglyceride-rich lipoprotein levels is needed for clomipramine medication. These findings provide useful information for the proper use of clomipramine in patients with hyperlipidaemia.

## Declarations

### Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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