Regular Article

Telmisartan Exacerbates Cisplatin-Induced Nephrotoxicity
in a Mouse Model

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Cisplatin (CDDP; cis-diamine dichloroplatinum)-induced nephrotoxicity is the main reason for dose limitations, which can reduce the efficacy of cancer treatment. Lower blood pressure and administration of renin angiotensin system (RAS) inhibitors have been reported as factors that exacerbate CDDP-induced nephrotoxicity; however, the detailed mechanisms remain unknown and the results of previous studies are conflicting. In this study, we examined the influence of various hypotensive drugs, including RAS inhibitors and calcium channel blockers, on CDDP-induced nephrotoxicity in BALB/c mice. The mice were divided into nine groups: (1) CDDP group (15 mg/kg CDDP), (2) AML group (5 mg/kg amlodipine), (3) ENA group (2.5 mg/kg enalapril), (4) telmisartan (TEL) group (10 mg/kg telmisartan), (5) LOS group (10 mg/kg losartan), (6) CDDP + AML group, (7) CDDP + ENA group, (8) CDDP + TEL group, and (9) CDDP + LOS group. Nephrotoxicity was evaluated by measuring serum creatinine (CRE) and blood urea nitrogen (BUN) levels. In addition, the kidney sections were stained with Masson's trichrome and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) to assess the renal fibrosis area and apoptotic area. Serum CRE and BUN levels were increased in the CDDP + ENA, CDDP + LOS, and CDDP + TEL groups compared to those in the CDDP alone group, and the CDDP + AML group showed a significant increase in the renal fibrosis area. These results suggest that exacerbation of CDDP-induced nephrotoxicity is not correlated with systolic blood pressure but is associated with administration of RAS inhibitors, particularly TEL.

Key words cisplatin; nephrotoxicity; telmisartan; renin angiotensin system inhibitor; fibrosis

INTRODUCTION

A cisplatin (CDDP; cis-diamine dichloroplatinum)-containing chemotherapy regimen is commonly used in the treatment of various cancers. However, adverse effects occur frequently, primarily exemplified by nausea, ototoxicity, myelosuppression, and nephrotoxicity, with the latter as the main dose-limiting adverse effect. For example, Jongh et al.1 reported an increase in the serum creatinine (CRE) level in 41% of 400 patients treated with high-dose CDDP (70–85 mg/m²), and Sheron et al.2 reported that 245 of 777 patients treated with CDDP developed acute kidney injury (AKI). Hydration, diuretic administration, and reduction in the CDDP dose are all common methods of preventing AKI and CDDP-induced nephrotoxicity; however, the preventive effect of these approaches is limited, requiring alternative options and a better understanding of the underlying mechanisms.

The main pathophysiological mechanisms contributing to CDDP-induced nephrotoxicity are considered as direct tubular epithelial cell toxicity and reduced renal blood flow as a consequence of endothelial dysfunction and vasoconstriction.3 Komaki et al.4 reported that lower blood pressure and concomitant administration of renin-angiotensin system (RAS) inhibitors were also associated with CDDP-induced nephrotoxicity in patients treated with CDDP-containing chemotherapy. In contrast, Saleh et al.5 reported that another RAS inhibitor, losartan, had protective effects on CDDP-induced nephrotoxicity in rats.

To help resolve this controversy and further elucidate the molecular mechanism of CDDP-induced nephrotoxicity, we investigated the influence of various antihypertensive drugs, including enalapril (ENA), an angiotensin-converting enzyme inhibitor, telmisartan (TEL) and losartan (LOS), angiotensin receptor blockers, and the calcium channel blocker amlodipine (AML), on CDDP-induced nephrotoxicity in mice.

MATERIALS AND METHODS

Animals and Grouping Six-week-old male BALB/c mice were purchased from Japan SLC (Shizuoka, Japan), and were bred and maintained under conventional conditions (23 ± 1°C and 47–67% humidity, 12:12-h light:dark cycle) with ad libitum access to food (RFC-1; Oriental Bio Co., Kyoto, Japan) and water. The study protocol was approved by the Institutional Animal Care Committee of Setsunan University (Nos. K17-15, K18-15, and K19-15), and all efforts were made to minimize animal suffering and the number of animals used in all experiments. The mice were divided into ten groups with five mice per group, except for the AML group, which contained six mice, and control group, which contained seven mice: (1) control group, no treatment; (2) CDDP group [15 mg/kg CDDP ∆7] (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) was provided as intraperitoneal (i.p.) injection on day 0; (3) CDDP + AML group [15 mg/kg i.p.
CDDP on day 0 and 5 mg/kg amlodipine besilate\(^8\) (Norvasc\(^\circledast\), Pfizer Japan, Inc., Tokyo, Japan) orally from days −3 to 4; (4) CDDP + ENA group (15 mg/kg CDDP i.p. on day 0 and 2.5 mg/kg enalapril maleate\(^9\) (Renivace\(^\circledast\), MSD K.K. a subsidiary of Merck & Co., Inc., Kenilworth, NJ, U.S.A.) orally from days −3 to 4; (5) CDDP + TEL group (15 mg/kg CDDP i.p. on day 0 and 10 mg/kg telmisartan\(^10,11\) (Micardis\(^\circledast\), Nippon Boehringer Ingelheim Co., Ltd., Tokyo, Japan) orally from days −3 to 4; (6) CDDP + LOS group (15 mg/kg CDDP i.p. on day 0 and 10 mg/kg losartan\(^11,12\) (Nu-lotan\(^\circledast\), MSD K.K.) orally from days −3 to 4); (7) AML group (5 mg/kg amlodipine besilate orally from days −3 to 4); (8) ENA group (2.5 mg/kg enalapril maleate orally from days −3 to 4); (9) TEL group (10 mg/kg telmisartan orally from days −3 to 4); and (10) LOS group (10 mg/kg losartan orally from days −3 to 4). Treatment was continued up to day 4. The antihypertensive drugs were administered before CDDP to decrease the blood pressure to levels similar to those observed in the clinical situation. The dose of antihypertensive drugs was determined based on the previous reports that each drug reduced blood pressure in mice.\(^8\)\textendash\(^12\)

In these reports, blood pressure was significantly reduced in antihypertensive drug-treated mice compared with that in untreated mice. The dose of CDDP (15 mg/kg) used in this study was higher than that used in a previous study on rats.\(^3\) However, although Peima et al.\(^5\) reported that administration of 15 mg/kg CDDP to mice resulted in a significant increase in CRE and blood urea nitrogen (BUN) levels at day 3 of administration compared with levels in the untreated group, Sasaki et al.\(^7\) reported that no death occurred in mice within 5 d after administering this dose of CDDP. Based on the above, we chose the 15 mg/kg dose of CDDP for this study.

Systolic blood pressure (sBP) was examined on days −3, 0, and 5 with a Blood Pressure Monitor for Mouse and Rats (MK-2000, Muromachi Kikai Co., Ltd., Tokyo, Japan). The kidney was then excised from each mouse under medetomidine (0.75 mg/kg), midazolam (4.0 mg/kg), and butorphanol (5.0 mg/kg) anesthesia after the observation period.

**Measurement of Serum CRE and BUN Levels**

On days −3, 0, 3, and 5 relative to CDDP administration, approximately 60 µL of peripheral blood samples were collected, and the serum CRE and BUN levels were measured with the SPOTCHEM system (Arkray, Kyoto, Japan).

**Assessment of Fibrosis Area**

The kidney was fixed with 4% paraformaldehyde in phosphate buffer solution (FUJIFILM Wako Pure Chemical Corporation), embedded in paraffin according to conventional methods, and cut into 3-μm sections using a microtome (ROM-380, Yamato Kohki Industrial Co., Ltd., Saitama, Japan). The kidney sections were stained with Masson's trichrome as follows. The sections were deparaffinized using xylene and incubated with the first mordant (Muto Pure Chemicals Co., Ltd., Tokyo, Japan) for 5 min. After submerging the sample into 1% acetic acid solution, the sections were incubated with 2.5% phosphotungstic acid solution (Muto Pure Chemicals Co., Ltd.) for 15 min, submersed in 1% acetic acid solution, and incubated with aniline blue solution (Muto Pure Chemicals Co., Ltd.) for 30 min. The kidney sections were dehydrated in ethanol, mounted with Canada balsam (FUJIFILM Wako Pure Chemical Corporation), and examined under a microscope (OLYMPUS BX50, Tokyo, Japan). The renal fibrosis areas were extracted with following method. The glomerulus areas were removed from each section, and renal fibrosis areas that were stained by the aniline blue dye were extracted using ImageJ (ver.1.41) software (NIH, Bethesda, MD, U.S.A.). The ratio of renal fibrosis areas to renal interstitial areas was calculated as the renal fibrosis area. Three fields of view per mouse were examined, and the average values were used for statistical analysis.

**Assessment of Apoptotic Area**

Kidney sections were prepared as described above to assess the fibrosis area and then stained with terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) using an ApopTag\(^\circledast\) Red in Situ Apoptosis Detection Kit (Merck Millipore, Billerica, MA, U.S.A.). The sections were deparaffinized in xylene and washed in phosphate-buffered saline (PBS). The tissue sections were incubated with 20 µg/mL of Proteinase K (Boehringer, Mannheim, Germany) for 15 min at room temperature (approx. 25°C), washed with PBS, and then incubated with equilibration buffer for approximately 10 s at room temperature. The sections were incubated with terminal deoxynucleotidyl transferase enzyme (Merck Millipore) for 60 min at 37°C. After incubation with Stop/Wash Buffer for 10 min at room temperature, the sections were washed with PBS and incubated with anti-digoxigenin conjugate for 30 min at room temperature in the dark. After washing with PBS, the nuclei were counterstained with Hoechst 33342 (Lonz, Basel, Switzerland). The sections were mounted with glyceral (FUJIFILM Wako Pure Chemical Corporation) and examined under a fluorescence microscope (KEYENCE BZ-X800, Osaka, Japan). The area of renal cortex containing TUNEL-positive cells was calculated using BZ-X800 Analyzer software (KEYENCE).

**Statistical Analysis**

Data are expressed as the mean ± standard deviation (S.D.). The significance of the differences in CRE and BUN levels among groups was evaluated using the Steel-Dwass test; \(p < 0.05\) was considered as statistically significant. The significance of the correlation between CRE or BUN and sBP was evaluated using Spearman’s rank correlation coefficient. Correlation coefficients were classified as follows: 0.4–0.7, correlation; 0.7–0.9, strong correlation; 0.9–1.0, absolute correlation. Nominal variables were compared using Fisher’s exact tests. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).

**RESULTS**

**Influence of Concomitant Administration CDDP and Antihypertensive Drugs on Serum CRE and BUN Levels**

To confirm the effects of the combination of CDDP and antihypertensive drugs on renal function, serum CRE and BUN levels were measured. The serum CRE level was not increased in the AML, CDDP + AML, LOS, or CDDP + LOS
groups during the treatment period. Although there were no significant differences in each group (Steel–Dwass test), the following trends were observed. In the CDDP + ENA and CDDP + TEL groups, the serum CRE level on day 5 tended to be increased as compared with that measured on day 0 or 3 (Fig. 1A). The serum BUN level was higher in all combination groups compared with that of the respective antihypertensive drug-alone groups on days 3 and 5, with a particularly strong effect noted in the CDDP + TEL group (Fig. 1B).

**Correlation between sBP and Biochemical Parameters**

To confirm that reduced blood pressure is associated with renal dysfunction induced by the combination of CDDP and antihypertensive drugs, we first calculated the difference in the serum CRE and BUN as well as sBP between day -3 and day 5 as ΔCRE, ΔBUN, and ΔsBP, respectively, and then examined the correlation between ΔsBP and Δserum CRE (Figs. 2A, C) and ΔBUN (Figs. 2B, D). No significant correlation was observed in the antihypertensive drug alone groups (Figs. 2A, B), and in the combination groups (serum CRE: $r = 0.11$, $p = 0.60$, Fig. 2C; serum BUN: $r = -0.31$, $p = 0.15$, Fig. 2D).

**Impact of Concomitant Administration of CDDP and Antihypertensive Drugs on Fibrosis Area**

Masson’s trichrome staining demonstrated an increase in the renal fibrosis area in the CDDP, CDDP + ENA, and CDDP + TEL groups as compared with the control or antihypertensive drug alone groups (Fig. 3A). Calculation of the ratio of the renal fibrosis area to the total renal interstitial area on the images showed an increased ratio in the CDDP group as compared with the control group and antihypertensive drug alone groups, and there was no difference in the ratio between the latter two groups.
groups. Moreover, this ratio was slightly increased in the CDDP + AML, CDDP + ENA, and CDDP + LOS groups and was significantly increased in the CDDP + TEL group compared with the CDDP group (Fig. 3B).

**Impact of Concomitant Administration CDDP and Antihypertensive Drugs on Apoptosis** TUNEL staining showed an increase in the renal apoptotic area in all groups administered CDDP as compared with the control group and antihypertensive drug-alone groups (Figs. 4A, B).

**Impact of Concomitant Administration CDDP and Antihypertensive Drugs on the Prevalence of Renal Dysfunction** The prevalence of renal dysfunction was also compared between the combination groups and CDDP alone group by assessing the 95% confidence intervals (CIs) of the serum CRE levels (0.43–1.13), BUN levels (33.4–161), fibrosis area (5.37–7.15), and ratio of the apoptotic area (0.14–7.06) in the CDDP alone group. In the CDDP + ENA and CDDP + LOS groups, three of five mice and one of four mice showed a serum CRE level higher than the 95% CI, demonstrating a renal dysfunction prevalence of 60 and 25%, respectively. In addition, two and one mice showed a BUN level higher than the 95% CI for a prevalence of 40 and 25% in the CDDP + ENA and CDDP + LOS groups, respectively. In the CDDP + TEL group, the prevalence of renal dysfunction was 75% based on both the serum CRE level and BUN level ($p<0.05$, Table 1). With respect to the fibrosis area, values higher than the 95% CI of the CDDP group were observed in two of the five animals in the CDDP + LOS group, but in four of the five animals (80%) in the CDDP + AML and CDDP + ENA groups. The prevalence was 100% in the CDDP + TEL group (Table 1). Similarly, two of the five animals in the CDDP + AML, CDDP + ENA, and CDDP + LOS groups showed a higher apoptotic ratio than the 95% CI of the CDDP group, with a prevalence of 20%. However, the prevalence of renal dysfunction was two of the five mice (40%) in the CDDP + TEL group according to apoptosis induction (Table 1).

**DISCUSSION**

The factors contributing to CDDP-induced nephrotoxicity are unclear. Here, we showed that antihypertensive drugs,
Fig. 3. (A) Renal Fibrosis Area at Day 5 Assessed by Masson’s Trichrome Staining; (B) The Ratio of Renal Fibrosis Area to Renal Interstitial Area for Each Group

(A) Magnification, 400×. (B) *p < 0.05 vs. CDDP group.

Fig. 4. (A) Renal Apoptotic Area Assessed by TUNEL Staining at Day 5; (B) Ratio of Renal Apoptotic Area to Renal Interstitial Area for Each Group
particularly RAS inhibitors, can exacerbate CDDP-induced nephrotoxicity in mice, which agrees with a previous study.\textsuperscript{4} The levels of serum CRE and BUN and prevalence of renal dysfunction tended to increase with concomitant administration of two drugs, specifically in the CDDP + TEL group. Therefore, our results suggest that telmisartan is a higher risk factor for exacerbation of CDDP-induced nephrotoxicity. Al-Husseiny et al.\textsuperscript{14} reported that RAS inhibitors reduce renal perfusion by dialysis of efferent arterioles and cause normotensive ischemic AKI,\textsuperscript{15} suggesting that the decrease of renal perfusion by TEL had some effect on CDDP-induced tubular atrophy, and that the CDDP + TEL group showed a significant increase in renal fibrosis area. ENA was assumed to exacerbate nephrotoxicity by a similar mechanism, but there was no significant difference in its effect compared with that of TEL. Further studies are needed to confirm the difference between TEL and ENA. On the other hand, unlike the RAS inhibitor combination group, nephrotoxicity was not observed in the CDDP + AML group. Hayashi et al.\textsuperscript{16} reported that L-type Ca channel blockers mainly dilate imported arterioles, and this mechanism seems to influence the difference in renal function deterioration.

Although a previous study found an association between lower blood pressure and CDDP-induced nephrotoxicity,\textsuperscript{4} we found no significant correlation between sBP and CRE or BUN serum levels. Therefore, factors other than blood pressure, such as those mentioned above, are likely responsible for exacerbating CDDP-induced nephrotoxicity. In this study, we thought that sBP in the CDDP alone group and CDDP plus antihypertensive drugs was lower than that in the antihypertensive drug alone groups because CDDP-treated mice had reduced activity levels, food intake and body weight.

Cisplatin induces apoptosis of renal cells as one of the mechanisms of CDDP-induced nephrotoxicity.\textsuperscript{17} In fact, Terada et al.\textsuperscript{18} reported that mice lacking the gene for apoptosis signal-regulating kinase 1, as an apoptosis-promoting gene, showed significantly lower levels of serum CRE and BUN compared to wild-type mice in an ischemia-induced AKI model. In addition, Kuwana et al.\textsuperscript{19} reported that mice lacking the gene for phosphoinositide-3 kinase gamma, as an apoptosis-suppressing gene, showed significantly higher levels of CRE and BUN compared to wild-type mice in a CDDP-induced nephrotoxicity model. In our study, the renal apoptotic area showed a tendency to increase with CDDP treatment alone and in combination with the antihypertensive drugs. Furthermore, this area tended to be increased in the combination groups compared with that of the CDDP alone group. As mentioned above, in our study, the combination of CDDP and RAS inhibitor tended to increase BUN and CRE. Collectively, these findings suggest that using a combined regimen of CDDP with an antihypertensive drug such as a RAS inhibitor can enhance the apoptotic effect to normal renal cells. However, further experiments are required to validate this hypothesis.

CONCLUSION

In conclusion, our study showed that using a combination of antihypertensive drugs could exacerbate CDDP-induced nephrotoxicity in cancer treatment. In addition, the mechanism is independent of the change in sBP. Notably, telmisartan showed a higher risk of exacerbating CDDP-induced nephrotoxicity. We suggest that administration of antihypertensive drugs before CDDP-based chemotherapy should be avoided to prevent exacerbation of CDDP-induced nephrotoxicity, which
would result in dose limitation or treatment discontinuation.

**Conflict of Interest** The authors declare no conflict of interest.

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