Effects of a Selective Inhibitor of Inducible Nitric Oxide Synthase, ONO-1714, on Experimental Hemodialysis-Related Hypotension in Renal Dysfunctional Dogs

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Abstract. The effect of a selective inducible nitric oxide synthase inhibitor, ONO-1714 ((1S,5S,6R,7R)-7-chloro-3-imino-5-methyl-2-azabicyclo[4.1.0]heptane hydrochloride), on hemodialysis-related hypotension was investigated using a canine model of renal dysfunction. Renal dysfunction was induced in dogs by complete bilateral ligation of renal arteries. On performing hemodialysis with ultrafiltration, the blood pressure of the renal dysfunction dogs gradually decreased and persisted at reduced levels until completion. ONO-1714 ameliorated the hemodialysis-induced hypotension in the renal dysfunction dogs at a dose that did not influence blood pressure in non-hemodialysis dogs with normal renal function. The above findings indicated that ONO-1714 may elicit beneficial effects on hemodialysis-related hypotension.

Keywords: ONO-1714, hemodialysis-related hypotension, ultrafiltration

Hemodialysis is an established therapy for renal dysfunction, although many complications (especially hypotension) are encountered (1). Previous reports have suggested that nitric oxide (NO) may contribute to hemodialysis-related hypotension (2 – 4). It is known that plasma levels of interleukin-1 (IL-1) and tumor necrosis factor (TNF) are chronically elevated in patients undergoing hemodialysis therapy (5 – 7). Since these cytokines are powerful inducers of inducible NO synthase (iNOS) (8), hemodialysis-associated hypotension may be attributable to the NO production by iNOS. We have reported that ONO-1714 is a selective inhibitor of iNOS compared with endothelial constitutive NO synthase (ecNOS) (9). In the present study, we attempted to elucidate the effect of ONO-1714 on hemodialysis-related hypotension in a canine experimental model with symptoms resembling clinical conditions of humans.

Six male beagles (CSK Research Park, Nagano) were used. The conditions for accommodation of dogs were consistently maintained (temperature: 23 ± 2°C, humidity: 55 ± 15%, lighting: fluorescent lamps with a 12-h illumination period). The solid pellets (CD-5; Cler Japan, Tokyo) were fed at 250 g/day. However, renal dysfunction dogs were force-fed with alimentary supplements (Elental; Ajinomoto, Tokyo) once daily at 80 g/day. An automatic device supplying drinking water ad libitum was installed in each cage. All experimental procedures described here were done according to the ‘Guidelines for Animal Studies’ stipulated by the Research Headquarters of Ono Pharmaceutical Co., Ltd.

Dogs were anesthetized with sodium pentobarbital at 25 – 30 mg/kg (i.v.). A subcutaneous implantation catheter system (Vascular Access Ports; Access Technologies, Skokie, IL, USA) was embedded subcutaneously under sterile conditions; the catheter end was inserted into a branch of the femoral artery. An arteriovenous shunt catheter (Showa Ika Kogyo, Nagoya) was connected to the carotid artery and jugular vein via a vessel-chip (Showa Ika Kogyo). The tube of the shunt system was exposed to the atmosphere from below the skin. Postoperative antibiotic (sodium ampicillin 250 mg/animal and sodium cloxacillin 250 mg/animal; Meiji Seika Kaisha, Tokyo) treatment was administered i.m. once daily.

The visceral sectioning of dogs was performed under anesthesia, and bilateral renal arteries were completely
ligated under sterile conditions. After 1-day hemodialysis acclimatization, renal dysfunction animals were subjected to experiments, where an artificial renal device (Fresenius 4008B; Fresenius Medical Care Japan, Tokyo) installed with a hemodialyzer (triacetate hollow fiber: FB-70U; Nipro, Osaka) was employed. Animals were positioned in a standing position, and the shunt catheter was then connected to the extracorporeal circulation of animals. The hemodialysis conditions were as follows: blood flow rate was 50 mL/min with a hemodialysis flow rate of 500 mL/min at 39°C, yielding ultrafiltration at 50 mL/kg per 3 h. ONO-1714, (1S,5S,6R,7R)-7-chloro-3-imino-5-methyl-2-azabicyclo[4.1.0]heptane hydrochloride, was supplied by Ono Pharmaceutical Co., Ltd. (Osaka). Etilefrine hydrochloride (Boehringer Ingelheim Seiyaku Co., Ltd., Migashine) is an α1-receptor agonist that is used for the treatment of hemodialysis-related hypotension in humans. Drug administration at 1 mL/kg was initiated within 5 min in the venous side of extracorporeal circulation at 110 min after initiating ultrafiltration. Heparin treatment was initiated with a bolus infusion of 25 U/kg, followed by an infusion rate of 20 U/kg per hour.

Changes in blood pressure were monitored via an amplifier (AP-601G; Nihon Kohden Co., Ltd., Tokyo) connected to a pressure transducer linked to the port of the catheter system by an inserted injection needle. The mean ± S.E.M of any change (compared with the pre-dosing mean blood pressure) for each measurement point was derived before the changes of each measurement point were compared between the ONO-1714-treated and vehicle-treated groups with the one-way ANOVA test. In cases where the differences were significant (P<0.05), differences between the vehicle-treated group and the ONO-1714-treated groups at the various doses were then verified by the Dunnett’s test for multiple comparison. In addition, differences between the vehicle-treated and etilefrine-treated groups were compared with the Student’s t-test. In such cases, the F-test was first performed followed by the Student’s t-test in cases where the F-value was 5% or less, while in cases where F-value was 5% or more, the Welch t-test was applied.

Since BUN levels of dogs with ligated renal arteries (33 – 132 mg/dL) were higher than those of normal dogs (9.3 – 14.9 mg/dL), renal dysfunction was thus established. The range of mean blood pressure of renal dysfunction dogs was high (139 to 160 mmHg) compared with normal renal function dogs. The extracellular volume expansion induced by anuria is the main pathophysiological determinant of hypertension in hemodialysis patients (10). In addition, the norepinephrine concentration in dogs with experimentally induced renal vascular hypertension tends to increase (11). Therefore, it seems likely that hypertension in renal dysfunction dogs was induced by extracellular volume expansion and changes in sympathetic nervous activity. On performing hemodialysis and ultrafiltration, the mean blood pressure gradually decreased, and a constant level (107 – 128 mmHg) was almost established by 90 min after initiating ultrafiltration. We have demonstrated in our previous study that hemodialysis-related hypotension is merely a transient event, using a dialysis device for animal use (12). However, the hemodialysis-related hypotension has been reported to behave in a persistent fashion in a clinical study (13). The ultrafiltration performed for human hemodialysis was used in the present study, and decreases in the blood pressure were consistent. In animals treated with the vehicle for 5 min, the mean blood pressure decreased by 11.7 mmHg at 60 min after administration. At doses of 0.03 and 0.3 mg/kg, ONO-1714 produced significant dose-dependent increases in the mean blood pressure compared with vehicle-treated controls. This effect was persistently observed from immediately after ONO-1714 treatment (Fig. 1). Etilefrine induced a significant increase in the mean blood pressure at 0.1 mg/kg compared with vehicle-treated controls, registering a peak value of 14.8 mmHg at 1 min after administration. However, the increasing effect rapidly decayed after infusion (Fig. 1).

ONO-1714 at 0.03 mg/kg did not elicit significant changes in the mean blood pressure of normal renal function dogs compared with controls (Fig. 2). Although significant increases in the blood pressure were achieved when the dosage was elevated to 0.3 mg/kg, the peak was a mere 8.2 mmHg. In the case of etilefrine, administration with a dose of 0.1 mg/kg significantly increased the mean blood pressure to a peak of 16.5 mmHg, registering a high level even at post-administration 60 min compared with controls (Fig. 2).

We have previously demonstrated in a study using a non-selective inhibitor of NOS that an increase in NO contributed to hemodialysis-related hypotension (12). In the present study, ONO-1714, which displays a tenfold specificity in the inhibitory activity on iNOS compared with ecNOS (9), reversed hemodialysis-related hypertension of renal dysfunction dogs with doses of 0.03 mg/kg or more in a dose-dependent fashion. In contrast, ONO-1714 at 0.03 mg/kg did not affect the blood pressure and produced slight increases in the blood pressure of normal renal function dogs at a higher dosage of 0.3 mg/kg. When changes in the plasma concentration of ONO-1714 were monitored in normal renal function and renal dysfunction dogs with hemo-
Hemodialysis Hypotension and iNOS Inhibitor

Fig. 1. Changes in mean blood pressure during HD in conscious renal dysfunctional dogs. After the mean blood pressure was measured for 30 min, shunt catheters of the carotid artery and jugular vein were connected to the extracorporeal circulation. Mean blood pressure during HD including ultrafiltration (50 mL/kg per 3 h) was measured for 3 h. Each point represents the mean value and the vertical bars show S.E.M. of 6 dogs. ONO-1714 or etilefrine was administered via the jugular vein from 110 to 115 min after initiating ultrafiltration. Open circles: vehicle control treatment group, closed circles: ONO-1714 (0.03 mg/kg) group, closed squares: ONO-1714 (0.3 mg/kg) group, open diamonds: etilefrine hydrochloride (0.1 mg/kg). Differences where $P<0.05$ (* or #) and $P<0.001$ (***).vs the vehicle group were considered significant.

Fig. 2. Changes from pre-dosing values for the mean blood pressure in conscious normal renal functional dogs. Values are the mean, and vertical bars show S.E.M. of 6 dogs. ONO-1714 or etilefrine was administered for 5 min via the cephalic vein. Open circles: vehicle control treatment group, closed circles: ONO-1714 (0.03 mg/kg) group, closed square: ONO-1714 (0.3 mg/kg) group, open diamonds: etilefrine hydrochloride (0.1 mg/kg). Differences where $P<0.05$ (* or #), $P<0.01$ (##) and $P<0.001$ (###) vs the vehicle group were considered significant.
dialysis, no pharmacokinetic differences between the dogs were detected (data not shown). These results suggest that the dosages of ONO-1714 used in the present study may have negligible inhibitory activity on ecNOS and iNOS-derived NOs could have induced hemodialysis-related hypotension.

Etilerine, an $\alpha_1$-adrenoceptor agonist, elicits a hypertensive effect that clinically reverses hemodialysis-related hypotension. In the present study, etilerine consistently increased blood pressure in normal renal function dogs. However, the effects of etilerine on the experimental model of hemodialysis-related hypotension have not been attempted as yet. In fact, the present study, to our knowledge, is the first report on the effects of etilerine on experimental hemodialysis-related hypotension. The present results indicated that etilerine induced transient effects on hemodialysis-related hypotension.

From the above findings, we suggest that efficacy of the selective iNOS inhibitor ONO-1714 in attenuating hemodialysis-related hypotension is highly plausible.

References