Original Article

TRPA1 promotes cisplatin-induced nephrotoxicity through inflammation mediated by the MAPK/NF-κB signaling pathway

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Background: The nephrotoxicity induced by cisplatin (DDP) has been a severe obstacle for its clinical use in anticancer treatment. The apoptosis and inflammation induced by DDP are the main causes of the nephrotoxicity. Transient receptor potential ankyrin 1 (*TRPA1*) is a non-selective cation ligand-gated channel that is involved in the inflammation progress.

Methods: The apoptosis, inflammation, MAPK/NF-κB signaling pathway, and *TRPA1* expression were assessed after HEK293 cells had been induced by DDP, and the role of *TRPA1* in apoptosis and inflammation of DDP-induced HEK293 cells treated with *TRPA1* antagonist HC-030031 was also evaluated by quantitative reverse transcription-polymerase chain reaction (qRT-PCR), flow cytometry, and western blot assays.

Results: The cell viability was reduced by DDP in both a time-dependent and dose-dependent manner with a minimal cytotoxic concentration of 10 μM. Moreover, DDP induced an enhancement of the apoptosis and inflammation in a dose-dependent manner, as indicated by the increase of the relative protein level of cleaved-caspase3 (cleaved-cas3), the cleavage product of caspase-3 substrate poly-ADP-ribose polymerase (cleaved-PARP) and inducible nitric oxide synthase (iNOS), and the messenger RNA (mRNA) expression level of interleukin (*IL*)*-1β*, *IL-6*, tumor necrosis factor-α (*TNF-α*), and interferon-γ (*INF-γ*). Additionally, DDP treatment increased the protein phosphorylation expression of IKKβ, JNK, ERK, and p38 in a dose-dependent manner, which was antagonized by the treatment of NF-κB-specific inhibitor BAY 11-7082 and pan-MAPK inhibitor U0126. It was also found that DDP upregulated the expression of *TRPA1* at both the mRNA and protein levels in a dose-dependent manner. Besides, block of *TRPA1* with HC-030031 relieved the apoptosis, diminished the level of *IL-1β*, *IL-6*, *TNF-α*, and *INF-γ*, reduced the level of cleaved-cas3, cleaved-PARP, and iNOS, decreased the p-IKKβ, p-JNK, p-ERK, and p-p38 expression, and enhanced the expression of IκBα.

Conclusions: Taken together, these results indicate that *TRPA1* regulates DDP-induced nephrotoxicity via inflammation mediated by the MAPK/NF-κB signaling pathway in HEK293 cells.

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Keywords: Cisplatin (DDP); nephrotoxicity; transient receptor potential ankyrin 1 (*TRPA1*); apoptosis; MAPK/NF-κB signaling pathway

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Introduction

Cisplatin (DDP) is a leading chemotherapy drug in the treatment of a variety of malignant solid tumors (1-3), such as non-small cell lung carcinoma, ovarian, head and neck, testicular, cervical, and numerous other cancers (2-5). Although DDP has become a mainstay for cancer therapy, its side effects are also non-negligible in clinical practice, and its nephrotoxicity has been a particularly dominating obstacle that restricts the use and efficacy of DDP in tumor therapy (4,6-10). Currently, about one-third of patients have been shown to experience nephrotoxicity with DDP treatment (4,11), among which acute kidney injury (AKI) is the most common and serious manifestation of nephrotoxicity (12). Renal apoptosis (13) and inflammation (14) have been recognized as the most important mechanism underlying DDP-induced nephrotoxicity.

The nephrotoxicity induced by DDP principally exists in proximal tubule epithelial cells (15). A vital factor underlying DDP-induced cellular apoptosis and inflammation is the massive production of oxygen free radicals. Atessahin *et al.* (16) showed that DDP-treated male rats have singlet oxygen, and other study also found that DDP can cause the increase of 0_2 of renal cells in male rats (17). The lack of antioxidant protection also plays an important role in DDP-induced cellular apoptosis and inflammation. The most effective cellular antioxidant system is the glutathione (GSH) oxidation cycle. As it passes through tubule epithelial cells, DDP depletes the level of endogenous oxide scavenger GSH, which leads to the imbalance of intracellular oxidation and accumulation of reactive oxygen species (ROS) to induce a series of stress responses (18,19). The ROS can activate a signaling cascade, such as the MAPK (20) and NF-κB (21) signal pathway, which triggers the production and release of numerous pro-inflammatory cytokines, such as interleukin (*IL*)-*1β*, *IL-6*, tumor necrosis factor-α (*TNF-α*), and interferon-γ (*INF-γ*) (22), as well as the inflammatory mediator inducible nitric oxide synthase (iNOS) (23). Consequently, the production and release of the pro-inflammatory cytokines and inflammatory mediator cause renal apoptosis and inflammation, and eventually lead to renal failure.

Transient receptor potential ankyrin 1 (*TRPA1*) is a non-selective cation ligand-gated channel belonging to the family of transient receptor potential (TRP) ion channels (24). Beside the major function of thermosensation and nociception (25,26), the role of inflammation that *TRPA1* plays has also attracted extensive research (25). The upregulation of *TRPA1* function can maintain or even aggravate the inflammatory response (27). A previous study has also suggested that *TRPA1* can contribute to the inflammation of the carrageenan-induced paw edema in mice via the pharmacological method (28). Meanwhile, the genetic deletion of *TRPA1* reduced nociception and inflammation in monosodium urate crystal-induced gouty arthritis (29) and monosodium iodoacetate-induced arthritis (30). Recently, our lab has suggested that *TRPA1* is expressed in human renal tubular epithelial cells and the hypoxia and reoxygenation that can imitate AKI significantly increases the expression of *TRPA1* (unpublished data), but its function in renal tubular epithelial cells is still unclear. Thus, in this study, we explored the role of *TRPA1* in DDP-induced nephrotoxicity. We hope our study can lay a foundation of the molecular regulation mechanism of *TRPA1* in DDP-induced nephrotoxicity. We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/atm-21-5125).

Methods

Reagents

Capsaicin, HC-030031, BAY 11-7082, and U0126 were all purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). The concentrations of the BAY11-7082, HC-030031, and U0126 used in the present study were 25, 10, and 10 μM, respectively. The remaining reagents utilized in the present study were of analytical purity and

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Table 1 The primary antibody used in the present study

Reagent or resource	Source	Identifier	Dilution concentration
Rabbit anti-caspase-3	Abcam	ab4051	1:1,000
Rabbit anti-cleaved-cas3	Abcam	ab2302	1:1,000
Rabbit anti-TRPA1	Sigma	SAB1411593	1:1,000
Rabbit anti-PARP	Abcam	ab74290	1:1,000
Rabbit anti-cleaved-PARP	Abcam	ab4830	1:1,000
Rabbit anti-iNOS	Abcam	ab178945	1:1,000
Rabbit anti-IKBα	Abcam	ab 7217	1:1,000
Rabbit anti-p-IKBα	Abcam	ab 24783	1:1,000
Rabbit anti-IKKβ	Abcam	ab 124957	1:1,000
Rabbit anti-p-IKKβ	Abcam	ab 38515	1:1,000
Rabbit anti-JNK	Abcam	ab 112501	1:1,000
Rabbit anti-p-JNK	Abcam	ab4821	1:1,000
Rabbit anti-ERK	Abcam	ab17942	1:1,000
Rabbit anti-p-ERK	Abcam	ab201015	1:1,000
Rabbit anti-P38	Abcam	ab170099	1:1,000
Rabbit anti-p-P38	Abcam	ab4822	1:1,000
Rabbit anti-β-actin	Abcam	ab8227	1:2,000
Goat anti-rabbit IgG H&L	Abcam	ab6721	1:5,000

commercially available.

Cell culture

The HEK293 cells (obtained from Punosai Life Technology Co., Ltd., Wuhan, China) were cultured in Dulbecco's modified Eagle medium (DMEM; Sigma, Germany) supplemented with 0.1% fetal bovine serum (FBS), 100 U/mL penicillin (Sigma, Germany), and 100 g/mL streptomycin (Sigma, Germany). Cultures were incubated at 37 ℃ with 5% carbon dioxide (CO_2) (31).

Cell viability assay

The HEK293 cells were inoculated in 96-well plates at a density of 1×10^5/well and maintained for 24 h. Then, cells were incubated with different concentrations of DDP (0, 5, 10, 20, 40, and 80 μM) for an accessional 24, 36, and 48 h respectively. Next, 10 μL of Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan) was appended for an incubation of 2 h. The results were analyzed by a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) at 450 nm.

Detection of apoptosis

After treatment with the given drugs for 48 h, the HEK293 cells were stained with 5 μL allophycocyanin (APC; Sigma, Germany) and 5 μL Annexin V-PE (Sigma) for 25 min. The flow cytometry (Becton, Dickinson, and Co., Franklin Lakes, NJ, USA; FACSVerse) was used to detect the cellular apoptotic rate.

Western blot analysis

Total protein from the cell samples was extracted, and the concentration of protein was determined by the bicinchoninic acid (BCA) protein quantification kit. The assays were executed according to the previous report (32). In brief, the protein samples were separated and electrically transferred to a polyvinylidene fluoride (PVDF) membrane. The membrane was maintained with the primary antibody (*Table 1*) overnight at 4 ℃ after pre-blocking with tris-buffered saline with Tween 20 [TBST; containing 3% bovine serum albumin (BSA)] at room temperature for 2 h. After 3 washes with TBST, the membrane was hatched with goat-anti-mouse IgG (H&L)-HRP or goat-anti-rabbit IgG (H&L)-HRP (1: 5,000; Abcam, Cambridge, UK) for 2 h at 37 ℃. An enhanced chemiluminescence kit (ECL; Affinity, San Francisco, CA, USA, KF001) was used to visualize the reaction for 1 min.

Reverse transcriptase-polymerase chain reaction (RT-PCR)

Cell samples were used to obtain the total RNA by Animal Total RNA Isolation Kit (Foregene, Chengdu, China; RE-03014) based on the operating instruction. Complementary DNA (cDNA) was synthesized with a PrimeScript RT reagent Kit (Takara, Kusatsu, Shiga, Japan; RR047A) according to the operating instruction. Quantitative RT-PCR (qRT-PCR) was executed according to the A PIKORed 96 (Thermo Fisher, USA) with the TB Green TM Premix Ex Taq^{TM} II (Tli RNaseH Plus) (Takara, RR820A) using primers listed in *Table 2* based on the previous study (33).

Statistical analysis

The one-way analysis of variance (ANOVA) and Duncan's test were utilized to analyze all the data in the present study

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Table 2 Primers used in this study

Primer name	Forward primer (5'-3')	Reverse primer (5'-3')
β-actin	GAAGATCAAGATCATTGCTCC	TACTCCTGCTTGCTTGCGATCCA
IL-1β	ATCCTCTCCAGTCAGGCTTCCTTGTG	AGCTCTTGTCGAGATGCTGCTGTGA
IL-6	ACAGAGGATACCACCCACAACAGACC	CGGAACTCCAGAAGACCAGAGCAGAT
TNF-α	TGCCTGATATCGACCGAACAGCCAAC	ACAGATAGGGTCACAGCCAGTCCTCT
INF-γ	CAACCCACAGATCCAGCACAAAGC	CCCAGAATCAGCACCGACTCCTT

IL-1β, interleukin-1β; *IL*-6, interleukin-6; *TNF-α*, tumor necrosis factor-α; *INF-γ*, interferon-γ.

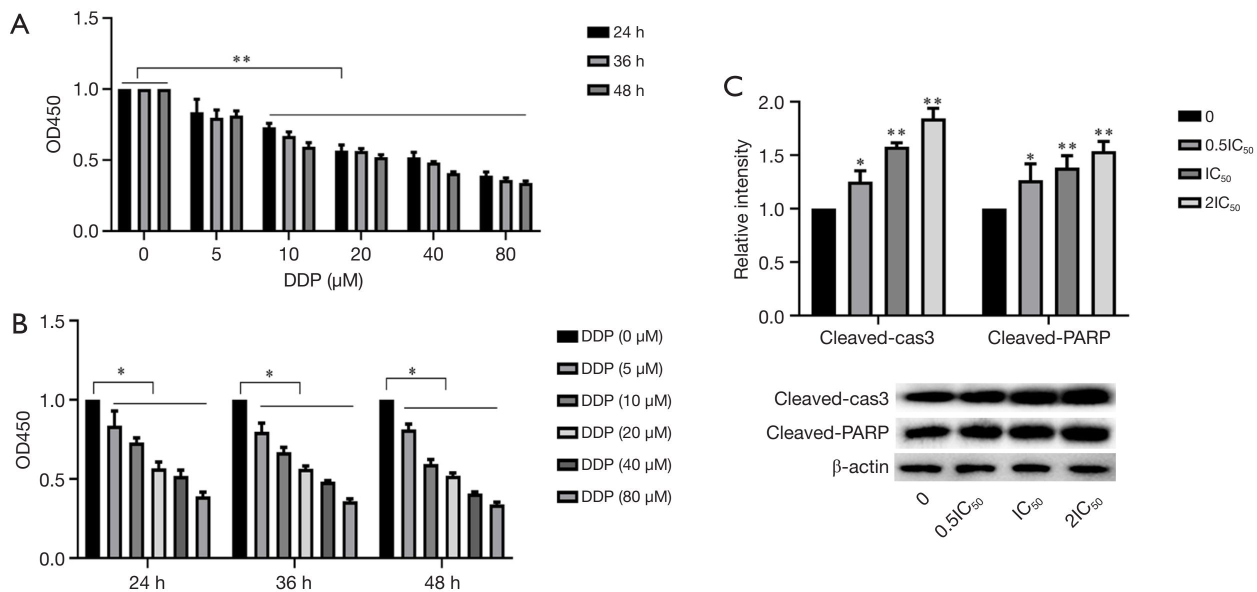

Figure 1 DDP inhibits the HEK293 cells viability and increases apoptosis. HEK293 cells were administrated with DDP with the concentration of 0, 5, 10, 20, 40, and 80 μM for 24, 36, and 48 h, respectively. DDP reduced the HEK293 cells viability in a time-dependent (A) and dose-dependent way (B). The cleaved-cas3 and cleaved-PARP protein levels were measured using western blot (C). The means ± SD of three independent samples were present. *P<0.05 and **P<0.01, compared with 0 group. DDP, cisplatin; SD, standard deviation; cleaved-cas3, cleaved-caspase3; cleaved-PARP, cleavage product of caspase-3 substrate poly-ADP-ribose polymerase.

using the software SPSS 20.0 package (SPSS Inc., Chicago, IL, USA). All data were exhibited as the means ± standard error of the mean (SEM), and the differences were thought statistically significant and extremely significant when P<0.05 and P<0.01, respectively.

Results

DDP decreased cell viability and increased apoptosis of HEK293 cells

To detect the HEK293 cells viability caused by DDP, HEK293 cells were treated with DDP at the concentrations of 0, 5, 10, 20, 40, and 80 μM for 24, 36, and 48 h, respectively. Then the cell viability was evaluated using the CCK-8 kit. The results revealed that 10 μM DDP is obviously cytotoxic for HEK293 cells, and DDP declined the cell viability both in a time-dependent and dose-dependent manner (*Figure 1A,1B*). Meanwhile, the IC_{50} of DDP for HEK293 cells was 25.03 μM at 48 h. To deeply evaluate the effect of DDP on HEK293 cells, we assessed the apoptosis of HEK293 cells treat with DDP with the concentration of 0, $0.5IC_{50}$, IC_{50}, and $2IC_{50}$ using the apoptosis-related protein. The results showed that

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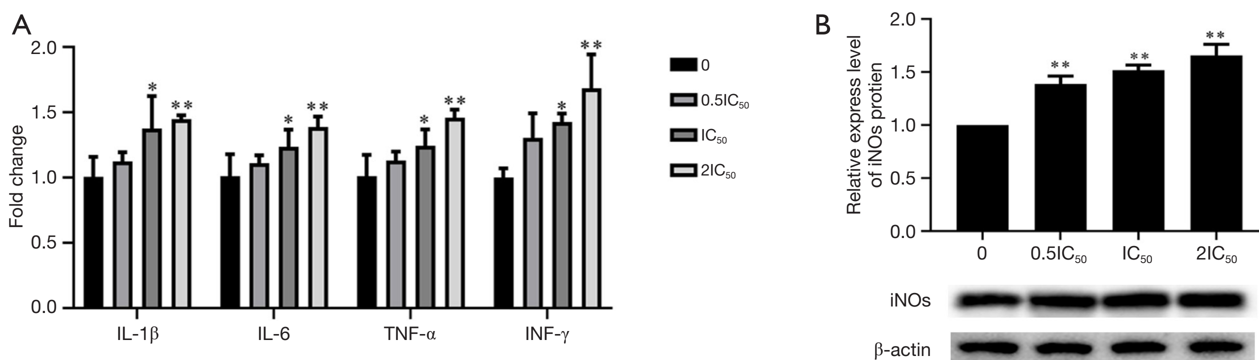

Figure 2 DDP boosted the inflammatory of HEK293 cells. HEK293 cells were administrated with DDP with the concentration of 0, $0.5IC_{50}$, IC_{50} and $2IC_{50}$ respectively. The expression level of *IL-1β*, *IL-6*, *TNF-α*, and *INF-γ* was detected by qRT-PCR (A). Also, the level of iNOS (B) was examined using western blot analysis respectively. The means ± SD of three independent samples were shown. The results were exhibited after being normalized to β-actin. *P<0.05 and **P<0.01, compared with 0 group. DDP, cisplatin; *IL-1β*, interleukin-1β; *IL-6*, interleukin-6; *TNF-α*, tumor necrosis factor-α; *INF-γ*, interferon-γ; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; iNOS, inducible nitric oxide synthase; SD, standard deviation.

the expression of cleaved-caspase3 (cleaved-cas3) and the cleavage product of caspase-3 substrate poly-ADP-ribose polymerase (cleaved-PARP) were elevated after HEK293 cells were treated with DDP in a dose-dependent way (*Figure 1C*).

DDP promoted HEK293 cells inflammation

Beside the renal apoptosis (13), renal inflammation (14) has also been noted as one of major factors of DDP-induced AKI. Thus, we explored the effect of DDP on the inflammation of HEK293 cells. Firstly, the expression of some typical pro-inflammatory factors such as *IL-1β*, *IL-6*, *TNF*-α, and *INF*-γ was detected in DDP-treated HEK293 cells using qRT-PCR. The results revealed that DDP treatment enhanced the *IL-1β*, *IL-6*, *TNF-α*, and *INF-γ* expression in a dose-dependent manner (*Figure 2A*). Subsequently, as one of most important inflammatory mediators, iNOS level also was determined using western blot analysis. Consistently, the expression of iNOS was improved after HEK293 cells were treated with DDP in a dose-dependent fashion (*Figure 2B*).

DDP acted on the MAPK/NF-κB signaling pathway

Due to its significance in the apoptosis- and inflammation-related signaling pathway, the expression of IKKβ involved in NF-κB signaling pathway was evaluated after HEK293 cells were treated with DDP using western blot analysis. As shown in *Figure 3A*, the phosphorylation expression of IKKβ as well as the ratio of phosphorylation expression of IKKβ and total expression of IKKβ (p-IKKβ/IKKβ) were increased in a dose-dependent manner. As one of most vital transducers of upstream signaling of NF-κB, the expression of proteins involved in the MAPK signaling pathway was determined after HEK293 cells were induced with DDP using western blot analysis. Equally, the phosphorylation level of JNK, ERK and p38, and p-JNK/JNK, p-ERK/ERK and p-p38/p38 were enhanced in a dose-dependent manner. These results indicated that DDP treatment activated the NF-κB and MAPK signaling pathways in HEK293 cells, respectively.

To further confirm the inhibitory effect of DDP on the NF-κB signaling pathway, we detected the expression of *IL-1β*, *IL-6*, *TNF-α*, and *INF-γ* by qRT-PCR, and the level of iNOS by western blot analysis after HEK293 cells had been incubated with the specific inhibitor of NF-κB, BAY 11-7082. As shown in *Figure 3B*,*3C*, for both the expression level of *IL-1β*, *IL-6*, *TNF-α*, and *INF-γ* and the expression of iNOS protein, BAY 11-7082 alone could obviously inhibit the expression, and BAY 11-7082 combined with DDP further diminished the expression compared with DDP alone. To verify that MAPK signaling occurred upstream of the NF-κB signaling pathway, the expression of IKKβ was detected after HEK293 cells had been incubated with the pan-MAPK inhibitor, U0126. As shown in *Figure 3D*,*3E*, for both the expression level of *IL-1β*, *IL-6*, *TNF-α*, and *INF-γ* and the expression

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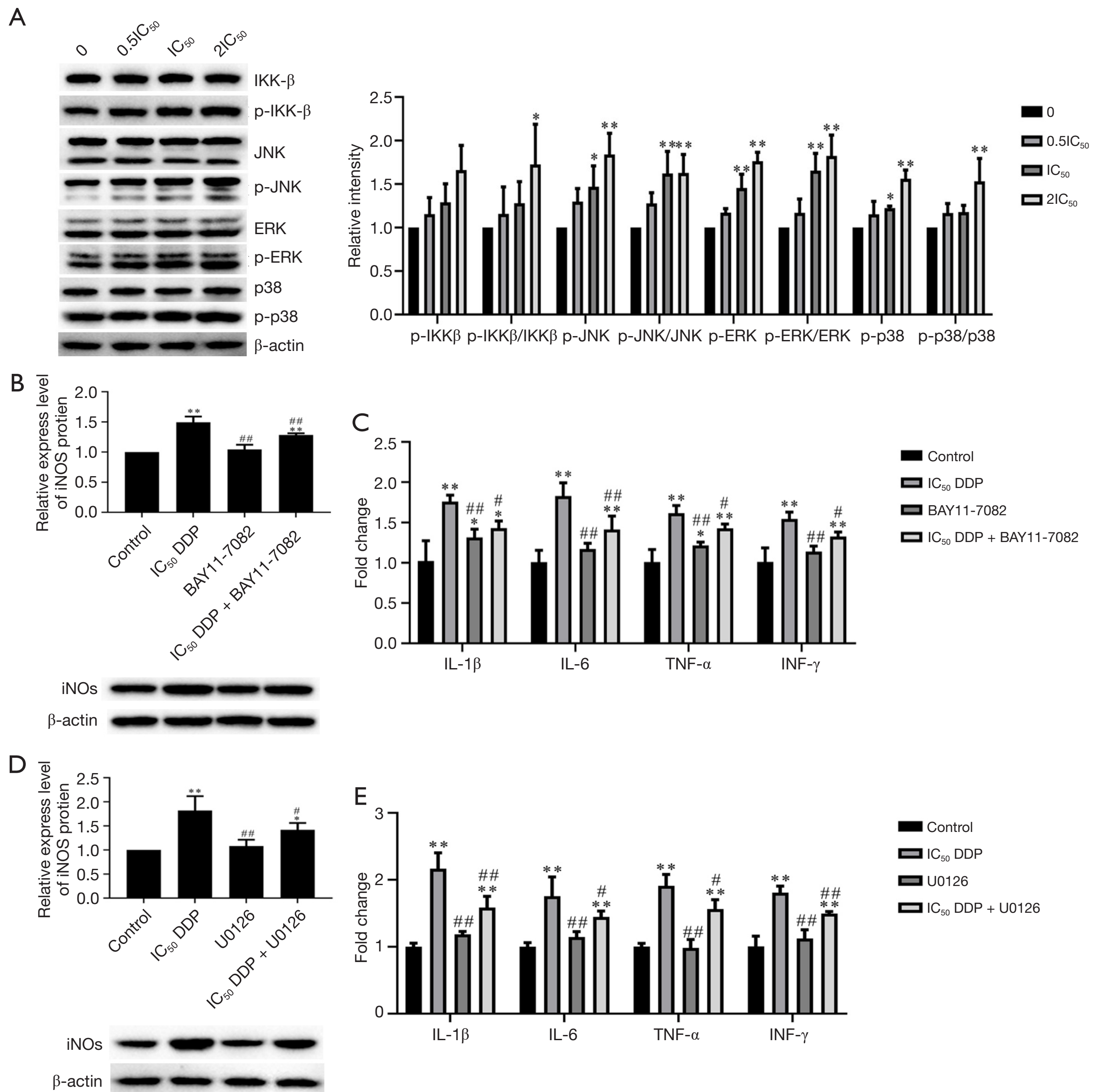
of iNOS protein, U0126 alone could obviously inhibit the expression, and U0126 combined with DDP further diminished the expression compared with DDP alone. In addition, the results revealed that U0126 treatment inhibited the activity of MAPKs and decreased the phosphorylation expression of IKKβ, while increased the phosphorylation level of IκBα (*Figure 3F*). These results showed that DDP activated the MAPK/NF-κB signaling pathway in HEK293 cells.

DDP enhanced the level of TRPA1

Since our previous study exhibited that the hypoxia and reoxygenation notably enhances the level of *TRPA1*, we detected the *TRPA1* expression using western blot. Consistently, the messenger RNA (mRNA) and protein level of *TRPA1* were elevated in a dose-dependent way after HEK293 cells were treated with DDP (*Figure 4A*). Moreover, BAY 11-7082 combined with DDP reduced



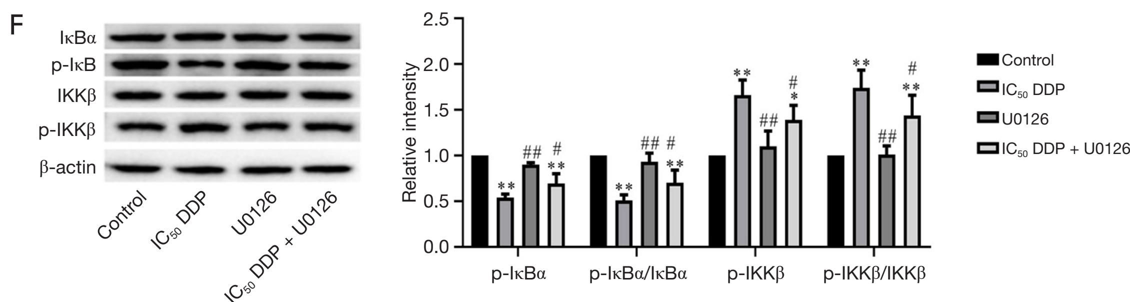


Figure 3 DDP influenced the MAPK/NF-κB signaling pathway. HEK293 cells were treated with DDP at the concentrations of 0, 0.5IC_{50}, IC_{50}, and 2IC_{50}, respectively. The expression level of IKKβ, p-IKKβ, JNK, p- JNK, ERK, p-ERK, p38, and p-p38 (A) were examined by western blot assay. Then, HEK293 cells were treated with IC_{50} DDP, BAY 11-7082, IC_{50} DDP + BAY 11-7082 or PBS (control) respectively. The expression of iNOS protein (B) and the expression of *IL-1β*, *IL-6*, *TNF-α*, and *INF-γ* (C) was examined by western blot and qRT-PCR analysis. After HEK293 cells were treated with IC_{50} DDP, U0126, IC_{50} DDP + U0126 or PBS (control) respectively, the expression of iNOS protein (D), the expression level of *IL-1β*, *IL-6*, *TNF-α*, and *INF-γ* mRNA (E), and (F) the expression of IκBα, p-IκBα, IKKβ, and p-IKKβ protein was determined using western blot analysis and qRT-PCR analysis. The means ± SD of three independent samples were exhibited. The data were analyzed after being normalized to β-actin. *P<0.05 and **P<0.01, compared with 0/control group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01, compared with IC_{50} DDP group. DDP, cisplatin; PBS, phosphate-buffered saline; iNOS, inducible nitric oxide synthase; *IL-1β*, interleukin-1β; *IL-6*, interleukin-6; *TNF-α*, tumor necrosis factor-α; *INF-γ*, interferon-γ; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; mRNA, messenger RNA; SD, standard deviation.

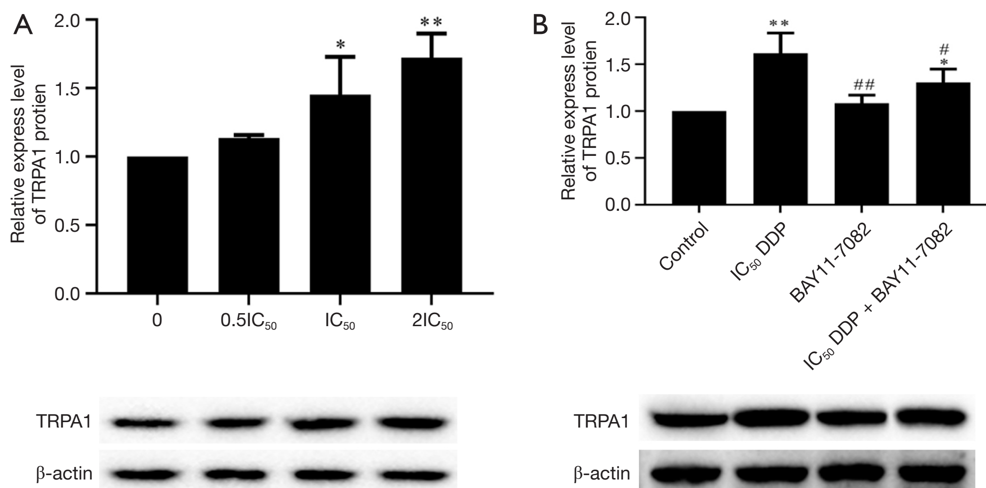


Figure 4 The *TRPA1* expression was increased both at mRNA and protein levels in a dose-dependent manner. HEK293 cells were administrated with DDP with the concentration of 0, 0.5IC_{50}, IC_{50}, and 2IC_{50} (A), or IC_{50} DDP, BAY 11-7082, IC_{50} DDP + BAY 11-7082 or PBS (control) (B) respectively. The *TRPA1* expression was examined using western blot analysis. The means ± SD of three independent samples were exhibited. The results were analyzed after being normalized to β-actin. *P<0.05 and **P<0.01, compared with 0/control group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01, compared with IC_{50} DDP group. DDP, cisplatin; PBS, phosphate-buffered saline; SD, standard deviation.

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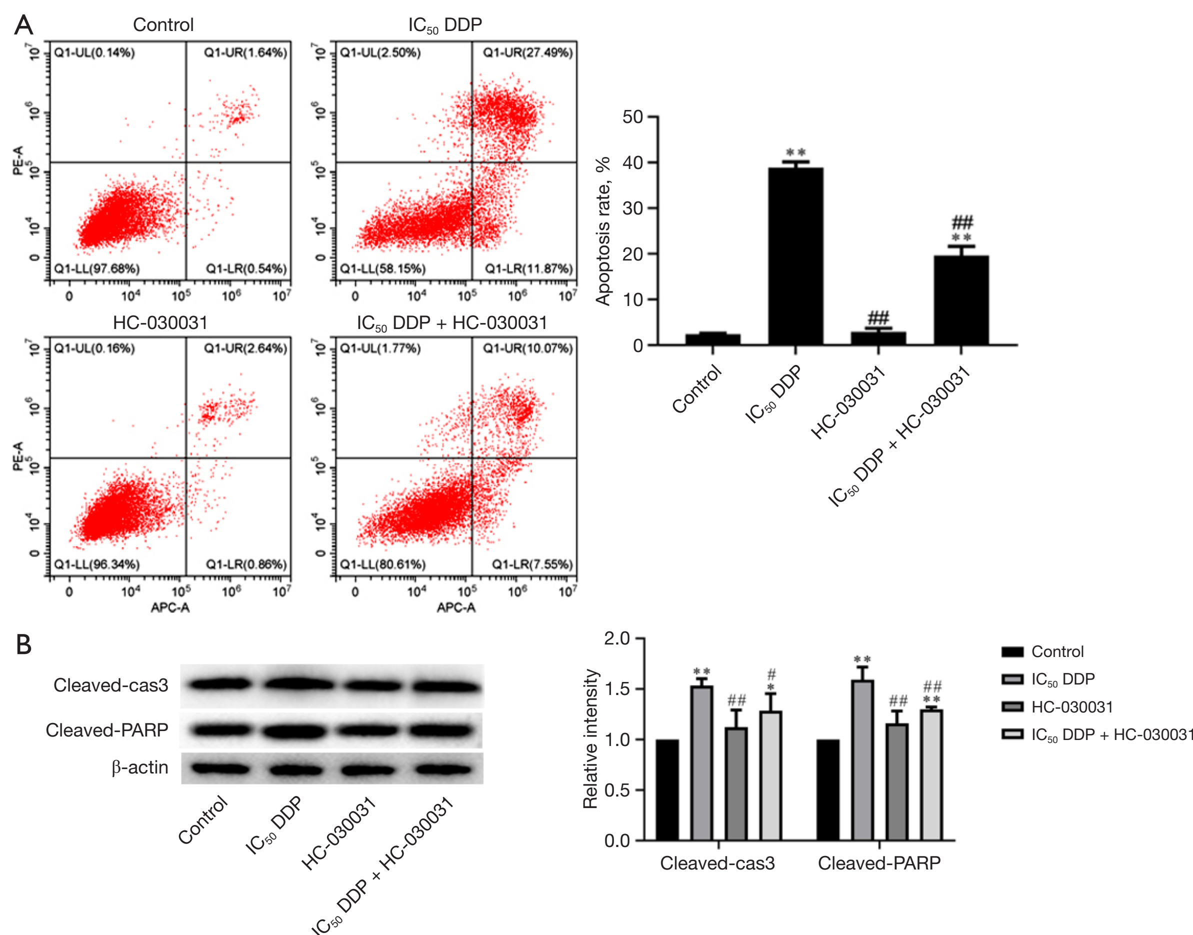

Figure 5 HC-030031 relieved the apoptosis and the expression level of apoptosis-related proteins. HEK293 cells were treated with IC_{50} DDP, HC-030031, IC_{50} DDP + HC-030031, or PBS (control), respectively. The apoptotic of the HEK293 cells was determined via flow cytometry after PE and APC co-staining (A), and the expression level of cleaved-cas3 and cleaved-PARP was examined by western blot (B). The means ± SD of three independent samples were shown. The results were shown after being normalized to β-actin. β-actin. *P<0.05 and **P<0.01, compared with control group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01, compared with IC_{50} DDP group. DDP, cisplatin; PBS, phosphate-buffered saline; PE, phycoerythrin; APC, allophycocyanin; cleaved-cas3, cleaved-caspase3; cleaved-PARP, cleavage product of caspase-3 substrate poly-ADP-ribose polymerase; SD, standard deviation.

TRPA1 expression compared with DDP alone (*Figure 4B*).

TRPA1 antagonist HC-030031 alleviated DDP-induced apoptosis

To elucidate the effects of *TRPA1* on DDP-induced apoptosis, the pharmacological blocker was incubated with the HEK293 cells that had been treated with DDP. The *TRPA1* antagonist, HC-030031, could distinctly alleviate the HEK293 cells apoptosis caused by DDP (*Figure 5A*). Additionally, HC-030031 also reduced the up-regulation of caspase3, cleaved-cas3, PARP, and cleaved-PARP induced by DDP (*Figure 5B*). Certainly, the HC-030031 alone had no effect on both the HEK293 cells apoptosis and the protein expression compared with control.

TRPA1 antagonist HC-030031 relieved DDP-induced inflammation

The effect of HC-030031 on DDP-induced inflammation also was assessed with the HEK293 cells induced with DDP. It was revealed that HC-030031 reduced the upregulation of

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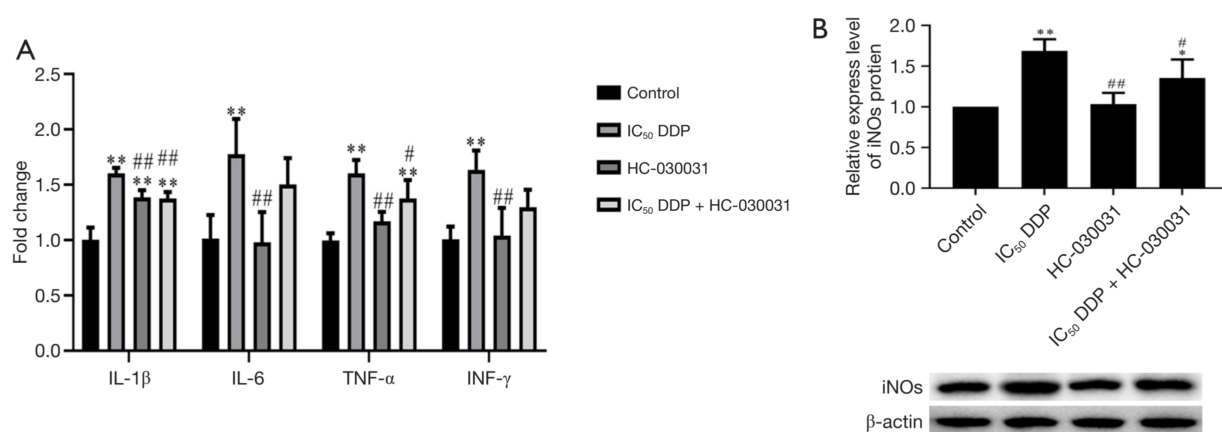

Figure 6 HC-030031 alleviated the inflammation. HEK293 cells were treated with IC_{50} DDP, HC-030031, IC_{50} DDP + HC-03003, or PBS (control), respectively. The expression level of *IL-1β*, *IL-6*, *TNF-α*, and *INF-γ* of the HEK293 cells was determined by qRT-PCR (A), and the expression of iNOS was determined via western blot (B). The means ± SD of three independent samples were present. The results were shown after being normalized to β-actin. *P<0.05 and **P<0.01, compared with control group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01, compared with IC_{50} DDP group. DDP, cisplatin; PBS, phosphate-buffered saline; *IL-1β*, interleukin-1β; *IL-6*, interleukin-6; *TNF-α*, tumor necrosis factor-α; *INF-γ*, interferon-γ; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; iNOS, inducible nitric oxide synthase; SD, standard deviation.

IL-1β, *IL-6*, *TNF-α*, and *INF-γ* induced by DDP (*Figure 6A*), and also reduced the up-regulation of iNOS induced by DDP (*Figure 6B*).

TRPA1 antagonist HC-030031 inhibited the MAPK/NF-κB signaling pathway

As shown in *Figure 7*, the expression of IκBα, IKKβ, JNK, ERK, and p38 that are involved in the MAPK/NF-κB signaling pathway did not reduce with HC-030031 treatment compared with those treated with DDP. However, the phosphorylation expression of IKKβ, JNK, ERK, and p38 decreased, while that of IκBα increased compared with DPP treatment.

Discussion

Due to its remarkable effect on a series of malignant solid tumors, DDP has been one of most commonly drugs used in cancer therapy. Nevertheless, adverse effects including nephrotoxicity have become the main issue restricting its use and efficacy in cancer chemotherapy. Our previous study exhibited that the hypoxia and reoxygenation which can stimulate AKI prominently enhanced the expression of *TRPA1*, which indicated that *TRPA1* might play an important role in DDP-associated nephrotoxicity. In this study, we first reported that *TRPA1* mediated DDP-induced cellular inflammation and apoptosis via the MAPK/NF-κB signal pathway in HEK293 cells *in vitro*.

TRPV1 is a powerful non-selective Ca^{2+} channel, thus our recent study has showed that *TRPV1* mediates DDP-induced apoptosis in renal tubular cells via calcium-dependent signaling pathway (34). Besides, Ta *et al.* (35) has shown that DDP induced up-regulation of *TRPA1* mRNA both *in vitro* and *in vivo*. However, more molecular mechanism related in the role of *TRPV1* in DDP-induced apoptosis also need further researches. Prominently, oxidative stress is closely related in the pathogenesis of DDP-induced nephrotoxicity and extremely drives to apoptotic cell death both *in vivo* (36,37) and *in vitro* (38). Furthermore, a large numbers of studies have showed that inflammation response is also involved in the pathogenesis of DDP-induced nephrotoxicity (39-41).

In our study, we found that DDP could induce HEK293 cell apoptosis while decreasing the cell viability and increasing the nuclear degradation in a dose-dependent manner. Activation of caspase3 can cleave its substrate protein PARP to lead to protein disintegration and apoptosis (42). Thus, caspase3 and PARP play an important role in apoptosis (43). Consequently, the expression of cleaved-cas3 and cleaved PARP also increased after HEK293 cells were treated with DDP in a dose-dependent way. Therefore, DDP induced

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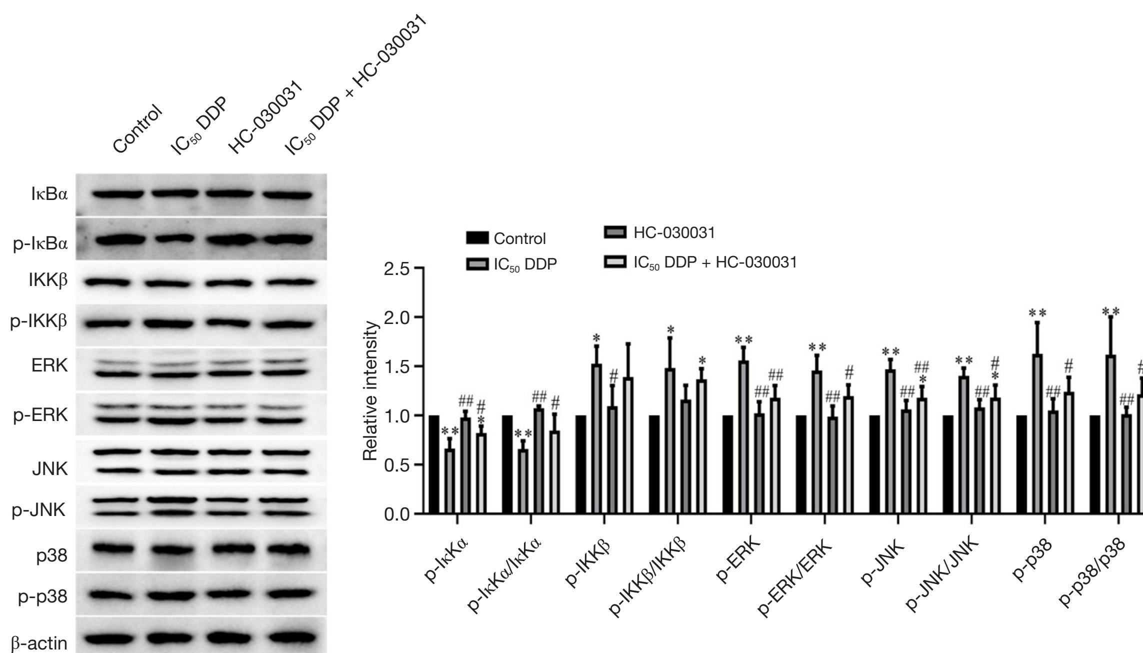

Figure 7 HC-030031 functioned on the MAPK/NF-κB signaling pathway. HEK293 cells were dealt with IC_{50} DDP, HC-030031, IC_{50} DDP + HC-030031, or PBS (control) respectively. The expression of IκBα, p-IκBα, IKKβ, p-IKKβ, JNK, p-JNK, ERK, p-ERK, p38 and p-p38 was examined using western blot assay. The means ± SD of three independent samples were shown. The data were exhibited after being normalized to β-actin. *P<0.05 and **P<0.01, compared with control group; #P<0.05 and ##P<0.01, compared with IC_{50} DDP group. DDP, cisplatin; PBS, phosphate-buffered saline; mRNA, messenger RNA; SD, standard deviation.

apoptosis of HEK293 cells, which is in line with earlier reports (44,45). Additionally, treatment with the *TRPA1* antagonist HC-030031 also decreased the apoptosis and expression of cleaved-cas3 and cleaved PARP, which indicated that TRPA1 mediates the apoptosis induced by DDP.

Earlier research has suggested that apoptosis is closely connected with the production and release of a series of inflammatory cytokines and mediators, including IL-1β, IL-6, TNF-α, INF-γ, and iNOS (46,47). Our results verified the DDP-induced inflammatory response through the rise of expression level of IL-1β, IL-6, TNF-α, and INF-γ, and iNOS protein. More importantly, the previous research indicated that the NF-κB signaling pathway is involved in inflammation induced by DDP (48). Upon the specific inhibitor IκB activation, NF-κB is activated with the removal of IκB by IKKα/β (49,50). The results revealed that the phosphorylation of IKKβ was activated with DDP treatment. The use of BAY371 11-7082 further supported that the release of IL-1β, IL-6, TNF-α, and INF-γ, and iNOS is NF-κB signaling pathway-dependent. More importantly, the *TRPA1* antagonist HC-030031 treatment also decreased the expression level of IL-1β, IL-6, TNF-α, and INF-γ, and iNOS protein, which suggested that *TRPA1* is associated with the NF-κB signaling pathway. As one of the most important signaling pathways in upstream signaling of NF-κB, the MAPK signaling pathway has been reported as associated with DDP-induced renal cell death (6). Among the MAPK signaling pathway, JNK leads to inflammation, apoptosis, and even kidney dysfunction upon activation by DDP (51), and ERK declines the level of apoptosis-related protein during DDP-induced renal cell death (52), additionally p38 mediates inflammation, oxidative stress, and apoptosis after the initiation of DDP-induced renal damage (53). Similarly, the phosphorylation JNK, ERK, and p38 were activated with DDP treatment. Treatment with U0126 inhibits the phosphorylation activity of p65 and IKKβ, suggesting that the MAPK signaling pathway is associated with the NF-κB signaling pathway-dependent inflammatory progress. Moreover, the *TRPA1* antagonist HC-030031 treatment also decreased the expression of phosphorylation IκBα, IKKβ, JNK, ERK, and p38 compared with DPP treatment, which indicated that *TRPA1* is associated with DDP-induced nephrotoxicity via the MAPK/NF-κB signaling pathway.

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In summary, this study demonstrated that *TRPA1* regulates phosphorylation of the MAPK/NF-κB signaling pathway to promote the production and release of inflammatory cytokines and mediators, which causes apoptosis and eventually nephrotoxicity. However, there are also some limitations of this article, for example, the role of *TRPA1* in DDP-induced nephrotoxicity in the animal models will consider in future research. I n brief, our study may provide a novel insight into the molecular mechanism of *TRPA1* in DDP-induced nephrotoxicity.

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