Effect of Glycerol-Induced Acute Renal Failure on the Pharmacokinetics of Lidocaine after Transdermal Application in Rats

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In this study, the effect of glycerol-induced acute renal failure (ARF) on the pharmacokinetics of lidocaine after transdermal application was investigated in rats. Microdialysis method was applied in vitro and in vivo to the abdominal skin of rats. After topical application of 1% lidocaine, the cumulative amount of lidocaine permeated through the excised rat abdominal skin showed parallel effect between normal and ARF rats with no significant difference in the in vitro permeation coefficient of lidocaine between them, while area under the plasma concentration versus time curve of lidocaine in ARF rats increased significantly. The protein binding rate of lidocaine in ARF plasma and the blood vessel permeability to muscle tissues, assessed by β-D-glucopyranosyl fluorescein isothiocyanate-labeled (FITC) albumin, increased significantly. After intravenous infusion of 5 mg/kg lidocaine, both of the total body clearance and the volume of distribution of lidocaine in the ARF rats decreased significantly. These results suggested that renal dysfunction did not have any effect on the skin permeability of lidocaine, but might change the plasma protein binding of drug and blood vessel permeability which led to high plasma concentration of lidocaine.

Key words  lidocaine; acute renal failure; transdermal drug delivery; pharmacokinetics

Lidocaine, a local anesthesia, is one of the drugs being applied by a transdermal formulation, and is used to avoid discomfort or pain associated with the curettage of molluscum contagiosum or the insertion of venous cannulation.1—3) Particularly for pediatric patients with hemodialysis, lidocaine is used to avoid pain during vascular access as a topical administration for a long time.4) Recently, the local anesthetics with lidocaine induce the apoptosis of human renal cell by increasing caspase activity and inhibiting the pro-survival signaling pathways.5) It is, therefore, important to take the disposition of lidocaine during renal dysfunction into consideration for the optimal use of transdermal application of lidocaine. However, there have been few reports on the pharmacokinetic profiles of lidocaine after transdermal administration during renal failure. This study was designed to obtain the basic information on the pharmacokinetics of lidocaine when it was applied percutaneously in glycerol-induced acute renal failure (ARF) rats.

MATERIALS AND METHODS

Chemicals  Lidocaine hydrochloride was purchased from ICN Biomedicals Inc. (Ohio, U.S.A.). β-D-Glucopyranosyl fluorescein isothiocyanate-labeled (FITC) albumin was purchased from Sigma Chemical Co. (St. Louis, U.S.A.). Acetonitrile used for a high-performance liquid chromatography (HPLC) and other chemicals were of HPLC and of analytical grade, respectively, and were used without further purification.

Animal Preparation and Induction of Acute Renal Failure  Male Sprague–Dawley rats, 300—350 g, were obtained from Nippon SLC Co. (Hamamatsu, Japan). They were housed for at least 2 d in a clean room with general food freely available. Previously dehydrated for 24 h, they received 50% (v/v) glycerol in 0.9% saline intramuscularly (10 ml/kg) in divided doses into the hind limbs under a light anesthesia with ether. Water was freely available thereafter. These ARF rats were studied 48 h after the injection of 50% glycerol. To assess degree of nephrotoxicity, creatinine and blood urea nitrogen (BUN) levels in plasma were measured with the standard spectrophotometric assay kits. Normal rats (control) received the same volume of 0.9% saline instead of 50% glycerol, and were treated by the same manner with ARF rats. All experiments were performed in accordance with the Guideline for Animal Experimentation in Kyoto Pharmaceutical University.

Microdialysis System  The microdialysis system consisted of a SP200 syringe pump (World Precision Instruments, Inc., U.S.A.) and CMA/20 microdialysis probes (CMA Microdialysis Research AB, Stockholm, Sweden). Briefly, the semi permeable membrane (length 4 mm, o.d. 0.5 mm) of the microdialysis probe is made of a polycarbonate polymer with a molecular cut-off level at 20 kD. Before experiments, the microdialysis probe was conditioned by passing through an appropriate buffer for 1 h.

In Vitro and in Vivo Recoveries with Microdialysis  In vitro recovery (outlet dialysate concentration/surrounding concentration) was determined by placement of the probe in a vial containing 1% of lidocaine in phosphate buffered saline (PBS, pH 7.4). For in vivo recovery (1—outlet dialysate concentration/inlet dialysate concentration), a microdialysis probe was inserted into the rat abdominal skin. After a microdialysis probe was inserted into the rat abdominal skin, Tyrode solution (NaCl 0.8%, dextrose 0.1%, NaHCO3 0.1%, CaCl2 0.02%, KCl 0.02%, MgCl2 0.01%, NaH2PO4 0.0066%) containing 0.5, 1.5, 5 μg/ml or 1% of lidocaine, respectively, was passed through the microdialysis probe, and the concentration of lidocaine in the outlet was measured. In both in vitro and in vivo experiments, a perfusion rate was set at 3 μl/min.

In Vitro Permeation Experiments with Microdialysis  The in vitro permeation procedures were determined using a side-by-side glass diffusion cell. The abdominal fur of the rat was shaved and incised. The incised skin was sandwiched between the donor and receptor chambers. Twenty milliliters of 1% lidocaine in PBS and only PBS were introduced into

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the donor and receptor chambers, respectively. Both of the chambers were occluded by parafilm. The permeation study was conducted at 37 °C. A microdialysis probe was inserted into the receptor cell directly and perfused with PBS at 3 μl/min. Samples were collected every 1 h for 6 h in total following application. Samples were directly injected into the HPLC system for lidocaine determination.

**In Vivo Transdermal Experiments with Microdialysis**

After rats were anaesthetized by intraperitoneal injection of sodium pentobarbital (40 mg/kg for normal rats, 20 mg/kg for ARF rats), the abdominal skin was incised over the dermis in the shaved abdominal region, and a microdialysis probe was inserted in the dermis in the same manner as reported by Matsuyama et al. After implantation, a glass reservoir was fixed above the tip of the probe on skin with medical adhesive. The microdialysis system was perfused with Tyrode solution at a flow rate of 3 μl/min. After 1 h equilibration, 1% lidocaine in Tyrode solution was introduced into the reservoir and the dialysate was collected every 1 h for 10 h in total. Simultaneously, the blood samples were taken from the jugular vein at 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5, 7.5, 8.5 and 9.5 h after the start of dialysis. The concentrations of lidocaine in dialysate and plasma were analyzed by HPLC.

**In Vivo Intravenous Infusion**

After rats were anaesthetized by intraperitoneal injection of sodium pentobarbital (40 mg/kg for normal rats, 20 mg/kg for ARF rats), lidocaine was infused from femoral vein at the dosing rate of 5 mg/kg/h over 2 h by a syringe pump. Thereafter, the infusion was stopped. The blood samples were taken from the jugular vein at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 6.0 h after the start of infusion. The concentrations of lidocaine in plasma were analyzed by HPLC.

**Protein Binding and Tissue Permeability**

The plasma samples from control and ARF rats containing 0.5 μg/ml of lidocaine were loaded onto the Nanosep (30 kD) concentrator (Pall Filtron CO., Dreieich, Germany) and centrifuged at 9000×g for 10 min at 37 °C. Aliquots of plasma water in the filtrate receiver were extracted using acetonitrile. The sample was determined for the lidocaine concentration with HPLC, and then the percentage of protein binding was calculated. The FITC-albumin (10 mg/kg) was injected into the normal rats intravenously. After 1 h, the rat was sacrificed by means of MULTI program using the equation of $P = J_d/C_{in}$, where $C_{in}$ is the concentration of lidocaine in donor solution. Lidocaine concentrations in the in vivo dialysate were used as they were. Area under the concentration versus time curve of lidocaine (AUC) after transdermal administration was calculated by a linear trapezoidal approximation up to the last measured point. For the plasma concentration–time data (C) of intravenous infusion, one-compartment open model was fitted by means of MULTI program using the equation of $C = (k_o/V_d) \cdot (1 - e^{-k_o \cdot t}) \cdot e^{-k_t \cdot t}$, where $k_o$, $V_d$, and $k_t$ represent infusion rate of lidocaine, the volume of distribution and the elimination rate constant, respectively. $t$ and $T$ represent the time up to stopping the infusion and the time thereafter. The total body clearance ($CL_t$) and the concentration at steady state ($C_{ss}$) after the infusion were calculated by the equation $CL_t = k_o \cdot V_d$ and $C_{ss} = k_o/C_{in}$, respectively.

**Data Analysis**

Lidocaine concentrations in the in vivo dialysate were corrected by the value of in vivo recovery ratio based on the zero-net-flux method. Permeability coefficient ($P$) of lidocaine through the skin was calculated from the steady-state flux ($J_s$) which derived from the slope of the cumulative amount-time profile using the equation of $P = J_s/C_{in}$, where $C_{in}$ is the concentration of lidocaine in donor solution. Lidocaine concentrations in the in vivo dialysate were used as they were. Area under the concentration versus time curve of lidocaine (AUC) after transdermal administration was calculated by a linear trapezoidal approximation up to the last measured point. For the plasma concentration–time data (C) of intravenous infusion, one-compartment open model was fitted by means of MULTI program using the equation of $C = (k_o/V_d) \cdot (1 - e^{-k_o \cdot t}) \cdot e^{-k_t \cdot t}$, where $k_o$, $V_d$, and $k_t$ represent infusion rate of lidocaine, the volume of distribution and the elimination rate constant, respectively. $t$ and $T$ represent the time up to stopping the infusion and the time thereafter. The total body clearance ($CL_t$) and the concentration at steady state ($C_{ss}$) after the infusion were calculated by the equation $CL_t = k_o \cdot V_d$ and $C_{ss} = k_o/C_{in}$, respectively.

**Statistics**

Statistical analysis was performed by Student’s t-test; results were expressed as the mean±standard error (S.E.). The p-values less than 0.05 were considered to be significant.

**RESULTS**

Creatinine and BUN in plasma were used to assess the degree of ARF induced by glycerol injection. Forty-eight hours after glycerol injection, the values of creatinine in ARF rats increased from 1.54±0.10 to 4.35±0.06 mg/dl ($n=3$), and BUN increased from 20.68±1.60 to 218.66±2.00 mg/dl ($n=3$). Thus acute renal failure was clearly evident.

To confirm the performance of the microdialysis probe, we checked the in vitro and in vivo recoveries of lidocaine using the microdialysis system by passing the 1% lidocaine solution through the microdialysis probe. As shown in Fig. 1, the recovery of lidocaine, which was given as 100×(outlet concentration/ (inlet concentration + peak concentration)), was not significantly changed in both the in vitro and in vivo microdialysis with the mean value over sampling period of 11.4±0.7% (Fig. 1A) and 11.6±2.2% (Fig. 1B), respectively. Figure 1C shows the in vivo result of the experiment to assess linearity between the $C_{in}$ and $C_{ss}$ over a wide range of concentration. The concentration cleared through the probe ($C_{in} - C_{out}$) increased linearity as the $C_{in}$ increased. The regression equation between $C_{in}$ and ($C_{in} - C_{out}$) was $Y = 0.133X - 0.0065$ ($r^2 = 0.999, p<0.01$). Thus, the slope of this regression line was equal to the experimental results of Figs. 1A and B, sug-
gesting that the drug recovery from the tissue to the dialysate is the same with the drug loss from the dialysate to the tissue, across the membrane. Therefore, using the value of 0.133, we corrected the lidocaine concentration in the outlet dialysate through the skin.

The in vitro penetration profiles of lidocaine through the normal and ARF rat skins with microdialysis are shown in Fig. 2. There was no significant difference in the cumulative amount of lidocaine after percutaneous penetration between normal and ARF rats. The permeability coefficients in control and ARF rat skin were 4.08±2.08 and 5.17±2.54 cm/s×10^6, respectively (not significant, n=3).

The in vivo concentration–time profile of lidocaine in the skin dialysate and plasma during 10 h skin microdialysis after topical application of 1% lidocaine in normal and ARF rats are shown in Fig. 3. The AUC values of skin dialysate in the normal and ARF rats were 43.07±7.01 and 41.88±6.56 μg·h/ml, respectively, and there was no significant difference (n=5). On the other hand, the AUC value of plasma in the ARF rat increased significantly from 2.18±0.13 to 4.87±0.17 μg·h/ml (p<0.01, n=5).

The in vivo concentration–time profile of lidocaine in plasma after intravenous infusion in normal and ARF rats are shown in Fig. 4. The pharmacokinetic parameters estimated by fitting the plasma concentration–time data to a one-compartment model are listed in Table 1. After stopping the infusion, the maximum lidocaine concentrations in plasma in normal and ARF rats immediately reached 0.82±0.05 and 1.47±0.18 μg/ml, respectively, and the values of C_{ss} estimated in normal and ARF rats were 0.93±0.04 and 1.24±0.20 μg/ml, respectively. The value of V_d also significantly decreased from 5.40±0.21 to 2.71±0.39 l/kg (p<0.01), and the value of CL_t also significantly decreased from 43.07±7.01 to 41.88±6.56 μg·h/ml, respectively, and there was no significant difference (n=5). On the other hand, the AUC value of plasma in the ARF rat increased significantly from 2.18±0.13 to 4.87±0.17 μg·h/ml (p<0.01, n=5).

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in both normal and ARF rats. The percentage of plasma protein binding of lidocaine in ARF rats increased significantly from 56.6±6.5 to 74.2±8.4% (p<0.05, n=3). The fluorescence intensity based on the FITC-albumin in the abdominal muscle but not in the epidermis increased significantly from 18.5±1.7 to 21.6±0.3 (p<0.01, n=3).

DISCUSSION

Microdialysis is a new method to perform pharmacokinetic studies of many drugs. Results in this study also show the usefulness of microdialysis and provide positive outcomes for transdermal delivery of lidocaine. Since the concentration of analyte in the dialysate is less than that of in the extracellular fluid surrounding the microdialysis probe, the knowledge of the fractional recovery is a prerequisite for calculating tissue extracellular concentration of the analyte. The in vivo calibration experiments in this study clearly indicate that the process of diffusion through the microdialysis membrane is concentration independent over a wide range of concentration for lidocaine (Fig. 1). Similar observations have been reported for an array of different analytes.

The main purpose of our study is to explore the influence of acute renal failure on lidocaine transdermal delivery. As shown in Fig. 2, ARF did not lead to significant changes in lidocaine percutaneous penetration. This can be explained by the unchanged epidermis, dermis and hypodermis or subcutis. The outmost layer of epidermis is called stratum corneum which is composed of corneocytes embedded in lipid domains consisting of alternately hydrophilic and lipophilic layers and is considered to be the primary barrier. ARF will not have any effect on the barrier characteristics of epidermis because the hydration, composition and thickness of epidermis and arrangement of lipid are not changed. However, the permeability study using the FITC-albumin showed that the permeability of molecules between blood vessel and muscle tissues was enhanced in the presence of ARF but not between blood vessel and epidermis (Table 2). Hyaluronic acid, collagen and polysaccharide together form the structural network of blood vessel and muscle tissues and have good characteristics of water accumulation. They can absorb extra fluid, and regulate equilibrium between tissue edema and vascular permeability. It is generally hypothesized that most drug molecules absorbed into the skin are transferred to the cutaneous blood flow and are transported to the rest of the body through systemic circulation. Therefore, the hemodynamic factor, such as blood vessel permeability, will influence the lidocaine systemic concentration. Our results, therefore, indicate that the ARF condition enhances the influx of lidocaine just after penetrated the skin into the blood vessel.

The present studies also suggested that changes of other physiological conditions, such as renal blood flow and uremic state might affect the pharmacokinetic profiles of lidocaine. Renal failure has been shown to decrease the plasma protein binding of acidic drugs and the presence of binding inhibitors in uremic plasma has been suggested. Increase in the hepatic blood flow was shown in rats with glycerol-induced ARF. Moreover, the uremic state may have effects on the metabolic transformation of lidocaine. Basically, lidocaine is a basic drug and classified as a flow-limited drugs. It, therefore, binds mainly to an inflammatory protein in plasma, α1-acid glycoprotein, which is induced by glycerol injection, and the hepatic clearance of lidocaine depends on the liver blood flow. Accordingly, if hepatic clearance does change with the dependence on hepatic blood flow, the total body clearance after the intravenous infusion should increase. However, in this study, the value of CL of lidocaine after intravenous infusion decreased significantly (Fig. 3, Table 2), suggesting that an increase in the bound fraction of lidocaine in plasma might mainly regulate the disposition of lidocaine in ARF condition. In addition, the fact that the volume of distribution, Vd, significantly decreases in ARF rats in spite of enhancement of blood vessel permeability shows that an increase in protein binding rate is the primary factor to affect the disposition of lidocaine in ARF condition.

In summary, it was proven that the pharmacokinetics of lidocaine after skin permeation was remarkably influenced in the presence of ARF condition, though ARF did not affect cutaneous permeability itself of the lidocaine. In clinical practice, several formulations of lidocaine (0.5—10%) are used to achieve topical anesthesia. In this study, even though at 1% of lidocaine solution, we recognized that the pharmacokinetic profiles of lidocaine after penetrating the skin were altered in the presence of ARF condition. Although the application time is actually short at the local site, we must pay attention to avoid side effects for the patients who has a higher sensitivity for lidocaine.

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