The leukotriene B₄–leukotriene B₄ receptor axis promotes cisplatin-induced acute kidney injury by modulating neutrophil recruitment

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Cisplatin is an effective chemotherapeutic agent that is widely used in the treatment of various solid organ malignancies, including head and neck, ovarian, and testicular cancers. However, the induction of acute kidney injury (AKI) is one of its main side effects. Leukotriene B₄ receptor 1 (BLT1) mediates the majority of physiological effects of leukotriene B₄ (LTB₄), a potent lipid chemoattractant generated at inflammation sites, but the role of the LTB₄–BLT1 axis in cisplatin-induced AKI remains unknown. Here we found upregulated LTB₄ synthesis and BLT1 expression in the kidney after cisplatin administration. Cisplatin was found to directly upregulate gene expression of leukotriene A₄ hydrolase and stimulate LTB₄ production in renal tubular epithelial cells. Reduced kidney structural/functional damage, inflammation, and apoptosis were observed in BLT1⁻/⁻ mice, as well as in wild-type mice treated with the LTA₄H inhibitor SC-57461A and the BLT1 antagonist U-75302. Neutrophils were likely the target of this pathway, as BLT1 absence induced a significant decrease in infiltrating neutrophils in the kidney. Adoptive transfer of neutrophils from wild-type mice restored kidney injury in BLT1⁻/⁻ mice following cisplatin challenge. Thus, the LTB₄–BLT1 axis contributes to cisplatin-induced AKI by mediating kidney recruitment of neutrophils, which induce inflammation and apoptosis in the kidney. Hence, the LTB₄–BLT1 axis could be a potential therapeutic target in cisplatin-induced AKI.


KEYWORDS: acute kidney injury; cisplatin nephrotoxicity; leukotriene B₄; leukotriene B₄ receptor 1; neutrophils

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SC-57461A, and the BLT1 antagonist U-75302. The results indicate that BLT1 induces specific chemotactic signals that guide neutrophils into an injured kidney after cisplatin administration, resulting in exacerbated apoptosis and renal inflammation. Our study highlights the potential importance of the inflammatory reaction in the pathogenesis of cisplatin-induced AKI and suggests that the LTB4-BLT1 inflammatory circuit is a potential therapeutic target for AKI.

RESULTS
Cisplatin administration caused AKI and upregulated LTB4 that is primarily produced by kidney epithelial cells

To investigate the role of the LTB4-BLT1 axis in cisplatin-induced AKI, we first measured concentrations of BLT1 ligand LTB4 in kidneys after exposure to cisplatin. AKI was induced in mice by i.p. injection of cisplatin (15 mg/kg). As shown in Figure 1a, LTB4 levels were elevated in the kidneys on day 3 after cisplatin injection, coinciding with significantly elevated blood urea nitrogen and serum creatinine levels (Supplementary Figure S1). The mRNA expression of LTA4H in the kidneys, which is critical for the synthesis of LTB4, was also significantly upregulated on day 3 after cisplatin-induced AKI.

To investigate the cell source of LTB4 in AKI, we assessed the mRNA expression of LTA4H in the cells sorted from injured kidney tissues (Supplementary Figure S2A). Surprisingly, LTA4H mRNA was significantly elevated in CD45- cells, which prompted us to speculate that nonleukocyte cells, rather than leukocytes, regulated LTB4 biosynthesis in

Figure 1 | Leukotriene B4 is upregulated after cisplatin-induced injury. (a) Leukotriene B4 (LTB4) levels in the kidney were examined by enzyme immunoassay (EIA). Kidney mRNA expression of leukotriene A4 hydrolase (LTA4H) was determined by real-time polymerase chain reaction (RT-PCR). (b) Renal CD45+ cells, CD45- cells, EpCAM+ cells, and CD31+ cells were sorted from mouse kidneys after cisplatin exposure by using a fluorescence-activated cell sorter. LTA4H expression of different types of cells was examined by RT-PCR. (c) HK-2 cells were exposed to cisplatin for 12 hours and 24 hours. Supernatants were collected and assessed for LTB4 levels by ELISA. LTA4H mRNA expression was examined by RT-PCR. *P < 0.05, **P < 0.01, and ***P < 0.001. Data are expressed as means ± SEM. Similar results were obtained in 3 independent experiments with 6 to 7 mice per group or in triplicate culture. HK-2, human kidney-2.
cisplatin-induced AKI. Furthermore, we found increased LTA₄H mRNA in the kidney resulted largely from epithelial cell adhesion molecule (EpCAM⁺) epithelial cells rather than CD45⁻ CD31⁺ endothelial cells (Figure 1b). Moreover, human proximal tubular epithelial line HK-2 cells treated with 10 μM cisplatin caused an obviously elevated LTA₄H mRNA level, and LTB₄ levels in supernatant were also markedly increased (Figure 1c). These results suggest that renal tubular epithelial cells may represent a primary source of LTB₄ in cisplatin-induced AKI.

**BLT1⁻/⁻ mice exhibit attenuated cisplatin-induced AKI**

For the purpose of analyzing the role of the LTB₄-BLT1 axis in cisplatin-induced AKI, we administered cisplatin to BLT1⁻/⁻ mice and wild-type (WT) mice. Compared with WT mice, BLT1⁻/⁻ mice had a significant reduction in renal injury. Functional injury, assessed by blood urea nitrogen and creatinine levels (Figure 2a), was decreased in BLT1⁻/⁻ mice compared with WT mice. Periodic acid-Schiff (PAS) staining revealed severe tubular injury with cast formation, loss of brush border membranes, sloughing of tubular epithelial...
cells, dilation of tubules, and increased infiltrating neutrophils in WT mouse kidneys, whereas in BLT1−/− mice, kidney damage was limited to mild swelling of the tubular epithelial cells and neutrophil infiltration (Figure 2b). This result was also confirmed by an evaluation of kidney injury score and calculation of the number of infiltrating neutrophils (Figure 2b).

BLT1−/− mice exhibited greatly reduced kidney infiltration of neutrophils, inflammatory cytokine expression, and cell apoptosis in cisplatin-induced AKI

BLT1 was previously demonstrated to be predominantly expressed on neutrophils, macrophages, eosinophils, and activated T cells.4 Our flow cytometric results confirmed that CD45+ leukocytes in the kidney mainly expressed BLT1 on days 2 and 3 after cisplatin injection, consistent with the timing of CD45+ infiltration of immune cells into the kidneys (Supplementary Figure S3).

We next assessed the composition of immune cells in the kidneys to elucidate the underlying mechanism by which BLT1 deficiency caused alleviation of AKI. Flow cytometric results showed that BLT1 deficiency resulted in impaired infiltration of CD45+ cells in the kidneys (Figure 3a). No differences were found in the percentages of kidney macrophages, CD4+ T cells, and CD8+ T cells in cisplatin-treated WT mice and BLT1−/− mice (Figure 3b and c). A significantly decreased percentage of neutrophils in the kidneys was found in BLT1−/− mice. The results are consistent with the decreased number of neutrophils observed in kidney PAS-staining sections (Figure 2b).

Excessive secretion of proinflammatory cytokines is associated with exacerbated kidney injury. We therefore investigated whether the LTB4-BLT1 axis influenced cytokine secretion in cisplatin-induced AKI. We found that cisplatin treatment increased the concentrations of tumor necrosis factor-α and interleukin 1β in WT kidney tissues, which were reduced in BLT1−/− mice (Figure 3d). Meanwhile, significantly increased concentrations of CXC ligand 1 (CXCL1) and CXC ligand 2 (CXCL2), both of which are key murine neutrophil chemokines, were greatly impaired in BLT1−/− mice (Figure 3d).

Next, we measured the cleaved caspase-3 level in the kidneys to assess whether BLT1 deficiency influenced cisplatin-induced apoptosis. The cleaved caspase-3 level was significantly upregulated by cisplatin treatment in WT mice (Supplementary Figure S4). However, the level was reduced in kidneys of BLT1−/− mice, implying that BLT1 signaling is important for cisplatin-induced apoptosis in the kidney (Figure 3e). Taken together, these data suggest that BLT1 is required for the development of cisplatin-induced kidney inflammation and apoptosis.

Adoptive transfer of WT neutrophils restored kidney injury in BLT1−/− mice

Previous studies have shown that generation of neutrophil-derived reactive oxygen intermediates could induce renal epithelial cell apoptosis.10 Therefore we measured principal parameters including oxidative stress malondialdehyde and myeloperoxidase activity in the kidneys. Consistent with decreased neutrophil accumulation, BLT1−/− mice had significantly lower kidney malondialdehyde levels and myeloperoxidase activity than WT mice, which may explain attenuated AKI in the absence of BLT1 (Figure 4a).

To confirm that the renal protection seen in BLT1−/− mice was a direct result of reduced infiltrating neutrophils in the kidneys, we reconstituted cisplatin-treated BLT1−/− mice with bone marrow–derived neutrophils. Neutrophils from bone marrow of WT or BLT1−/− mice were purified (Supplementary Figure S2B) and adoptively transferred to BLT1−/− mice separately on day 2 after cisplatin treatment. Mice were killed 20 hours later. We demonstrated that BLT1−/− mice that received WT neutrophils developed renal functional and histologic injuries similar to those observed in WT mice on day 3 (Figure 4b and c), indicating that neutrophils directly mediated cisplatin-induced AKI. In contrast, BLT1−/− mice that received BLT1−/− neutrophils failed to restore the kidney injury, indicating that the effect of the neutrophil adoptive transfer was dependent on neutrophil BLT1 expression, which mediates kidney recruitment of neutrophils (Figure 4b and c). Flow cytometric analysis showed restored infiltrating neutrophils in mice that received WT neutrophils, but not in those that received BLT1−/− neutrophils (Figure 4d). We also showed the preferential infiltration of injected WT neutrophils into kidneys compared with BLT1−/− neutrophils through co-transfer experiment (Supplementary Figure S5). Importantly, adoptive transfer of WT neutrophils restored the upregulation of kidney malondialdehyde and cleaved caspase-3 level in cisplatin-treated BLT1−/− mice, suggesting that neutrophils are required for renal injury in a proapoptotic manner (Figure 4e and f). These results demonstrate that BLT1 expression is required for neutrophils trafficking to the kidney and subsequent development of kidney injury in cisplatin-induced AKI.

Administration of BLT1 antagonist (U75302) attenuated cisplatin-induced AKI

We used U75302, a specific BLT1 antagonist, to further confirm the role of BLT1 in cisplatin-induced AKI. U75302 was administered i.p. to WT mice on the day of cisplatin administration, with another dose 2 days later. Mice treated with U75302 showed less functional and histologic renal damage on day 3 compared with mice that received vehicle treatment (Figure 5a and b). Moreover, BLT1 blockade by U75302 resulted in markedly reduced infiltration of neutrophils, proinflammatory cytokines, and chemokines CXCL1/CXCL2 in kidneys (Figure 5c and d). Furthermore, treatment with U75302 significantly decreased kidney apoptosis (Figure 5e).

Blockade of LTB4 synthesis with SC-57461A attenuated cisplatin-induced AKI

We next investigated whether inhibition of LTB4 synthesis could block neutrophil influx into kidneys and further kidney
injury by SC-57461A, a specific LTA4H inhibitor. SC-57461A effectively decreased LTB4 levels within the kidneys of cisplatin-treated mice (Supplementary Figure S6). We found that oral administration of SC-57461A markedly attenuated cisplatin-induced AKI, evidenced by significant decreases in blood urea nitrogen and creatinine levels, as well as kidney injury score compared with vehicle treatment (Figure 6b). Kidney infiltration by neutrophils, inflammatory cytokine expression, and apoptosis were also suppressed by SC-57461A treatment (Figure 6c–e). Moreover, another LTA4H inhibitor, captopril, was also renoprotective (Supplementary Figure S7). These results suggest that inhibition of LTB4 production prevents cisplatin-induced AKI.

**DISCUSSION**

In this study, we demonstrate that the LTB4-BLT1 axis contributes to cisplatin-induced AKI by mediating recruitment of neutrophils to the kidney, which amplifies renal inflammation, thereby leading to apoptosis of renal tubular epithelial cells. Blocking the LTB4-BLT1 axis significantly attenuated kidney injury, indicating that the LTB4-BLT1 axis could be a potential therapeutic target for human drug-induced AKI. Furthermore, we found that renal tubular epithelial cells act as a major cell source of upregulated LTB4 levels on cisplatin stimulation.

Accumulating evidence indicates that the LTB4-BLT1 axis plays an important role in renal diseases. The gene expression of key enzymes in LTB4 synthesis, 5-lipoxygenase and LTA4H has been reported in renal tissues of patients with nephrotic syndrome. In animal experiments, induced LTB4 production was found in hyperlipidemic renal injury, chronic renal failure, and several forms of glomerular immune injury. The inhibition of LTB4 biosynthesis or the addition of BLT1 antagonist reduced the formation of crescentic glomeruli in nephrotoxic serum nephritis and reduced glomerular damage in hyperlipidemic renal injury. Moreover, Eisei et al. showed that the BLT1 antagonist ONO-4057 protected against a decrease in renal function in a mouse model of ischemia-reperfusion injury–induced AKI. Given the increasing recognition of the importance of inflammation in the pathogenesis of cisplatin nephrotoxicity, our study reveals the important role of the LTB4-BLT1 axis in cisplatin-induced AKI.

Interestingly, we found that renal tubular epithelial cells, but not leukocytes, act as the major cellular source of the increased LTB4 in the kidney, although leukocytes mostly secrete LTB4 in other types of tissue inflammation. This is consistent with a previous study in which the ubiquitous localization of LTA4H in rat tubular epithelial cells was reported. Moore et al. also showed that kidney tissue can produce leukotrienes independent of the circulating cell. It is important to note that infiltrating immune cells can also produce LTB4, setting up a positive-reinforcing vicious cycle. Thus tissue cells, such as tubular epithelial cells, produce LTB4 on cisplatin stimulation, initiating the recruitment and activation of neutrophils, which in turn produce more LTB4, further propagating the kidney inflammatory response and subsequent injury. This could be a new mechanism for cisplatin-induced kidney inflammation.

Adaptive transfer of WT neutrophils restored kidney injury in cisplatin-treated BLT1-/- mice, indicating that neutrophils are the key drivers of the pathogenic role of BLT1 in cisplatin nephrotoxicity. Nevertheless, previous studies on the role of neutrophils in cisplatin-induced AKI are conflicting, although most studies have demonstrated that kidney recruitment of neutrophils increases after cisplatin administration. Interruption of neutrophil recruitment to the kidney (CXCR2-/- mice and BLT1-/- mice) can relieve kidney injury, while systemic neutrophil depletion has yielded variable results. Moore et al. found that preemptive neutrophil depletion with anti-Ly6G antibody resulted in a trend toward attenuated kidney injury; however, this effect reached statistical significance only after additional interleukin-17A inhibition. Another experiment in which the same antibody was used revealed no effect on nephrotoxicity. Administration of anti-Gr-1 antibody also did not offer protection against AKI induced by a high dose (30 mg/kg) of cisplatin. We speculate that inflammation provoked by injury to renal epithelial cells serves to amplify kidney injury, but a high dose of cisplatin leads to irreversible severe tubular necrosis, a condition in which the role of neutrophils and even inflammation in AKI could be limited. Thus targeting BLT1-neutrophil can be effective in our moderate AKI model induced by cisplatin (15 mg/kg). Meanwhile, systemic neutrophil depletion may have possible adverse effects, which could obscure its beneficial effects in the kidney, as circulating neutrophils have other roles in the body’s defense. Another possibility could be multiple targeting of the anti-Gr-1 antibody. The anti-Gr-1 antibody used in many studies can bind to both Ly6G and Ly6C, which is not specific for neutrophils. Thus it may have depleted monocytes, which could obscure the effects of neutrophil depletion. Collectively, it could be useful in the

![Figure 3](image-url) **Figure 3** | Leukotriene B4 receptor 1 (BLT1) is required for the development of inflammation and apoptosis in cisplatin-induced acute kidney injury. (a) Flow cytometry was performed on kidney-derived single-cell suspensions to analyze intrarenal CD45+ leukocytes on day 3. Gr-1+CD11b+ cells were considered neutrophils; F4/80+CD11b+, CD4+, and CD8+ cells were considered macrophages, CD4+ T cells, and CD8+ T cells, respectively. (b) Representative gating strategy for flow cytometric analysis of renal tissue. The numbers indicated are the percentage of cells in CD45+ cells. (c) The percentages of indicated cells in total kidney-derived single cells. (d) Kidney levels of cytokines were examined by enzyme-linked immunosorbent assay. (e) Cleaved caspase-3 expression in kidneys was examined by Western blot. Densitometric analysis is shown. *P < 0.05, **P < 0.01, ***P < 0.001, and ns = not significant. Data are expressed as the mean ± SEM. Similar results were obtained in three independent experiments with 6 to 7 mice per group. Cis, cisplatin; FSC, forward scatter; PMN, polymorphonuclear neutrophils; SSC, side scatter; Veh, vehicle; WT, wild-type.
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treatment of cisplatin-induced AKI by reducing activated infiltrating neutrophils in the local kidney.

Neutrophil BLT1 expression is required for multiple chemokines, which amplify the inflammatory response in various inflammatory diseases.\textsuperscript{26,27,29} In this cisplatin-induced AKI model, we found that blockade of the LTB\(_4\)-BLT1 axis was accompanied by significantly decreased intrarenal levels of interleukin-1β, tumor necrosis factor-α, CXCL1, and CXCL2, which are critical in the progression of kidney injury.\textsuperscript{30} The effect may depend on BLT1-mediated early recruitment of neutrophils, acting as the primary cellular source of cytokines.\textsuperscript{31} Apart from chemokine production and enhanced inflammation, BLT1-mediated neutrophil recruitment can directly result in cell apoptosis. Activated neutrophils accelerate renal epithelial cell apoptosis through increased activity of caspases 8 and 3.\textsuperscript{10} Another study showed that co-culture with activated neutrophils from both WT and BLT1\(^{-/-}\) mice results in significant neural cell death.\textsuperscript{32} Consistent with these experiments, reduced levels of oxidant and apoptosis in cisplatin-treated kidneys of BLT1\(^{-/-}\) mice could result from decreased infiltration of neutrophils.

In consideration of the clinical application, an ideal approach of renoprotection is to protect the kidneys yet enhance the therapeutic effects of cisplatin in tumors. LTB\(_4\) signaling has been associated with cancer cell proliferation and tumor metastasis.\textsuperscript{33,34} Interruption of the LTB\(_4\)-BLT1 axis has been proven to decrease proliferation of colon cancer cells and the incidence of squamous-cell carcinoma.\textsuperscript{34-36} Meanwhile, Yosuke et al. found that BLT1 receptor deficiency induced long-term anti-tumor memory responses in leukemia.\textsuperscript{37} Recently, neutrophils have been identified as a main driver of metastatic establishment within the metastatic lung microenvironment in mouse breast cancer models. Inhibition of leukotriene (including LTB\(_4\)) production abrogates neutrophil pro-metastatic activity and consequently reduces metastasis.\textsuperscript{33} Taken together, these findings indicate an anti-tumor role of blocking LTB\(_4\)-BLT1 signaling for new cancer therapeutics. Thus inhibition of the LTB\(_4\)-BLT1 axis may not only protect the kidneys but enhance the cancer therapy effects of cisplatin.

In summary, our study reveals the important proinflammatory and proapoptotic role of the LTB\(_4\)-BLT1 axis in the development of cisplatin-induced AKI by attracting neutrophils to injured kidneys. We also describe a new mechanism by which inflammation mediates cisplatin nephrotoxicity and identify the importance of renal tubular epithelial cells in kidney inflammation by generating LTB\(_4\). Given the predictable occurrence of AKI, the LTB\(_4\)-BLT1 axis could be a potential therapeutic target in cisplatin-induced AKI.

**MATERIALS AND METHODS**

**Experiment design and ethics**

Male C57BL/6 mice (8–10 weeks old) were purchased from the Chinese Academy of Sciences (Shanghai, China). Breeding pairs of BLT1\(^{-/-}\) mice on the C57BL/6 background were purchased from the Jackson Laboratory (Bar Harbor, ME) and bred in the animal facility of Fudan University (Shanghai, China). All mice used were housed in a specific pathogen-free environment.

A moderate mouse model of cisplatin-induced AKI was induced with cisplatin (15 mg/kg) (Sigma, St Louis, MO), injected i.p. U 75302 (10 μg/mouse) (Cayman, Ann Arbor, MI) or vehicle was injected i.p. 1 hour before cisplatin challenge, and an additional same dose was injected on day 2.\textsuperscript{27} Two oral LTA\(_4\)H inhibitors, purchased from Sigma, SC-57461A (5 mg/kg), were administered twice a day, and captoapril (30 mg/kg) was administered daily. The LTA\(_4\)H inhibitors and captoapril were administered for 3 days, beginning on the day of cisplatin administration.\textsuperscript{38,39} All animal experiments were performed according to the guidelines of the Animal Care and Use Committee at Fudan University.

**Assessment of renal injury**

Blood urea nitrogen and serum creatinine levels in serum collected at the end of experiments were measured by using an autoanalyzer. The kidney injury score was calculated as previously described. Details can be found in the Supplementary Methods.

**Cell culture and treatment**

Human kidney-2 (HK-2) cells (ATCC, Manassas, VA), from an immortalized human proximal tubular epithelial cell line, were maintained in Dulbecco modified Eagle medium F12 (Invitrogen, Waltham, MA) supplemented with 10% fetal bovine serum (Gibco, Thermo Fisher Scientific). For the cisplatin treatments, HK-2 cells were seeded at 6 × 10\(^4\) cells/ml in 24-well plates and treated with cisplatin (10 μmol/L) for the periods of time (0 h, 12 h, and 24 h). After incubation, the supernatants and cells were collected.

**Flow cytometry analysis**

Flow cytometry was used to analyze the mean intensity of fluorescence of BLT1 expression and quantify infiltrating leukocyte subsets in the injured kidneys.\textsuperscript{34,41} Details can be found in the Supplementary Methods.

**Neutrophil preparation from bone marrow and adoptive transfer**

Mouse neutrophils were prepared from the femur and tibia of WT or BLT1\(^{-/-}\) mice using magnetic separation columns (Miltenyi Biotec, San Diego, CA). Neutrophil enrichment was achieved according to protocol, which has been shown not to activate the cells or affect cell

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**Figure 4** | Adoptive transfer of wild-type (WT) neutrophils restored kidney injury in BLT1\(^{-/-}\) mice. (a) Malondialdehyde content and myeloperoxidase activity were assessed 3 days after cisplatin administration. (b) Blood urea nitrogen (BUN) and serum creatinine (SCr) levels were examined 20 hours after adoptive transfer. (c) Histopathologic examinations of the kidneys were assessed with periodic acid-Schiff stain (original magnification × 400). Bar = 100 μm. (d) Kidney-derived single-cell suspensions were analyzed by flow cytometry gating on CD45\(^{+}\) leukocytes or total cells for Gr-1\(^{+}\) neutrophils. (e,f) Malondialdehyde content and cleaved caspase-3 in mouse kidneys were assessed. Densitometric analysis of Western blots is shown. *P<0.05, **P<0.01. Data are expressed as the means ± SEM. Similar results were obtained in three independent experiments with 6 to 7 mice per group. BLT1, leukotriene B\(_4\) receptor 1; PMN, polymorphonuclear neutrophils.
Figure 5 | Leukotriene B4 receptor 1 (BLT1) antagonist (U75302) attenuated cisplatin-induced acute kidney injury. (a) Blood urea nitrogen (BUN) and serum creatinine (Scr) levels after U75302 administration. (b) Representative photomicrographs of kidney sections with periodic acid-Schiff staining (original magnification ×400). Bar = 100 μm. (c) Proportion of neutrophils in kidney-derived single-cell suspensions. (d) Kidney levels of cytokines were determined by enzyme-linked immunosorbent assay. (e) Western blotting of cleaved caspase-3 in mouse kidneys. Densitometric analysis is shown. *P<0.05, **P<0.01, and ***P<0.001. Data are expressed as means ± SEM. Similar results were obtained in 3 independent experiments with 6 to 7 mice per group. Cis, cisplatin; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; Veh, vehicle.
Figure 6 | Blockade of leukotriene B4 (LTB4) synthesis with SC-57461A attenuated cisplatin-induced acute kidney injury. (a) Blood urea nitrogen (BUN) and serum creatinine (Scr) after SC-57461A administration. (b) Representative photomicrographs of kidney sections with periodic acid-Schiff staining (original magnification ×400). Bar = 100 μm. (c) Proportion of neutrophils in kidney-derived single-cell suspensions. (d) Kidney levels of cytokines were determined by enzyme-linked immunosorbent assay. (e) Western blotting of cleaved caspase-3 in mouse kidneys. Densitometric analysis is shown. *P<0.05, **P<0.01, and ***P<0.001. Data are expressed as means ± SEM. Similar results were obtained in three independent experiments with 6 to 7 mice per group. Cis, cisplatin; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; Veh, vehicle.
viability. Details can be found in the Supplementary Methods. Neutrophils were >95% viable as determined by trypan blue exclusion. Preparations were >90% pure (Supplementary Figure S2). For the neutrophil transfer experiments, 5 × 10^6 neutrophils in Hank’s balanced salt solution were injected i.v. into cisplatin-treated BLT1-/- recipients.5

**Measurement of myeloperoxidase and malondialdehyde levels**

Myeloperoxidase activity and malondialdehyde content in kidney homogenate were measured by biochemical assay kits (Nanjing Jiancheng, Nanjing, China) according to the manufacturer’s instructions.43,44

**Western blotting analysis and quantitative real-time polymerase chain reaction**

Western blotting was performed as described previously.45 The primary antibodies used were against cleaved caspase-3 and β-actin (Cell Signaling Technology, Beverly, MA). Quantitative real-time polymerase chain reaction (RT-PCR) was performed to analyze the relative gene expression level in kidneys and cells. Details can be found in the Supplementary Methods. The primer sequences of all genes for PCR are listed in Supplementary Table S1.

**Enzyme-linked immunosorbent assay**

Cytokines and chemokines were measured in kidney homogenates. Details can be found in the Supplementary Methods.

**Statistical analysis**

A two-tailed Student t test or one-way analysis of variance was used for determining statistical differences between groups. A P value less than 0.05 was considered statistically significant.

**DISCLOSURE**

All the authors declared no competing interests.

**ACKNOWLEDGMENTS**

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**AUTHOR CONTRIBUTIONS**

FD, RH and CH conceived and designed the research; BD, YL, SM, YZ, XY, BL, WY, QX, and TL performed the research; BD, YL, and FD analyzed the data; and BD and YL wrote the paper.

**SUPPLEMENTARY MATERIAL**

**Supplementary Methods.**

**Supplementary Table 1.** The primer sequences of all genes for polymerase chain reaction are listed.

**Figure S1.** Renal function was assessed after cisplatin administration. Blood urea nitrogen (BUN) and serum creatinine (SCR) levels were measured by using an autoanalyzer on days 1, 2, and 3 after cisplatin administration. *P < 0.05 versus day 0; **P < 0.01 versus day 0; **P < 0.001 versus day 0; ns = not significant. The data are expressed as means ± SEM. Similar results were obtained in three independent experiments with six to seven mice per group.

**Figure S2.** Bone marrow neutrophil purification for adoptive neutrophil transfer. (A) Kidney CD45-CD45+CD31-, and EpCAM+ cells were sorted by using a fluorescence-activated cell sorter. (B) Mouse neutrophils were prepared using magnetic separation columns. The purification was confirmed by flow cytometry analysis. The numbers indicated are the percentage of cells in total acquired cells. FSC, forward scatter; SSC, side scatter.

**Figure S3.** CD45+ cells and leukotriene B4 receptor 1 (BLT1) expression were assessed after cisplatin administration. Mouse kidneys were harvested on days 1, 2, and 3 after cisplatin administration. (A) The percentage of CD45+ cells in total renal cells after cisplatin administration was examined by flow cytometric analysis. (B) BLT1 mRNA expression in kidney tissues after cisplatin exposure was examined by real-time polymerase chain reaction (RT-PCR). (C) Renal CD45- cells and CD45+ cells were sorted from mouse kidneys after cisplatin exposure using a fluorescence-activated cell sorter. BLT1 mRNA expression in CD45- cells and CD45+ cells after cisplatin exposure was determined by RT-PCR. (D) BLT1 protein expression on CD45- or CD45+ cells on day 3 after cisplatin administration was determined by flow cytometric analysis. *P < 0.05 versus day 0; **P < 0.01 versus day 0; ***P < 0.001 versus day 0; ns = not significant. The data are expressed as means ± SEM. Similar results were obtained in three independent experiments with six to seven mice per group.

**Figure S4.** Cleaved caspase 3 expression were assessed after cisplatin administration. Mouse kidneys were harvested on day 3 after cisplatin administration. Cleaved caspase-3 level expression in mouse kidneys was determined by Western blot analysis. Anti-β-actin antibody was used as a loading control.

**Figure S5.** Co-transfer of wild-type (WT) and BLT1-/- neutrophils. In a co-transfer experiment, neutrophils from WT and BLT1-/- mice were labeled with 1 μM CellTracker Green CMFDA and 15 μM CellTracker Red CMTMR (Invitrogen), respectively, at 37°C for 10 minutes. 5 × 10^6 WT neutrophils loaded with CMFDA dye were mixed with BLT1-/- neutrophils loaded with CMTMR dye at a ratio of 1:1 and adoptively transferred by i.v. injection into cisplatin-treated BLT1-/- recipients. Four hours later, fluorescent cells in the kidney were determined. The numbers indicated are the percentage of cells in acquired CD45+ cells. BLT1, leukotriene B4 receptor 1; FSC; forward scatter; ISO, isotope control; SSC, side scatter.

**Figure S6.** Leukotriene B4 (LTB4) levels in kidneys were assessed after cisplatin administration. LTB4 levels in kidneys were determined by enzyme-linked immunosorbent assay. *P < 0.05; **P < 0.001. The data are expressed as means ± SEM. Similar results were obtained in three independent experiments with six to seven mice per group. Cis, cisplatin; Veh, vehicle.

**Figure S7.** The leukotriene A4 hydrolase (LTA4H) inhibitor captopril was renoprotective in cisplatin-induced renal injury. (A) Blood urea nitrogen (BUN) and serum creatinine (SCR) levels were measured after captopril administration. (B) Representative photomicrographs of kidney sections with periodic acid-Schiff staining (original magnification ×400). Bar = 100 μm. (C) Proportion of neutrophils in kidney-derived single-cell suspensions was determined by flow cytometry. (D) Tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), CXCL1, and CXCL2 mRNA expression in kidneys were assessed by real-time polymerase chain reaction (RT-PCR). (E) Cleaved caspase-3 in mouse kidneys was determined by Western blot analysis. *P < 0.05;
**P < 0.01; ***P < 0.001. The data are expressed as means ± SEM. Similar results were obtained in three independent experiments with six to seven mice per group. Cis, cisplatin; Veh, vehicle.

Supplementary material is linked to the online version of the paper at http://www.nature.com/ki

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