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Protection from toxicant-mediated renal injury in the rat with anti-CD54 antibody

K.J. Kelly, Shane M. Meehan, Robert B. Colvin, Winfred W. Williams, jr., and Joseph V. Bonventre

Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio, and Medical and Pathology Services, Massachusetts General Hospital, Departments of Medicine and Pathology, Harvard Medical School, Boston, Massachusetts, USA

Protection from toxicant-mediated renal injury in the rat with anti-CD54 antibody.

Background. The benefit of the potent chemotherapeutic agent cisplatin in treating neoplasms is limited by nephrotoxicity. We tested the hypothesis that CD54 [intercellular adhesion molecule-1 (ICAM-1)] is an important mediator in cisplatin-mediated renal failure.

Methods. The effect of a monoclonal anti-CD54 antibody was evaluated in a rat model of cisplatin toxicity. Renal function, histopathology, renal myeloperoxidase activity, and mortality were determined in the anti-CD54 and placebo groups.

Results. Renal CD54 mRNA expression was markedly increased by 24 hours after exposure to cisplatin in mice. An improvement in renal function, mortality, and histological abnormalities was evident in animals exposed to cisplatin and treated with anti-CD54 antibody (mAb). Seven days after the administration of cisplatin, the mean creatinine was 0.65 ± 0.05 mg/dl in the rats that received anti-CD54 mAb and 4.76 ± 1.42 in control animals ($P < 0.02$). Mortality was lower in experimental animals (0 vs. 29% in control rats seven days following cisplatin, $P < 0.04$). Histological evidence of cell injury was markedly attenuated ($P < 0.04$) in the treated compared with the control rats.

Conclusion. CD54 may be critical in the pathophysiology of renal injury following cisplatin, perhaps by its effects on leukocyte–endothelial interactions.

The potent antineoplastic agent cisplatin (cis-diamminedichloroplatinum II) is among the most commonly used drugs in the therapy of cancer and has markedly improved the prognosis of patients with germ cell and other tumors. Nephrotoxicity is frequent and is the major limitation to the use of cisplatin [1]. Several mechanisms are believed to contribute to renal dysfunction following exposure to cisplatin: direct toxicity of the agent on tubular cells [2], vascular factors [3, 4], reactive oxygen species [5], and inflammatory cells [2]. We sought to investigate the role of the adhesion receptor CD54 [intercellular adhesion molecule-1 (ICAM-1)] in an animal model of cisplatin-mediated nephropathy.

Adhesion receptors mediate leukocyte adhesion to other leukocytes, endothelial cells, and cell matrix components, and are thought to be important in the localization of white cells to sites of inflammation. CD54 (ICAM-1) is a member of the immunoglobulin superfamily. It is expressed on vascular endothelium, and its expression is regulated by stimuli such as interleukin-1 and tumor necrosis factor-α. CD54 serves as a ligand for the β_2 integrins, LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18), on leukocytes. To evaluate the possible role of CD54 in cisplatin nephropathy, we administered a monoclonal antibody (mAb) against CD54 to rats exposed to cisplatin.

METHODS

Animal protocols

All animal experimentation was conducted in conformity with the "Guiding Principles for Research Involving Animals and Human Beings." Male Sprague-Dawley rats weighing 180 to 240 g and male Swiss Webster mice weighing 20 to 25 g (Charles River Breeding Laboratories, Wilmington, MA, USA) were allowed free access to standard rodent chow and water. The animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (65 mg/kg). The animals were placed on a homeothermic table to maintain the core body temperature at approximately 37°C during the period of anesthesia. Anti-CD54 mAb (IgG_1; IA29 [6]), placebo (5 mg/kg bovine serum albumin; Sigma Chemical Co., St. Louis, MO, USA), control antibody (IgG_1 antimonkey CD3), or vehicle (0.9% NaCl) alone was administered via the

Key words: nephrotoxicity, acute renal failure, intercellular adhesion molecule-1, adhesion receptors, cisplatin toxicity, leukocyte.

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Table 1. Experimental groups

Group	Treatment	Time of administration[a]
I	Placebo	0 hours
II	Anti-CD54 mAb	0 hours
III	Placebo	0, 8 and 24 hours
IV	Anti-CD54 mAb	0, 8 and 24 hours
V	Placebo	−2 hours
VI	Anti-CD54 mAb	−2 hours
VII	Placebo	1 hour
VIII	Anti-CD54 mAb	1 hour

[a] Times are relative to the time of administration of cisplatin (0 hours)

tail vein in a total volume of 500 μl. In some experiments, the placebo consisted of 500 μl of ascites from an animal injected with incomplete Freund's adjuvant (Sigma Chemical Co.) but no hybridoma cells. Experiments performed with bovine serum albumin, the ascites placebo, and vehicle (0.9% NaCl) yielded equivalent results; thus, the results were combined. Cisplatin (Sigma) was administered intraperitoneally at a dose of 7.5 mg/kg. Blood samples for blood urea nitrogen (BUN) and creatinine determination were obtained from the tail vein or via cardiac puncture at baseline and at several time points (Figures 1–9) following the administration of cisplatin. Some animals were sacrificed at predetermined time points in order to obtain tissue for histopathology and determination of myeloperoxidase (MPO) activity. All experiments were blinded, that is, the investigator administering the mAb was not aware of the identity of the substance administered to each animal until all of the results were obtained.

Experimental groups

Animals in group I received placebo, vehicle, or control antibody immediately prior to cisplatin (Table 1). Those in group II received anti-CD54 mAb immediately prior to the administration of cisplatin. Group III received three doses of placebo. The initial dose was administered immediately prior to cisplatin, and subsequent doses were given 8 and 24 hours later. Group IV received three doses of anti-CD54 mAb administered at the same time points. Group V received one dose of placebo two hours prior to cisplatin. Group VI received one dose of anti-CD54 mAb two hours prior to cisplatin. Group VII received one dose of placebo one hour after the administration of cisplatin. Group VIII received one dose of anti-CD54 one hour after cisplatin.

Northern analysis

Total mRNA was prepared, and blots were probed with ^{32}P-labeled full-length mouse CD54 cDNA [7] using standard techniques [8]. Briefly, kidney tissue was homogenized in guanidine isothiocyanate, and RNA was isolated by centrifugation in a cesium chloride gradient. Hybridization was carried out in formamide at 42°C, and the blots were washed in 2 × SSPE [SSPE = (in mM) 150 NaCl, 10 NaH_2PO_4, 1.0 ethylenediaminetetraacetic acid (EDTA)] at room temperature twice, 2 × SSPE at 65°C twice, and then 0.5 × SSPE twice at room temperature. Autoradiograph densities were compared using Kodak digital science image analysis software 2.0.2 (Eastman Kodak Company, Rochester, NY, USA).

Monoclonal antibodies

Cell culture supernatant or mouse ascitic fluid from hybridoma cell lines producing mouse IgG_1 anti-rat CD54 (anti–ICAM-1) mAb (IA29) [6] was filtered prior to injection. The adhesion of activated (but not resting) lymphocytes to the cultured high endothelial cell line, Ax, is inhibited by 1A29 antibodies [6]. This antibody has also been shown to inhibit phorbol 12-myristate 13-acetate–induced aggregation of rat phytohemagglutinin blasts *in vitro* [6]. In rats, Sasso et al found 28.3 ± 4.1% of injected indium-111–labeled 1A29 present in the blood at 15 minutes [9]. This decreased to 8.5 ± 0.9% at 18 hours. At 15 minutes, 5.0 ± 0.2% of the injected antibody was found in the kidney and at 18 hours 6.7 ± 0.2% was localized in the kidney [9]. The placebo consisted of an equivalent volume (500 μl) and protein concentration of bovine serum albumin (Sigma) or ascites from an animal injected with incomplete Freund's adjuvant (Sigma) but no hybridoma cells. Ascitic fluid containing the irrelevant control antibody (anti-monkey CD3) was the generous gift of Drs. Paul Russel and A.B. Cosimi (Massachusetts General Hospital, Boston, MA, USA).

Light microscopy

At 4 hours or 3, 4, or 7 days following cisplatin administration, rat kidneys were removed from animals anesthetized with pentobarbital (100 mg/kg) and were formalin fixed, paraffin embedded, sectioned at 4 microns, and stained using hematoxylin and eosin and periodic acid-Schiff stains. The percentage of tubules in the outer medulla that showed epithelial necrosis with luminal necrotic debris and tubular dilation was each quantitated as follows: 0 = none, 1+ = <10%, 2+ = 10 to 25%, 3+ = 26 to 75%, 4+ = >75%. The extent of infiltration of the outer medulla by inflammatory cells was graded similarly. The estimates were performed on coded sections without the knowledge of the experimental group to which the animals belonged.

Myeloperoxidase activity

Myeloperoxidase activity, used as an indicator of neutrophil infiltration [10], was measured in coded kidney homogenates four days after cisplatin administration. Aliquots (0.2 ml) of 40,000 × *g* supernatants of kidney homogenates, prepared as previously described [11],

were added to 0.8 ml of reaction mixture containing 60 mM potassium phosphate buffer, pH 6.0, 0.20 mg/ml *o*-dianisidine dihydrochloride (Sigma), and 0.0006% H_2O_2. The change in absorbance was measured at 460 nm. MPO activity was normalized to the protein content of the supernatant [11] and was expressed as the percentage increase over animals subjected to sham surgery.

Fractional excretion of sodium

Serum and urine sodium were measured using a flame photometer (Instrumentation Laboratory, Lexington, MA, USA), and fractional excretion of sodium (FE_{Na}) was calculated as: ($Urine_{Na} \times Serum_{BUN}/Urine_{BUN} \times Serum_{Na}$) $\times$ 100.

Statistics

Creatinine and BUN values are expressed as means $\pm$ 1 SE. Analysis of variance was used to determine if differences among mean values of BUN, creatinine, and MPO levels reached statistical significance. Student's *t*-test was used for comparisons between groups. Fisher's exact test was used to determine if differences in mortality, histological parameters, autoradiographic densities, and FE_{Na} were significant. Mortality rates were calculated for the seven days following reversal of anesthesia. Animals sacrificed before seven days [for histological specimens, MPO activity, or Northern analysis] were not included in mortality statistics. A P value <0.05 was considered significant.

RESULTS

Effect of cisplatin on CD54 mRNA expression

In normal mice, renal CD54 mRNA levels were elevated at 24 hours after exposure to cisplatin (7.5 mg/kg) when compared with levels in kidneys from sham-injected animals (Fig. 1). Quantitation demonstrated that CD54 mRNA (normalized to GAPDH mRNA) was increased to twofold levels in vehicle-treated animals at four hours (P = NS) and 2.3-fold at 24 hours ($P < 0.02$; Fig. 1B).

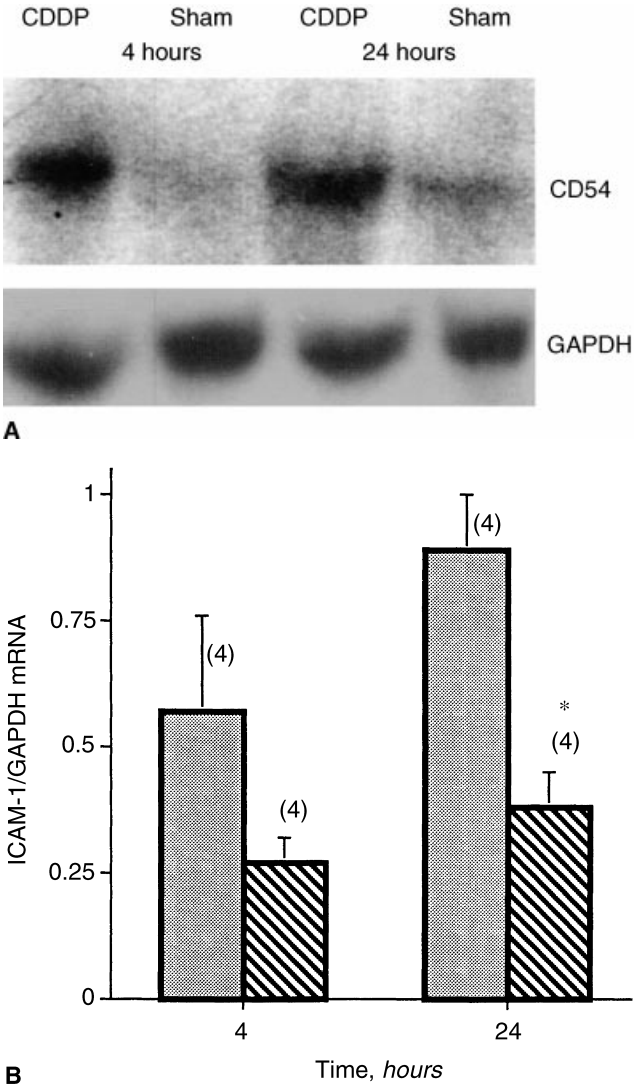


Fig. 1. Effect of cisplatin on renal CD54 mRNA expression. Northern analysis demonstrates increased CD54 mRNA expression in normal kidney tissue 4 and 24 hours following exposure to cisplatin. Little CD54 mRNA is detected following an injection of placebo. A representative blot is shown in (*A*). Hybridization with a full-length human GAPDH probe was used to demonstrate equal loading of lanes. Quantitation of ICAM-1 mRNA expression (normalized to GAPDH mRNA) is presented (*B*). Symbols are: (■) cisplatin; (▨) placebo; *$P < 0.02$.

Effect of anti-CD54 monoclonal antibody on renal function following cisplatin administration

In placebo-treated animals, serum creatinine increased approximately fourfold over baseline three days following the administration of cisplatin (7.5 mg/kg; Fig. 2). The mean creatinine levels increased further to approximately 10-fold baseline by seven days after cisplatin. Creatinine levels from three to seven days following the induction of nephropathy were significantly lower ($P < 0.05$) following cisplatin in animals (group II) treated with anti-CD54 mAb at the time of administration of cisplatin when compared with the mean creatinine levels in rats receiving placebo (group I; Fig. 2). A similar pattern of protection is seen when urea nitrogen values are examined (Fig. 3).

Effect of different dosing regimens of anti-CD54 monoclonal antibody on renal function following cisplatin administration

Rats that received multiple doses of anti-CD54 mAb (group IV) were also protected from renal dysfunction ($P < 0.01$) when compared with rats receiving placebo (group III; Fig. 4). A single dose of anti-CD54 mAb given two hours prior (group VI) to the administration of cisplatin resulted in protection from renal dysfunction

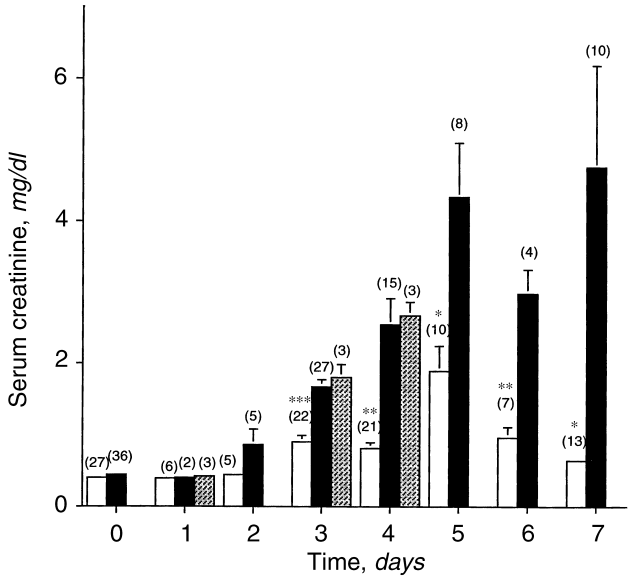


Fig. 2. Effect of treatment with anti-CD54 monoclonal antibody (mAb) on renal function as measured by serum creatinine following cisplatin administration. Creatinine values were measured prior to cisplatin and daily from days 1 through 7 following cisplatin administration. Symbols are: (□) anti-CD54; (■) placebo; (▨) control antibody; $*P < 0.05$; $**P < 0.01$; $***P < 0.0002$. In Figures 2, 3, 4, 5, 6, and 9, data are expressed as means ± 1 SEM. Numbers in parentheses represent the number of animals studied at each point. *P* values for comparisons between anti-CD54 mAb (group II) and placebo-treated (group I) animals are presented.

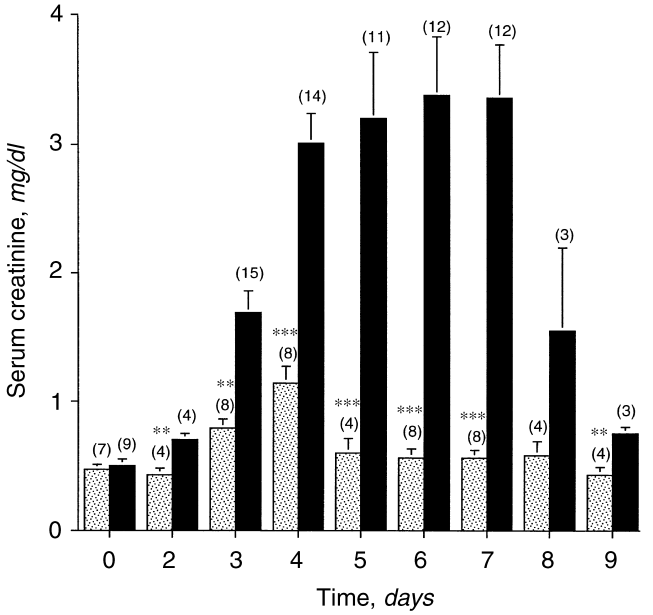


Fig. 4. Effect of different treatment regimens on renal function following cisplatin administration. Creatinine values prior to cisplatin and on days 1 through 9 following induction of nephropathy are presented. Animals received three doses of anti-CD54 monoclonal antibody (▨; group IV) or placebo (■; group III) at the time of cisplatin administration and 8 and 24 hours after cisplatin injection. *P* values are $**P < 0.01$ and $***P < 0.002$ for the comparison of anti-CD54 mAb and placebo-treated groups. In addition, mean creatinine values on days 4, 5, and 6 in the group receiving three doses of anti-CD54 mAb were significantly lower ($P < 0.03$) that the values in animals following a single dose of mAb.

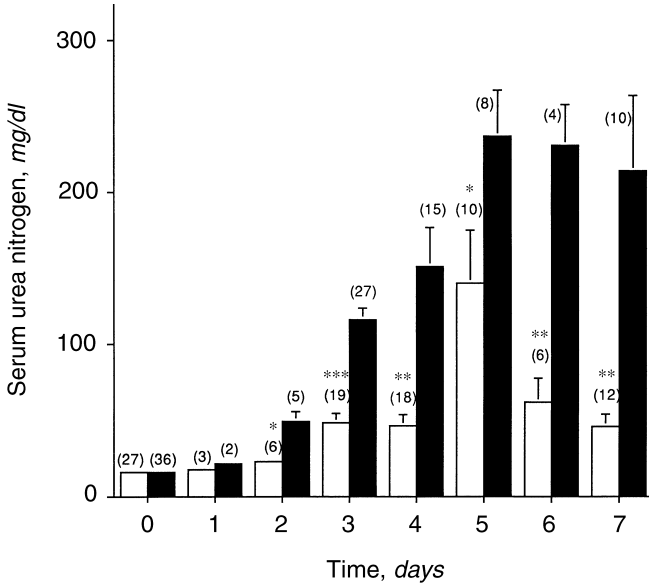


Fig. 3. Effect of treatment with anti-CD54 monoclonal antibody (mAb) on renal function as measured by serum urea nitrogen following cisplatin administration. Serum urea nitrogen values were measured prior to the induction of nephropathy and daily from days 1 through 7 after cisplatin. Symbols are: (□) anti-CD54; (■) placebo; $*P < 0.05$; $**P < 0.01$; $***P < 0.0002$.

that was no different than in animals treated with anti-CD54 at the time of cisplatin (Fig. 5). The administration of anti-CD54 mAb (group VIII) after the exposure to cisplatin did not result in protection from renal failure. The mean serum creatinine in group VIII was no different than that in the placebo group (VII) on days 0 through 7 following the administration of cisplatin (Fig. 6).

Mortality following cisplatin administration and treatment with anti-CD54 monoclonal antibody or placebo

Another indication of the protection against toxic nephropathy in the treated animals was reflected in the mortality rates (Table 2). None (0%) of the anti-CD54–treated rats (group II, $N = 13$) followed for seven days after cisplatin expired, whereas four (29%) of the control post-cisplatin (group I, $N = 14$) animals died ($P < 0.04$).

Effects of anti-CD54 monoclonal antibody on histological changes of cisplatin nephropathy

Histological examination of kidney sections three, four, and seven days following cisplatin administration revealed decreased tubular necrosis in kidneys from anti-

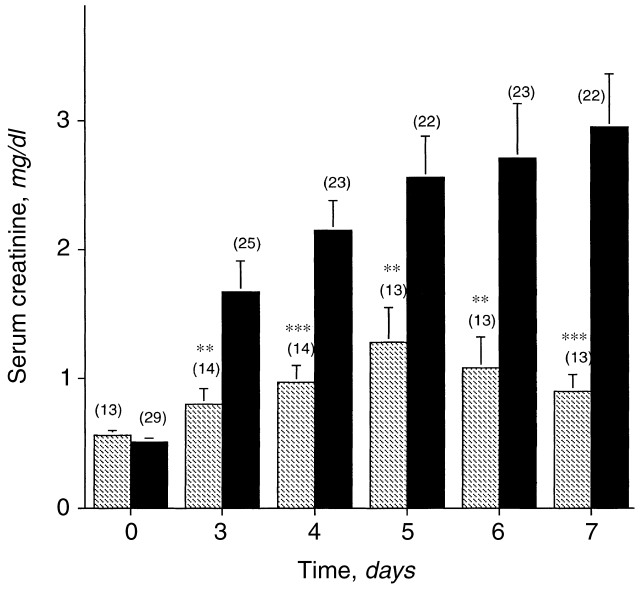


Fig. 5. Effect of administration of anti-CD54 monoclonal antibody (mAb) prior to cisplatin administration on renal function. Creatinine values zero to seven days following cisplatin are presented. Symbols are: (■) placebo; (▨) anti-CD54; $^{**}P < 0.01$; $^{***}P < 0.002$.

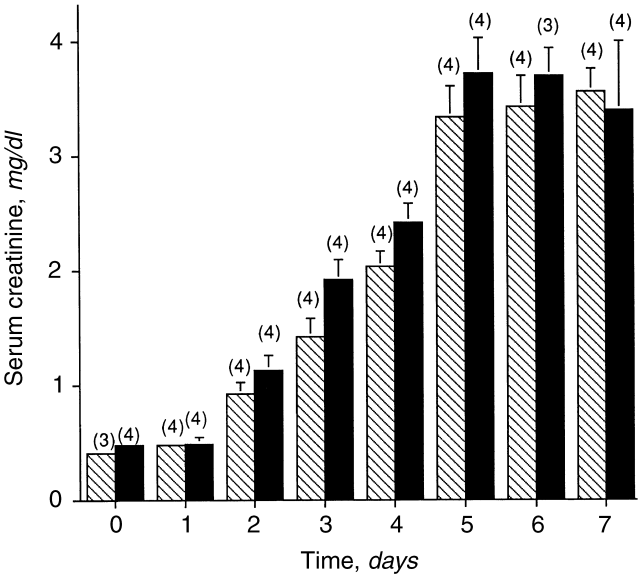


Fig. 6. Effect of administration of anti-CD54 monoclonal antibody (mAb) after cisplatin on renal function. Mean creatinine values prior to cisplatin and on days 1 through 7 after the administration of anti-CD54 (▧) or placebo (■) and cisplatin are shown. No statistically significant differences were observed between the groups at any of the time points evaluated.

CD54 mAb-treated animals (group II) as compared with the control rats (group I) at the early time point (days 3 and 4) and decreased dilation of tubules on day 7 (Table 3 and Fig. 7, $P < 0.04$). Fewer inflammatory cells were present in the kidneys from animals treated with anti–CD54 compared with the placebo group on day 4 ($P < 0.05$). Given the increases in the CD54 mRNA level observed after exposure to cisplatin (Fig. 1), histological sections were examined four hours after the administration of cisplatin. No morphological differences in the kidneys from the anti-CD54 and placebo groups were observed. At four hours, there were no differences between mean serum urea nitrogen, creatinine, or FE_{Na} in the anti-CD54 and placebo-treated animals (data not shown).

Table 2. Mortality rates[a]

Experimental group	Mortality	Number of animals
Placebo (group I)	4 (29%)	14
Anti-CD54 mAb (group II)	0 (0%)[b]	13

[a] Only animals followed for 7 days following exposure to cisplatin are included in the mortality statistics
[b] $P < 0.04$ vs. placebo

Effect of anti-CD54 monoclonal antibody on myeloperoxidase activity in cisplatin nephropathy

Myeloperoxidase activity, determined as an index of tissue neutrophil content [10], was increased over levels in kidneys from sham-injected animals by 24 hours following the exposure to cisplatin. MPO activity was significantly lower at 48 ($P < 0.05$) and 72 ($P < 0.01$) hours in animals treated with anti-CD54 mAb (group II) than in those treated with placebo (group I; Fig. 8).

Effect of anti-CD54 monoclonal antibody on the fractional excretion of sodium in cisplatin nephropathy

Fractional excretion of sodium (FE_{Na}) was increased at three days following the administration of cisplatin ($P < 0.01$ vs. day 1), but there was no difference between the mean FE_{Na} in the anti-CD54 and placebo-treated groups (Fig. 9).

DISCUSSION

Acute renal failure is a common clinical entity that most often results from ischemia/reperfusion injury and/or exposure to toxicants. Both exposure to cisplatin and ischemia/reperfusion produce acute tubular necrosis histologically. Alterations in the expression of several genes are also similar in the two conditions [12]. Cisplatin nephrotoxicity may be due to the induction of ischemia by the drug [13]. Because CD54 appears to be involved in the pathogenesis of ischemic acute renal failure [14], we sought evidence for its involvement in cisplatin-mediated renal injury.

The introduction of cisplatin approximately 25 years ago markedly improved the prognosis of patients with

Table 3. Histopathology following cisplatin

		Day 3 and day 4		Day 7	
		Anti-CD54	Placebo	Anti-CD54	Placebo
Outer medullary	<25%	13	12	9	6
tubular necrosis	≥25%	3	13	2	2
P value		0.033		0.72	
Outer medullary	<25%	14	19	5	0
tubular dilation	≥25%	2	6	6	8
P value		0.36		0.026	

The percent of tubules in the outer medulla that showed epithelial necrosis or tubular dilation was quantified on coded sections. The numbers shown represent the number of animals with kidney sections in each category.

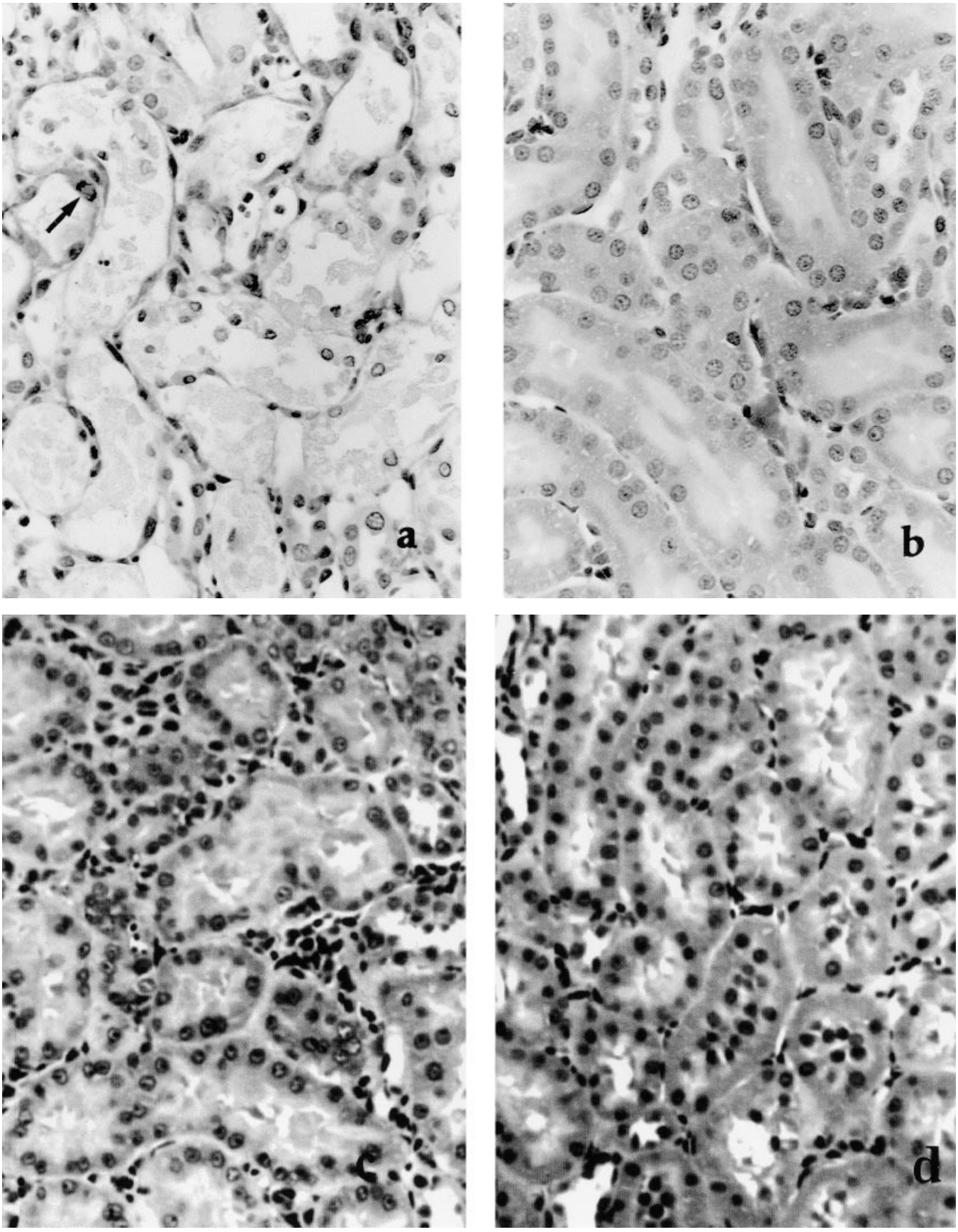
germ cell and other tumors [15]. The major limitation to the use of this agent is nephrotoxicity. Alterations in renal function occur commonly with doses in excess of 5 mg/kg [2, 16] and can contribute to mortality [17]. One dose of cisplatin may result in acute renal failure [18], and multiple doses can cause chronic impairment of renal function. In early clinical trials, azotemia occurred in approximately 25% of patients after a single dose of cisplatin [19].

Direct toxicity to renal tubular epithelial cells and hemodynamic alterations are thought to contribute to renal dysfunction following exposure to cisplatin [2, 3, 20]. Cisplatin induces DNA cross-links, which result in the inhibition of DNA synthesis and correlate with toxicity, although the precise relationship to nephrotoxicity is unknown [2]. Within the kidney, platinum is concentrated largely in the renal cortex and is found in both the cytoplasm and subcellular organelles. Ten minutes after an injection of cisplatin in rats, it is found primarily in the juxtamedullary and outer stripe regions of the kidney, with the highest concentration in the S3 segments of the proximal tubule [2].

Lam and Adelstein found tubular cell casts or tubular epithelial cells in 100% of 47 urine specimens from patients two to four days after treatment with cisplatin [21]. In animals treated with cisplatin, patchy necrosis of cells in the medulla with cellular debris in tubular lumens and dilated tubules has been described [22]. Impairment of proximal tubular function has been observed immediately after administration of the drug to dogs [15]. In patients, both decreased proximal absorption of sodium and water and increased excretion of the tubular marker N-acetyl-β-D-glucosamidase (NAG) have been observed [23]. However, significant decreases in renal function do not occur until several days after cisplatin administration, suggesting that factors other than tubular cell damage may be important in the development and perhaps the initiation of nephrotoxicity. Although CD54 can be induced on renal tubular epithelial cells following injury [24, 25], the data demonstrating no difference in FE_{Na} between the anti-CD54 and placebo-treated groups support another mechanism of protection with anti-CD54 in cisplatin nephropathy.

Renal vasoconstriction may be critical in the induction of nephrotoxicity by cisplatin. In cancer patients, progressive decreases in effective renal plasma flow have been demonstrated during an infusion of cisplatin and prior to any alteration in the glomerular filtration rate (GFR) [13]. Decreased renal blood flow (RBF) has been demonstrated in dogs [26] and rats [4] 48 to 72 hours after cisplatin administration. Improvement in GFR after volume expansion parallels the increase in RBF, suggesting that vasoconstriction is an important determinant of renal function following exposure to cisplatin [2]. The nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester inhibits the ability of glycine to protect against cisplatin-mediated nephrotoxicity in rats, again suggesting the importance of hemodynamic factors [27]. Other investigators have argued that RBF is not markedly decreased immediately after cisplatin administration and thus suggest that an alteration in RBF is not a primary mechanism of cisplatin-mediated nephrotoxicity [28].

Other mechanisms may contribute to renal dysfunction following exposure to cisplatin. The drug results in decreased mitochondrial respiratory function, respiratory chain enzyme activity, and glutathione peroxidase in rat kidneys, possibly resulting in free radical-mediated renal injury [29]. Cisplatin may also decrease the synthesis or release of vasopressin [30]. It has been postulated that the nephrotoxic effects of cisplatin can be attributed to its affinity for sulfhydryl groups of enzymes. Glutathione, a sulfhydryl-containing compound, may protect against cisplatin-induced renal dysfunction [31]. Cisplatin increases lipid peroxidation [31] and may alter prostaglandin synthesis or metabolism [2]. Calcium has been implicated in cisplatin nephrotoxicity by investigators who have found increases in renal calcium content and endoplasmic reticulum calcium pump activity prior to morphological evidence of injury following the administration of cisplatin [32]. In addition, calcium channel blockers may provide protection from cisplatin-mediated injury [33]. Increased Na^+,K^+-ATPase activity with ATP depletion has also been implicated in cisplatin nephrotoxicity [34].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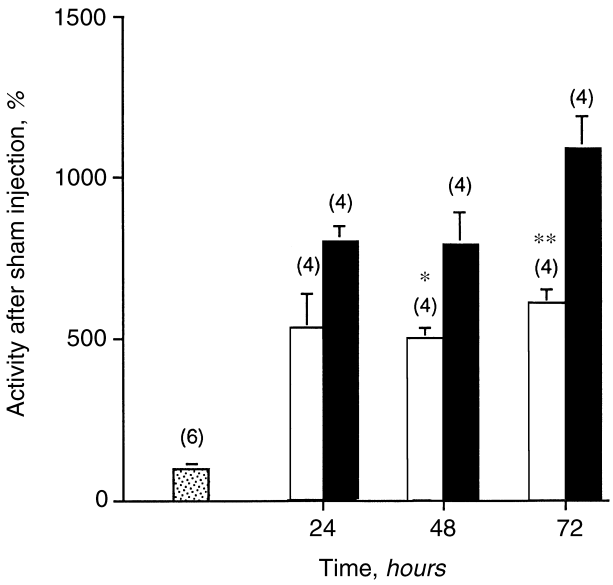

Fig. 8. Effect of anti-CD54 monoclonal antibody (mAb) on myeloperoxidase activity in cisplatin nephropathy. Myeloperoxidase (MPO) activity was measured in kidney tissue 24, 48, or 72 hours following exposure to cisplatin and anti-CD54 mAb (group II) or placebo (group I). MPO activity is presented as a percentage of activity at the same time following sham-injection in normal animals. Symbols are: (▨) sham-operated; (□) anti CD54; (■) placebo; $*P < 0.05$ and $**P < 0.01$ for comparison between MPO activity in kidneys from anti-CD54 mAb and placebo-treated rats.

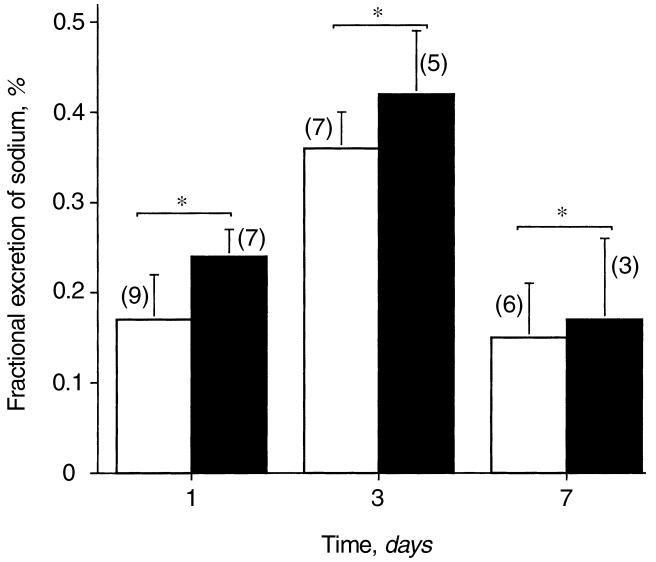

Fig. 9. Effect of anti-CD54 monoclonal antibody (mAb) on fractional excretion of sodium following exposure to cisplatin. Fractional excretion of sodium (FE_{Na}) was determined on days 1, 3, and 7 following cisplatin and anti-CD54 (□) or placebo (■). FE_{Na} is significantly higher on day 3 than on day 1, but (*) no statistically significant differences in FE_{Na} were observed between the anti-CD54–treated and placebo groups.

The data presented demonstrate marked protection from renal dysfunction following cisplatin with administration of anti-CD54 mAb to rats. Although several agents have recently shown promise in experimental models of toxic nephropathy, the treatment of cisplatin-mediated nephrotoxicity clinically remains largely supportive.

Hydration, mannitol, and/or furosemide are nonspecific measures commonly used in patients receiving cisplatin treatment [1], although clear protection of renal function has not always been seen with these interventions [15]. Sodium thiosulfate [35, 36] and metabolites of amifostine (WR-2721) may inactivate toxic platinum species and protect against nephrotoxicity [37]. Glutathione is also protective against cisplatin-induced nephrotoxicity, perhaps by replacing sulfhydryl groups of enzymes that have reacted with cisplatin [38]. Probenecid inhibits cisplatin secretion in renal tubules and has been shown to be protective in a phase I trial [39]. Other agents protect against toxicity in animal models or *in vitro* systems [40]. To our knowledge, no studies examining the role of adhesion receptors of cisplatin nephrotoxicity have been undertaken. These data provide evidence for the importance of the adhesion receptor CD54 (ICAM-1) in the functional and histological changes that follow the administration of cisplatin. We have shown increases in CD54 mRNA at early time points following the administration of cisplatin, before decreases in renal function and histological evidence of injury are seen. Although CD54 is expressed constitutively on the endothelium in kidneys [8], we have found that administered anti-CD54 mAb was localized largely to the vasa rectae of the outer medulla in a model of ischemia/reperfusion [11]. This area of the kidney may be particularly susceptible to injury given its basal hypoxia [41]. These studies also support the hypothesis that neutrophils contribute to renal dysfunction.

Leukocytes may cause renal dysfunction via several mechanisms. Following activation, polymorphonuclear neutrophils release many potentially destructive mediators (including reactive oxygen species, cytokines, prote-

Fig. 7. Effects of anti-CD54 monoclonal antibody (mAb) on histopathology in toxic nephropathy. Light microscopy of a representative section of kidney outer medulla four days after cisplatin administration (*a*) demonstrates extensive tubular epithelial cell necrosis and mitosis (arrow) in the control animal (group I). A comparable section from the kidney of an anti-CD54 mAb (group II)-treated rat (*b*) shows histologically normal tubules. Sections four hours after the administration of cisplatin and placebo (*c*) or anti-CD54 mAb (*d*) show little evidence of injury (hematoxylin and eosin ×200).

ases, elastases, MPO, and other enzymes). These substances attract additional inflammatory cells, increase vascular permeability, and alter expression of adhesion receptors. They may also damage tissue directly [42] and impair endothelial function [42–44]. Endothelial layer damage results in a decreased vasodilatory response to hypoxia and acetylcholine [45]. Together with increased vascular permeability, this can result in increased interstitial pressure and decreased capillary blood flow. Activated polymorphonuclear cells (PMNs) produce vasoconstrictive arachidonic acid metabolites, which may potentiate vascular obstruction [46]. Anti-CD54 mAb, by decreasing the infiltration or activation of neutrophils, may decrease the obstruction of renal vessels or release of reactive oxygen species and other mediators or decrease cellular damage and ameliorate cisplatin nephropathy. We have demonstrated decreased histological evidence of cisplatin-mediated tubular injury, medullary infiltration of inflammatory cells, and renal MPO activity in anti-CD54–treated animals.

In summary, rats treated with anti-CD54 mAb are protected from renal dysfunction following cisplatin administration. Histological evidence of cell injury and increases in tissue MPO are markedly reduced in the treated animals. These data suggest a critical role for CD54 in the pathophysiology of toxic nephropathy following cisplatin and may have important therapeutic implications for the treatment of cisplatin-mediated toxicity in humans. Anti-CD54 mAb has been administered safely to humans [47].

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Reprint requests to K.J. Kelly, M.D., University of Cincinnati College of Medicine, Division of Nephrology and Hypertension, 231 Bethesda Avenue, P.O. Box 670585, Cincinnati, Ohio, 45267–0585 USA.
E-mail: kellykj@email.uc.edu

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