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**Anti-proteinase 3 antibodies both stimulate and prime human neutrophils**

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**Abstract**

**Background.** Anti-neutrophil cytoplasmic antibodies (ANCA) against proteinase 3 (PR3) are postulated to injure vascular endothelium by inducing cytokine-primed neutrophils to release proteolytic enzymes and generate reactive oxygen species. Anti-PR3 induce exocytosis, and since priming is associated with upregulation of plasma membrane proteins by exocytosis of intracellular granules, we tested the hypothesis that anti-PR3 prime neutrophils in the absence of cytokines.

**Methods.** Isolated human neutrophils were incubated with or without anti-PR3. Superoxide release was determined by measuring the reduction of ferricytochrome C. Exocytosis of secretory vesicles and specific granules was determined by measuring the expression of CD35 and CD66b, respectively, using flow cytometry.

**Results.** Anti-PR3 (15 µg/mL) directly stimulated superoxide production and enhanced FMLP-stimulated superoxide production. Anti-PR3 (0.5 µg/mL) did not stimulate superoxide production but did enhance FMLP-stimulated superoxide production. Incubation of neutrophils with anti-PR3 resulted in time-dependent exocytosis of secretory vesicles and specific granules. Anti-PR3-induced exocytosis, but not superoxide production, was dependent on p38 mitogen-activated protein kinase.

**Conclusions.** These data demonstrate that anti-PR3 can directly stimulate production of reactive oxygen species by neutrophils without cytokine priming, and that anti-PR3 prime neutrophils for increased FMLP-stimulated reactive oxygen species production. Anti-PR3 also induce exocytosis via a mechanism separate from their effect on reactive oxygen species production. These findings suggest that anti-PR3 ANCA may activate neutrophils and cause endothelial cell injury by multiple pathways, including some that are independent of priming by a second agent.

**Keywords:** ANCA; exocytosis; neutrophil; priming; reactive oxygen species

**Introduction**

Anti-neutrophil cytoplasmic antibodies (ANCA) mediate small vessel injury associated with a group of diseases, including Wegener’s granulomatosis, microscopic polyangiitis, renal-limited vasculitis and Churg–Strauss syndrome. ANCA are directed against proteinase 3 (PR3) and myeloperoxidase (MPO), and are postulated to exert their pathogenic effect by binding to those proteins on the surface of neutrophils, interacting with FcγRs and activating the cells. Activated neutrophils are thought to adhere to vascular endothelial cells and induce injury through either production of reactive oxygen species or release of proteolytic enzymes stored in neutrophil granules.

While the role of ANCA in vascular injury is well established, the mechanisms through which ANCA activate neutrophils remain poorly defined. The most commonly invoked mechanism involves interaction of ANCA with target antigens on the surface of neutrophils primed by cytokines, such as tumor necrosis factor α (TNFα). Priming increases plasma membrane expression of ANCA target antigens

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and enhances neutrophil responses to activation by ANCA. Activation of primed neutrophils by ANCA increases expression of adhesion molecules, releases proteolytic enzymes and generates reactive oxygen species [1]. The need for a priming agent is supported by a report showing that mice treated intravenously with murine anti-PR3 followed by cutaneous injection of TNFα developed neutrophil infiltration and panniculitis at the site of TNFα injection that was significantly greater than that observed in mice receiving murine anti-PR3 or TNFα, alone [2].

Neutrophil priming is associated with phosphorylation of cytoplasmic components of the NADPH oxidase [3,4]; however, priming agents also increase plasma membrane expression of receptors and membrane components of the NADPH oxidase through exocytosis of intracellular granules [5]. Thus, the observations that ANCA enhance glomerular injury in models of anti-GBM antibody glomerulonephritis [6,7] and that ANCA induce neutrophil granule exocytosis [1,8–10] suggested an alternative mechanism of vascular injury in which ANCA prime neutrophil responses to a second stimulus, leading again to release of proteolytic enzymes, generation of reactive oxygen species and vascular endothelial damage. To evaluate these possible mechanisms of ANCA-induced disease, we examined the ability of anti-PR3 to directly stimulate neutrophil respiratory burst activity and exocytosis and to prime neutrophils for an enhanced response to formylated peptides.

Subjects and methods

Reagents

Anti-PR3 (clone CLB 12.8), which has been shown to be specific for human PR3 [11], was obtained from Accurate Chemical (Westbury, New York, USA), and isotype mouse IgG was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The kinase inhibitors, SB203580, PD980059, LY294002 and genistein, were obtained from Calbiochem (San Diego, CA, USA). FITC-conjugated monoclonal anti-CD35 and FITC-conjugated mouse IgG1 were obtained from Pharmingen (San Diego, CA, USA). FITC-conjugated monoclonal anti-CD66b was obtained from Accurate Chemical. Experiments were performed in the Krebs–Ringer phosphate buffer (pH 7.2) containing 0.2% dextrose (KRPB), which was filtered through an endotoxin-retentive filter and contained <0.06 EU/mL of endotoxin by the Limulus amebocyte lysate assay.

Neutrophils

Neutrophils were isolated from healthy donors using plasma-Percoll gradients [12]. Isolated neutrophils were washed and resuspended in KRPB. Microscopic evaluation showed that >97% of cells were neutrophils. Greater than 97% of cells were viable by trypan blue exclusion. The Human Studies Committee of the University of Louisville approved the use of human donors.

Results

Anti-PR3 stimulates production of reactive oxygen species by neutrophils

ANCA were reported to stimulate production of reactive oxygen species by normal human neutrophils [1,8,9,14]. However, these observations were made in cells pre-treated with cytochalasin B [1], TNFα [1,14] or a combination of the two agents [8,9], both of which enhance the respiratory burst. Little production of reactive oxygen species was reported without pre-treatment [14]. To determine if ANCA directly stimulate production of reactive oxygen species, neutrophils were incubated with 15 μg/mL of anti-PR3, isotype antibody or KRPB for 120 min in the presence of ferricytochrome C. The incubation time and concentration encompass conditions used by previous investigators [8,9,14]. Total superoxide production over the 120-min incubation period was significantly greater in cells incubated with anti-PR3 than in cells incubated with a buffer or isotype antibody (27.4 ± 3.2 nmol/2 × 10⁶ cells/120 min for anti-PR3 versus 11.7 ± 1.7 nmol/2 × 10⁶ cells/120 min

Respiratory burst activity

Respiratory burst activity was assessed as O2⁻− production, determined by measuring the reduction of ferricytochrome C [13]. Neutrophils (4 × 10⁶/mL) were suspended in KRPB containing calcium and magnesium and 1 mg/mL ferricytochrome C. Following stimulation of O2⁻− production, the reaction was stopped by placing the tubes on ice and pelleting the cells by centrifugation at 4°C. Superoxide production was quantified using the change in absorbance of the supernatant at 550 nm and expressed as nanomoles of O2− per 2 × 10⁶ cells using an extinction coefficient of 2.1 × 10⁶/M/cm. All assays of respiratory burst activity were performed in duplicate.

Exocytosis

Exocytosis of secretory vesicles and specific granules was assayed by measuring plasma membrane expression of CD35 and CD66b, respectively [5]. Briefly, neutrophils were incubated at 4°C for 30 min with FITC-conjugated monoclonal anti-CD35 or FITC-conjugated monoclonal anti-CD66b. FITC-conjugated mouse IgG1 was used as an isotype control. Fluorescence intensity was measured by flow cytometry (Coulter Epics XL Flow Cytometer, Miami, FL, USA). Exocytosis of gelatinase granules was determined by ELISA for MMP9 (R&D Systems, Minneapolis, MN, USA).

Statistical methods

Differences between experimental conditions were examined by analysis of variance (SPSS 14.0 for Windows, SPSS Inc., Chicago, IL, USA). Where significant differences were identified, differences between individual groups were assessed using the Student–Newman–Keuls post hoc test or a Bonferroni correction, as appropriate. Statistical significance was defined as P < 0.05.
Anti-PR3 primes FMLP-stimulated production of reactive oxygen species by neutrophils

The respiratory burst elicited by stimulation of normal circulating neutrophils may be enhanced by prior exposure to various pro-inflammatory agents at concentrations that do not stimulate respiratory burst activity. This process is referred to as priming. To determine if anti-PR3 primed or enhanced the respiratory burst, the impact of pre-treatment with anti-PR3 on FMLP-stimulated neutrophil superoxide production was examined. Neutrophils were incubated with 15 µg/mL of anti-PR3 or isotype antibody for up to 110 min. FMLP and ferricytochrome C were added for the final 10 min of incubation. The data in Figure 1 demonstrate that FMLP-stimulated superoxide release was enhanced by exposure to the isotype antibody for 10 min, but returned toward levels seen with isotype antibody, alone, thereafter. Compared to the isotype antibody, pre-treatment with anti-PR3 was associated with significantly more FMLP-stimulated superoxide production at all time points but 30 min. Superoxide production was greatest in cells where FMLP was added with the anti-PR3 (exposure time to anti-PR3 of 10 min); otherwise, the effect of anti-PR3 on FMLP-stimulated superoxide production was independent of the time of exposure to anti-PR3.

The increased FMLP-stimulated superoxide release associated with exposure to anti-PR3 does not necessarily indicate priming of the respiratory burst because anti-PR3 also increased basal superoxide production (Figure 1). To address this concern, we calculated the difference between basal and FMLP-stimulated superoxide production in the presence of anti-PR3 or isotype antibody. The FMLP-stimulated increase in superoxide production was greater in cells pre-treated with anti-PR3 for 50, 70, 90 and 110 min than in cells pre-treated for the same times with the isotype antibody suggesting that anti-PR3 prime, as well as stimulate, respiratory burst activity.

In an attempt to more clearly determine if anti-PR3 prime the respiratory burst, we examined the response of neutrophils to incubation with various concentrations of anti-PR3 with and without subsequent stimulation by FMLP. Neutrophils were incubated with anti-PR3 for 20 min, followed by the addition of FMLP and ferricytochrome C for a further 10 min. Anti-PR3 did not stimulate superoxide production at concentrations <1.0 µg/mL (Figure 2). However, pre-treatment with 0.5 µg/mL, or more, of anti-PR3 resulted in significantly more superoxide production following stimulation with FMLP than that obtained in the absence of pre-treatment.
The finding that 0.5 μg/mL of anti-PR3 enhanced FMLP-stimulated superoxide production, while failing to directly stimulate superoxide production, indicates that anti-PR3 are capable of priming neutrophil superoxide production.

**Priming of FMLP-stimulated superoxide production by anti-PR3 is independent of p38 MAPK, ERK and tyrosine kinases**

Various signalling pathways have been implicated in neutrophil respiratory burst priming, including the p38 and ERK mitogen-activated protein kinase pathways [5,15], phosphatidylinositol 3-kinase (PI3-kinase) [16] and tyrosine kinases [3]. To determine if any of those pathways were involved in anti-PR3 stimulation of respiratory burst activity or priming of FMLP-stimulated respiratory burst activity, we examined the ability of pharmacologic inhibitors to block FMLP-stimulated superoxide production in cells treated with 15 μg/mL anti-PR3 or isotype control antibody. The data in Figure 3 demonstrate that the enhanced FMLP-stimulated superoxide production in the presence of anti-PR3 was not affected by inhibition of p38 MAPK with SB203580 or by inhibition of tyrosine kinases with genistein. As we previously reported [17], inhibition of ERK with PD980059 reduced FMLP-stimulated superoxide production in the presence of isotype antibody. However, anti-PR3 retained its capacity to enhance FMLP-stimulated superoxide production in the presence of PD980039, suggesting that anti-PR3 activates signalling pathways that are independent of ERK. In contrast, pre-treatment with the PI3-kinase inhibitor, LY294002, completely blocked the ability of anti-PR3 to enhance FMLP-stimulated superoxide production.

**Anti-PR3 stimulates neutrophil exocytosis**

Rather than damaging vascular endothelium through release of reactive oxygen species, it has been suggested that ANCA-stimulated neutrophils damage endothelial cells by releasing proteases via exocytosis of intracellular storage granules [18]. ANCA were reported to stimulate exocytosis in normal human neutrophils [1,8–10], although as with ANCA-stimulated production of reactive oxygen species these observations were obtained in cells pre-treated with cytochalasin B or TNFα [1,8–10]. Therefore, we examined the ability of anti-PR3 to induce exocytosis in normal human neutrophils in the absence of pre-treatment with other agents. The data in Figure 4 demonstrate that anti-PR3 at a concentration of 15 μg/mL induce a significant time-dependent increase in plasma membrane expression of CD35 and CD66b and release of gelatinase signifying exocytosis of secretory vesicles and gelatinase and specific granules.

As demonstrated by the data in Figure 2, an anti-PR3 concentration of 0.5 μg/mL is insufficient to stimulate superoxide production. To examine if anti-PR3 is capable of inducing exocytosis at this concentration, normal neutrophils were incubated with 0.5 μg/mL of anti-PR3 for 75 min. As a positive control, neutrophils were incubated with 2 ng/mL of TNFα for 10 min. The data in Figure 5...
Anti-PR3-induced exocytosis is p38 MAPK dependent

As demonstrated by the data in Figure 3, only inhibition of PI3-kinase prevented priming of FMLP-stimulated superoxide production by anti-PR3. Previous studies suggested that exocytosis contributes to priming of respiratory burst activity by inducing translocation of membrane components of the NADPH oxidase to the plasma membrane [5]. To examine if exocytosis induced by anti-PR3 contributed to enhanced respiratory burst activity, we examined the ability of the same pharmacologic inhibitors to block anti-PR3-induced exocytosis. As shown by the data in Figure 6, inhibition of the p38 MAPK pathway with SB203580 significantly reduced the ability of 15 μg/mL anti-PR3 to induce exocytosis of secretory vesicles (CD35) and specific granules (CD66b). While inhibition of PI3-kinase with LY294002 also inhibited anti-PR3-induced exocytosis of specific granules, it did not prevent anti-PR3-induced exocytosis of secretory vesicles. Inhibition of the ERK pathway or tyrosine kinases did not significantly alter exocytosis of either granule subset.

Discussion

The data obtained in this study demonstrate that anti-PR3 can directly stimulate production of reactive oxygen species and exocytosis of secretory vesicles, gelatinase granules and specific granules in neutrophils without priming or treatment with an agent that disrupts the actin cytoskeleton. Pre-treatment with anti-PR3 also primed neutrophils for increased production of reactive oxygen species stimulated by FMLP. These observations suggest the potential for multiple roles for anti-PR3 in activating neutrophils and injuring endothelial cells.

Basal production of superoxide has been reported to be significantly greater in neutrophils from patients with anti-PR3-associated vasculitis than in neutrophils from healthy persons, particularly in the presence of active disease [19,20]. This observation is consistent with in vitro studies showing that anti-PR3 stimulated superoxide production in neutrophils primed by disruption of the actin cytoskeleton [21] or by cytokines [8–10,22,23]. Some investigators have found priming to be necessary for anti-PR3 to stimulate superoxide production [1,14], and administration of bacterial lipopolysaccharide was shown to markedly worsen renal injury in a murine model of anti-MPO-induced glomerulonephritis [24]. Priming was postulated to be required for translocation of PR3 from intracellular storage compartments to the plasma membrane, where it serves as the target for anti-PR3. In contrast to these reports, our data confirm the observation of Porges et al. [25] that incubation of neutrophils with anti-PR3 for 45–60 min results in direct stimulation of respiratory burst activity without prior disruption of the cytoskeleton or priming. Recent studies indicated that PR3 is expressed on the plasma membrane
of at least a subset of circulating neutrophils [26,27]. Our data suggest that this PR3 expression is sufficient to initiate cell activation by anti-PR3, which then may be amplified through expression of additional PR3.

Pre-treatment with anti-PR3 at a concentration that did not directly stimulate respiratory burst activity primed neutrophils for enhanced FMLP-stimulated superoxide production. This finding agrees with a report that anti-PR3 can prime FMLP-induced production of reactive oxygen species in monocytes [28]. However, Hattar and colleagues reported that FMLP-stimulated superoxide production was significantly decreased in isolated neutrophils pre-treated with 1 µg/mL anti-PR3, even though FMLP-induced leukotriene generation and chemotaxis were enhanced [29]. The reasons for these discordant findings are unclear. Other agents that enhance neutrophil chemotaxis toward FMLP, such as TNFα and β-glucan, also prime FMLP-stimulated production of reactive oxygen species [30,31]. Our data suggest that priming is not required for ANCA to activate neutrophils. Additionally, ANCA are able to prime neutrophils for an enhanced response to a subsequent stimulus.

Although PR3 is usually considered a component of the azurophil granules of neutrophils, significant stores of PR3 also exist in specific granules and secretory vesicles [26,32]. It has been postulated that anti-PR3 activates circulating neutrophils [33], possibly by interacting with PR3 expressed on the plasma membrane [34]. Our finding that anti-PR3 can directly stimulate reactive oxygen species production and induce exocytosis of secretory vesicles, gelatinase granules and specific granules, even at low concentrations, is consistent with that hypothesis. Exocytosis of these granule subsets increases membrane expression of integrin receptors involved in neutrophil adhesion to vascular endothelium, and anti-PR3 was reported to increase integrin-mediated PMN adhesion [33]. Exocytosis of specific granules and secretory vesicles could result in increased plasma membrane expression of PR3, raising the possibility that anti-PR3 is capable of inducing a positive feedback loop by upregulating the amount of antigen expressed on the neutrophil surface. That anti-PR3 is capable of inducing exocytosis in unprimed neutrophils is consistent with previous reports. Tanaka and colleagues demonstrated that anti-PR3 induced an increase in CD66b expression and α-defensin release in neutrophils that were first incubated at 37°C for 40 min to increase membrane-associated PR3 [35]. In those experiments, the magnitude of the increase in CD66b was dependent on the level of PR3 expression. In addition, Muller Kobold and colleagues reported that neutrophils from patients with active ANCA-associated vasculitis had increased CD66b expression compared to healthy subjects and patients with quiescent disease [36].

Anti-PR3 signalling leading to the production of reactive oxygen species is mediated through co-ligation of PR3 and FcγRIIa on the plasma membrane [25] and has been shown to be PI3-kinase dependent [37,38]. Our results confirm this PI3-kinase dependence and demonstrate that it extends to anti-PR3-induced exocytosis. Priming of FMLP-stimulated production of reactive oxygen species by TNFα and GM-CSF is p38-MAPK- and PI3-kinase dependent [15]. Two mechanisms of priming neutrophil reactive oxygen species production have been proposed, upregulation of flavocytochrome b558 expression in the plasma membrane via exocytosis [5] and phosphorylation of cytosolic components of the NADPH oxidase [3,4]. The observation that low concentrations of anti-PR3 both induced exocytosis of specific granules and primed FMLP-stimulated superoxide production is consistent with a role for exocytosis in priming of the respiratory burst by ANCA. However, while pharmacologic inhibition of p38 MAPK reduced anti-PR3-induced exocytosis of specific granules and secretory vesicles, it had no effect on FMLP-stimulated production of reactive oxygen species in neutrophils pre-treated with anti-PR3. The effect of anti-PR3 on reactive oxygen species production is mediated by FcγRIIa [25], however, and we have previously shown that reactive oxygen species production stimulated by ligation of FcγRIIa does not depend on p38 MAPK [39]. Thus, while anti-PR3 both induce exocytosis and prime FMLP-stimulated respiratory burst activity, the differences in signal transduction pathways and time courses between anti-PR3-mediated priming and exocytosis indicate that exocytosis does not contribute to anti-PR3-mediated priming. The failure of p38 MAPK inhibition to alter the effect of anti-PR3 on FMLP-stimulated superoxide production also suggests that our finding that anti-PR3 directly stimulates reactive oxygen species production is not an artifact of contamination with lipopolysaccharide, which is a p38 MAPK-dependent priming agent.

Our study has limitations. It is possible that the neutrophils were primed during isolation and handling. However, we found no difference in either basal or FMLP-stimulated superoxide production between neutrophils isolated as described above and neutrophils present in leukocytes obtained from whole blood by dextran sedimentation of red blood cells (data not shown). In addition, Stie and Jesaitis reported that the method we used to isolate neutrophils yields cells that are structurally and functionally unprimed compared to other methods of isolation [40]. Moreover, the data in Figure 2 show that the cells remained capable of being primed by anti-PR3. Finally, our observations were made with anti-PR3 and it may not be possible to generalize our findings to anti-MPO. MPO is predominantly stored in azurophil granules. Although some MPO has been detected in specific and gelatinase granules [32], it is unclear that sufficient MPO is expressed on the plasma membrane of resting neutrophils to serve as a target for anti-MPO in the same way as membrane-bound PR3 serves as a target for anti-PR3 [26].

The pathogenetic events leading to ANCA-associated vasculitis and glomerulonephritis are postulated to begin with priming of circulating neutrophils by cytokines or other factors typically generated by an infection (reviewed in 41–43). Priming is accompanied by exocytosis of intracellular granules, resulting in increased plasma membrane expression of the ANCA antigens, MPO and PR3, and adhesion molecules. The primed neutrophils are then activated by ANCA through interaction with these newly expressed ANCA antigens and Fc receptors. The activated neutrophils adhere to endothelial cells and induce injury through release of granular enzymes and reactive oxygen species. This hypothesis is supported by in vitro studies indicating that priming of isolated neutrophils was required for
ANCA stimulation of respiratory burst activity [1,22,23], by in vivo studies showing that concomitant administration of ANCA and LPS to mice resulted in significantly greater vascular injury than administration of ANCA, alone [24], and by observations in humans that an infection frequently precedes development of ANCA-associated vasculitis [44]. Our data suggest, however, that the sequence of events leading to ANCA-associated vasculitis may be more varied. The ability of ANCA to activate unprimed neutrophils in our studies may explain previous reports that passive transfer of anti-MPO ANCA or anti-MPO splenocytes into mice produces varying degrees of systemic vasculitis in the absence of priming [45], and neutrophils from patients with ANCA-associated vasculitis, even those in remission, have increased basal respiratory burst activity [19,20]. Our data showing that low doses of ANCA prime neutrophils suggest the possibility that exposure to ANCA is the initial event priming circulating neutrophils, and a subsequent neutrophil stimulus leads to clinical disease. Thus, the hypothesis that ANCA may play one of several roles in different individuals with ANCA-associated vasculitis needs to be tested.

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Conflict of interest statement. None declared.

References

2. Pfister H, Ollert M, Fröhlich LF et al. Antineutrophil cytoplasmic autoantibodies against the murine homolog of proteinase 3 (Wegener autoantigen) are pathogenic in vivo. Blood 2004; 104: 1411–1418
30. Bajaj MS, Kew RR, Webster RO et al. Priming of neutrophil functions by tumor necrosis factor: enhancement of superoxide anion
Serum cystatin C in mouse models: a reliable and precise marker for renal function and superior to serum creatinine

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Abstract

Background. Serum creatinine (SCR) and blood urea nitrogen (BUN) determine the glomerular filtration rate (GFR) improperly in acute renal failure. Serum cystatin C (CYS) has the potential to be a more precise marker for GFR. The aim of this study was to compare the sensitivity of SCR, BUN and CYS with respect to the detection of acute renal failure in mice.

Methods. In an ischaemia reperfusion (I/R) injury model, mice suffered 60-min left kidney ischaemia and right nephrectomy. In a nephrectomy model, mice were nephrectomized to a different extent: from unilateral (3/6Nx) to bilateral nephrectomy (BiNx). Blood samples were collected 2, 12 or 24 h post-op.

Results. SCR, BUN and CYS increased significantly in the I/R-model in comparison to sham mice and 3/6Nx mice at 12 and 24 h post-op (SCR P = 0.009; BUN P < 0.001 and CYS P < 0.004). There were no significant differences in all three markers between 3/6Nx and sham-operated mice. In graded nephrectomy, BUN and CYS showed already significantly the loss of kidney in 4/6Nx mice 12 h post-op [BUN (mg/dl): sham 26.4 ± 3.5, 4/6Nx 52.3 ± 13.4, P < 0.01; CYS (mg/l): sham 0.08 ± 0.03, 4/6Nx 0.15 ± 0.04, P < 0.01], whereas SCR was only significantly increased in 5/6Nx and BiNx mice 24 h post-op [SCR (mg/dl): sham 0.39 ± 0.05, 4/6Nx 0.52 ± 0.07, P = 0.13, 5/6Nx 1.00 ± 0.29, P < 0.01]. In the longitudinal experiment, CYS showed the renal damage significantly earlier and to a larger extent (2 h: SCR 57 ± 15%, BUN 40 ± 16%, CYS 295 ± 143%, P < 0.001).

Conclusions. CYS can be used as a reliable and precise marker for renal function in mouse models. CYS is more sensitive than SCR, and it shows renal damage earlier than SCR and BUN.

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