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Preventive effect of zelandopam, a dopamine D1 receptor agonist, on cisplatin-induced acute renal failure in rats

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Abstract

To elucidate the role of peripheral dopamine D1 receptors in cisplatin-induced acute renal injury, effect of zelandopam (YM435, (−)-(*S*)-4-(3,4-dihydroxyphenyl)-7,8-dihydroxy-1,2,3,4-tetrahydroisoquinoline hydrochloride hydrate), a selective renal dopamine D1 receptor agonist, on cisplatin-induced acute renal failure in rats was studied. Rats were divided into six groups: control, cisplatin and cisplatin plus zelandopam (30, 100, 300 mg/kg p.o. twice, 75 and 15 min before cisplatin injection) or the free radical scavenger CV-3611 (2-*O*-octadecylascorbic acid, 10 mg/kg p.o., 75 min before cisplatin injection) treated groups. Rats received intraperitoneal injection of cisplatin at a dose of 5 mg/kg. Four days after cisplatin injection, plasma creatinine, blood urea nitrogen and body weight were measured and the kidneys were removed for histological examination. Cisplatin induced acute renal failure characterized by the increases in plasma creatinine and blood urea nitrogen with tubular damage, and decreased body weight. Zelandopam dose-dependently prevented all these changes. The free radical scavenger CV-3611 significantly attenuated a decrease in body weight and renal dysfunction without reducing tubular damage. The present study is the first demonstration for that a selective dopamine D1 receptor agonist is effective in preventing acute renal failure induced by cisplatin.

Keywords: Renal failure, acute; Cisplatin; Dopamine D1 receptor; Zelandopam

1. Introduction

Zelandopam (YM435) is a selective dopamine D1 receptor agonist with potent renal vasodilation property (Yatsu et al., 1997a,b,c). Zelandopam has also been shown to provide significant renal protection in a canine model of ischemic acute renal failure (Yatsu et al., 1998). The beneficial effects of zelandopam in ischemic acute renal failure were, for the most part, accompanied by an increase in renal blood flow. Cisplatin-induced acute renal failure in experimental animals is accompanied by the reduced renal blood flow associated with increased renal vascular resistance (Matsushima et al., 1998; Winston and Safirstein, 1985) and histologic damage to proximal tubular cells (Chopra et al., 1982; Dobyan et al., 1980; Jones et al., 1985). Oxygen free radical is well known to play a role in renal dysfunction by cisplatin (Baliga et al., 1998; Matsushima et al., 1998; Sugihara et al., 1987). The free radical scavengers are reported to provide partial protection against cisplatin-induced structural and functional alterations in rats (Dobyan et al., 1986; Saad et al., 2000). The induction of nephrotoxicity by cisplatin is assumed to be a rapid process involving reaction with proteins and renal tubules (Heidemann et al., 1985). This renal tubular damage is caused in the first hour after injection of cisplatin (Elferink et al., 1986). Hence, it is important that protective agents must be present in the renal tissue before the cisplatin administration (Rao et al., 1999). Based on these, the present study was designed to investigate the role of peripheral dopamine D1 receptors in renal functional and structural alterations by cisplatin in rats and to evaluate the potential therapeutic effect of dopamine D1 receptor agonist on cisplatin-induced acute renal failure. In addition, we have compared the preventive effects of zelandopam and the free radical scavenger CV-3611 (Kato et al., 1988; Kuriyama et al.,

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2001; Kuzuya et al., 1989) on renal functional and structural alterations in rats with cisplatin-induced acute renal failure.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (4 weeks old) were obtained from Japan SLC (Shizuoka, Japan). All animals were housed in communal cages and maintained on a 12-h light/dark cycle with food and water available ad libitum. All experimental procedures involving animals conformed to the regulations of the Animal Ethical Committee of Yamanouchi Pharmaceutical and "The Guide for the Care and Use of Laboratory Animals" (U.S. Department of Health and Human Services, 1985 NIH Publication No. 85-23).

2.2. Experimental design

Sixty rats (5 weeks old) weighing 196–212 g were employed and divided in six groups: group 1 ($n=10$), cisplatin-untreated rats; group 2 ($n=10$), cisplatin-injected rats; group 3 ($n=9$), cisplatin-injected rats treatment with zelandopam at a low dose; group 4 ($n=10$), cisplatin-injected rats treatment with zelandopam at a middle dose; group 5 ($n=11$), cisplatin-injected rats treatment with zelandopam at a high dose; and group 6 ($n=10$), cisplatin-injected rats treatment with CV-3611.

Fifty rats received intraperitoneal injection of cisplatin at a dose of 5 mg/kg. In the preliminary study, one dose of zelandopam was not fully effective in this model, so we performed the administration of two doses of the selective dopamine D1 receptor agonist. Zelandopam was orally administered at doses of 30, 100, 300 mg/kg twice 75 and 15 min before the injection of cisplatin. CV-3611, a free radical scavenger, was orally administered at a dose of 10 mg/kg 75 min before the injection of cisplatin. Ten rats served as the control (group 1). Four days after the injection of cisplatin, animals were anesthetized with ether, and blood was taken from the abdominal aorta for measurement of plasma creatinine and blood urea nitrogen. The plasma was separated by centrifugation. Plasma creatinine and blood urea nitrogen were measured with an automatic analyzer (Model 7250, HITACHI, Tokyo, Japan). Body weight was determined before and 4 days after the injection of cisplatin.

2.3. Histological studies

After blood sampling, the kidneys were removed for histological examination. The kidneys were fixed in 10% buffered formaldehyde, dehydrated in graded alcohols, and then embedded in paraffin. The kidney tissue block was cut at 3–4 μm and stained with hematoxylin and eosin. The kidneys were examined by light microscopy using coded slides by investigators who were blinded to treatment group. Tubular damage was evaluated by tubular epithelial cell alterations and intratubular casts. Score was graded on a scale from 0 to 3 as follows: scale 0, normal; scale 1, slight; scale 2, moderate; and scale 3, severe.

2.4. Statistical analysis

Data are expressed as mean ± S.E.M. of 9–11 experiments. Data were analyzed by one-way analysis of variance followed by Tukey's multiple comparison test. Histological data were analyzed by nonparametric testing (Steel–Dwass test). $P<0.05$ was considered statistically significant.

2.5. Drugs

Zelandopam, (−)-(*S*)-4-(3,4-dihydroxyphenyl)-7,8-dihydroxy-1,2,3,4-tetrahydroisoquinoline hydrochloride hydrate, and CV-3611, 2-*O*-octadecylascorbic acid, were synthesized by Yamanouchi Pharmaceutical (Tokyo, Japan). Cisplatin was purchased from Bristol Pharmaceuticals (Tokyo, Japan). Zelamdopam was dissolved in distilled water and administered in a volume of 5 ml/kg by gastric gavage. CV-3611 was suspended in 5% gum arabic in water and administered in a volume of 5 ml/kg by gastric gavage.

3. Results

There were no differences in initial body weight among the six experimental groups (group 1, 204 ± 1 g; group 2, 203 ± 1 g; group 3, 203 ± 1 g; group 4, 204 ± 2 g; group 5, 205 ± 1 g; group 6, 205 ± 1 g). The injection of cisplatin induced significant increases in plasma creatinine and blood urea nitrogen and a decrease in body weight by 4 days (Figs. 1 and 2). Histological examination revealed tubular damage as assessed by tubular epithelial cell alterations and intratubular cast formation by 4 days (Table 1).

Treatment with zelandopam dose-dependently prevented cisplatin-induced elevations in plasma creatinine and blood urea nitrogen (Fig. 1). Treatment with CV-3611 also significantly prevented cisplatin-induced elevations in plasma creatinine and blood urea nitrogen. Treatment with the selective dopamine D1 receptor agonist dose-dependently attenuated cisplatin-induced reductions in body weight (Fig. 2). Treatment with the free radical scavenger also significantly attenuated cisplatin-induced reductions in body weight. Values for body weight in the zelandopam (300 mg/kg)-treated group were not significantly different from those observed in the group of cisplatin-untreated animals (Fig. 2).

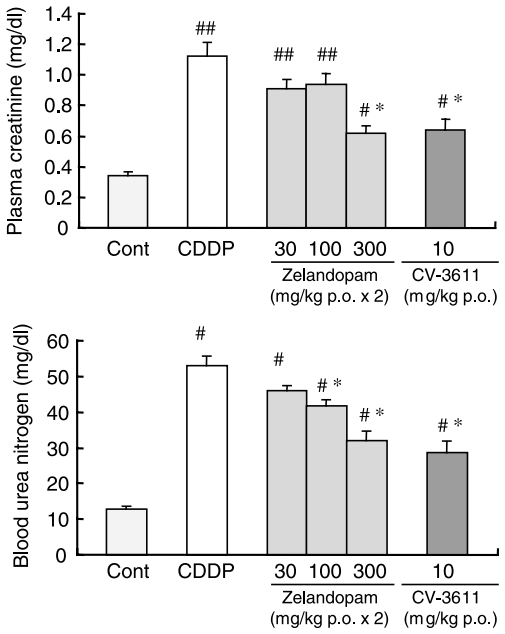

Fig. 1. Effects of zelamdopam and CV-3611 on cisplatin-induced changes in plasma creatinine (upper panel) and blood urea nitrogen (lower panel) in rats. Values are mean ± S.E.M. of 9–11 animals per group. $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs. Cont; $*P<0.01$ vs. CDDP. Cont, cisplatin-untreated rats; CDDP, cisplatin (5 mg/kg i.p.)-injected rats.

Score of tubular epithelial cell alterations and intratubular casts was significantly elevated in cisplatin-injected compared with cisplatin-untreated rats. The selective dopamine D1 receptor agonist dose-dependently attenuated tubular damage (Table 1). Score of intratubular casts in cisplatin-injected rats with zelandopam at a high dose significantly lowered as compared with that in cisplatin-injected rats. Zelandopam tended to inhibit the increase in score of tubular epithelial cell alterations; however, this effect was not statistically significant. Score of tubular epithelial cell alterations and intratubular casts in cisplatin-injected rats with CV-3611 were not statistically different from that observed in cisplatin-injected rats.

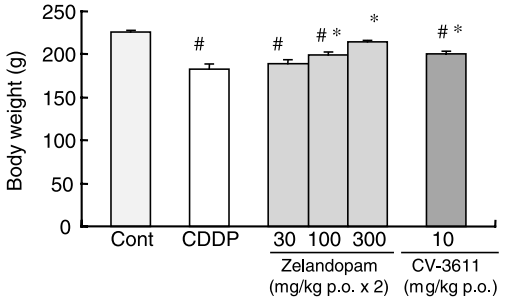

Fig. 2. Effects of zelamdopam and CV-3611 on cisplatin-induced changes in body weight in rats. Values are mean ± S.E.M. of 9–11 animals per group. $^{\#}P<0.01$ vs. Cont; $*P<0.01$ vs. CDDP. Cont, cisplatin-untreated rats; CDDP, cisplatin (5 mg/kg i.p.)-injected rats.

Table 1
Histological score at 4 days after the injection of cisplatin

	Tubular epithelial cell alterations	Intratubular casts
Cont	0 ± 0	0 ± 0
CDDP	2.50 ± 0.32[a]	1.68 ± 0.28[a]
ZD-L	2.94 ± 0.08[a]	1.39 ± 0.20[a]
ZD-M	2.85 ± 0.12[a]	1.30 ± 0.21[a]
ZD-H	2.25 ± 0.34[a]	0.67 ± 0.19[a,b,c]
CV	2.85 ± 0.12[a]	1.60 ± 0.22[a]

Values are mean ± S.E.M.
Cont, cisplatin-untreated rats; CDDP, cisplatin-injected rats; ZD-L, cisplatin-injected rats treated with zelandopam at a low dose (30 mg/kg p.o. twice); ZD-M, cisplatin-injected rats treated with zelandopam at a middle dose (100 mg/kg p.o. twice); ZD-H, cisplatin-injected rats treated with zelandopam at a high dose (300 mg/kg p.o. twice); CV, cisplatin-injected rats treated CV-3611 (10 mg/kg p.o. once).
[a] $P<0.05$ vs. Cont.
[b] $P<0.05$ vs. CDDP.
[c] $P<0.05$ vs. CV.

4. Discussion

This is the first study to assess the role of peripheral dopamine D1 receptors on renal functional and structural alterations by cisplatin in rats. Single injection of cisplatin in rats resulted in deterioration of renal function as assessed by plasma creatinine and blood urea nitrogen and tubular damage as assessed by score of tubular epithelial cell alterations and intratubular casts. These results are consistent with previous studies that cisplatin induced acute renal failure in laboratory animals (Alfieri and Cubeddu, 2000; Miyaji et al., 2001) and human beings (Madias and Harrington, 1978; Weiner and Jacobs, 1983). The pathogenesis of acute renal failure by cisplatin is considered to be reduced renal blood flow and/or proximal tubular damage. Zelandopam has been shown to produce a dose-dependent increase in renal blood flow via dopamine D1 receptors (Yatsu et al., 1997a,b,c). In the kidney, dopamine D1 receptors have been identified not only in the renal vasculature (Yasunari et al., 2000) but also in the renal proximal tubules (Uh et al., 1998; Vachvanichsanong et al., 1995). This study indicated that the renal dopamine D1 receptor agonist provided significant protection against cisplatin-induced acute renal failure in rats. Based on these findings, it is suggested that dopamine D1 receptors play an important role in the prevention of cisplatin-induced acute renal failure in rats. Recently, it has been reported that another D1 receptor agonist, fenoldopam, prevents acute renal failure induced by contrast dye in animal models (Chu and Cheng, 2001) and in man (Tumlin et al., 2002). These findings also provide some support that dopamine D1 receptors may have a role in the management of drug-induced nephrotoxicity.

Body weight changes are generally an important factor in toxicological studies. In the present study, a reduction of body weight was observed in cisplatin-injected rats. Zelandpoman produced a dose-dependent attenuation of decrease in body weight. The radical scavenger CV-3611 also showed significant blunting of that. This result is in agreement with reports that the free radical scavengers resulted in significant protection against the cisplatin-induced reduction of body weight (Matsushima et al., 1998; Rao et al., 1999; Sugihara and Gemba, 1986).

In this study, we did not determine renal blood flow in rats with cisplatin-induced acute renal failure. However, others have demonstrated that cisplatin induced a significant reduction of renal blood flow in rats (Winston and Safirstein, 1985) and lecithinized superoxide dismutase, a superoxide anion scavenger, prevented cisplatin-induced acute renal failure through preservation of renal blood flow (Matsushima et al., 1998). In addition, zelandopam has been shown to produce a dose-dependent increase in renal blood flow via dopamine D1 receptors (Yatsu et al., 1997a, b,c). Furthermore, zelandopam and CV-3611 showed beneficial effects on renal function as assessed by plasma creatinine and blood urea nitrogen in this study. Thus, it is conceivable that beneficial effect of zelandopam against cisplatin-induced acute renal failure may be mainly attributable to its renal vasodilatory action mediated by stimulated dopamine D1 receptors. Clarification of this possibility must await further investigation.

Zelandopam attenuated cisplatin-induced tubular damage as assessed by score of tubular epithelial cell alterations and intratubular casts. On the other hand, CV-3611 did not affect tubular damage. This result is consistent with a previous report that lecithinized superoxide dismutase, a superoxide anion scavenger, prevented cisplatin-induced acute renal failure through preservation of renal blood flow without modification of tubular damage (Matsushima et al., 1998). However, higher doses (e.g. 30 or 100 mg/kg p.o.) of CV-3611 may prevent tubular damage. Further study is required to clarify the effect of CV-3611 on cisplatin-induced tubular damage. In the kidney, dopamine D1 receptors have been identified not only in the renal vasculature (Yasunari et al., 2000) but also in the renal proximal tubules (Uh et al., 1998; Vachvanichsanong et al., 1995). Therefore, there is a possibility that favorable effect of zelandopam against cisplatin-induced tubular damage may be caused by its direct tubular action. However, additional study would be necessary to determine the effect of zelandopam in an in vitro model of cisplatin-induced cytotoxicity in renal tubular epithelial cells.

In conclusion, cisplatin produced renal dysfunction and tubular damage after 4 days, evidenced by elevations in plasma creatinine and blood urea nitrogen, and by significant tubular epithelial cell alterations and intratubular casts. Zelandopam, a selective dopamine D1 receptor agonist, provided significant protection against cisplatin-induced acute renal failure. Our findings suggest that renal dopamine D1 receptors play an important role in the prevention of cisplatin-induced acute renal failure in rats.

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References

Alfieri, A.B., Cubeddu, L.X., 2000. Role of NK_1 receptors on cisplatin-induced nephrotoxicity in the rat. Naunyn-Schmiedeberg's Arch. Pharmacol. 361, 334–338.

Baliga, R., Zhang, Z., Baliga, M., Ueda, N., Shah, S.V., 1998. In vitro and in vivo evidence suggesting a role for iron in cisplatin-induced nephrotoxicity. Kidney Int. 53, 394–401.

Chopra, S., Kaufman, J.S., Jones, T.W., Hong, W.K., Gehr, M.K., Hamburger, R.J., Flamenbaum, W., Trump, B.F., 1982. *Cis*-diamminedichlorplatinum-induced acute renal failure in the rat. Kidney Int. 21, 54–64.

Chu, V.L., Cheng, J.W., 2001. Fenoldopam in the prevention of contrast media-induced acute renal failure. Ann. Pharmacother. 35, 1278–1282.

Dobyan, D.C., Levi, J., Jacobs, C., Kosek, J., Weiner, M.W., 1980. Mechanism of *cis*-platinum nephrotoxicity: II. Morphologic observations. J. Pharmacol. Exp. Ther. 213, 551–556.

Dobyan, D.C., Bull, J.M., Strebel, F.R., Sunderland, B.A., Bulger, R.E., 1986. Protective effects of *O*-(beta-hydroxyethyl)-rutoside on *cis*-platinum-induced acute renal failure in the rat. Lab. Invest. 55, 557–563.

Elferink, F., van der Vijgh, W.J., Klein, I., Pinedo, H.M., 1986. Interaction of cisplatin and carboplatin with sodium thiosulfate: reaction rates and protein binding. Clin. Chem. 32, 641–645.

Heidemann, H.T., Gerkens, J.F., Jackson, E.K., Branch, R.A., 1985. Attenuation of cisplatinum-induced nephrotoxicity in the rat by high salt diet, furosemide and acetazolamide. Naunyn-Schmiedeberg's Arch. Pharmacol. 329, 201–205.

Jones, T.W., Chopra, S., Kaufman, J.S., Flamenbaum, W., Trump, B.F., 1985. *Cis*-diamminedichloroplatinum (II)-induced acute renal failure in the rat. Correlation of structural and functional alterations. Lab. Invest. 52, 363–374.

Kato, K., Terao, S., Shimamoto, N., Hirata, M., 1988. Studies on scavengers of active oxygen species: 1. Synthesis and biological activity of 2-*O*-alkylascorbic acids. J. Med. Chem. 31, 793–798.

Kuriyama, H., Waki, M., Nakagawa, M., Tsuda, M., 2001. Involvement of oxygen free radicals in experimental retinal ischemia and the selective vulnerability of retinal damage. Ophthalmic Res. 33, 196–202.

Kuzuya, T., Hoshida, S., Nishida, M., Kim, Y., Fuji, H., Kitabatake, A., Kamada, T., Tada, M., 1989. Role of free radicals and neutrophils in canine myocardial reperfusion injury: myocardial salvage by a novel free radical scavenger, 2-octadecylascorbic acid. Cardiovasc. Res. 23, 323–330.

Madias, N.E., Harrington, J.T., 1978. Platinum nephrotoxicity. Am. J. Med. 65, 307–314.

Matsushima, H., Yonemura, K., Ohishi, K., Hishida, A., 1998. The role of oxygen free radicals in cisplatin-induced acute renal failure in rats. J. Lab. Clin. Med. 131, 518–526.

Miyaji, T., Kato, A., Yasuda, H., Fujigaki, Y., Hishida, A., 2001. Role of the increase in p21 in cisplatin-induced acute renal failure in rats. J. Am. Soc. Nephrol. 12, 900–908.

Rao, M., Kumar, M.M., Rao, M.A., 1999. In vitro and in vivo effects of phenolic antioxidants against cisplatin-induced nephrotoxicity. J. Biochem. 125, 383–390.

Saad, S.Y., Najjar, T.A., Al-Sohaibani, M.O., 2000. The effect of rebamipide on cisplatin-induced nephrotoxicity in rats. Pharmacol. Res. 42, 81–86.

Sugihara, K., Gemba, M., 1986. Modification of cisplatin toxicity by antioxidants. Jpn. J. Pharmacol. 40, 353–355.

Sugihara, K., Nakano, S., Koda, M., Tanaka, K., Fukuishi, N., Gemba, M., 1987. Stimulatory effect of cisplatin on production of lipid peroxidation in renal tissues. Jpn. J. Pharmacol. 43, 247–252.

Tumlin, J.A., Wang, A., Murray, P.T., Mathur, V.S., 2002. Fenoldopam mesylate blocks reductions in renal plasma flow after radiocontrast dye infusion: a pilot trial in the prevention of contrast nephropathy. Am. Heart J. 143, 894–903.

Uh, M., White, B.H., Sidhu, A., 1998. Alteration of association of agonist-activated renal $D1_A$ dopamine receptors with G proteins in proximal tubules of the spontaneously hypertensive rat. J. Hypertens. 16, 1307–1313.

Vachvanichsanong, P., Kimura, K., Sidhu, A., 1995. Differences in photoaffinity labeling of DA_1 receptors in renal proximal tubules from normotensive rat and SHR. Am. J. Physiol. 268, F1009–F1016.

Weiner, M.W., Jacobs, C., 1983. Mechanism of cisplatin nephrotoxicity. Fed. Proc. 42, 2974–2978.

Winston, J.A., Safirstein, R., 1985. Reduced renal blood flow in early cisplatin-induced acute renal failure in the rat. Am. J. Physiol. 249, F490–F496.

Yasunari, K., Kohno, M., Kano, H., Minami, M., Yoshikawa, J., 2000. Dopamine as a novel antioxidative agent for rat vascular smooth muscle cells through dopamine D_1-like receptors. Circulation 101, 2038–2302.

Yatsu, T., Arai, Y., Takizawa, K., Kasai-Nakagawa, C., Takanashi, M., Uchida, W., Inagaki, O., Tanaka, A., Asano, M., Honda, K., Takenaka, T., 1997a. Renal effect of YM435, a new dopamine D_1 receptor agonist, in anesthetized dogs. Eur. J. Pharmacol. 322, 45–53.

Yatsu, T., Takizawa, K., Kasai-Nakagawa, C., Uchida, W., Tanaka, A., Asano, M., Honda, K., Takenaka, T., 1997b. Hemodynamic characterization of YM435, a novel dopamine DA_1 receptor agonist, in anesthetized dogs. J. Cardiovasc. Pharmacol. 29, 382–388.

Yatsu, T., Uchida, W., Inagaki, O., Tanaka, A., Takenaka, T., 1997c. Dopamine DA_1 receptor agonist activity of YM435 in the canine renal vasculature. Gen. Pharmacol. 29, 229–232.

Yatsu, T., Arai, Y., Takizawa, K., Kasai-Nakagawa, C., Takanashi, M., Uchida, W., Inagaki, O., Tanaka, A., Takenaka, T., 1998. Effect of YM435, a dopamine DA_1 receptor agonist, in a canine model of ischemic acute renal failure. Gen. Pharmacol. 31, 803–807.