Efficacy of diphenhydramine as a preventive medicine against cisplatin-induced nephrotoxicity

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Abstract

Cisplatin is widely used as an anti-tumor drug for the treatment of solid tumors. Unfortunately, it causes nephrotoxicity as a critical side effect, limiting its use, given that no preventive drug against cisplatin-induced nephrotoxicity is currently available. This study identified that a previously developed drug, diphenhydramine, may provide a novel treatment for cisplatin-induced nephrotoxicity based on the results of the analysis of medical big data. We evaluated the actual efficacy of diphenhydramine via *in vitro* and *in vivo* experiments in a mouse model. Diphenhydramine inhibited cisplatin-induced cell death in renal proximal tubular cells. Mice administered cisplatin developed kidney injury with renal dysfunction (plasma creatinine: 0.43 ± 0.04 mg/dl vs 0.15 ± 0.01 mg/dl, $p<0.01$) and showed augmented oxidative stress, increased apoptosis, elevated inflammatory cytokines, and mitogen-activated protein kinases activation; however, most of these symptoms were suppressed by treatment with diphenhydramine. Further, the renal concentration of cisplatin was attenuated in diphenhydramine-treated mice (platinum content: 70.0 ± 3.3 μg/g dry kidney weight vs 53.4 ± 3.6 μg/g dry kidney weight, $p<0.05$). Importantly, diphenhydramine did not influence or interfere with the anti-tumor

effect of cisplatin in any of the *in vitro* or *in vivo* experiments. Moreover, a retrospective clinical study of 1467 cancer patients treated with cisplatin showed that patients who had used diphenhydramine exhibited less acute kidney injury than patients who had not used diphenhydramine (6.1 % vs 22.4 %, $p<0.05$). Thus, diphenhydramine demonstrated efficacy as a novel preventive medicine against cisplatin-induced nephrotoxicity.

Keywords: cisplatin, nephrotoxicity, diphenhydramine

Translational Statement

Cisplatin-induced nephrotoxicity remains an unresolved condition, with no preventive medicine available. We identified that an existing antihistamine, diphenhydramine, is a candidate preventive medicine for cisplatin-induced nephrotoxicity based on the results of the analysis of medical big data. In a mouse study, cisplatin-induced nephrotoxicity was suppressed by diphenhydramine pre-treatment, while diphenhydramine did not interfere with the cisplatin anti-tumor effects. A retrospective clinical study showed that patients with cancer who had used diphenhydramine before cisplatin treatment exhibited less renal dysfunction. Thus, diphenhydramine is a novel drug effective against cisplatin-

induced nephrotoxicity, contributing to improved prognosis of cancer patients undergoing cisplatin therapy.

Introduction

Cisplatin (cis-diamminedichloroplatinum; CDDP) is a major anti-tumor drug used as a chemotherapeutic agent for a wide spectrum of human malignancies worldwide. Despite its beneficial effects against various cancers, patients treated with cisplatin suffer severe side effects; nephrotoxicity is a well-known side effect reported to occur in 25% of patients undergoing cisplatin chemotherapy[1]. Although the molecular mechanism of cisplatin-induced nephrotoxicity (CIN) involves multiple factors including inflammation, apoptosis, and oxidative stress[2-4], no preventive drugs for CIN are available for clinical use. Instead, only the promotion of hydration or the administration of diuretics such as furosemide are widely used as preventive measures prior to cisplatin-treatment[5].

The Food and Drug Administration (FDA) Adverse Events Reporting System (FAERS) database is one of the largest global databases, containing millions of case reports on drug-associated adverse events; is used widely to supply real clinical data for pharmacovigilance[6-9]. Recently, the FAERS database has also been utilized to research drug repositioning against various diseases; indeed, certain candidate drugs have already been found for hypertension[10] and depression[11]. This database also allows the assessment

the clinical implications based on basic experimental studies. We confirmed that the drug effect evaluated by the FAERS database analysis is consistent with the results of our experimental study using a mouse model[12, 13]. Therefore, combined analysis using both the FAERS database and conventional experimental techniques is thought to be advantageous for the confirmation of the "novel efficacy" of existing drugs.

In the present study, we identified that the histamine H1 receptor (H1R) antagonist diphenhydramine (DPH) confers a preventive effect against CIN, based on information from the FAERS database, conventional experimental studies, and a retrospective clinical study.

Results

The effect of H1R antagonist on CIN as per the FAERS database

The FAERS database was used to evaluate the occurrence of CIN in patients concomitantly treated with the H1R antagonist. As expected, as per the FAERS database, cases undergoing cisplatin treatment were associated with the increased occurrence of nephrotoxicity [ROR 3.90 (3.75–4.06)]. Next, we performed a comprehensive analysis of 1534 drugs used in combination with cisplatin to seek candidate drugs that reduce the

occurrence of nephrotoxicity. Except for anti-cancer drugs and general infusion components, four drugs were detected as candidates for the prevention of CIN (Table 1). To further explore the drug candidates, we focused on DPH, a first-generation H1R antagonist. H1R is ubiquitously expressed in many tissues and H1R activation is involved in processes including inflammation[14], oxidative stress[15], and apoptosis[14]; therefore, the H1R antagonist has the potential to exert a preventive action against CIN. In addition to DPH, we analyzed the occurrence of nephrotoxicity in cases treated with nine additional concomitantly administered first-generation H1R antagonists. As shown in Table 2, only DPH-treated patients showed a significantly lower prevalence of CIN. Consequently, we focused on DPH as a candidate drug for the prevention of CIN in subsequent experiments.

Effects of DPH against cisplatin-induced cell death in renal proximal tubular cells

We investigated whether DPH exerted a protective effect against CIN using HK-2 and LLC-PK1 cells in an *in vitro* screening experiment. As expected, the MTS assay showed that cisplatin treatment augmented cell death, which was significantly suppressed by the treatment with DPH in both cell lines (Figure 1). Similar to our findings, a conference abstract reported that DPH treatment would protect against CIN, as per *in*

vitro data in the context of renal proximal tubule cells[16]. Upon determining that DPH prevented renal tubular cell death induced by cisplatin, we moved on to *in vivo* experiments.

Effects of DPH against cisplatin-induced kidney injury in a mouse model

We examined the preventive effect of DPH against CIN in an *in vivo* mouse model. Mice treated with cisplatin exhibited reduced body weight, while no change in kidney weight was found (Table 3). In a histological analysis, kidney injury was initially induced in mice with cisplatin administration; however, this condition was alleviated by the concomitant treatment with DPH (Figure 2A and B). The mRNA expression of KIM-1 and LCN2 (markers of renal tubular damage) and the plasma BUN and creatinine levels were upregulated in cisplatin-administered mice, except in those co-treated with DPH (Figure 2C and Table 3). The cisplatin-induced mRNA upregulation of inflammatory cytokines such as TNF-α, IL-1β, IL-6, and MCP-1 was also inhibited by the treatment with DPH (Figure 2D). Of note, the preventive effect of DPH against CIN was observed in a dose-dependent manner; however 20 md/kg DPH led to almost the same protective degree as 40 mg/kg DPH (Supplemental Figure S1). The mRNA expression of the

histamine H1 receptor was reduced by cisplatin treatment and was unchanged after DPH co-administration (Supplemental Figure S2). Dihydroethidium (DHE) staining and 4-hydroxynonenal (HNE) expression, induced by cisplatin, were also inhibited by the administration of DPH (Figure 3A and C). In terms of apoptosis, cisplatin augmented TUNEL positive cells and cleaved caspase-3 expression in the kidneys; this expression declined under DPH treatment (Figure 3B and C). Moreover, cisplatin-induced phosphorylation in the context of the JNK and ERK1/2 signaling pathways was suppressed by the DPH treatment (Figure 3D). Cisplatin also increased the phosphorylation of p38MAPK, which was not inhibited by DPH (Figure S3). These results suggest that the preventive effect of DPH in cisplatin-induced kidney injury involve the inhibition of the inflammatory response, oxidative stress, and apoptosis. Additionally, we evaluated leukocytes populations via IHC using macrophage (F4/80), neutrophil (Ly-6G/C), lymphocyte (T cell, CD3) and eosinophil (toluidine blue and c-kit) markers. Te accumulation of macrophages and lymphocytes was decreased in the kidneys of cisplatin-treated mice with or without DPH. On the other hand, the accumulation of neutrophils was increased in the kidneys of cisplatin-treated mice, and suppressed by

DPH. Mast cells were almost not detected in the kidneys of mice treated (or not) with cisplatin/DPH by c-kit IHC as per toluidine blue staining (Supplemental Figure S4). The mRNA expression levels of *F4/80, Ly-6g, CD3, c-kit* were also not elevated by cisplatin administration (Supplemental Figure S5). The preventive effect of DPH on inflammation might partly involve the prevention of cisplatin-induced neutrophils accumulation in addition to the direct inhibition of oxidative stress via platinum uptake into renal tubules. Importantly, the administration of cisplatin caused a reduction in the survival rate of mice; all mice died by day 6. On the other hand, DPH-treated mice showed a higher survival rate compared to that of vehicle treated mice 14 days after cisplatin administration (73% vs. 0 %, $P < 0.01$) (Supplemental Figure S6).

Involvement of the histamine H1 receptor in CIN

We checked the role of the H1R against CIN using H1R gene knockout KO (KO) mice. The increased BUN and plasma creatinine levels as well as renal injury (assessed by histological changes and *KIM-1* and *LCN2* mRNA expression) induced by CIN were not evident in H1RKO mice (compared to wild-type (WT) mice; Table 4 and Figure 4A, B, and C). The cisplatin-induced *IL-6* and *IL-1β* mRNA levels were also

inhibited in H1RKO mice; however, the expression of *TNF-α* and *MCP-1* was not inhibited (Figure 4D). Cisplatin-induced oxidative stress and apoptosis, as well as ERK1/2 and JNK activation, were also attenuated in H1RKO mice (versus WT mice; Figure 4E-H). Importantly, the degree of prevention against CIN was more effective in WT mice under DPH treatment than in H1RKO mice. Therefore the action of DPH against CIN is probably not only mediated by the H1R pathway, but also by other mechanisms.

The effect of DPH on the platinum levels in the kidneys, plasma, whole blood, and urine

In the kidneys, the cisplatin uptake into renal tubular cells is mediated by organ cation transporter 2 (OCT2); cisplatin accumulates, resulting in nephrotoxicity[17, 18]. Interestingly, DPH has previously been reported to inhibit OCT2[19, 20]. To evaluate the DPH action on cisplatin pharmacodynamics within the body, we measured the platinum levels in whole blood, plasma, and the kidneys following cisplatin administration. As shown in Tables 5 and 6, renal and plasma platinum levels were reduced by approximately 75% at both 10 min and 8 h after cisplatin treatment in mice treated with DPH. The plasma-free platinum concentration was also lower after cisplatin

administration in mice under DPH treatment. Conversely, no differences in platinum concentration were found in the whole blood of both control and DPH-treated mice, 10 min and 8 h after cisplatin administration. On the other hand, the concentration of platinum in clots (contained more than 90% red blood cells) was higher in mice under DPH treatment at both 10 min and 8 h after cisplatin administration. These findings suggest that DPH might promote the binding of cisplatin to blood cells, contributing to the reduction in the platinum incorporated into the kidneys. In addition, there was no significant differences in urinary platinum excretion regardless of DPH treatment, indicating no effect of DPH on the urinary excretion of cisplatin.

Effects of DPH against CIN in H1RKO mice

We examined whether DPH could exert further preventive action against CIN in H1RKO mice. DPH still alleviated the cisplatin-induced aggravation of BUN, plasma creatinine, renal injury, and inflammatory cytokines including TNF-α and MCP-1 in H1RKO mice (Figure 5A-D). Of note, there was no difference in the renal OCT2 expression and renal cisplatin content between WT and H1RKO mice (Figure 5E and Supplemental Table S1). Moreover, cisplatin-induced DNA damage, as well as the

increment of the platinum content, were also suppressed by DPH treatment in H1RKO mice (Figure 5F and G). These findings suggest that the action of DPH against CIN is mediated by both the H1R-dependent and -independent mechanisms.

Effect of DPH in tumor-bearing mice under cisplatin treatment

DPH suppressed CIN; however, it was unclear whether DPH would diminished the cytotoxic effects of cisplatin in a cancer model. To address this, we tested the effect of DPH on the cisplatin anti-tumor action using cancer cell lines. As depicted in Figure 6, cisplatin-induced cell death was not inhibited by the concomitant treatment with DPH in 3LL cells, MKN45 cells, colon26 cells, and HeLa cells, suggesting no inhibitory effect of DPH on the anti-tumor properties of cisplatin *in vitro*. OCT2 was not expressed in these tumor cell lines, indicating the intake of cisplatin can be mediated by a transporter other than OCT2 (Supplemental Figure S7). We further examined the effect of DPH on the anti-tumor action of cisplatin using a 3LL-cell *in vivo* xenograft mouse model. During the four week observation period, the saline control group showed continuous tumor growth, whereas the two drug-treated groups exhibited suppression of tumor growth; however, no difference in the cisplatin anti-tumor effect was found for in mice with or

without DPH treatment (Figure 7A). Moreover at the end of the four week study-period, mice under the vehicle treatment showed a significant increase in tumor weight and volume compared to mice treated with cisplatin and cisplatin + DPH. Of note, no differences in tumor volume and weight were observed between the cisplatin-treated and the cisplatin + DPH-treated animals (Figure 7B). Despite tumor growth, mice in the vehicle treatment group did not develop renal dysfunction as evidenced by the plasma creatinine levels. In contrast, the plasma creatinine levels of the cisplatin-treated group nearly doubled, whereas the group treated with cisplatin plus DPH exhibited a significantly improved renal function (Table 7). Furthermore, cisplatin-induced kidney injury as indicated by histology and the mRNA levels of renal damage markers was attenuated by the DPH treatment (Figure 7C and D). Repeated cisplatin administration induced renal fibrosis, which was also inhibited in the context of the concomitant treatment with DPH (Figure 7E and F). Therefore, DPH prevents CIN without affecting the anti-tumor action of cisplatin.

Retrospective clinical study of cancer patients receiving DPH before cisplatin-treatment

A total of 1467 patients, consisting of 1416 DPH non-users and 51 DPH users, were enrolled in this study (Supplemental Figure S8). The clinical characteristics of patients included in the analysis are listed in Table 8. The characteristics of patients differed in the non-DPH and the DPH groups. Particularly, the main problem was the difference in creatinine clearance profiles, mainly due to the fact that almost all DPH users were female patients. Of note, in the FEARS analysis, there was no significant differences in RORs between males and females with respect to the preventive effect of DPH on CIN (Supplemental Table S2). As opposed to the entire population, the propensity-matched patients (49 in each group) showed similar group characteristics. The incidence of AKI was 6.1% and 22.4% in patients undergoing and not-undergoing DPH treatment, respectively, before the administration of cisplatin (P = 0.04) (Table 9). Overall, these results suggest that cisplatin-induced AKI is suppressed in patients under diphenhydramine treatment.

Discussion

We identified DPH as a preventive drug for CIN using the FAERS database. DPH was also confirmed to prevent CIN in experimental studies; of note DPH was

confirmed to prevent CIN without affecting the anti-tumor effect of cisplatin. Moreover, the clinical cohort study revealed that the previous usage of DPH suppressed acute kidney injury in patients undergoing cisplatin treatment. Thus, our results identify DPH as a potential drug candidate for the prevention of CIN.

Previous studies have shown the involvement of inflammation, apoptosis, and oxidative stress in CIN; their inhibition led to the amelioration of CIN[2-4]. The present study clarified that DPH pre-treatment reduced cisplatin-induced renal damage via the inhibition of the increase of inflammatory cytokines, apoptosis, and oxidative stress in a mouse model. Cisplatin also activated the MAP kinase pathways including ERK1/2, JNK, and p38MAPK. On the other hand, DPH treatment inhibited the cisplatin-induced activation of ERK1/2 and JNK, but not of p38MAPK. Thus, the inhibition of the MAPK pathways was probably behind the reduction of apoptosis and inflammation, contributing to the suppression of CIN[21-23].

H1R is ubiquitously expressed in many tissues and cells, mediating numerous histamine-induced symptoms via various signaling pathways[24]. In fact, the histamine-histamine receptor mediated pathway plays a crucial role in the progression of end-organ

tissue injury, including in kidneys[25]. The administration of platinum agents also causes hypersensitivity reactions[26], suggesting an action of cisplatin on H1R-mediated responses. H1R activation is also involved in the following processes: inflammation[14], oxidative stress[15], and apoptosis[14]. Therefore, the H1R antagonist might exert a preventive action against CIN through the inhibition of inflammation, apoptosis, and oxidative stress. Indeed, H1R-deficient mice exhibited lower degrees of CIN compared to those in WT mice; nevertheless no difference was seen with respect to the renal platinum content, thereby indicating the preventive action of the H1R antagonist against CIN. Therefore, pharmacological inhibition or gene knockout of H1R alleviated CIN through the suppression of inflammation, oxidative stress, and apoptosis. However, the degree of CIN prevention was stronger in H1RKO mice under DPH treatment; DPH exerted increased preventive effect against CIN in H1RKO mice. Thus, DPH may also induce an alternative effect not dependent on the inhibition of H1R.

CIN is caused by the accumulation of cisplatin in the proximal tubules of the kidneys; it accumulates in the renal cortex in concentrations five-fold higher than those in serum[27] and locates primarily in the S3 segment of renal proximal tubules[28]. OCT2 is

specifically expressed in the basal membranes of proximal tubular cells[29] and is responsible for the uptake of cisplatin[18]. CIN was inhibited in OCT1/2-deficient mice[30], indicating a target for the prevention of CIN. Interestingly, DPH has been shown to inhibit OCT2[19, 20]. Therefore, we surmised that the suppressive effect of DPH was mediated through the prevention of the uptake of cisplatin via OCT2. As expected, DPH pretreatment reduced approximately 75% of the platinum concentration in the kidneys of mice under cisplatin treatment. However, the plasma platinum concentration, as well as the free platinum concentration, were also lower in mice under DPH treatment, 10 min after cisplatin administration, whereas no differences in the platinum concentration were found in whole blood samples between both groups, suggesting that DPH induced cisplatin to bind to blood cells.

Once cisplatin is administered, approximately 98% of the drug immediately binds to plasma proteins including transferrin, γ-globulin, and albumin[31]. However, other studies reported that the severity of CIN was related to the peak plasma cisplatin concentration and/or the area under the plasma cisplatin concentration-time curve for unbound cisplatin[32-34]. In cancer patients treated with cisplatin, the concentration of

cisplatin in whole blood was 10, 500, and 100 ng Pt/mL in plasma, plasma proteins, and hemoglobin, respectively[35]. Thus, cisplatin was also distributed to the blood cell compartment (nearly all red blood cells). We analyzed platinum levels of the clot as an alternative to red blood cells because the clots contain more than 90% red blood cells. The concentration of clot platinum was higher in mice under DPH treatment at both 10 min and 8h after cisplatin administration. These findings indicate that DPH promotes the binding of cisplatin to red blood cells. Moreover, no differences in urinary platinum excretion were seen in mice administered cisplatin with or without DPH treatment. DPH may facilitate the binding of cisplatin to the red blood cell compartment, thereby attenuating the plasma cisplatin concentration as well as the intake of cisplatin to the proximal tubules without affecting urinary excretion.

The anti-tumor effect of cisplatin was reported to be exerted in its free form, unbound to proteins[36]. However, another study reported that transferrin-bound cisplatin showed an enhanced anti-tumor efficacy and produced fewer side effects compared to free cisplatin[37]. Although it remains controversial whether the free form or the protein-bound form of cisplatin exerts the primary anti-tumor effect, in the present study, DPH

did not prevent the anti-tumor effect of cisplatin in either *in vitro* or *in vivo* experiments. Especially in the *in vivo* experiment, the unfettered anti-tumor effect of cisplatin may be due to the stability of its concentration in whole blood with or without DPH. Our findings suggest that DPH prevents CIN without affecting the anti-tumor efficacy of cisplatin.

We further investigated the effects of DPH in the context of CIN in a retrospective clinical study and found that DPH treatment significantly reduced the occurrence of AKI following cisplatin treatment in patients with cancer. Antihistamine drugs have been used as premedication for the prevention of chemotherapy-related hypersensitivity reactions in clinical settings worldwide[38]; in fact, DPH has been used clinically as a premedication drug for the combination therapy regimen of cisplatin and paclitaxel in patients with advanced-stage epithelial ovarian cancer[39] or head and neck squamous cell carcinoma[40]. Therefore, our results suggest that the premedication with DPH brings the additional advantage of the prevention of CIN. This said, there were limitations in the present study. The DPH user group included a greater number of young females, with lower serum creatinine levels at baseline. Therefore, we used a propensity score analysis to exclude the potential confounding factors and selection biases in sample

size including marked gender differences between the groups. At the same time, the FEARS analysis showed no significant differences in ROR between males and females in the preventive effect of DPH on CIN. Although our results support the preventive effect of DPH against CIN, as per the findings of the FAERS database analysis and basic experimental studies, further research is necessary to confirm the preventive effect of DPH against CIN.

In conclusion, a novel activity was revealed for DPH, a potential preventive medicine against CIN; therefore drug repositioning should be considered (and is expected to be easy since DPH is already administered to cancer patients undergoing chemotherapy). A prospective study is required to clarify the potential of DPH as a preventive treatment for CIN in the future.

Methods

Analysis of the FAERS database

Adverse event records from January 2004 to September 2017 were obtained from the FDA web site (www.fda.gov). The adverse event terms corresponded to the

Medical Dictionary for Regulatory Activities (MedDRA). The MySQL software (version 5.7.21) was used to build a database integrating the FAERS data[1]. The adverse event risk signal was evaluated via the calculation of the reporting odds ratio (ROR) with a 95% confidence interval (CI) [12, 41-44]. The inverse risk signal was considered significant when the ROR and the upper limit of the corresponding 95% CI were <1.

Cell culture and cell death assay

Proximal tubule cell lines were obtained from the American Type Culture Collection (Virginia, USA) and the Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan). Cancer cell lines were obtained from the Japanese Collection of Research Bioresources Cell Bank. Cell death was assessed as described previously [45].

Animal model of cisplatin-induced nephrotoxicity

Seven to eight-week-old male C57BL/6J mice were purchased from Nippon CLEA (Tokyo, Japan). H1RKO mice (genetic background: C57BL/6J) were purchased from Oriental Bio Service Inc.(Kyoto, Japan)[46]. Mice with nephrotoxicity induced by cisplatin (20 mg per kg) were administered with DPH (20 mg per kg) or vehicle.

Histological analysis

The histological evaluation of renal tubular damage has been described previously [47]. The picrosirius red staining was used for the evaluation of renal fibrosis as previously described [45].

Immunohistochemistry

Frozen sections were used for the detection of Platinum-(GpG) DNA adducts and visualized using immunofluorescence (Alexa fluor; Life Technology, Tokyo, Japan) as previously described in detail[44]. Paraformaldehyde-fixed paraffin-embedded sections were used for immunohistochemistry of leukocytes as previously described [48].

Measurement of the platinum concentration

The kidney, plasma, whole blood, clot, and urine samples were incubated at 95°C for 1 h with 60% nitric acid (28163-1B, Kanto Kagaku Co., Tokyo, Japan). The lysate was centrifuged and the supernatant was used for the measurement of platinum content.

Tumor-bearing mouse model

3LL cells (5×10^6 cells per site) were subcutaneously injected on the right flank of mice, and the tumor growth was routinely monitored using a vernier caliper. The mice

were randomly divided into three groups for weekly intraperitoneal treatment with cisplatin, cisplatin plus DPH, or vehicle (control).

Retrospective clinical study

We retrospectively reviewed the medical charts of patients who were administered cisplatin at Tokushima University Hospital between 2008 and 2019. All patients had received their first course of chemotherapy with cisplatin.

Statistical analysis of experimental studies

Data are presented as the mean ± standard error of the mean (mean ± SEM) in the form of dot plots. For data not normally distributed, data are presented as the median with the interquartile range. The Mann–Whitney U test or t-test were used for comparison between two groups. The Kruskal–Wallis test was used for comparisons between more than two groups, and the statistical significance of each difference was evaluated. Survival curves were created using the Kaplan-Meier method and tested using a log-rank test. Incidence was compared by means of the Fisher's exact test. Statistical significance was indicated by $P < 0.05$.

Study Approval

All experimental procedures on mice were performed in accordance with the guidelines of the Animal Research Committee of the Tokushima University Graduate School and the protocol was approved by the Institutional Review Board of the Tokushima University Graduate School (Permit Number: T30-74, T30-99). The retrospective clinical study and all protocols were reviewed and approved by the Ethics Committee of the Tokushima University Hospital (approval number: 3331-2).

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drafting/revising the work for intellectual content and context: H.H., Y.I., M.G., K.F., S.K., M.C., M.Y., T.N., K.Ta., M.I., Y.Z., Y.H., Y I-I, L.M., K.I., H.F., T.T., K-I.A., K.Ts.; final approval and overall responsibility for the published work: Y.I. All authors read and approved the final manuscript. **Competing interests**: There are no relevant competing interests to declare.

Supplementary Materials

1. Supplemental materials and methods

2. Supplemental Tables

Supplemental Table S1. Effect of each drugs on the occurrence of cisplatin-induced renal disorder by FAERS analysis.

Supplemental Table S2. Search terms for renal disorder-related adverse events in the analysis of FAERS.

Supplemental Table S3. Platinum content in kidney, plasma, whole blood at 10m after CDDP treatment in WT mice and Hrh1KO mice.

Supplemental Table S4. Primer sequences.

3. Supplemental Figures

Supplemental Figure S1. Dose-dependency of DPH against CIN. Quantitative analysis of mRNA expression in the kidneys of mice in each group. Values are expressed as mean ± SEM. *P < 0.05, **P < 0.01, n =5-9 in each group.

Supplemental Figure S2. Quantitative analysis of mRNA expression, immunohistological analysis, and protein expression in the kidneys of mice in each group. Values are expressed as mean ± SEM. *P < 0.05, **P < 0.01, n =5-9 in each group.

Supplemental Figure S3. Left panel: Representative protein bands of phospho-p38MAPK, and total p38MAPK, and β-actin in the kidneys of mice. Right panel: Semi-quantitative analysis of densitometry for p38MAPK phosphorylation. Values are expressed as mean ± SEM, *P < 0.05, **P < 0.01, n = 5 in each group.

Supplemental Figure S4. The effect of cisplatin with or without DPH on leukocytes populations in the kidney. Left panel: representative immunohistochemistry of F4/80, Ly-6g, CD3, toluidine blue and c-kit in the kidneys of mice in each group. Right panel: semi-quantitative analysis of F4/80, Ly-6g, and CD3 positive cells. Values are expressed as mean ± SEM, *P < 0.05, n = 4–5 in each group. Arrow heads; c-kit positive cells.

Supplemental Figure S5. mRNA expression levels of F4/80, Ly-6g, CD3, and c-kit in the kidneys of mice in each group. Values are expressed as mean ± SEM. *P < 0.05, **P < 0.01, n = 8–9 in each group.

Supplemental Figure S6. Survival rates of mice with vehicle or DPH treatment after cisplatin administration. Survival rates were calculated by the Kaplan-Meier method and compared by the log-lank test. n=11 in each group.

Supplemental Figure S7. Left panel; Representative PCR product bands of OCT2, H1R, and 36B4 in KH-2 and LLC-PK1. Right panel; Representative PCR product bands of OCT2 and 36B4 in human kidney, mouse kidney, and various cancer cell lines.

Supplemental Figure S8. Flow chart of patient selection.

Supplementary information is available on Kidney International's website.

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Figure legends

Figure 1. Inhibitory effect of diphenhydramine (DPH) on cisplatin-induced renal proximal tubular cell death. Cisplatin-induced cell death was attenuated by DPH treatment in HK-2 and LCC-PK1 cells. Values are expressed as the mean ± SEM, n = 8–12 in each group. $^{**}P < 0.01$.

Figure 2. DPH inhibits cisplatin-induced nephrotoxicity in mice. (A) Representative hematoxylin and eosin (H&E) staining of kidney sections from control mice, cisplatin-injected mice with vehicle or DPH treatment. (B) Quantitative analysis of the renal tubular damage scores. Values are expressed as the mean ± SEM. *P < 0.05, **P < 0.01; n = 5 in each group. (C) mRNA expression levels of kidney injury markers (KIM-1 and lipocalin-2) in the kidneys of mice in each group. Values are expressed as the mean ± SEM. *P < 0.05, **P < 0.01, n = 8–9 in each group. (D) DPH prevents the cisplatin-induced upregulation of renal inflammation. Quantitative analysis of mRNA expression of inflammatory cytokines in the kidneys of mice in each group. Values are expressed as the mean ± SEM. *P < 0.05, **P < 0.01, n =8–9 in each group. The effect of DPH against cisplatin-induced DNA damage. (E) Left panel: Representative immunohistological images of DNA platination product Pt-(GpG) and DAPI in the kidney sections from cisplatin-injected mice with vehicle and DPH treatment. Right panels: Semi-quantitative analysis of Pt-(GpG) in DNA. Values are expressed as the mean ± SEM. *P < 0.05, **P < 0.01; n = 5 in each group.

Figure 3. The effect of DPH on oxidative stress, apoptosis, and the mitogen-activated protein kinase pathway induced by cisplatin. (A) Left panel: representative images of dihydroethidium (DHE) staining in the kidneys of mice in each group. Right panel: semi-quantitative analysis of DHE fluorescence intensity. Values are expressed as the mean ± SEM, *P < 0.05, n = 4–5 in each group. (B) Left panel: representative images of TdT-mediated dUTP nick end labeling (TUNEL) staining in the kidneys of mice in each group. Right panel: semi-quantitative analysis of TUNEL positive cells. Values are expressed as the mean ± SEM, *P < 0.05, n = 4–5 in each group. (C) Left panel: representative protein bands of 4-hydroxynonenal (HNE), cleaved caspase-3, and β-actin in the kidneys of mice. Right panel: semi-quantitative analysis of densitometry for 4-HNE and cleaved caspase-3. Values are expressed as the mean ± SEM, *P < 0.05, **P < 0.01, n = 5 in each group. (D) Left panel: representative protein bands of phospho-c-jun N-terminal kinase (JNK), total JNK, phospho-extracellular signal-regulated kinase (ERK) 1/2, total ERK1/2, and β-actin in the kidneys of mice. Right panel: semi-quantitative analysis of densitometry for JNK and ERK1/2 phosphorylation. Values are expressed as the mean ± SEM, *P < 0.05, **P < 0.01, n = 5 in each group.

Figure 4. Attenuated cisplatin-induced nephrotoxicity in H1R-deficient mice. (A) Representative hematoxylin and eosin (H&E) staining of the kidney sections from WT mice, H1RKO mice, cisplatin-injected WT mice, and cisplatin-injected H1RKO mice. (B) Quantitative analysis of renal tubular damage scores. Values are expressed as the mean ± SEM. *P < 0.05, **P < 0.01; n

= 7 in each group. (C) mRNA expression levels of kidney injury markers (KIM-1 and lipocalin-2) in the kidneys of mice in each group. Values are expressed as the mean ± SEM. *P < 0.05, **P < 0.01, n = 7–10 in each group. (D) Effect of H1R-deficiency on cisplatin-induced upregulation of renal inflammatory cytokines. Quantitative analysis of mRNA expression of inflammatory cytokines in the kidneys of mice in each group. Values are expressed as the mean ± SEM. *P < 0.05, **P < 0.01, n = 7–10 in each group. (E) Left panel: representative images of dihydroethidium (DHE) staining in the kidneys of mice in each group. Right panel: semi-quantitative analysis of DHE fluorescence intensity. Values are expressed as the mean ± SEM, *P < 0.05, n = 4–7 in each group. (F) Left panel: representative images of TdT-mediated dUTP nick end labeling (TUNEL) staining in the kidneys of mice in each group. Right panel: semi-quantitative analysis of TUNEL positive cells. Values are expressed as the mean ± SEM, *P < 0.05, n = 4–5 in each group. (G) Upper panel: representative protein bands of 4-hydroxynonenal (HNE), cleaved caspase-3, and β-actin in the kidneys of mice. Right panel: semi-quantitative analysis of densitometry for 4-HNE and cleaved caspase-3. Values are expressed as the mean ± SEM, *P < 0.05, **P < 0.01, n = 7–10 in each group. (H) Upper panel: representative protein bands of phospho-JNK, total JNK, phospho- ERK1/2, total ERK1/2, and β-actin in the kidneys of mice. Right panel: semi-quantitative analysis of densitometry for JNK and ERK1/2 phosphorylation. Values are expressed as the mean ± SEM, *P < 0.05, **P < 0.01, n = 7–10 in each group.

Figure 5. Effect of DPH on cisplatin-induced nephrotoxicity in H1R-deficient mice. (A) Plasma BUN and creatinine levels. Values are expressed as the mean ± SEM. *P < 0.05, **P < 0.01; n = 9–11 in each group. (B) Left panel: representative hematoxylin and eosin (H&E) staining of kidney sections in H1RKO mice with cisplatin or cisplatin plus DPH treatment. Right panel: quantitative analysis of renal tubular damage scores. Values are expressed as the mean ± SEM. *P < 0.05, **P < 0.01; n = 7 in each group. (C) mRNA expression levels of kidney injury markers (KIM-1 and lipocalin-2) in the kidneys of mice in each group. Values are expressed as the mean ± SEM. *P < 0.05, **P < 0.01, n = 7 in each group. (D) Effect of DPH on cisplatin-induced upregulation of renal inflammatory cytokines in H1RKO mice. Quantitative analysis of mRNA expression of inflammatory cytokines in the kidneys of mice in each group. Values are expressed as the mean ± SEM. *P < 0.05, **P < 0.01, n = 7 in each group. (E) Left panel: representative protein bands of OCT2 and β-actin in the kidneys of mice. Right panel: semi-quantitative analysis via densitometry of OCT2 protein expression. Values are expressed as the mean ± SEM, n = 5 in each group. (F) Cisplatin-induced DNA damage in the kidney from cisplatin-injected H1RKO mice, and cisplatin-injected H1RKO mice with DPH treatment. Left panel: Representative images of DNA platination product Pt-(GpG) and DAPI in the kidney sections. Right panels: Semi-quantitative analysis of Pt-(GpG) in DNA. Values are expressed as the mean ± SEM. **P

< 0.01; n = 5 in each group. (F) Platinum content in kidneys 10 min after cisplatin treatment. Values are expressed as the mean ± SEM. *P < 0.05; n =9 in each group.

Figure 6. DPH does not inhibit cisplatin-induced cell death in various cancer cell lines. Cisplatin-induced cell death was not influenced by the DPH treatment in (A) 3LL mice lung carcinoma cells, (B) MKN45 human gastric cancer cells, (C) colon26 mouse colon cancer cells and (D) Hela human cervical tumor cells. Values are expressed as the mean ± SEM, *P < 0.05, **P < 0.01, n = 6–12 in each group.

Figure 7. DPH ameliorates cisplatin-induced kidney injury without blocking the therapeutic effects in mice bearing Lewis lung carcinoma cells. (A) Changes in tumor volume during the observation period. Tumors were measured to determine tumor volume. *P < 0.05, **P < 0.01 vs. other groups; n = 6–11 in each group. (B) Upper panel: representative extracted tumors. Lower panels: tumor volume and weight at the end of the 4 week-period. Mean ± SEM. **p < 0.01 vs. vehicle group; n = 6–11 in each group (C) Left panel: representative hematoxylin and eosin (H&E) staining of kidney sections at 4 weeks after vehicle, cisplatin, or cisplatin-plus-DPH treatment. Right panel: quantitative analysis of renal damage scores. Values are expressed as the mean ± SEM. *P < 0.05, **P < 0.01; n = 6–9 in each group. (D) mRNA expression levels of kidney injury markers (KIM-1 and lipocalin-2) in the kidneys of mice in each group. Values are expressed as the mean ± SEM. *P < 0.05, **P < 0.01; n = 6–9 in each group. (E) mRNA expression levels of renal fibrosis markers (collagen I and III) in the kidneys of mice in each group.

Values are expressed as the mean ± SEM. *P < 0.05, **P < 0.01; n = 6–9 in each group. (F) Left panel: representative picrosirius red staining of kidney sections at 4 weeks after vehicle, CDDP, or CDDP-plus-DPH treatment. Right panel: quantitative analysis of renal fibrosis area. Values are expressed as the mean ± SEM. *P < 0.05, **P < 0.01; n = 5 in each group.

Figure 1 Hamano and Ikeda, et al.

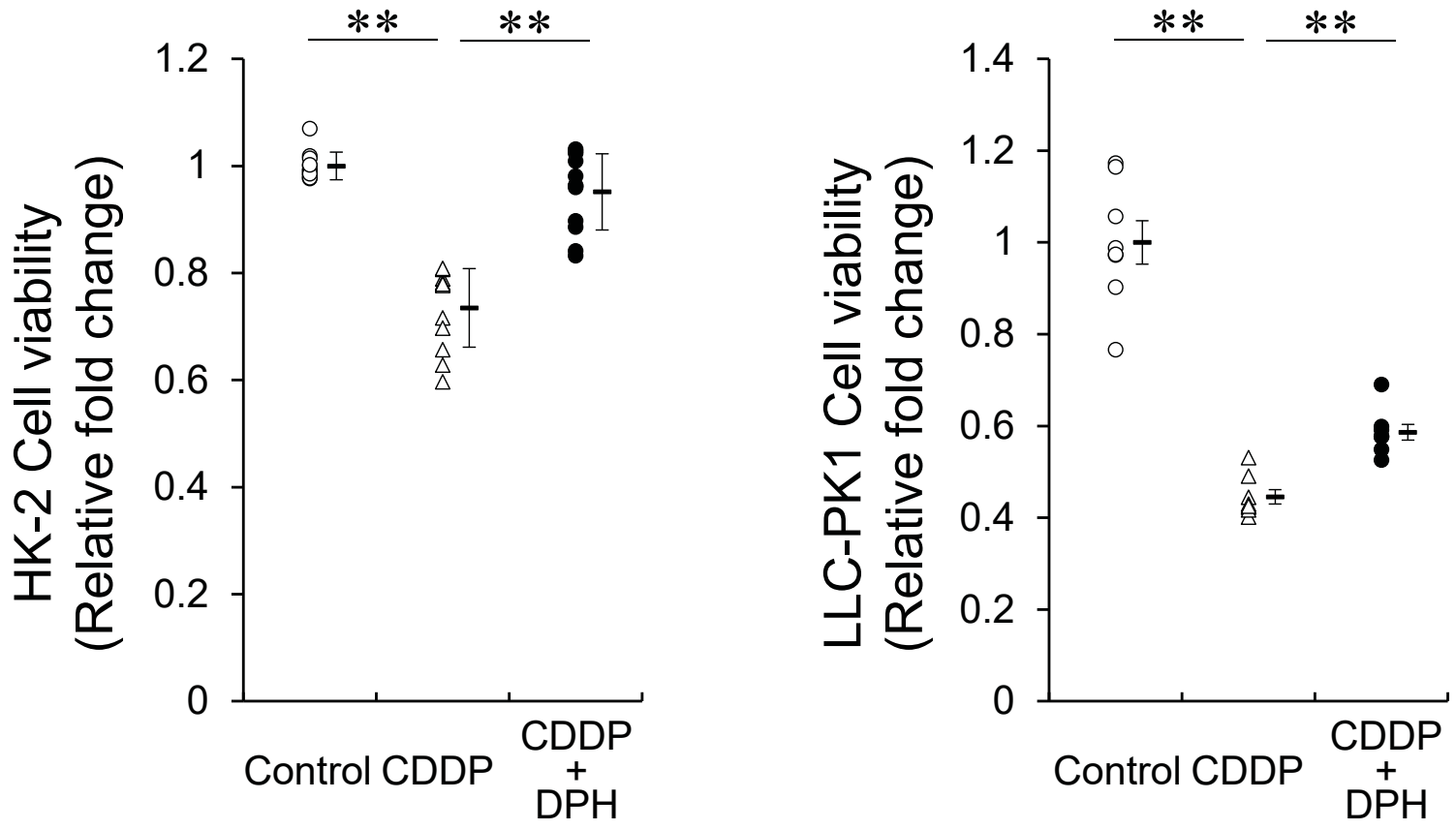


Figure 2 Hamano and Ikeda, et al.

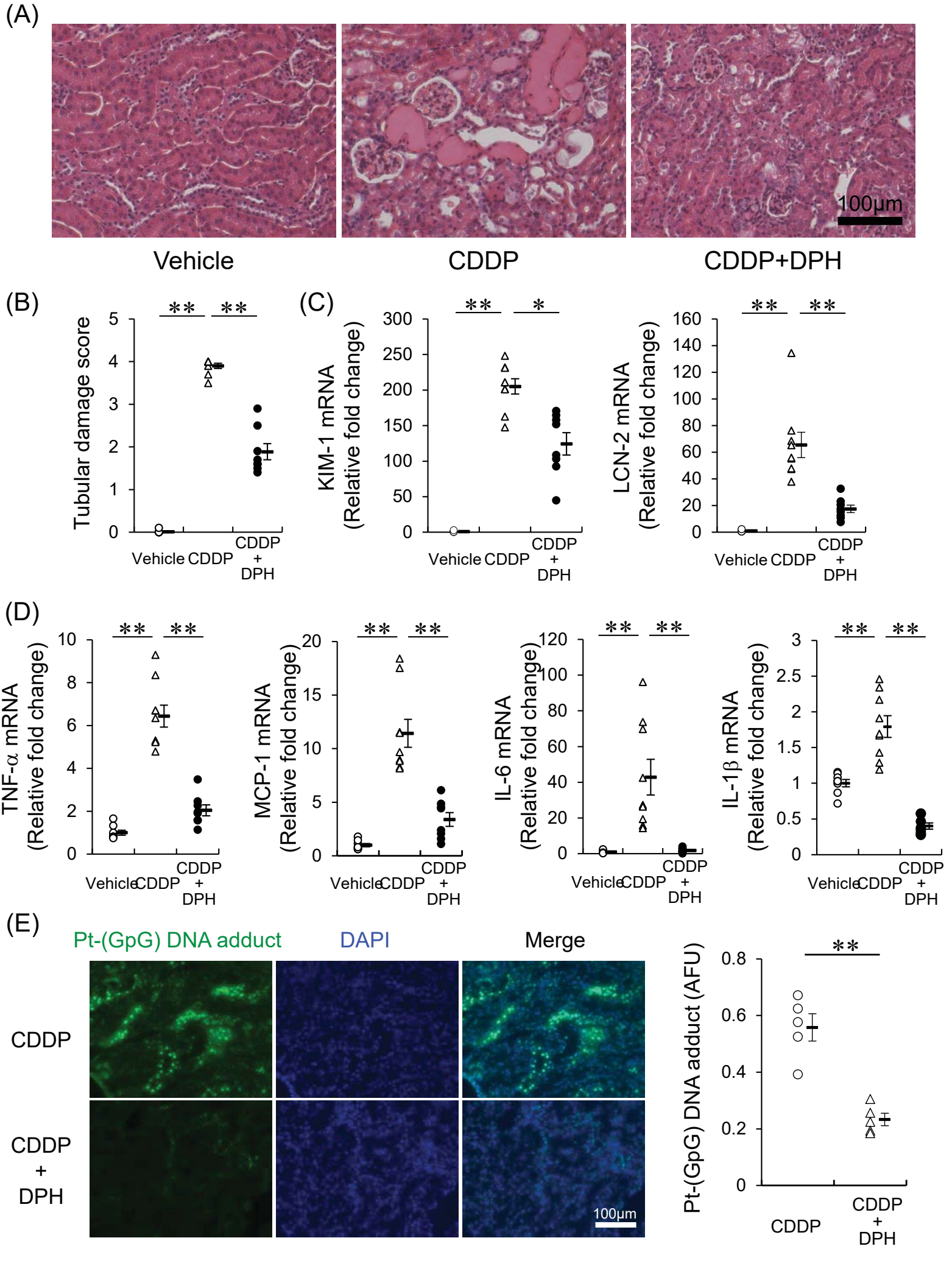
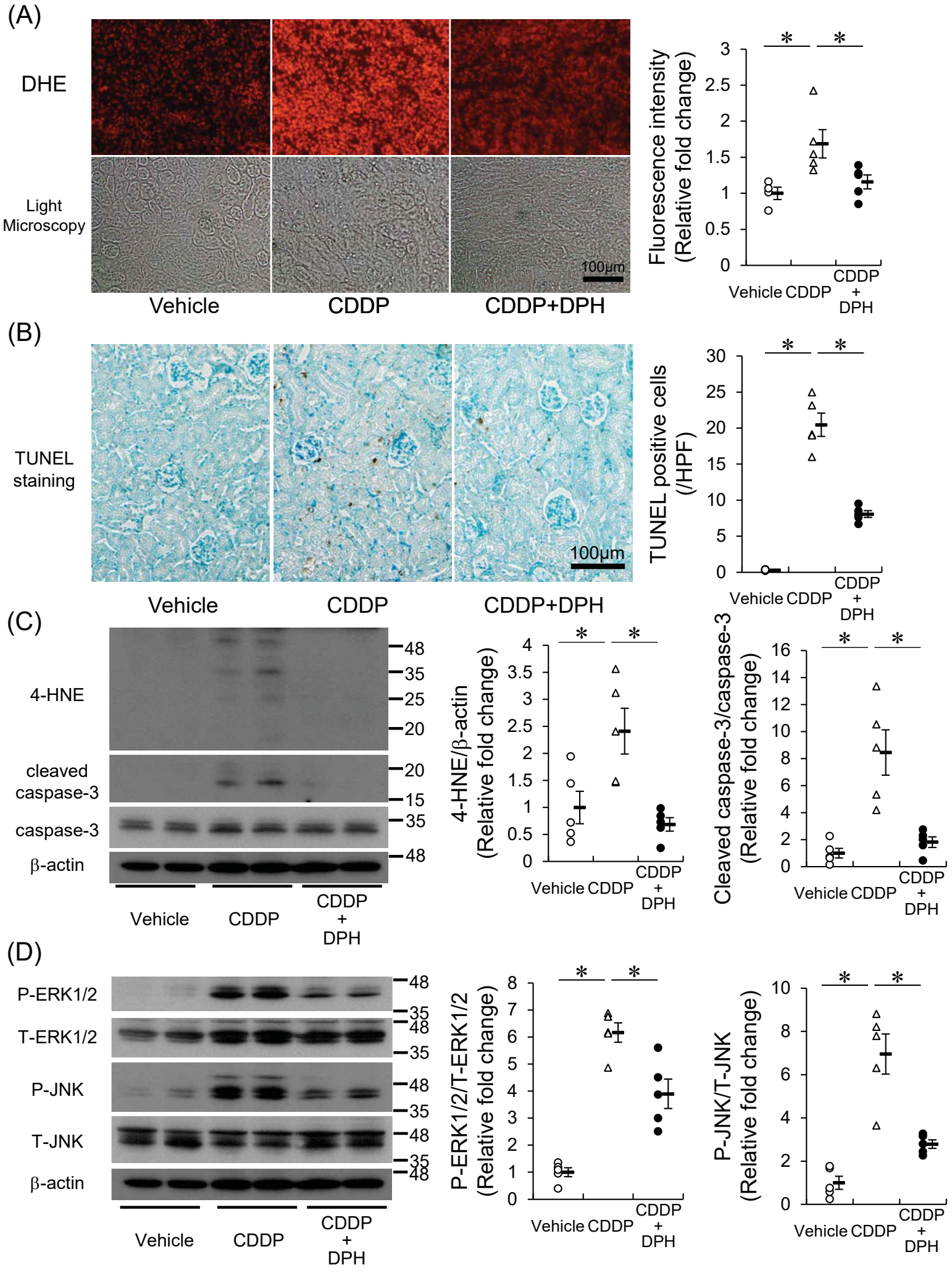


Figure 3 Hamano and Ikeda, et al.



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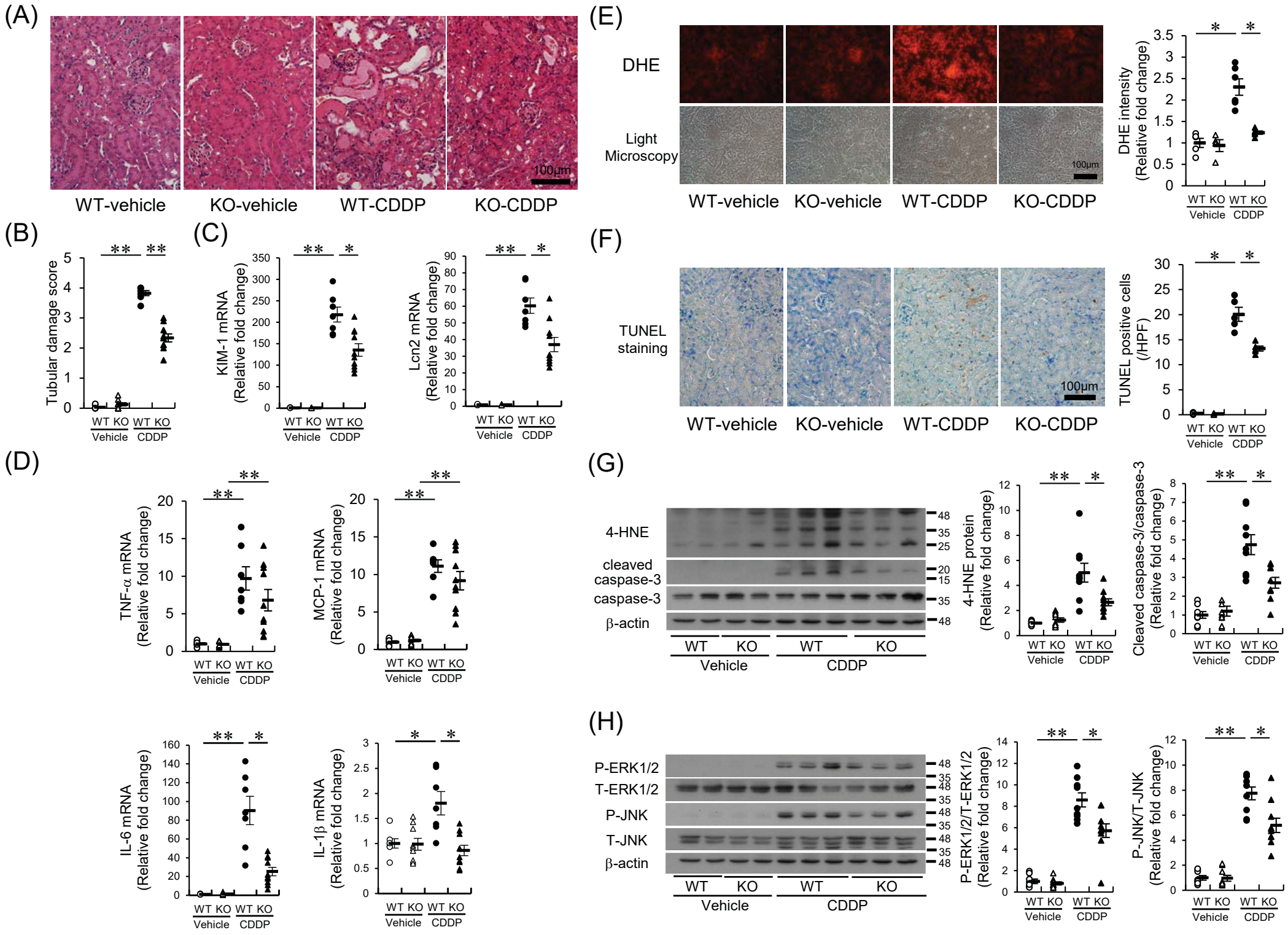

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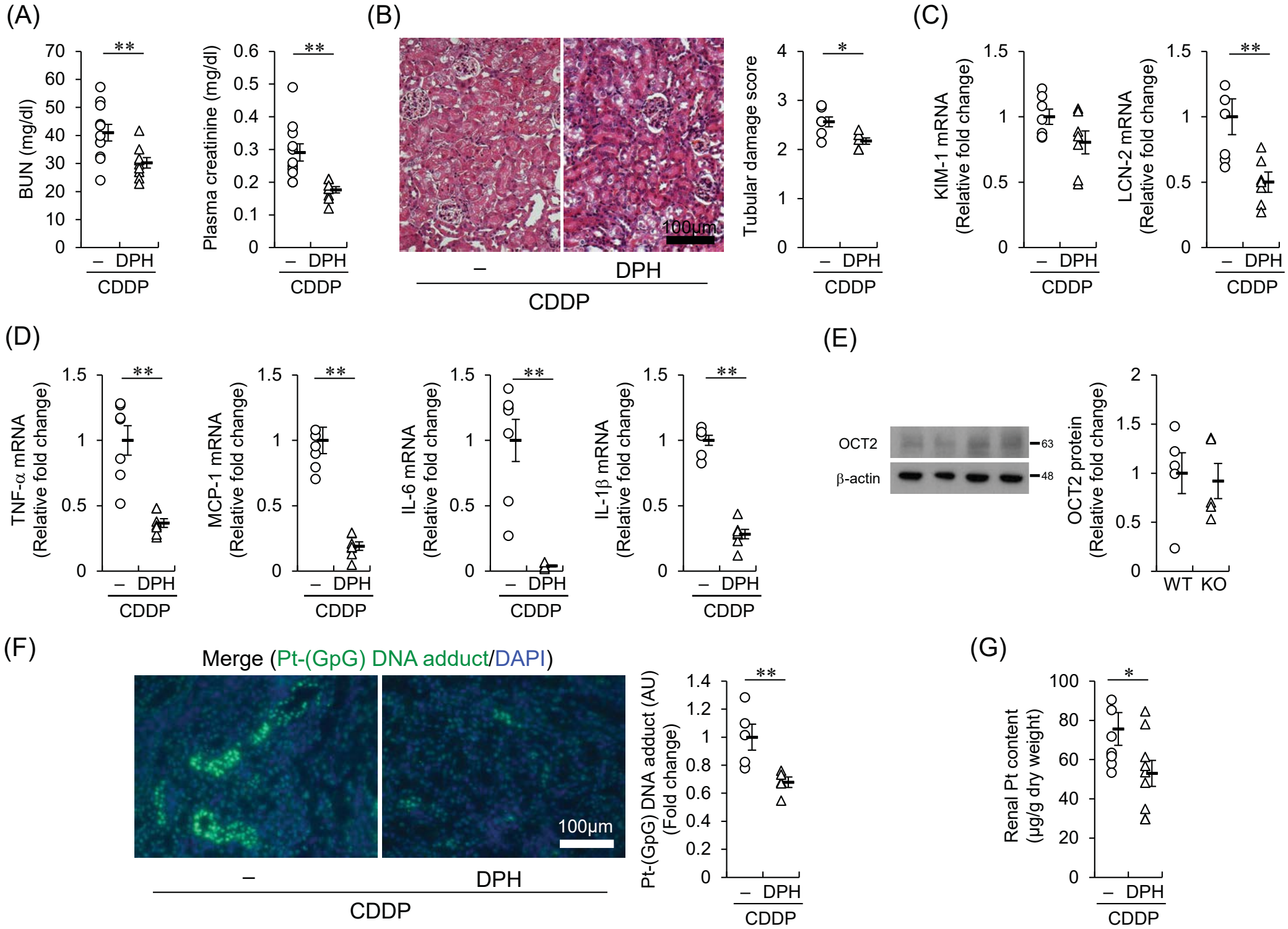


Figure 6 Hamano and Ikeda, et al.

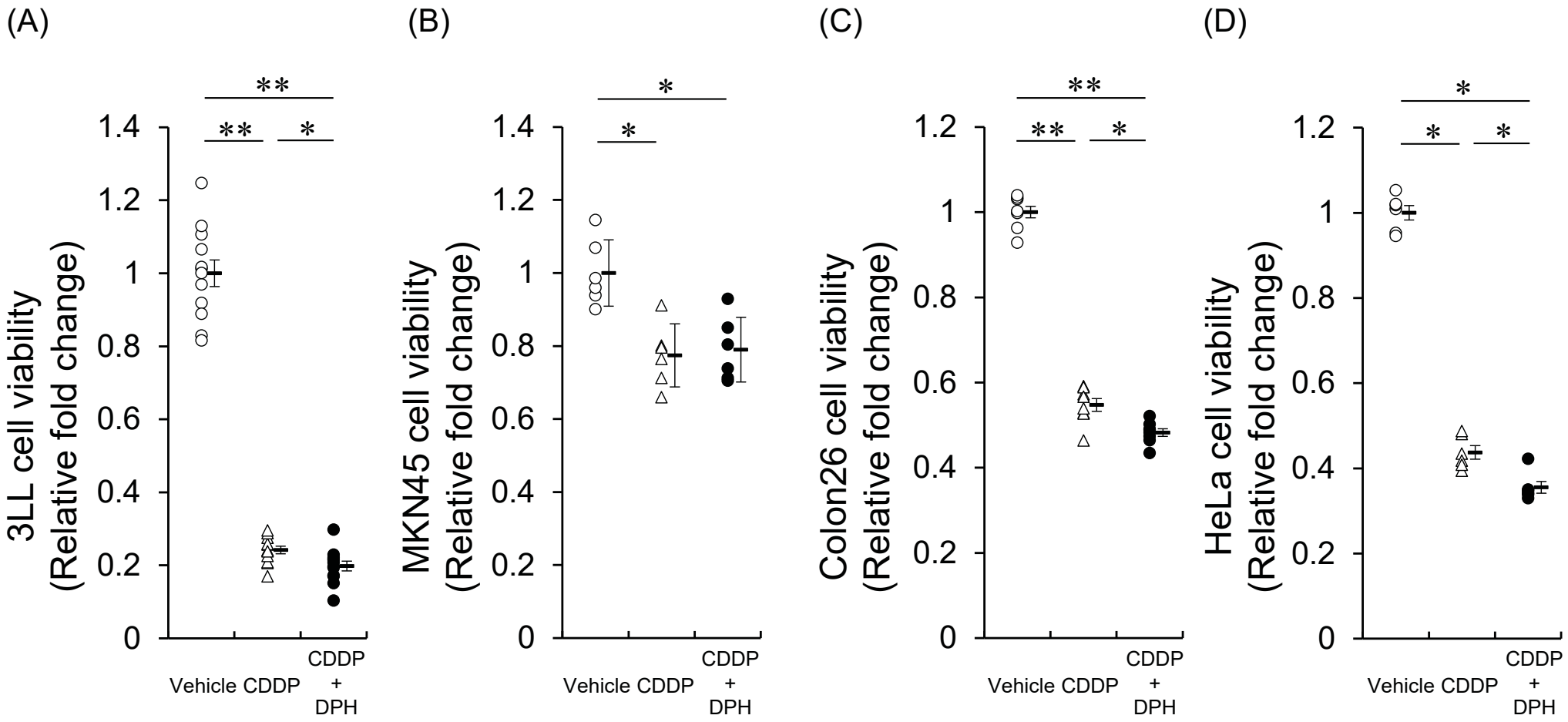
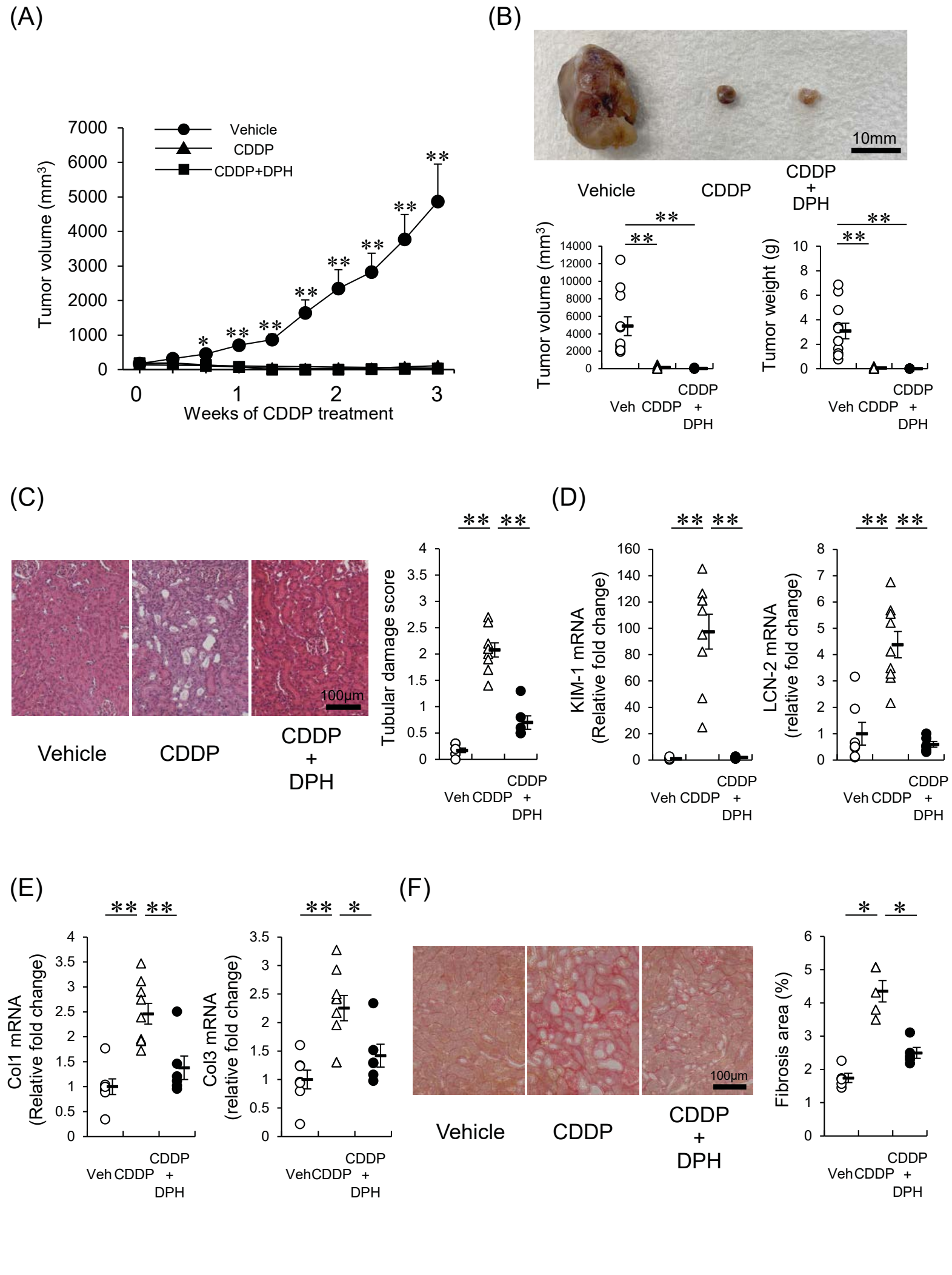


Figure 7 Hamano and Ikeda, et al.



Efficacy of diphenhydramine as a preventive medicine against cisplatin-induced nephrotoxicity

Medical big data research

Search for existing drugs used concomitantly with cisplatin

Drug identification

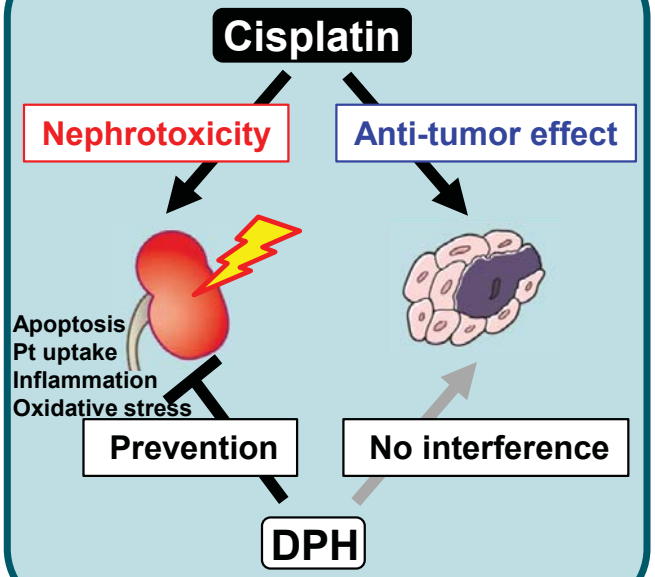
Comprehensive analysis of 1543 drugs used with cisplatin

Occurrence of CIN

Drug name	ROR
Diphenhydramine	0.60 (0.41-0.89)
Metronidazole	0.40 (0.18-0.90)
Fluconazole	0.66 (0.44-0.97)
Lorazepam	0.74 (0.57-0.98)

Diphenhydramine (DPH) as a candidate for the prevention of cisplatin-induced nephrotoxicity (CIN)

Basic research



Clinical research

Reduction of CIN in cancer patients under DPH treatment: a retrospective cohort study

Incidence rate of CIN

1416 DPH Non-users	51 DPH users
(−) DPH use	(+)
22.4%	6.1%

CONCLUSION:

DPH is effective as a novel preventive medicine against CIN.

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