Research Square

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β2 integrin-induced neutrophil activation mediates trichloroethylene-sensitized mice kidney injury: an in vivo study

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Research Article

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Abstract

Background

Trichloroethylene (TCE) is widely used in the cleaning of metal surfaces and in organic synthesis. Occupational exposure to TCE in environmental contexts has been linked to the onset of occupational medicamentosa-like dermatitis due to trichloroethylene (OMDT). However, the precise mechanism by which activated vascular endothelial cells contribute to TCE-induced renal damage remains inadequately understood.

Methods and Results

TCE-sensitized mouse model was developed, and both β2 integrin-neutralizing antibody and the peptidylarginine deiminase 4 (PAD4) inhibitor GSK484 hydrochloride were utilized to elucidate the relationship between neutrophil extracellular traps (NETs) and renal injury and to ascertain whether the release of NETs is mediated by β2 integrin. Our study demonstrates that the expression levels of neutrophil extracellular trap (NET) marker proteins, such as myeloperoxidase (MPO), PAD4, and neutrophil elastase (NE), were significantly elevated in the kidneys of TCE-sensitized mice, which exhibited pronounced renal damage. Importantly, the inhibition of PAD4 led to a substantial reduction in renal injury. Additionally, β2 integrin expression was notably upregulated in TCE-sensitized mice. Neutralizing β2 integrin in peripheral blood effectively inhibited NET release in these mice.

Conclusions

These findings suggest that the formation of the neutrophil extracellular trap network in the kidneys of TCE-sensitized mice is mediated by β2 integrin, which plays a pivotal role in the renal damage associated with TCE sensitization.

Introduction

Trichloroethylene (TCE) is a chlorinated volatile organic solvent widely used in industrial metal degreasing, precision electronics cleaning, and dry cleaning. In 2021, the global production of TCE exceeded 700,000 tons [1], with China's annual consumption surpassing 70,000 tons. This extensive utilization has led to significant environmental pollution and potential health hazards, necessitating urgent attention [2]. In occupational settings, exposure to TCE can induce a systemic disease affecting multiple organs, particularly the liver and kidneys, known as occupational medicamentosa-like dermatitis due to trichloroethylene (OMDT). Patients diagnosed with OMDT often require prolonged treatment and face elevated mortality rates, as no specific pharmacological treatments are currently available. Current clinical management primarily focuses on symptomatic relief and the use of glucocorticosteroids.

Consequently, the clinical management of OMDT poses a considerable challenge within the domain of occupational health in China.

Renal injury is a common clinical manifestation in patients with OMDT. Renal vascular endothelial cells (ECs), which are highly specialized within the kidney, may become activated by immune signaling molecules present in the bloodstream when immune system dysfunction occurs. This activation is marked by increased expression of vascular endothelial cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), endothelin-1 (ET-1), and E-selectin, all of which contribute to the initiation and progression of renal inflammatory responses. These processes accelerate disease progression, thereby positioning activated ECs as both a driving factor and a potential therapeutic target [3, 4]. Our previous research demonstrated that TNF-α can induce the activation of renal ECs and exacerbate renal damage in TCE-sensitized mice. Nonetheless, the specific pathway by which activated vascular endothelial cells contribute to renal damage remains to be elucidated.

Neutrophils, also known as polymorphonuclear cells, are the most abundant type of circulating leukocytes in the human body [5]. Extensive research has demonstrated that neutrophils serve as the primary cellular defense against invading pathogens and are integral to the innate immune response. As the understanding of neutrophil functions has evolved, numerous studies have increasingly emphasized their significant role in tissue injury [6]. The recruitment of neutrophils into tissues is a complex, multi-step biological process termed the neutrophil extravasation cascade. Initially, neutrophils in the bloodstream are captured by activated ECs. These initial adhesive interactions are predominantly mediated by selectins expressed by ECs. Subsequently, chemokines present on the EC surface activate integrins on the neutrophil surface, promoting the firm adhesion of neutrophils to the activated ECs. Ultimately, neutrophils undergo morphological changes that enable them to traverse the endothelial lumen, thereby completing their migration to the site of inflammation and contributing to the inflammatory response [7].

Integrins serve as the primary transmembrane receptors facilitating cell adhesion, playing a crucial role in the activation and migration of neutrophils [8]. In particular, β2-integrin, which is exclusively expressed on the surface of leukocytes and commonly referred to as leukocyte integrins [9], is indispensable not only for neutrophil adhesion, migration, and extravasation but also for various cellular biological processes as a signaling molecule [10]. Studies have shown that mice deficient in β2-integrin exhibit impaired leukocyte recruitment during inflammatory conditions such as arthritis and peritonitis, highlighting the critical role of β2-integrin in neutrophil recruitment [11]. Among the β2-integrins expressed on neutrophils, macrophage-1 antigen (MAC-1) and lymphocyte function-associated antigen-1 (LFA-1) are predominant. These integrins facilitate the processes of slow rolling, firm adhesion, and transient crawling of neutrophils by interacting with activated endothelial cells expressing the ICAM-1 and VCAM-1 ligands. Specifically, LFA-1 is responsible for mediating slow rolling and firm adhesion, whereas MAC-1 is involved in luminal crawling [12, 13].

Research has demonstrated that activated neutrophils undergo chromatin depolymerization, a process facilitated by histone deiminases that catalyze histone guanylation. This process ultimately results in the release of neutrophil extracellular traps (NETs), which are instrumental in combating pathogenic microorganisms and recruiting immune cells [14]. NETs consist of an extracellular DNA network and various granule proteins, including citrullinated histone H3 (CitH3), neutrophil elastase (NE), myeloperoxidase (MPO), Cathepsin-G, and metalloproteinases (MMP) [15]. However, in the context of inflammation and immune disorders, neutrophils can become excessively activated, leading to the production of aberrant NETs that mediate inflammatory responses and exacerbate tissue and organ damage. The excessive activation of neutrophils and subsequent NETs production have been implicated in a variety of allergic and autoimmune diseases, such as asthma, rhinitis, sepsis, and systemic lupus erythematosus. Consequently, the investigation of NETs production and its regulatory mechanisms as potential therapeutic strategies for autoimmune diseases and allergic reactions constitutes a significant focus of contemporary research within the field of immunology. In the context of renal damage in TCE - sensitized mice, a thorough examination of the involvement of NETs in renal injury is of substantial scientific importance for elucidating the pathological mechanisms underlying immune-mediated renal injury in patients with OMDT.

In this study, we developed a TCE-sensitization mouse model and assessed the expression of endothelial cell activation markers, such as ET-1and VCAM-1, along with the expression of β2 integrin in neutrophils, to investigate the pathway of neutrophil recruitment by endothelial cells. We evaluated renal injury and the level of NETs release in the mice to determine the role of neutrophil activation in the TCE sensitization process. Furthermore, by inhibiting NETs secretion, we assessed the recovery of kidney damage in mice to explore the specific mechanisms of neutrophil activation in TCE-induced renal damage.

Materials and Methods

Experimental reagents

Mouse blood urea nitrogen (BUN) (C013-2-1), creatinine (Cre) (C011-2-1) and Cystatin C (Cys-c) assay kit (H336-1-1), periodic acid-schiff (PAS) staining reagent (D004-1-2) were purchased from Nanjing Jiancheng Bioengineering Research Institute, Nanjing, China. GSK484 hydrochloride (HY-100514) was purchased from MedChemExpress, New Jersey, USA. Mouse anti-CD18 antibody (SAB4700620) was purchased from Merck KGaA, Hessen, Germany. Rabbit anti-MPO antibody (BA0544) was purchased from BOSTER Biological Technology co.ltd, Wuhan, China. Rabbit anti-NE antibody (ab314916), rabbit anti-CitH3 antibody (Ab5103), mouse anti-GAPDH antibody (ab8245), mouse anti-β Actin antibody (ab8226), rabbit anti-Ly6G antibody (ab238132), mouse anti-ET-1 antibody (ab2786), rabbit anti-peptidyl arginine deiminase 4 (PAD4) antibody (ab96758) were purchased from Abcam, Cambridge, England. Rabbit anti-β2 integrin antibody (73663S) was purchased from Cell Signaling Technology, Danvers, Massachusetts, USA. Mouse anti-VCAM1 antibody (sc-13160) was purchased from Santa Cruze, California, USA.

Animal model establishment

A total of 140 specific pathogen free (SPF)-grade female BALB/c mice, aged 6–8 weeks and weighing 18.5 ± 1.5 g, were procured from the Experimental Animal Centre at Anhui Medical University. These mice were maintained on a standard diet with unrestricted access to water, and their bedding was replaced every two days. Environmental conditions were regulated with a manually controlled temperature range of 20℃-24℃ and a relative humidity of 50%±5%. The mice were allocated into six groups: blank control group (n = 10), solvent control group (n = 10), TCE-treated group (n = 30), TCE + β2 integrin neutralizing antibody co-treated group (n = 30), TCE + IgG co-treated group (n = 30), and TCE+GSK484 co-treated group (n = 30). Prior to toxicological staining, the dorsal hair and skin of the mice were shaved. On day 1, the mice received a subcutaneous injection of 100 μl of 25% TCE (formulated as Fuchs' complete adjuvant:TCE:acetone:olive oil = 10:5:3:2, V/V/V/V) in the dorsal skin. Subsequently, on days 4, 7, and 10, 100 μl of 50% TCE (TCE:acetone:olive oil = 5:3:2, V/V/V) was applied topically to the back, followed by 100 μl of 30% TCE (TCE:acetone:olive oil = 3:2:5, V/V/V) on days 17 and 19. Specifically, mice in the TCE and β2 integrin-neutralizing antibody co-treatment group received an additional tail vein injection of the β2 integrin-neutralizing antibody at a dosage of 3 mg/kg on days 17 and 19. Similarly, mice in the TCE and IgG co-treatment group were administered an additional tail vein injection of IgG at 3 mg/kg on the same days. In contrast, mice in the TCE and GSK484 co-treatment group received an additional intraperitoneal injection of GSK484 at a dosage of 4 mg/kg on days 17 and 19 of the toxicity treatment. On day 20, dorsal skin sensitization was assessed, with mice exhibiting a score of ≥ 1 being classified as the sensitization-positive group, while those without any sensitization symptoms were categorized as the sensitization-negative group. Based on the scoring results, the TCE-treated group could then be divided into TCE sensitisation-negative group (20 animals) and TCE sensitisation-positive group (10 animals); the TCE + β2-integrin neutralising antibody co-treatment group could be further divided into TCE + β2-integrin sensitisation-negative group (21 animals), and TCE + β2-integrin sensitisation-positive group (9 animals); TCE + IgG isotype control sensitization negative group (22 mice), TCE + IgG isotype control sensitisation positive group (8 mice); TCE+GSK484 sensitisation negative group (20 mice), TCE+GSK484 sensitisation positive group (10 mice). The mice were sacrificed after scoring on day 20, and the organs and blood were aseptically isolated and collected.

Detection of kidney injury

Hematoxylin and eosin (HE) staining was employed to examine the pathological alterations in kidney tissue. Paraffin-embedded sections were initially dewaxed, followed by sequential staining with hematoxylin and eosin solutions. Post-staining, the sections were sealed and examined microscopically to identify pathological changes. Additionally, renal PAS staining was utilized to assess renal tissue damage. The paraffin sections underwent deparaffinization and were subsequently stained with Schiff's reagent and hematoxylin. After sealing, these sections were also observed under a microscope. Furthermore, serum levels of cys-c, Cre, and BUN were measured to evaluate changes in renal function in mice, with all procedures conducted according to the specified protocols.

Mouse kidney ECs isolation

Mouse kidney ECs were isolated utilizing the magnetic bead technique according to previous study described [16, 17]. Briefly, the mouse was undergoing perfusion with 10 ml of sterile phosphate-buffered saline (PBS) via the left ventricle, succeeded by perfusion with 10 ml of PBS containing 100 μl of magnetic beads (the beads were pre-incubated with an anti-CD31 antibody overnight). The kidneys were then harvested and subjected to enzymatic digestion using trypsin and collagenase type I. Thereafter, the ECs were allowed to adhere to the tube facilitated by a magnetic frame, after which the cells were washed and collected.

Western blot

Western blot
RIPA lysis solution was added, and after homogenization, the supernatant was collected. Protein concentration was determined using the BCA assay, and SDS-PAGE electrophoresis was performed. Proteins were transferred to membranes, which were then blocked with milk. Primary and secondary antibodies were incubated to develop and detect protein expression levels. Band intensity was analyzed using ImageJ software.

Transmission electron microscope observation of ultrastructure

The ultrastructure of renal vascular endothelial cells was examined using transmission electron microscopy. Kidney tissue underwent fixation, dehydration, embedding, and electron staining before being observed and photographed with transmission electron microscopy.

Immunohistochemical

Immunohistochemical analyses were conducted to assess the expression of β2 integrin and Ly6G in murine kidney tissues. The procedure involved deparaffinization and hydration of paraffin-embedded sections, blocking of endogenous peroxidase activity, antigen retrieval, serum blocking, incubation with primary and secondary antibodies, reaction with streptavidin-peroxidase (SP), and color development using diaminobenzidine (DAB). Hematoxylin was used for nuclear staining, followed by sealing of the sections for microscopic observation and imaging.

Immunofluorescence

Additionally, an immunofluorescence assay was employed to evaluate the expression level of MPO in kidney. This involved dewaxing, hydration, antigen retrieval, permeabilization with Triton-X100, serum

blocking, incubation with primary and secondary antibodies, and nuclear staining with DAPI. The sections were then observed and photographed using a fluorescence microscope.

Statistical analysis

The quantitative data obtained from this experiment were subjected to statistical analysis, with results expressed as mean ± standard deviation. Comparisons of group means were conducted using one-way analysis of variance (ANOVA) and post hoc least-significant difference (LSD) test, a significance threshold set at $\alpha = 0.05$. Differences were deemed statistically significant at $p < 0.05$. Statistical analyses were performed using SPSS version 22.0, and parametric tests were employed to assess differences between groups.

Results

NETs are abundantly deposited on renal vascular ECs

During the activation of neutrophils, DNA decondensation and histone deamination, facilitated by enzymes such as PAD4, lead to histone guanidinylation and the subsequent release of NETs into the extracellular milieu. The primary protein markers of NETs include MPO, CitH3, and PAD4, with their expression levels serving as indicators of NET formation. Immunofluorescence analysis demonstrated that MPO co-localized not only with Ly6G, a neutrophil marker (Fig. 1A), but also with CD31, a marker for vascular endothelial cells (Fig. 1B), in the mouse kidney. These findings suggest that NETs are released from neutrophils and ultimately deposited on endothelial surfaces. Western blot analysis of isolated renal vascular endothelial cells revealed a significant increase in TCE sensitization in positive mice. Moreover, inhibition of PAD4 enzymatic activity using GSK484 resulted in a reduction in the expression of NET-related proteins, including MPO, PAD4, CitH3, and neutrophil elastase (NE), thereby suppressing NETs formation (Fig. 1C).

Inhibiting the formation of NETs effectively reduces renal injury and inhibits ECs activation in TCE-sensitised mice

Results from HE staining revealed that TCE-sensitized positive mice showed tubular swelling and luminal narrowing, accompanied by significant pathological damage (Fig. 2A,B). Additionally, there were increased levels of serum Cre (Fig. 2C), BUN (Fig. 2D), and Cys-c (Fig. 2E). The administration of GSK484, an inhibitor of NETs formation, effectively mitigated renal injury in these mice. Furthermore, the expression of ET-1, a marker indicative of vascular endothelial cell activation, was elevated in the kidneys of TCE-sensitized positive mice (Fig. 2F). Inhibition of NETs formation also suppressed ET-1 expression. These findings suggest that GSK484-mediated inhibition of NETs formation can effectively prevent the activation of renal vascular endothelial cells and attenuate renal injury in mice.

Neutrophil infiltration exacerbates renal vascular endothelial cell injury in TCE-sensitized mice

Ly6G, a marker protein for neutrophils, was found to be abundantly expressed in renal neutrophils of TCE-sensitized mice, as demonstrated by immunohistochemical analysis (Fig. 3A). Similarly, β2 integrin showed elevated expression in the renal vascular endothelial cells (ECs) of TCE-sensitized mice (Fig. 3B). Upon administration of a β2 integrin-neutralizing antibody via tail vein injection, a significant downregulation of renal β2 integrin expression was observed, along with a concomitant reduction in VCAM-1 expression in ECs (Fig. 3C). These results indicate that inhibiting neutrophil infiltration may mitigate endothelial cell injury.

Inhibition of neutrophil adhesion inhibits NETs formation and alleviates renal injury

The administration of a β2 integrin-neutralizing antibody through tail vein injection in mice led to the suppression of NETs formation within the renal tissue (Fig. 4A). Correspondingly, electron microscopy analysis demonstrated a reduction in damage to vascular endothelial cells (Fig. 5A). Furthermore, there was a significant decrease in renal pathological damage (Fig. 5B and 5C), accompanied by reduced levels of blood Cre (Fig. 5D), BUN (Fig. 5E), and Cys-c (Fig. 5F). Collectively, these findings suggest an improvement in renal injury in the treated mice.

Discussion

TCE is a widely used organic solvent in industrial settings, valued for its exceptional chemical properties, relatively low cost, and diverse applications across various industrial processes. In China, the demand for TCE has remained substantial in recent years, significantly contributing to economic advancement. However, this extensive utilization has raised increasing concerns within the academic community regarding the potential health risks associated with occupational exposure to TCE. Individuals who work with TCE for extended periods are at risk of developing TCE-induced dermatitis, characterized by generalized skin rashes and multi-organ dysfunction, with kidney damage being particularly prevalent. This study investigates the underlying mecha-/nisms of TCE-induced renal injury, focusing on the role of neutrophil activation and NETs.

Neutrophils, a subtype of white blood cells also referred to as polymorphonuclear leukocytes, play a critical role in the immune system. Upon activation, neutrophils release NETs, which are web-like structures composed of DNA, histones, and antimicrobial proteins. Initially, NETs were believed to primarily function in the control of infectious pathogens. However, emerging evidence suggests that NETs may also contribute to tissue damage, exhibiting either pro-inflammatory or anti-inflammatory effects [18, 19]. In this study, we observed increased expression of NETs marker proteins, including MPO,

PAD4, and CitH3, in the kidneys of mice sensitized to TCE. These findings indicate that NETs formation is a key pathological feature in TCE-induced renal injury.

The PAD4 enzyme, a key regulator of the NETosis pathway, has emerged as a promising therapeutic target in renal disease research. PAD4-selective inhibitors, such as Cl-amidine, YW3-56, and GSK484, have shown significant anti-inflammatory potential in animal models [20–22]. These inhibitors effectively reduce neutrophil infiltration, thereby mitigating the inflammatory response, while also inhibiting the release of inflammatory mediators and the formation of NETs. In the present study, we employed intraperitoneal injection of GSK484, a PAD4 enzyme inhibitor known to impede the formation of NETs, to demonstrate a significant reduction in the expression of proteins such as MPO and PAD4 in the renal tissues of mice sensitized with TCE and GSK484. This intervention notably inhibited NETs production and ameliorated renal injury, highlighting the therapeutic potential of PAD4 inhibitors in TCE-induced renal damage.

NETs are generated through the excessive activation of neutrophils in response to inflammatory and immune disorders [23]. Previous research has indicated that β2 integrin plays a role in neutrophil activation [24]. In our study, we observed overexpression of β2 integrin in the kidneys of mice sensitized to TCE. Following the administration of a β2 integrin-neutralizing antibody, we noted a downregulation of its ligand, VCAM1, accompanied by a significant decrease in NETs formation and an attenuation of renal injury. These findings suggest that β2 integrin-mediated neutrophil activation is a critical pathway in TCE-induced renal injury.

In the investigation of the mechanisms underlying renal injury associated with TCE-sensitization, this study is pioneering in integrating β2 integrin activation of neutrophils, which release NETs, into the mechanistic framework of the TCE sensitization model. The research uncovers a novel mechanism of renal injury in TCE-sensitized mice, wherein β2 integrin-dependent activation of neutrophils and subsequent NETs release play a crucial role in renal tissue damage. This discovery significantly enhances the theoretical understanding of immunological kidney injury induced by environmental toxicants and provides a new perspective for the development of targeted therapies.

In summary, the present study elucidates the role of neutrophils in TCE-induced renal damage by employing a mouse model of TCE sensitization. The findings demonstrate that β2 integrin-mediated neutrophil activation in the kidneys of TCE-sensitized mice contributes to the formation of NETs, thereby exacerbating renal injury. Future research should focus on further characterizing the molecular pathways involved in β2 integrin-mediated neutrophil activation and NETs formation in TCE-induced renal injury. Additionally, the therapeutic potential of PAD4 inhibitors and β2 integrin-neutralizing antibodies should be explored in clinical settings to develop effective interventions for TCE-induced renal damage.

Declarations

Ethics approval

Studies were carried out in accordance with the Guidelines on the Operation of the Animals (Scientific Procedures) Act 1986 and associated guidelines. The animal experiments were approved by Anhui Medical University (ethical approval number: LLSC 20230343).

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contribution

Qixing Zhu, Haibo Xie and Jiaxiang Zhang conceived and designed the study. Ruijie Jiang and Jihong Gao performed the experiments. Ruijie Jiang and Jihong Gao analyzed the data and wrote the manuscript. Ruijie Jiang, Haibo Xie and Qixing Zhu revised the manuscript. All authors read and approved the final manuscript.

Data availability Statement

The data in this study are included in the article/supplementary materials. Contact the corresponding authors directly to request the underlying data.

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Figures

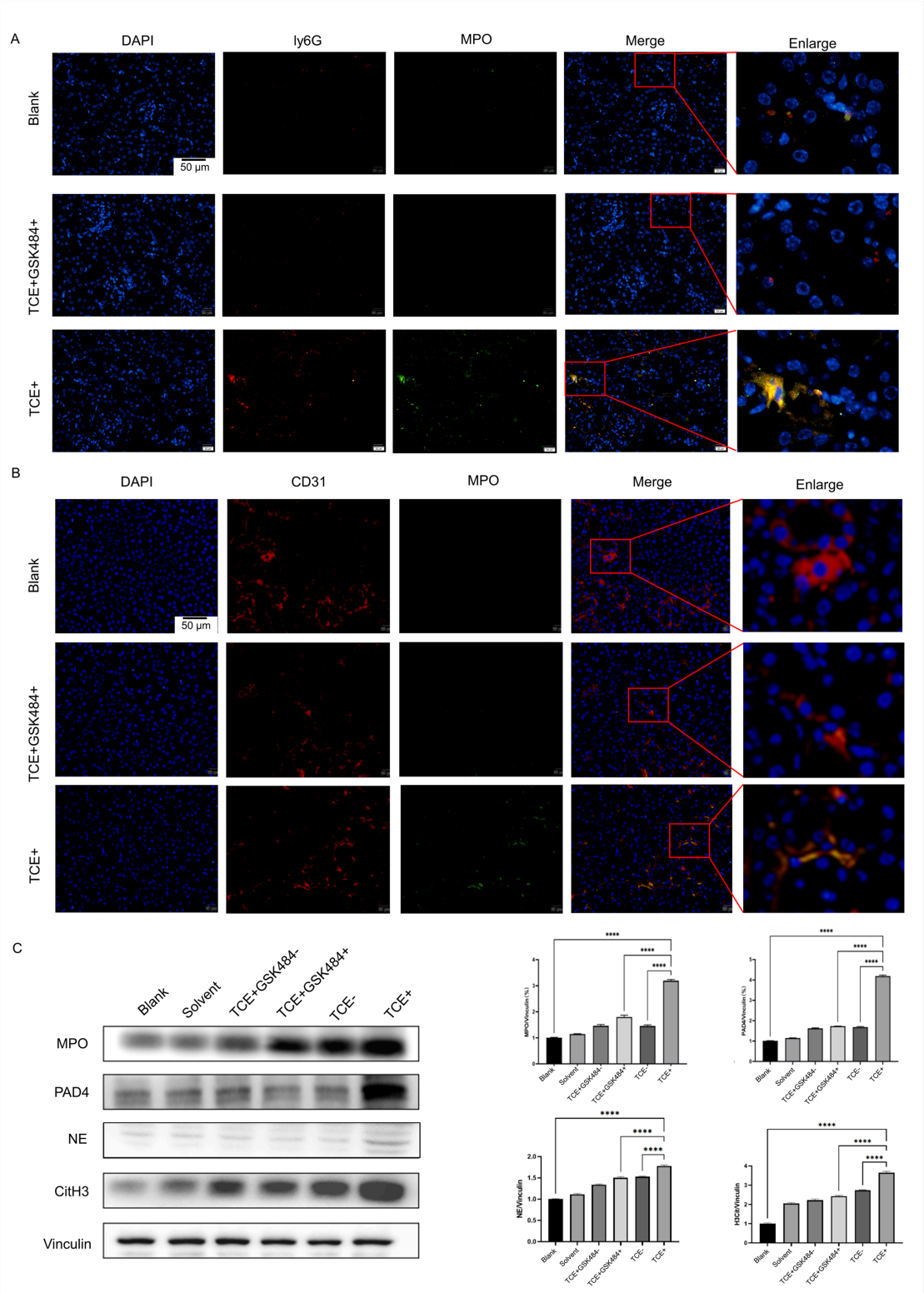


Figure 1

NETs are abundantly deposited on renal vascular endothelial cells.

(A) Immunofluorescence co-localization to detect MPO and Ly6G (neutrophil marker) (×400). (B) Immunofluorescence co-localization to detect MPO and CD31 (endothelial cell marker) (×400). (C) Western blot results of MPO, PAD4, NE and CitH3. Blank: Blank control group, N=10; Solvent: Solvent

control group, N=10; TCE+GSK484-: TCE+GSK484 sensitization negative group, N=20; TCE+GSK484+: TCE+GSK484 sensitization positive group, N=10; TCE-: TCE sensitization negative group, N=20; TCE+: TCE sensitization positive group, N=10; *P<0.05; **P<0.01; ***P<0.001; ****P< 0.0001.

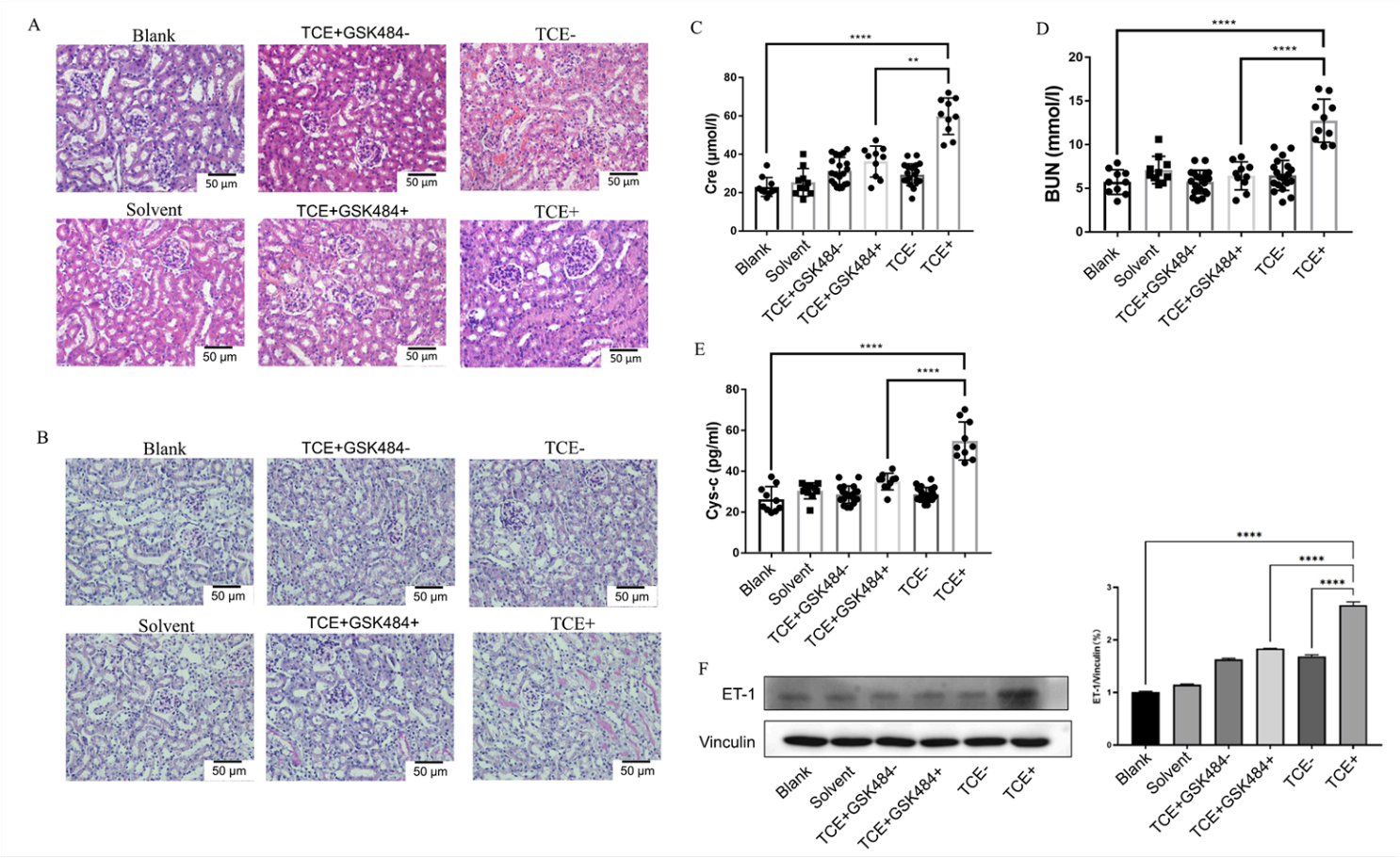


Figure 2

Inhibiting the formation of NETs effectively reduces renal injury and inhibits ECs activation in TCE-sensitised mice.

(A) HE staining results of mouse kidneys in each group (×400); (B) PAS staining results of mouse kidneys in each group (×400); (C) Serum Cre level; (D) Serum BUN level; (E) Serum Cys-c level; (F) Western blot results of endothelial cell ET-1. Blank: Blank control group, N=10; Solvent: Solvent control group, N=10; TCE+GSK484-: TCE+GSK484 sensitization negative group, N=20; TCE+GSK484+: TCE+GSK484 sensitization positive group, N=10; TCE-: TCE sensitization negative group, N=20; TCE+: TCE sensitization positive group, N=10; *P<0.05; **P<0.01; ***P<0.001; ****P< 0.0001.

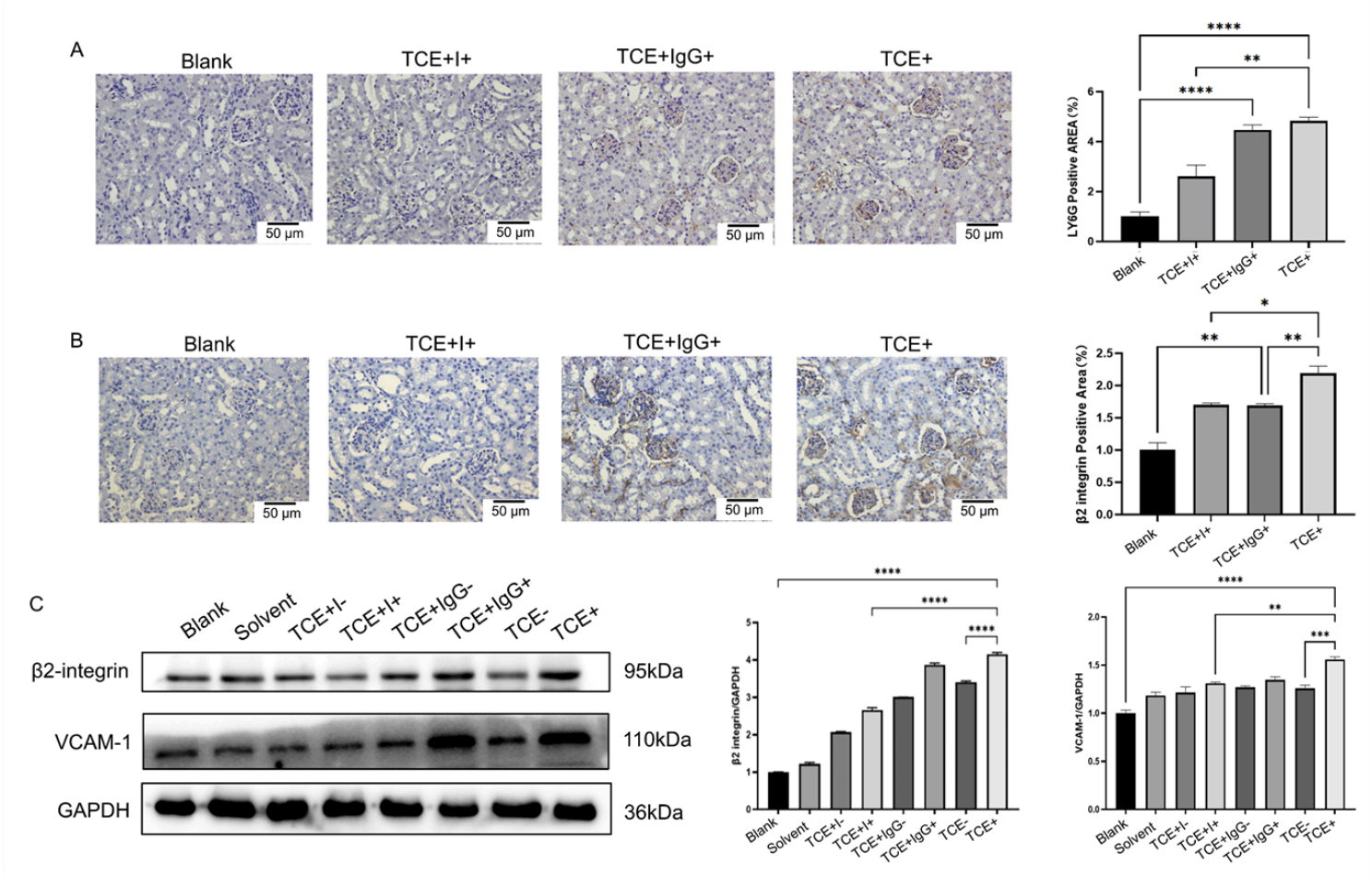


Figure 3

Elevated expression of β2integrin in renal vascular ECs of TCE-sensitised mice.

(A) Immunohistochemistry results of kidney LY6G (×400); (B) Immunohistochemistry results of kidney β2integrin (×400); (C) Western blot results of endothelial cell β2integrin and VCAM-1. Blank: Blank control group, N=10; Solvent: Solvent control group, N=10; TCE+I-: TCE+β2integrin sensitization negative group, N=21; TCE+I+: TCE+β2integrin sensitization positive group, N=9; TCE+IgG-: TCE+ IgG isotype control sensitization negative group, N=22; TCE+IgG+: TCE+ IgG isotype control sensitization positive group, N=8; TCE-: TCE sensitization negative group, N=20; TCE+: TCE sensitization positive group, N=10; *P<0.05; **P<0.01; ***P<0.001; ****P< 0.0001.

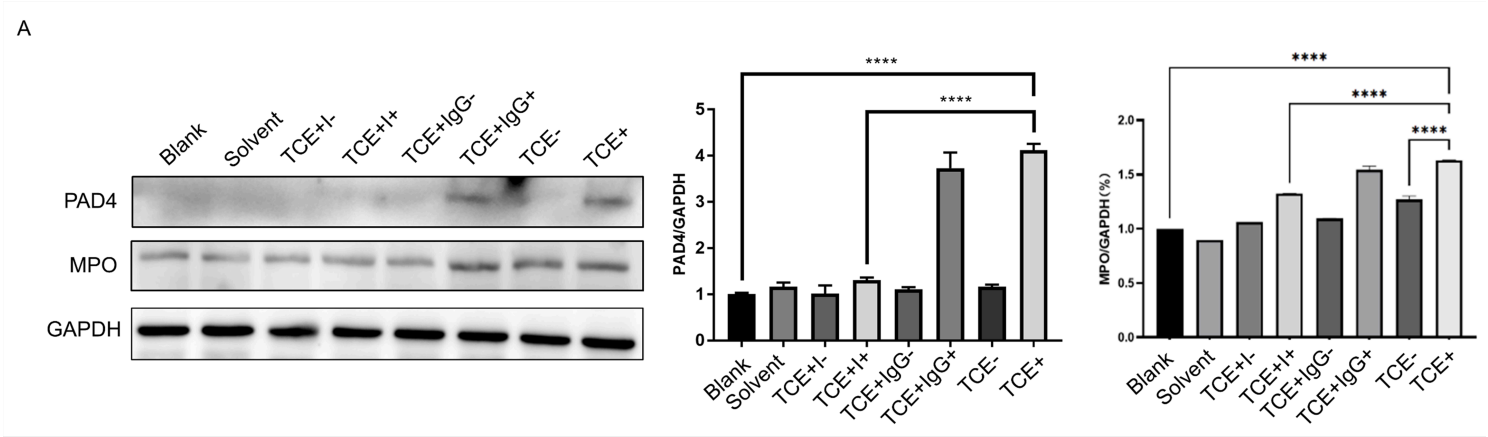


Figure 4

β2 integrin-neutralizing inhibits NETs formation.

(A) Western blot results of endothelial cell PAD4 and MPO.

Blank: Blank control group, N=10; Solvent: Solvent control group, N=10; TCE+I-: TCE+β2integrin sensitization negative group, N=21; TCE+I+: TCE+β2integrin sensitization positive group, N=9; TCE+IgG-: TCE+ IgG isotype control sensitization negative group, N=22; TCE+IgG+: TCE+ IgG isotype control sensitization positive group, N=8; TCE-: TCE sensitization negative group, N=20; TCE+: TCE sensitization positive group, N=10; *P<0.05; **P<0.01; ***P<0.001; ****P< 0.0001.

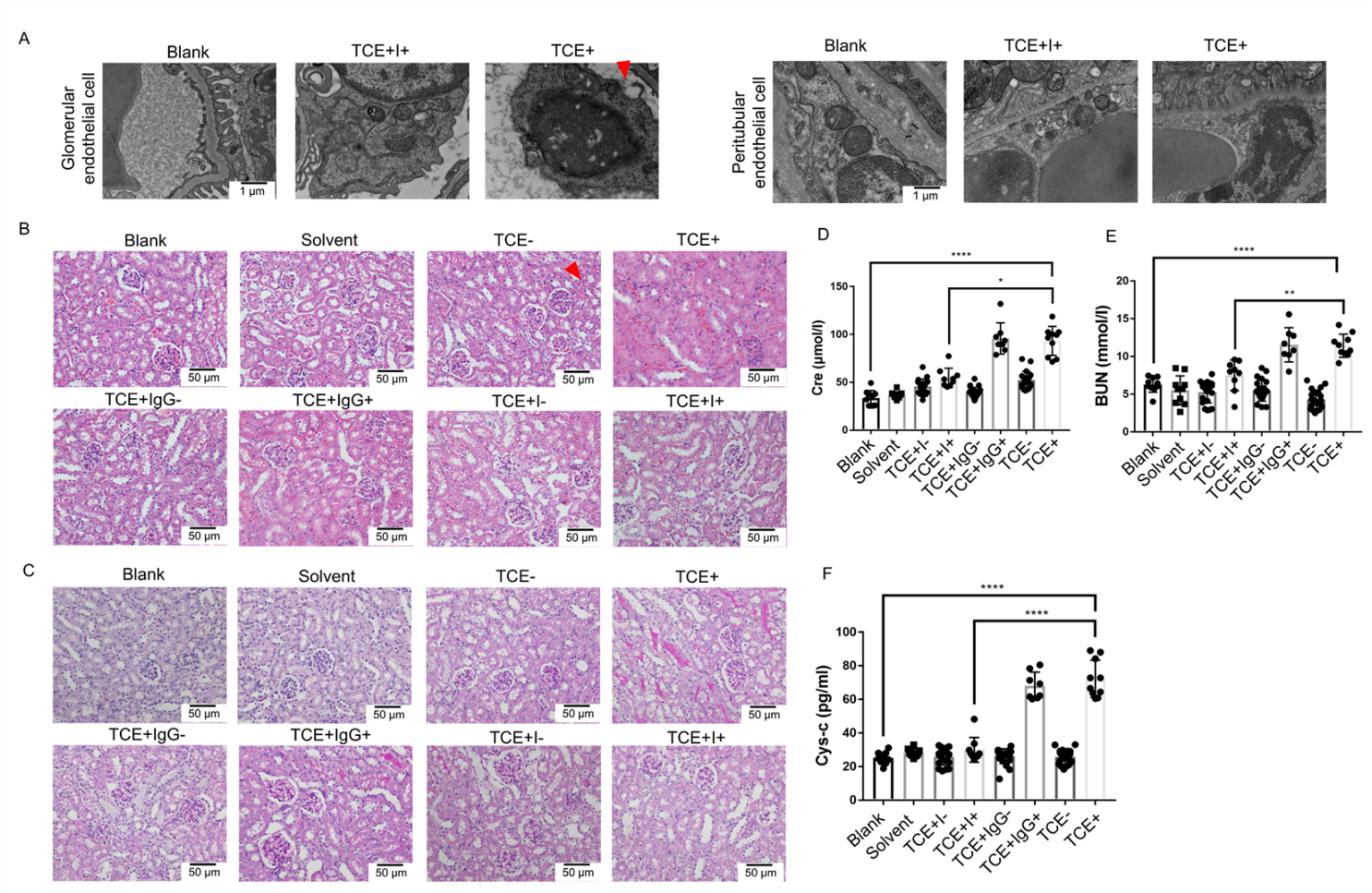


Figure 5

Inhibition of neutrophil adhesion alleviates renal injury.

(A) Transmission electron microscopy results of kidneys (×1000); (B) HE staining results of kidneys (×400); (C) PAS staining results of kidneys (×400); (D) serum level of Gre; (E) serum level of BUN; (F) serum level of Cys-C. Blank: Blank control group, N=10; Solvent: Solvent control group, N=10; TCE+I-: TCE+β2integrin sensitization negative group, N=21; TCE+I+: TCE+β2 integrin sensitization positive group, N=9; TCE+IgG-: TCE+ IgG isotype control sensitization negative group, N=22; TCE+IgG+: TCE+ IgG isotype control sensitization positive group, N=8; TCE-: TCE sensitization negative group, N=20; TCE+: TCE sensitization positive group, N=10; *P<0.05; **P<0.01; ***P<0.001; ****P< 0.0001.