Lactobacillus salivarius BP121 prevents cisplatin-induced acute kidney injury by inhibition of uremic toxins such as indoxyl sulfate and *p*-cresol sulfate via alleviating dysbiosis

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Abstract. The gut microbiota is important for maintaining the integrity of the intestinal barrier, promoting immunological tolerance and carrying out metabolic activities that have not evolved in hosts. Intestinal dysbiosis is associated with chronic kidney disease and probiotic supplementation has been shown to be beneficial. However, it is not known whether gut micro-organisms-specifically, lactic acid bacteria (LAB) can protect against acute kidney injury (AKI). To address this issue, the present study investigated the effects of *Lactobacillus salivarius* BP121, an intestinal LAB isolated from the feces of newborns, in a rat model of cisplatin-induced AKI and also in Caco-2 human intestinal epithelial cells. BP121 prevented cisplatin-induced AKI in rats, as demonstrated by decreases in inflammation and oxidative stress in kidney tissue and in serum levels of uremic toxins such as indoxyl sulfate (IS) and *p*-cresol sulfate (PCS). BP121 also reduced intestinal permeability, as determined using fluorescein isothiocyanate-dextran by immunohistochemical detection of tight junction (TJ) proteins such as zona occludens-1 and occludin. The abundance of *Lactobacillus* spp., which are beneficial intestinal flora, was increased by BP121; this was accompanied by an increase in the concentrations of short-chain fatty acids in feces. Additionally, H_2O_2-induced TJ protein damage was reduced in Caco-2 cells treated with BP121 culture supernatant, an effect that was reversed by the 5' AMP-activated protein kinase (AMPK) inhibitor Compound C and Toll-like receptor (TLR)4 inhibitor TLR4-IN-C34. In conclusion, this study demonstrated that *L. salivarius* BP121 protects against cisplatin-induced AKI by decreasing inflammation and oxidative stress and this renoprotective effect is partially mediated by modulating the gut environment and thereby suppressing IS and PCS production as well as by regulating AMPK and TLR4 dependent TJ assembly.

Introduction

Acute kidney injury (AKI) is a clinical syndrome characterized by a rapid decrease in renal function accompanied by accumulation of waste products such as urea (1,2). The incidences of non-dialysis-requiring and dialysis-requiring AKI in the U.S. are 522 and 29.5 per 100,000 people/years, respectively (3). Drug-induced AKI is a common occurrence in clinical practice because the kidney is a major target of various toxic chemicals (4,5). Although the incidence is difficult to estimate due to variable patient characteristics, many newly developed drugs have distinct mechanisms of nephrotoxicity, making it the second most common cause of hospital-acquired AKI (4). Recent studies have revealed a close relationship between the gut microbiome and various kidney diseases; however, the association between intestinal bacteria and nephrotoxic AKI remains unclear (6,7).

Cis-diammineplatinum (II) dichloride (cisplatin) is an inorganic platinum-based chemotherapy drug that is widely used in the treatment of solid tumors including testicular, ovarian, cervical and head and neck cancers (5,8). However, nephrotoxicity is major side effect of cisplatin (9,10), which binds to DNA to form inter- and intra-stand crosslinks that damage DNA templates or inhibit DNA synthesis and replication. The potential for damage is even greater in rapidly dividing cells such as cancer or epithelial cells (10); however, it can also disrupt the intestinal epithelium, thereby increasing

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intestinal permeability and causing systemic inflammation by permitting the egress of microbes and their toxins (11,12).

Probiotics are living, non-pathogenic microbes (bacteria or yeast) that provide beneficial nutrients to the host (13,14). Probiotics can directly inhibit the growth or promote death of pathogenic microorganisms by producing anti-microbial substances such as short-chain fatty acids (SCFAs) and have shown immunomodulatory effects in several chronic inflammatory disease models. *L. salivarius* BP121 (BP121) that was isolated from infant feces. *L. salivarius* has been shown to have remarkable probiotic efficacy because it had high survival rate in gastrointestinal tract conditions and produced antimicrobial compounds such as SCFAs (15). Previous studies have shown that *L. salivarius* produces abundant propionate/butyrate (16,17). Additionally, in previous studies, improved barrier function by SCFA was reported (18-20). SCFAs such as butyrate and acetate are known to protect intestinal barrier integrity by regulating through the activation of AMPK and TLR4. To date, there have been no studies investigating the possible benefits of probiotics including lactic acid bacteria (LAB) BP121 in the context of AKI.

AST-120 is an oral spherical carbon absorbent consisting of oral urea toxin remover and porous carbon particles. AST-120 reduces the accumulation of indoxyl sulfate in patients by adsorbing indole, a precursor of toxin sulfate. This function has the effect of slowing the progress of kidney disease (21).

To address this issue, the effect of BP121 treatment on a rat model of cisplatin-induced AKI was investigated, as well as the mechanistic basis for the observed effects of BP121 using Caco-2 human intestinal epithelial cells.

Materials and methods

Isolation and selection of strains from infant feces. LAB were isolated from fresh feces of new born infants (22). The samples were blended with 50 ml 0.85% NaCl solution and serially diluted 10-fold in 0.85% NaCl; each diluted solution was plated onto De Man, Rogosa, and Sharpe (MRS) agar (BD Biosciences; Becton, Dickinson and Company). The plates were incubated in an anaerobic jar with Anaeropack (BD Biosciences; Becton, Dickinson and Company) at 37°C for 72 h. White colonies on the plates were individually picked and streaked on fresh MRS agar plates. Isolated strains were identified based on the near-complete 16S rRNA gene sequence using the EZ-taxon server (http://www.ezbiocloud.net).

LAB screen for indoxyl sulfate (IS) inhibitory activity. LAB strains were screened for their ability to reduce IS levels as previously described (23). The strains were incubated in MRS broth with 60 μg/ml IS and the one with the highest inhibitory activity was selected. After incubation for 72 h, IS levels were measured with the Indican Assay kit (Sigma-Aldrich; Merck KGaA).

Analysis of SCFA in the probiotics conditioned medium and stool. *L. salivarius* BP121, *L. acidophilus* BP105 and *L. rhamnosus* GG were used for analysis of SCFA in conditioned medium. A total of three strains were incubated in MRS broth at 37°C for 48 h. SCFA analyzed using a high-performance liquid chromatography (HPLC; Hewlett-Packard HP1100 series; Agilent Technologies, Inc.), equipped with a UV-vis

detector and diode array detector. (Colum; YMC-Pack FA 250/6 mm I.D., Mobile phase; acetonitrile:water (85:15), pH 4.5 adjusted by 0.1% trifluoro acetic acid; flow rate, 1.2 ml/min, Temperature 35 UV at 400 nm). Culture medium was directly injected into the chromatograph after filtering with 0.2 um syringe filter. Also, 200 mg stool was mixed with 0.15 mM $HClO_4$ and then sonicated at 37°C and 70 KHz for 15 min. The prepared samples were filtered with 0.2 μm syringe filter. The filtered sample 20 μl was injected into the HPLC. Acetate, propionate and butyrate mixture were used to prepare a standard curve (range of 1-1,000 ppm).

Animals and cell line. A total of 48 male Sprague-Dawley rats (SantacoBioKorea, Inc.) weighing 187-221 g (8 weeks old) were housed two rats per cage in a room with controlled temperature (20-24°C) and humidity (40-70%) on a 12-h light/dark cycle with free access to food and sterile water. Experimental procedures involving the rats were approved by the Institutional Animal Care and Use Committee of Hoseo University [approval no. HSIACUC-18-097(1)]. Caco-2 cells were purchased from the Korean Cell Line Bank (cat. no. 30037.1) and were maintained as a monolayer in minimal essential medium (Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific, Inc.), 25 mM HEPES, and 25 mM $NaHCO_3$ at 37°C in a humidified atmosphere of 5% CO_2.

Experimental design. The 48 rats were randomly divided into six groups (n=8 each). The AKI model was established by single intraperitoneal (i.p.) injection of cisplatin (8 mg/kg body weight in 10 ml saline). BP121 was administered daily by oral gavage [i.e., per os (p.o.) administration] starting from 10 days prior to and continuing until 4 days after cisplatin injection. Kremezin fine granules (AST120; CJ Corp.) were orally administered at 3.6 g/kg body weight for 4 days after cisplatin injection. The groups were as follows: Normal control, saline (i.p.) + saline (p.o.); cisplatin, cisplatin (i.p.) + saline (p.o.); BP121-L, cisplatin (i.p.) + BP121 $1x10^8$ (p.o.); BP121-M, cisplatin (i.p.) + BP121 $1x10^9$ (p.o.); BP121-H, cisplatin (i.p.) + BP121 $1x10^{10}$ (p.o.), AST120, cisplatin (i.p.) + AST120 (p.o.; Fig. 1). BP121 was obtained from. Green Cross Wellbeing Corp., (Fig. 1).

Intestinal permeability. Intestinal permeability was measured by administering fluorescein isothiocyanate-labeled dextran (60 mg/kg body weight, MW = 4,000 kDa; Sigma-Aldrich; Merck KGaA) to rats by oral gavage on day 4 after cisplatin injection. At 4 h after gavage, 0.6 ml of blood was drawn via the jugular vein and the fluorescence intensity of the plasma was measured using a GloMax-Multi+ fluorophotometer (excitation/emission, 490/525 nm; Promega Corporation).

Histological analysis. Kidney and small intestine tissues were fixed at room temperature for 48 h in 10% neutral buffered formalin, embedded in paraffin, and sectioned at a thickness of 4 μm. Kidney tissue sections were stained at room temperature with periodic acid-Schiff (PAS) (periodic acid for 5 min and Schiff reagent for 15 min) and observed under a light microscope. The staining intensity (reflecting the area of tissue damage) was assessed in five randomly selected areas at x200 magnification as follows: None (score: 0) = normal,
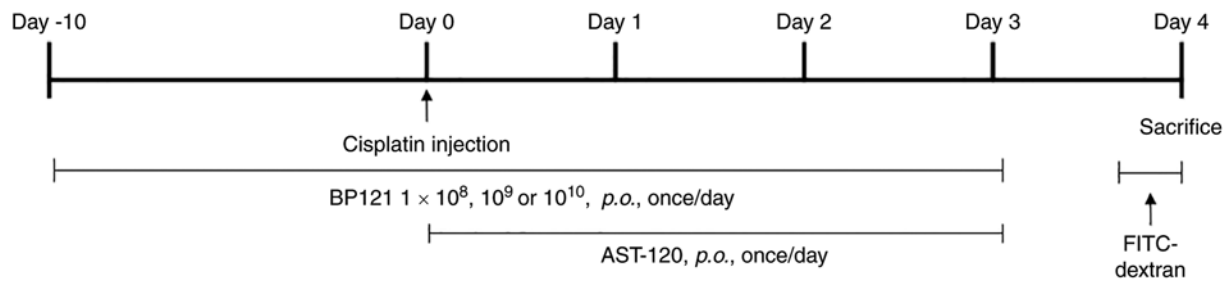
Figure 1. Diagram of the study design. P.o., per os; FITC, fluorescein isothiocyanate.
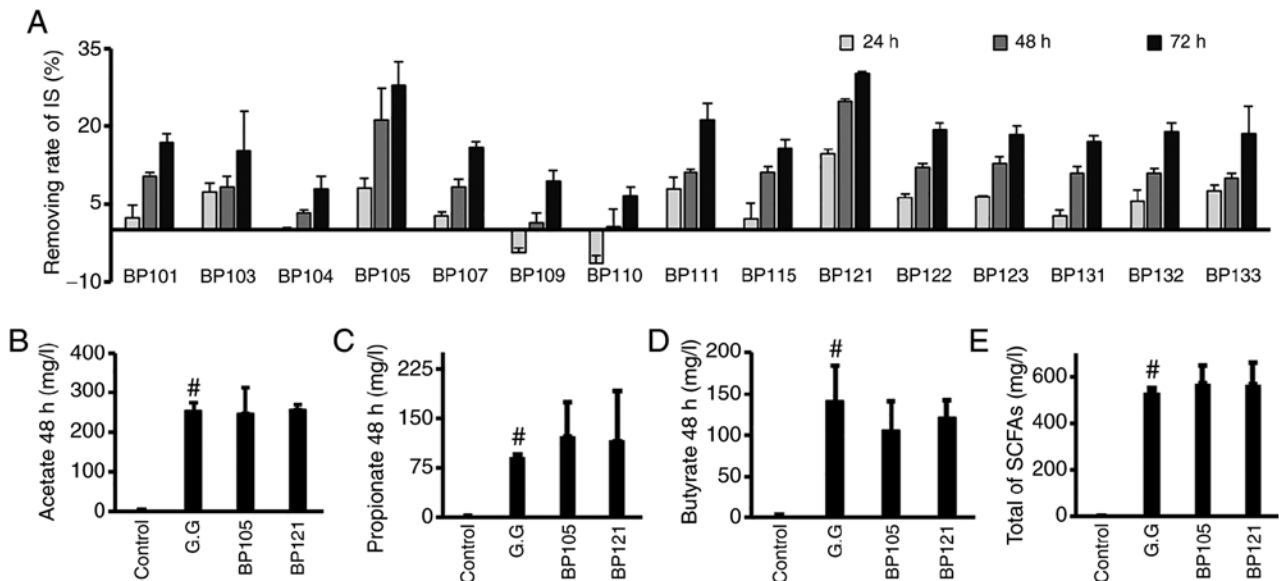

Figure 2. Effect of LAB strains on IS inhibiting effects and comparison of SCFA production capacity between *Lactobacillus salivarius* BP121, *Lactobacillus acidophilus* BP105 and *L. rhamnosus* GG. (A) Rate of IS removal. (B) Acetate, (C) propionate, (D) butyrate and (E) total of SCFAs. SCFA, short chain fatty acids. [#]P<0.05, normal vs. *Lactobacillus rhamnosus* GG.

weak (score: 1) = <10%, mild (score: 2) = 10-25%, moderate (score: 3) = 26-75% and strong (score: 4) = >75% (24). Immunohistochemical detection of monocyte chemoattractant protein (MCP)1, B cell lymphoma 2-associated X protein (Bax) in kidneys, zona occludins (ZO)-1, and occludin in small intestine was performed using a commercial kit (Abcam; cat. no. ab80436) and antibodies [all from Abcam; cat. nos. ab9668 (1:500), ab32503 (1:250), ab96587 (1:500) and ab216327 (1:500), respectively]. Apoptotic cells were detected with the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay using the TACS 2 TdT DAB (diaminobenzidine) kit (Trevigen, Inc.; cat. no. 4810-30-k) according to manufacturer's protocol. The average positive cell number was calculated by observing 10 fields at x200 magnification using a light microscope.

Biochemical parameters. Blood samples were collected from rats and serum levels of blood urea nitrogen (BUN), creatinine, and C-reactive protein (CRP) were measured with a Model 7020 automated biochemistry analyzer (Hitachi, Ltd.). IS and *p*-cresol sulfate (PCS) concentrations in serum were analyzed by liquid chromatography (ACQuity™) followed by tandem mass spectrometry (TSQ Quantum Ultra; Thermo Fisher Scientific, Inc.) (Data SI).

Evaluation of oxidative stress and inflammation. A 10-ml volume of phosphate-buffered saline was added to 0.5 g kidney tissue cut into 1-2 mm pieces and homogenized. The homogenate was centrifuged at 5,000 x g for 10 min at 4°C; the supernatant was collected and the levels of interleukin (IL)-6 (cat. no. MBS824560) and tumor necrosis factor (TNF)-α (cat. no. MBS175904) (both from MyBioSource); malondialdehyde (MDA; Cayman Chemical; cat. no. 10009055); and reduced to oxidized glutathione ratio (GSH/GSSG; Abcam; cat. no. ab138881) were analyzed.

Analysis of gut microbiota abundance. Fecal samples for gut microbiome profiling were collected at 4 days after cisplatin injection and stored at -70°C until analysis. Species were identified by 16S rRNA pyrosequecing using the EzTaxon database at Chunlab of Seoul National University (Seoul, South Korea). The procedure included sorting and quality pre-screening, trimming of primer sequences, schematic structure assignment, and chimera testing.

Effect of BP121 on tight junction (TJ) regulation in Caco-2 cells. Caco-2 cells were treated with 20 μM H₂O₂ (Volatile Free Acid Mix; Sigma-Aldrich; Merck KGaA; cat. no. CRM46975) to cause TJ protein damage; the culture supernatant of
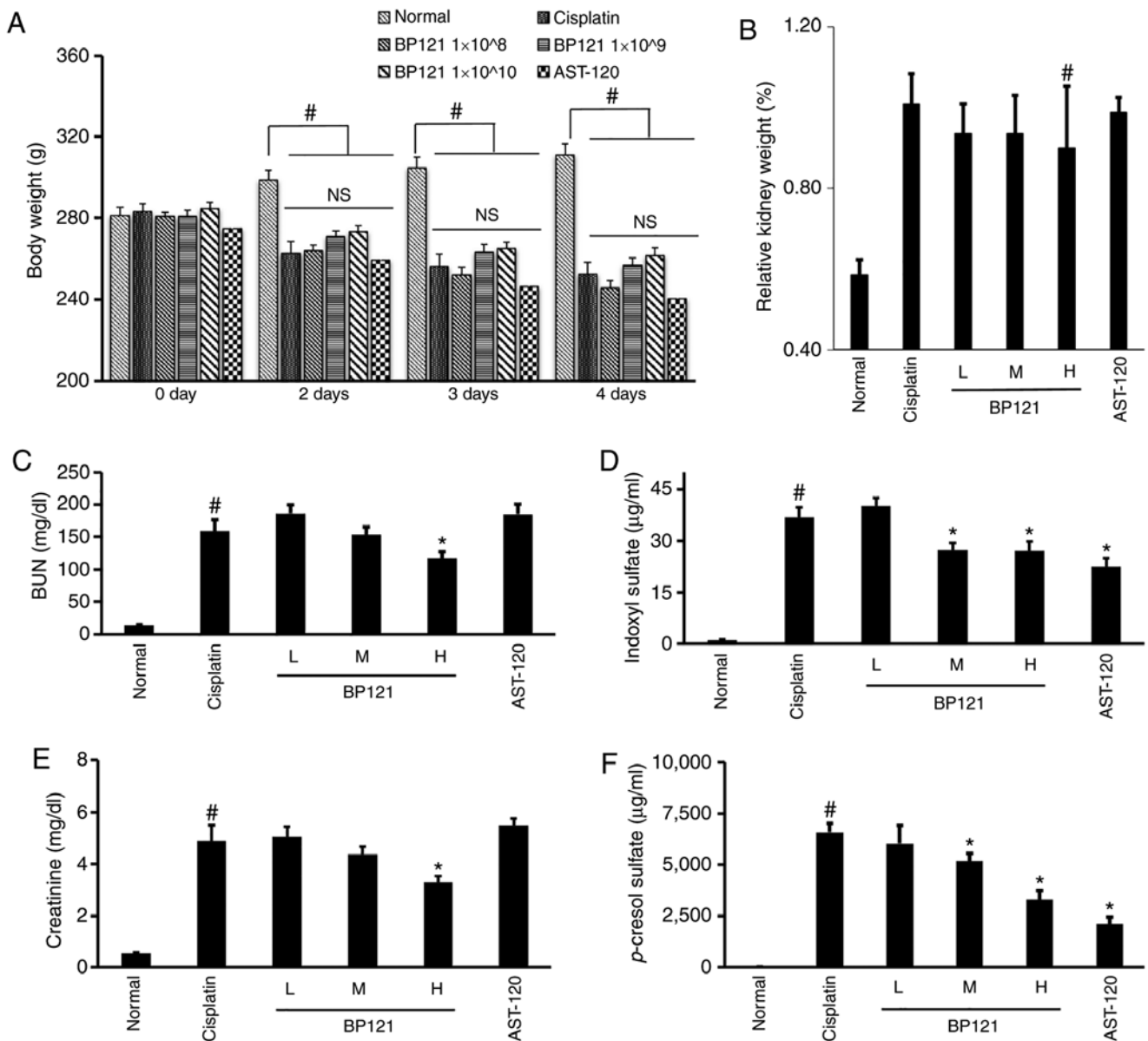
Figure 3. Physiological parameters in rats with cisplatin-induced AKI. Changes in (A) body weight and (B) relative kidney weight. Concentration of the urinary toxins (C) BUN, (D) IS, (E) CREA and (F) *p*-cresol sulfate in rats with cisplatin-induced AKI. L = BP121 1x10⁸, M = BP121 1x10⁹, H = BP121 1x10¹⁰. #P<0.05, normal vs. cisplatin; *P<0.05, cisplatin vs. treatment group. AKI, acute kidney injury; BUN, blood urea nitrogen; IS, indoxyl sulfate; CREA, creatinine; NS, not significant.

BP121 was used as a control. The 5' AMP-activated protein kinase (AMPK) inhibitor Compound C (cat. no. P5499) and the Toll-like receptor (TLR)4 inhibitor TLR-IN-C34 (cat. no. SML0832; both from Sigma-Aldrich; Merck KGaA) were used to evaluate the mechanism of TJ protein regulation. Each inhibitor was applied 30 min after H_2O_2 treatment. SCFAs and culture medium were added simultaneously with H_2O_2. ZO-1 and occludin were detected by immunofluorescence after incubation at 37˚C for 24 h; Alexa Fluor 488 (cat. no. ab150077; 1:400) and Alexa Fluor 647 (cat. no. ab150079; 1:400) (both from Abcam) were used as secondary antibodies and nuclei were stained with DAPI at 37˚C for 15 min (Sigma-Aldrich; Merck KGaA; cat. no. D9542).

Statistical analysis. Data are expressed as the mean ± standard error. Data were analyzed using SPSS (version 21.0; IBM Corp.) software. After homogeneity of variances using Levene's test, one way ANOVA analysis was performed to evaluate the significant differences, with Tukey's post-hoc test. P<0.05 was considered to indicate a statistically significant difference. Experiments were repeated 5-6 times for the *in vivo* investigations, or 3 times for the *in vitro* investigations.

Results

Isolation and selection of strains from infant feces. To screen for IS-inhibiting bacteria, bacteria obtained from fresh feces of newborn infants were grown on MRS agar plates. A total of 15 strains of LAB were selected and cultured in MRS broth with IS for 72 h; the strain with the highest inhibitory effect on IS was found to be BP121. This strain was able to reduce the levels of IS by >30% after 72 h. To identify this strain, analysis of
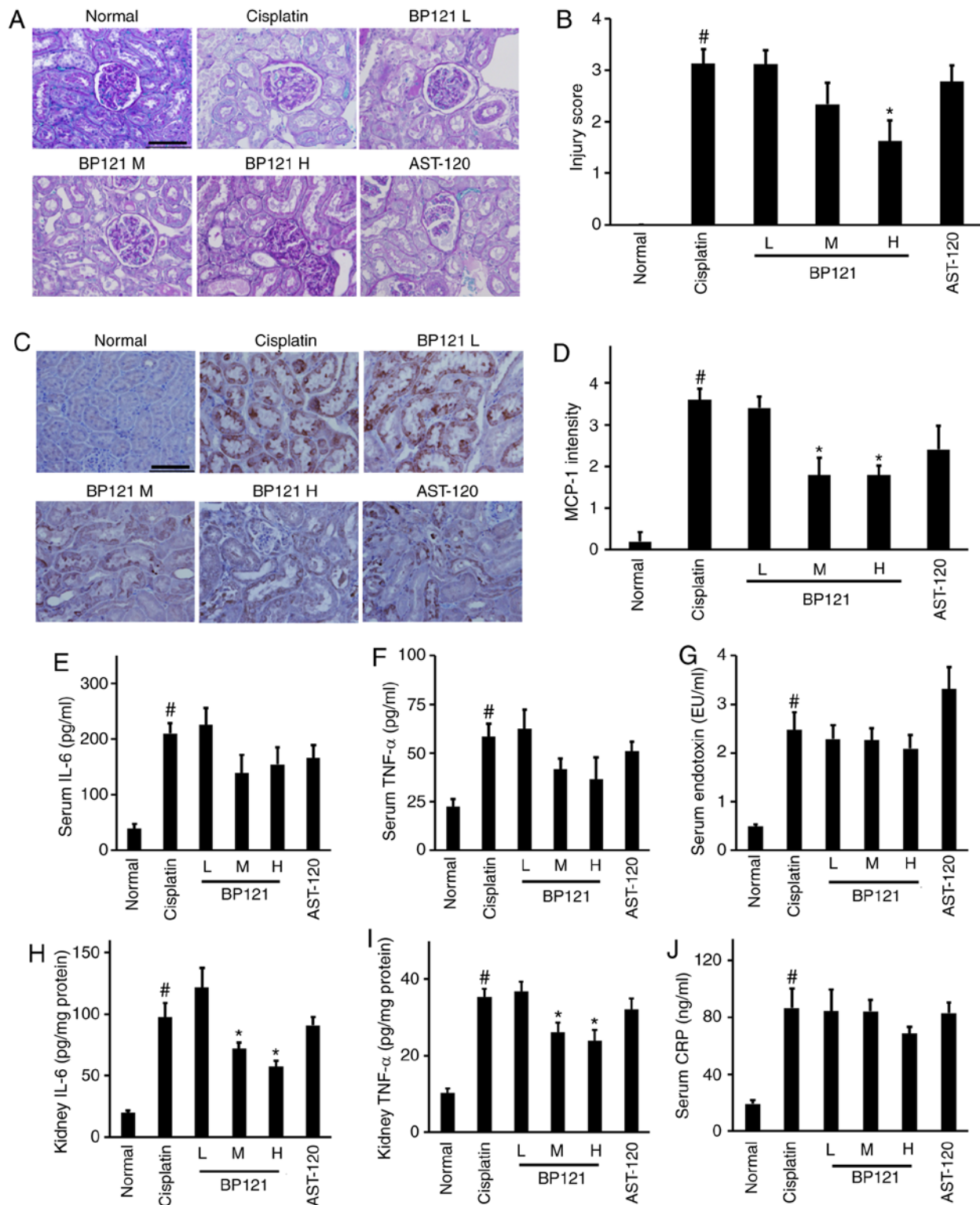
Figure 4. Histological analysis of the kidney and IL-6, TNF-α, and CRP expression following cisplatin-induced AKI. (A) PAS staining and (B) injury score. (C) MCP-1 immunoreactivity and (D) intensity. Concentrations of (E) serum IL-6, (F) TNF-α, (G) serum endotoxin, (H) kidney IL-6, (I) kidney TNF-α and (J) CRP levels were measured by ELISA. Scale bar=100 µm. #P<0.05, normal vs. cisplatin; *P<0.05, cisplatin vs. treatment group. IL, interleukin; TNF, tumor necrosis factor; CRP, c-reactive protein; PAS, periodic acid Schiff; AKI, acute kidney injury.

the 16S rRNA gene sequence of BP121 (Table SI) revealed that it is closely related to *Lactobacillus salivarius* JCM1231, with 99.93% sequence similarity. After culturing BP121 and BP105 for 48 h, SCFA production was analyzed. Both strains produced SCFA similar to *L. rhamnosus* GG (Fig. 2 B-E). Based on these results, BP121 was selected as the test strain, which showed the highest IS inhibitory effect and high SCFA production (Fig. 2).

BP121 restores kidney weight and reduces serum urinary toxin levels. Rats that were administered BP121 did not show
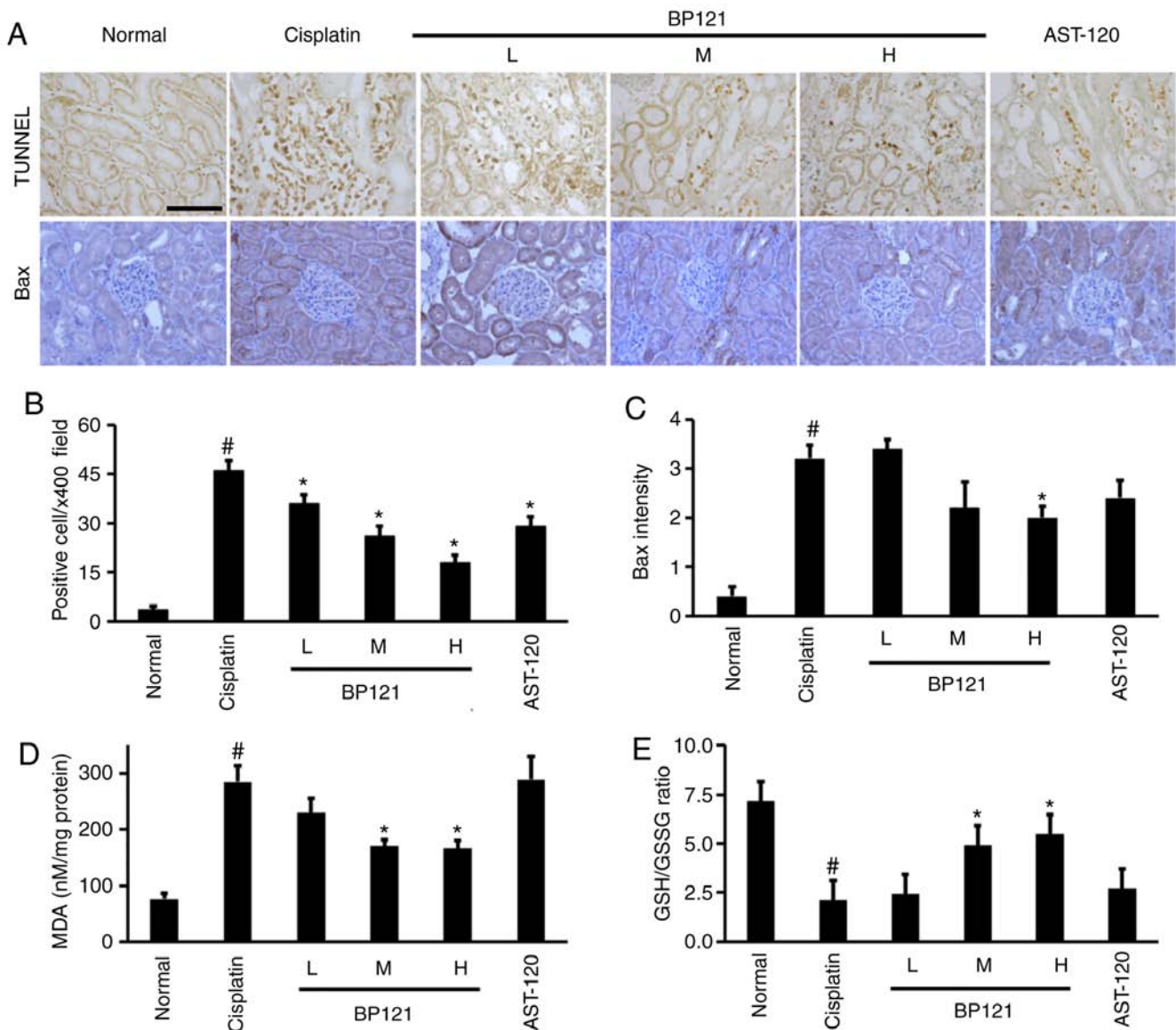
Figure 5. Histological analysis of kidney in rats with cisplatin-induced acute kidney injury. (A) TUNEL results are shown as (B) the number of positive cells per field at magnification, x400. (C) Bax immunolabeling. (D) MDA level and (E) GSH/GSSG ratio in kidney. Scale bar=100 μm. #P<0.05, normal vs. cisplatin; *P<0.05, cisplatin vs. treatment group. MDA, malondialdehyde.

any difference in body weight compared with those that were treated with cisplatin (Fig. 3A). On the other hand, the increase of relative kidney weight in cisplatin treated rats was partially decreased in rats who were administered a high dose of BP121 (Fig. 3B). Similar trends were observed for serum BUN and creatinine levels (Fig. 3C and E). In addition, BP121 caused a dose-dependent reduction in IS and PCS concentrations (Fig. 3D and F). AST-120 had no effect on body weight, relative kidney weight, or blood BUN and creatinine levels, but significantly reduced IS and PCS levels in the blood (Fig. 3).

BP121 suppresses inflammation and injury in kidney. PAS stained kidney tissue revealed extensive renal damage following cisplatin treatment; this was reduced by BP121-H administration (Fig. 4A and B). In addition, the increase in the number of MCP-1-positive cells induced by cisplatin was reversed by BP121 (Fig. 4C and D). Although there were no differences in serum levels of IL-6, TNF-α, endotoxin and CRP between

the cisplatin- and BP121-treated groups (Fig. 4E, G and J), IL-6 and TNF-α concentrations in kidney tissue homogenate were significantly reduced by BP121 treatment (Fig. 4H and I). Despite a substantial decrease of both IS and PC, AST-120 failed to show a renal protective effect (Fig. 4A-I).

BP121 inhibits apoptosis and oxidative stress in the kidney. The number of TUNEL positive apoptotic cells significantly increased in cisplatin induced AKI and BP121 treatment dose dependently reduced apoptosis.

A similar trend was observed in the expression of the pro-apoptotic protein Bax, which was upregulated in the kidney of cisplatin-treated rats and downregulated upon administration of a high dose BP121 (Fig. 5A-C). The MDA level and GSH/GSSG ratio were also measured as indices of oxidative damage and found that lipid peroxidation (MDA level) was increased whereas GSH/GSSG ratio was decreased by cisplatin injection; these changes were partially reversed by pretreatment
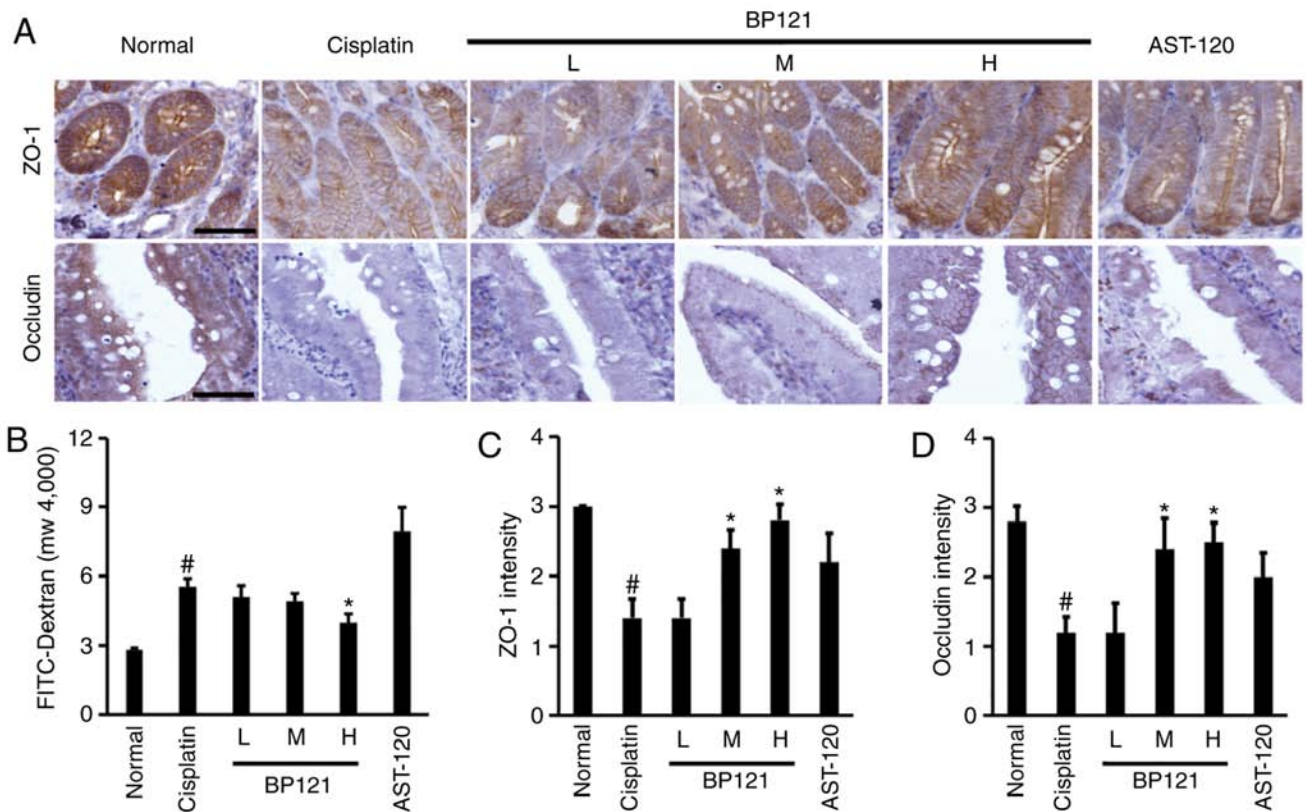
Figure 6. Histological evaluation of intestinal tissue in rats with cisplatin-induced acute kidney injury. (A) Immunohistochemical detection of ZO-1 and occludin. (B) Intestinal permeability as measured by FITC-dextran (MW=4,000 kDa). (C) Scoring of ZO-1 and (D) occludin immunoreactivity. Scale bar=100 μm. #P<0.05, normal vs. cisplatin; *P<0.05, cisplatin vs. treatment group. FITC, fluorescein isothiocyanate; ZO, zona occludens.
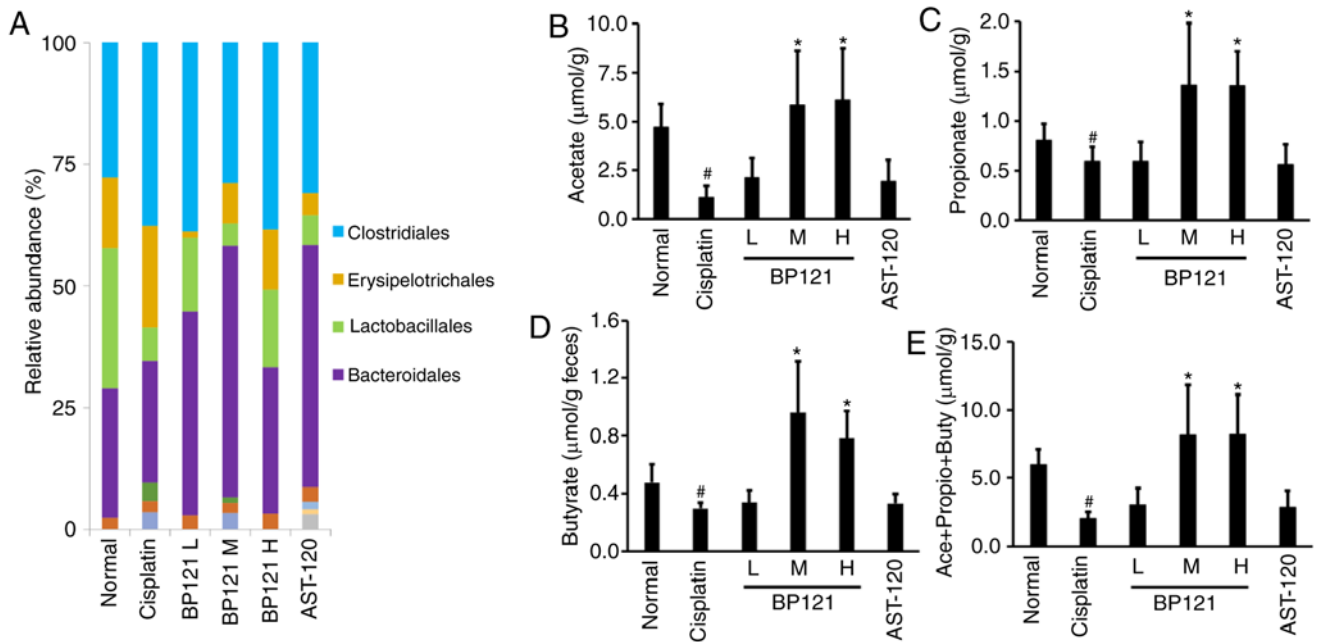

Figure 7. Change in microbiota abundance in the feces of rats with cisplatin-induced acute kidney injury following BP121 administration. (A) Relative abundance of gut microbiota under indicated conditions. (B) Acetate, (C) proprionate, (D) butyrate and (E) Ace+Propio+Buty concentrations in feces. #P<0.05, normal vs. cisplatin; *P<0.05, cisplatin vs. treatment group.

with medium and high concentrations of BP121 (Fig. 5D and E). No changes in oxidative stress relative to the cisplatin-treated group was observed upon AST-120 administration.

BP121 reverses cisplatin-induced increase in intestinal permeability. Intestinal permeability was increased by cisplatin; this was accompanied by downregulation of ZO-1
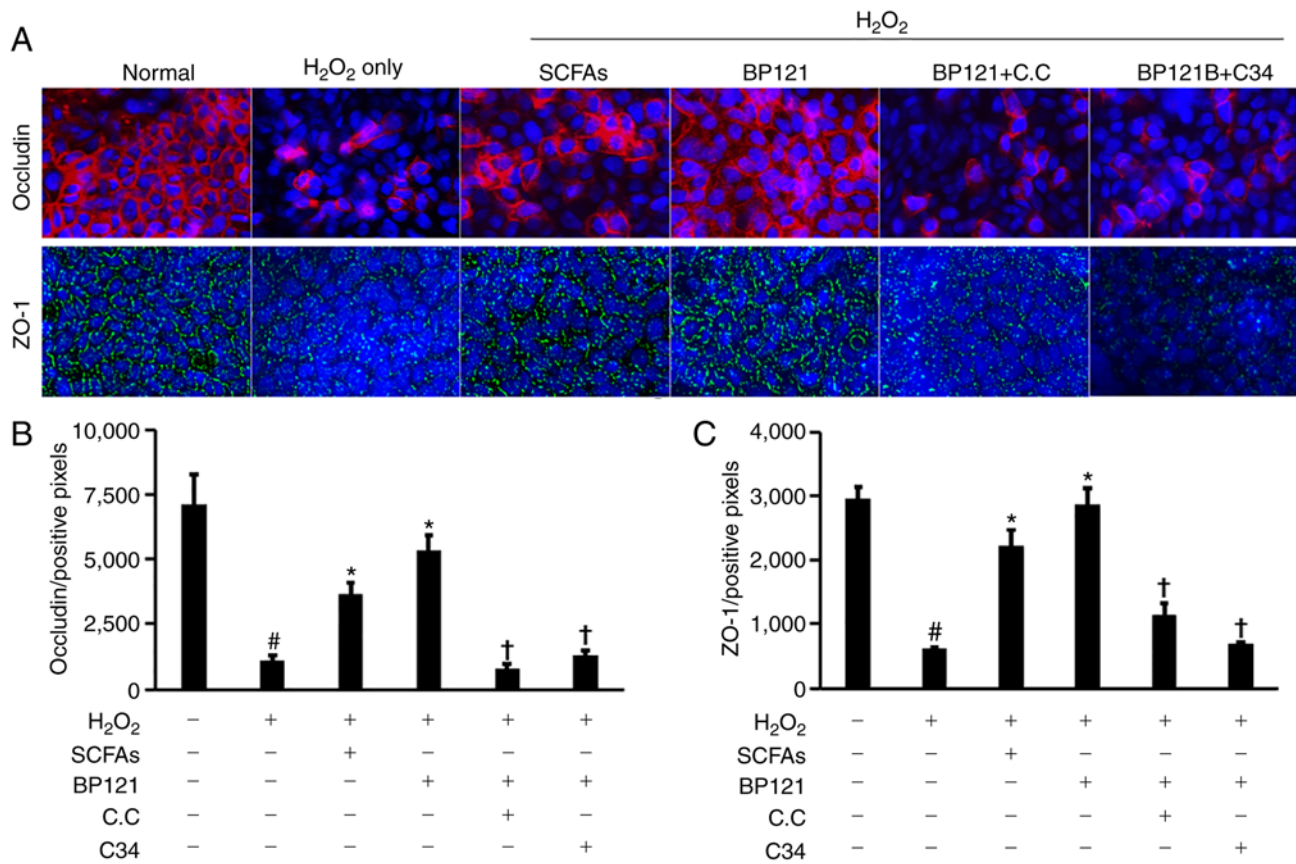
Figure 8. Expression of tight junction proteins in Caco-2 cells following H_2O_2 treatment. (A) Immunocytochemical detection of occludin and ZO-1 under indicated treatment conditions; nuclei were stained with DAPI. Quantitative analysis of (B) occludin and (C) ZO-1 immunoreactivity. BP121, treatment with BP121 culture supernatant for 48 h; C.C, Compound C (AMPK inhibitor); C34, TLR4-IN-C34 (TLR4 inhibitor). #$P<0.05$, untreated vs. H_2O_2-treated; *$P<0.05$, H_2O_2-treated vs. SCFA- and BP121 supernatant-treated; †$P<0.05$, BP121 supernatant-treated vs. C.C- and C34-treated. SCFA, short chain fatty acids; ZO-1, zona occludens; TLR, toll-like receptor.

and occludin (Fig. 6A-D). However, medium and high doses of BP121 restored the levels of these proteins and reduced intestinal permeability. Treatment with AST 120 did not affect TJ protein expression or intestinal permeability.

BP121 causes changes in intestinal flora and stimulates SCFA production. The 16S rRNA analysis showed that the proportion of *Lactobacillales*, a representative order of beneficial bacteria, was decreased by cisplatin; however, the abundance was restored upon BP121 administration. On the other hand, there were no changes in the proportion of *Clostridiales*, an order of harmful bacteria, across treatment groups (Fig. 7A). The concentrations of the representative SCFAs acetate, propionate and butyrate, which are known to regulate the expression of TJ proteins-in feces were examined and it was observed that they were decreased in cisplatin-treated rats. However, medium and high concentrations of BP121 increased the levels of all three SCFAs. AST-120 administration had no effect on SCFA levels (Fig. 7B-E).

BP121 increases TJ protein expression in Caco-2 cells. In vitro experiments were carried out using Caco-2 cells to clarify the mechanism by which BP121 improves intestinal permeability. H_2O_2 treated Caco-2 cells showed a marked decrease of both occludin and ZO-1 expression and pretreatment with SCFA or BP121 partially restored their expression. However, cotreatment

with inhibitors of AMPK or TLR4 blocked the beneficial effect of BP121, suggesting that BP121 mediated barrier modulation is dependent on AMPK and TLR4 signaling (Fig. 8A-C).

Discussion

The human intestine contains over 1,000 species of bacteria that interact dynamically with the host, which plays an important role in health and disease (25). The microbiota is important for maintaining the integrity of the intestinal barrier as well as for immunological tolerance and metabolic activities that have not evolved in the host (26,27). Intestinal dysbiosis is implicated in various pathological processes including inflammatory bowel disease, diabetes, obesity and AKI (27-29). Supplementing the gut microbiome with beneficial bacteria such as LAB can promote host health.

The present study investigated the effects of *L. salivarius* BP121, one of the 15 intestinal microorganisms isolated from the feces of newborns-on AKI. Previous studies have shown that the two-peptide bacteriocin ABP-118 produced by *L. salivarius* inhibits the growth of pathogenic bacteria but not most *Lactobacillus* species (30,31). The current study speculated that *L. salivarius* could contribute to the treatment of diseases caused by dysbiosis. Indeed, the present results showed that BP121 increased the abundance of *Lactobacillales* in rats with cisplatin-induced dysbiosis.

The intercellular junctional complex is an important component of the intestinal barrier. TJ proteins such as ZO-1 and occludin seal the paracellular space between epithelial cells (32), thus preventing the leakage of solutes such as microorganisms and antigens as they are transported across the epithelium (33). In this study, BP121 increased the expression of TJ proteins that were downregulated by H_2O_2 and cisplatin treatments. This is consistent with a previous study in which *Lactobacillus rhamnosus* GG increased the expression of human epithelial TJ proteins *in vivo*, which conferred protection against alcohol-induced epithelial barrier disruption (34). Moreover, *L. plantarum* administration increased ZO-1 and occludin levels in an animal model of alcoholic liver disease (34), and prevented the loss of TJ proteins and abolished the increase in epithelial permeability induced by phorbol ester in Caco-2 cells (35). Probiotics prevent damage to TJs by stimulating lipoxygenase activity via SCFAs (36). The maintenance of TJ proteins also involves activation of AMPK signaling (34,35). In the present study, a 48-h treatment with BP121 culture supernatant containing SCFAs alleviated TJ protein damage caused by H_2O_2 in Caco-2 cells; however, this effect was blocked by AMPK inhibitor. These results are in agreement with previous reports that SCFAs such as butyrate and acetate can preserve intestinal barrier integrity by SCFAs (37-39).

SCFAs are straight-chain saturated FAs with fewer than six carbon atoms; acetate, propionate and butyrate are the most abundant SCFAs in the human intestinal tract (11). SCFAs are the end products of fermentation of complex polysaccharides (40) including non-digestible dietary fibers such as inulin (41) and endogenous substrates such as epithelium-derived mucus (42). SCFAs are not only present in the gut, but can also be absorbed in the bloodstream (13) and protect against kidney injury by suppressing pro-inflammatory chemokines and cytokines such as IL-6, TNF-α, and nitric oxide along with the production of reactive oxygen species (ROS) and apoptosis (43,44), and/or by stimulating the production of the anti-inflammatory cytokine IL-10 (45) via G protein-coupled receptor activation (46) or histone deacetylase inhibition (47).

The antioxidant properties of probiotics have been confirmed and demonstrated by multiple studies (48,49). ROS were shown to mediate cisplatin-induced AKI (5,8) and it was demonstrated that H_2O_2 and OH radicals are involved in cisplatin-induced necrosis and apoptosis, respectively (49). In this study, BP121 decreased lipid peroxidation and increased the GSH/GSSG ratio; this was accompanied by reductions in the number of TUNEL- and Bax-positive cells and decreases in the levels of MCP-1 and the pro-inflammatory cytokines IL-6 and TNF-α in kidney tissue.

IS and PCS are uremic toxins that are not efficiently removed by hemodialysis (50). They are generated from bacterial fermentation of tryptophan, aromatic amino acids and tyrosine followed by sulfation in the liver or colonic mucosa (50). In the plasma, IS and PCS are protein-bound and accumulate upon kidney failure (51). The concentrations of both toxins are correlated with progression of chronic kidney disease and higher risk of mortality (52). The observed increases in serum IS and PCS levels could result from enhanced production of these toxins, presumably due to an increase in the abundance of specific microbiota and disruption of the intestinal barrier by IS itself or by a decline in the *Lactobacillales* population (53). In the present study, treatment of AKI rats with BP121 stabilized intestinal barrier function and reduced serum levels of IS and PCS, which suppressed in renal inflammation.

In conclusion, the current results demonstrate that the newly isolated *L. salivarius* strain BP121 protects against cisplatin-induced AKI by suppressing IS and PCS production through modulation of the gut environment and regulation of TJ protein assembly via AMPK activation. These findings suggest that probiotics including BP121 are an effective treatment for dysbiosis and can prevent AKI.

Availability of data and materials

All data generated or analyzed in the present study are included in this published article.

Authors' contributions

THL, DP and YJK contributed to the study conception and design, as well as the data acquisition and analysis, interpretation. THL, DP drafted the manuscript. IL performed the immunohistochemical staining and *in vitro* studies. SK, CTO and JYK contributed to data interpretation. JY and SKJ contributed in study conception and reviewing the draft. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Institutional Animal Care and Use Committee of Hoseo University [approval no. HSIACUC-18-097(1)].

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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