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Evaluation of uroprotective efficacy of amifostine against cyclophosphamide induced hemorrhagic cystitis

A Srivastava[1], SC Nair[1], VM Srivastava[2], AN Balamurugan[3], L Jeyaseelan[4], M Chandy[1] and S Gunasekaran[3]

Departments of [1]Hematology, [2]General Pathology, [3]Physiology and [4]Biostatistics, Christian Medical College Hospital, Vellore, India

Summary:

The role of amifostine in the prevention of cyclophosphamide-induced hemorrhagic cystitis (HC) was evaluated in the rat model. Urinary bladders from control rats that received no drugs (group I) were compared with those from rats receiving cyclophosphamide alone at a dose of 150 mg/kg (group II), and two other groups receiving amifostine at 100 mg/kg (group III) and 200 mg/kg (group IV), 15 min prior to cyclophosphamide. Bladders were assessed macroscopically and histologically at 24 h and after 7 days. All the animals that received cyclophosphamide alone developed severe HC. On the basis of the scores of macroscopic and histologic changes, animals that received amifostine showed excellent uroprotection. Only 2/6 rats in group III and 1/6 rats in group IV developed mild HC at 24 h. None of the rats in either of these groups showed any evidence of HC at 7 days. It is concluded that amifostine protects the urothelium against cyclophosphamide-induced HC.
Keywords: amifostine; cyclophosphamide; hemorrhagic cystitis; prevention

Hemorrhagic cystitis (HC) is a potentially life-threatening sequel of therapy with oxazaphosphorine agents (cyclophosphamide, ifosfamide). It tends to occur most frequently as a consequence of using high-dose cyclophosphamide as conditioning therapy for BMT. It has been noted in 40–50% of these patients and contributes to mortality in 2–4%.[1,2] The uro-toxicity of these drugs is related to contact of the lining epithelium with the renally excreted 4-hydroxy metabolites, particularly acrolein. Other factors including viral infections, radiation and drugs such as busulfan may also cause HC in patients undergoing BMT.[2]

Since there is no effective treatment for this condition, the emphasis has been on prevention. The most widely employed method for prevention is the combination of 2-mercaptoethane sodium sulfonate (MESNA) and hydration.[3] This thiol molecule has been shown to bind acrolein and reduce its toxicity. In spite of this, up to 18% of patients can develop severe manifestations (grade III to grade IV).[4] Very recently, the combination of mesna and hyperbaric oxygen was shown to completely eliminate HC in guinea pigs.[5]

Amifostine [S-2-(3-aminopropylamino) ethyl phosphorothioic acid], designated WR-2721, is an aminothiol that is now in clinical use as a protective agent against chemotherapy-related cytotoxicities.[6] Although all the mechanisms of cytoprotection have not been fully elucidated, the most significant seems to be that the active metabolite, WR-1065, which is a thiol, acts as an intracellular scavenger of free radicals.[7] Its cytoprotective potential has been established for various tissues including hematopoietic progenitor cells, renal cells, myocardium, intestinal epithelium and neuronal cells.[8,9] The possibility that amifostine may provide protection to the urothelium against cyclophosphamide-induced HC has not been explored.

In view of the fact that mesna and WR-1065, the active metabolite of amifostine, are both thiols, we postulated that amifostine could protect the urothelium against cyclophosphamide-induced HC. We evaluated this hypothesis in the rat model.

Materials and methods

A total of 48 albino rats, weighing 130–270 g, were allowed free access to food and water and were randomly assigned to one of four groups of 12 rats each: Group I: received no drugs at all; group II: received 150 mg/kg of cyclophosphamide alone by intraperitoneal injection; group III: received 100 mg/kg of amifostine, 15 min prior to cyclophosphamide as above; group IV: received 200 mg/kg of amifostine, 15 min prior to cyclophosphamide as above.

Induction of hemorrhagic cystitis

Cyclophosphamide (150 mg/kg) was administered intraperitoneally to consistently induce HC, as previously described.[10]

Administration of amifostine

Amifostine was administered intraperitoneally, 15 min prior to the administration of cyclophosphamide. Two different doses were evaluated – 100 mg/kg (group III) and 200 mg/kg (group IV). These doses were selected on the basis of previous studies on cytoprotection in rats.[11]

Correspondence: Dr A Srivastava, Department of Hematology, CMC Hospital, Vellore 632004, India
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Table 1 Comparison of scores on gross evaluation of bladder for hemorrhage (mean ± s.e.m.)

Group	*Treatment*	*Score day 1*	*P value*	*Score day 7*	*P value*
I	Control	1.0 ± 0.0		1.0 ± 0.0	
II	Cyclophosphamide	1.5 ± 0.2		2.66 ± 0.2	
III	Af-100 mg/kg and CY	1.0 ± 0.0	0.055	1.0 ± 0.0	0.001
IV	Af-200 mg/kg and CY	1.0 ± 0.0	0.055	1.0 ± 0.0	0.001

CY = cyclophosphamide; Af = amifostine.

Evaluation of hemorrhagic cystitis

In each group consisting of 12 rats, six were sacrificed 24 h after treatment and the rest after 7 days using a high intraperitoneal injection of 50 mg/kg of pentobarbital. The bladders were carefully dissected and fixed in 10% formalin. They were macroscopically assessed for HC and graded as normal (1+), telangiectasia (2+), mucosal hematoma (3+) and intravesical clots (4+). Standard paraffin blocks were prepared and sections cut for hematoxylin and eosin-stained slides. The pathologist evaluating these bladders had no knowledge of the treatment received by these rats. Histological damage was graded according to the following criteria: normal histology – normal epithelium, no edema, inflammatory cell infiltrate, ulceration or hemorrhage (1+); mild changes – mild edema and inflammation, no hemorrhage or ulceration (2+); moderate changes – moderate edema and inflammation, flattening of epithelium and regeneration, focal ulceration and mild hemorrhage (3+); severe changes – severe edema and inflammation, mucosal erosions, extensive ulceration and hemorrhage (4+).

Statistical analysis

All numeric data were analyzed using the Wilcoxon rank sum test.

Results

The scores of gross assessment for presence of hemorrhage and histologic evaluation of damage to the bladder are shown in Tables 1 and 2, respectively.

Table 2 Comparison of scores of histologic grading of bladder changes (mean ± s.e.m.)

Group	*Treatment*	*Score day 1*	*P value*	*Score day 7*	*P value*
I	Control	1.0 ± 0.0		1.0 ± 0.0	
II	Cyclophosphamide	3.66 ± 0.2		4.0 ± 0.0	
III	Af-100 mg/kg and CY	2.33 ± 0.2	0.066	2.0 ± 0.0	<0.001
IV	Af-200 mg/kg and CY	2.16 ± 0.1	0.003	2.0 ± 0.0	<0.001

CY = cyclophosphamide; Af = amifostine.

Macroscopic assessment

While bladders from animals in group I were normal, HC was obvious in all animals in group II. It was mild to moderate on the first day but became severe in all by the seventh day. Bladders from animals in groups III and IV did not show any evidence of hemorrhage (Table 1).

Histological grading

Bladders of animals in group I were normal (Figure 1). All the animals in group II (12/12) had evidence of HC. It was moderate to severe at 24 h (Figure 2a) and uniformly severe by day 7 (Figure 2b). In group III, only 2/12 animals showed mild to moderate histological changes of HC (2/6 on day 1 and 0/6 on day 7) (Figure 3a and b). The mean scores for bladder damage were significantly lower compared to group II ($P = 0.006$ on day 1 and <0.001 on day 7). In group IV, only 1/12 animals had mild changes suggestive of HC (1/6 on day 1 and 0/6 on day 7) (Figure 4a and 4b) Mild mucosal edema was noted in some of these bladders. The mean scores for bladder damage were even lower for this group when compared to group II ($P = 0.003$ on day 1 and <0.001 on day 7).

Discussion

The data presented clearly show that amifostine protects the urothelium from cyclophosphamide-induced HC. Though 2/6 animals in the group that received 100 mg/kg and 1/6 animals in the group that received 200 mg/kg of amifostine showed mild HC at 24 h, none of the animals in either group showed any evidence of HC by the seventh day. How this will translate to clinical protection of patients from cyclophosphamide-induced HC remains to be established, but these results are encouraging. A combination of mesna and hydration, which is the present standard for protection against HC, still results in clinically significant HC in about 20% of patients.[4] The recent report of the combination of mesna with hyperbaric oxygen providing complete protection from HC is an excellent experimental

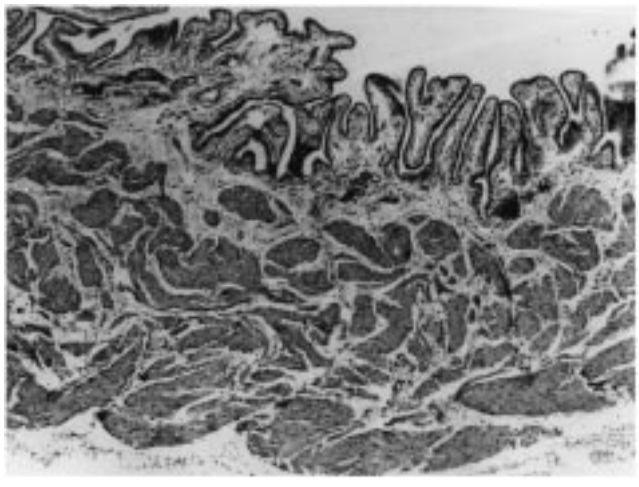

Figure 1 Normal rat bladder. H&E ×40.

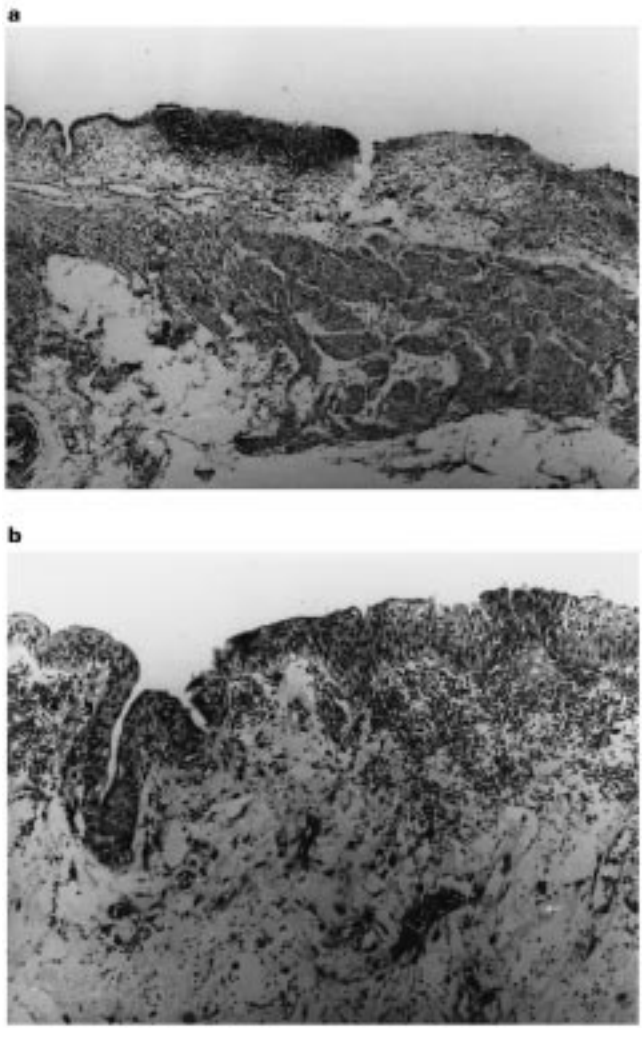

Figure 2 (**a**) Post-cyclophosphamide treatment – day 1. Extensive ulceration and severe acute inflammation with hemorrhage and edema. H&E ×40. (**b**) Post-cyclophosphamide treatment – day 7. Marked edema with extensive hemorrhage and inflammation. H&E ×90.

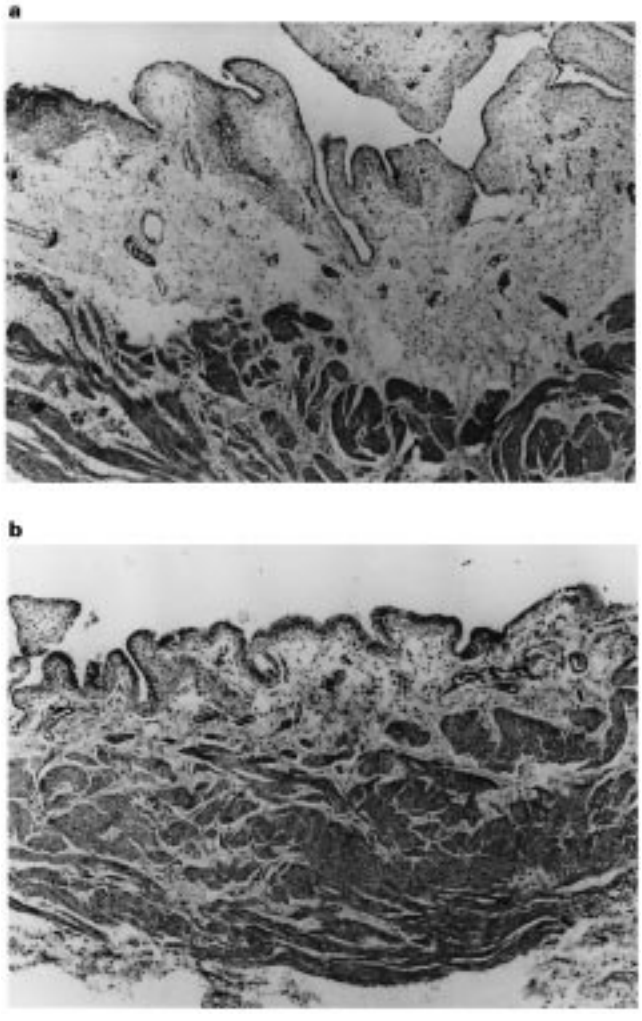

Figure 3 (**a**) Cyclophosphamide and amifostine (100 mg/kg) treated bladders – day 1. Marked edema with mild inflammation, congestion and focal ulceration. H&E ×40. (**b**) Cyclophosphamide and amifostine (100 mg/kg) treated bladders – day 7. Mild edema, congestion and inflammation. H&E ×40.

model,[5] but it is not practical for the majority of patients undergoing chemotherapy or BMT.[12]

The metabolites of cyclophosphamide are urotoxic and may persist in blood for 12–24 h after a single dose.[13] Mesna exerts its protective effect by binding with these toxic metabolites in the lumen of the urinary system. Since the half-life of mesna is very short, multiple doses are required for continued neutralization of these metabolites. It is likely that this mechanism does not completely prevent exposure of the urothelium to acrolein and other toxic metabolites. This would explain the occurrence of HC in some patients in spite of mesna.[4] Recent pharmacokinetic studies have shown that amifostine has a biphasic decrease in levels after intravenous administration with a final half-life of 0.8 h.[14] Its active metabolite, WR-1065, however, has a much longer plasma half-life of 7.3 ± 3.6 h. It is possible that the intracellular WR-1065 may persist for even longer in normal tissues because a single dose of amifostine appears to protect from both early and late (>48 h) HC. This could be the basis for continued and more effective protection of the urothelium until the disappearance of all metabolites of cyclophosphamide.

If clinical trials are to be initiated based on these data, then issues related to the dose and route of administration of amifostine need to be considered. The dose range of 100–200 mg/kg was used in this study because most of the pre-clinical data on cytoprotection of tissues by amifostine in mice and rats are based on doses ranging from 100–400 mg/kg.[15] The dose of 200 mg/kg in rats is equivalent to about 1180 mg/m^2.[11] The ideal dose of amifostine for different clinical situations remains to be defined, but doses of 740 mg/m^2 or 910 mg/m^2 have been shown to provide cytoprotection in most studies.[4] It would be reasonable to presume that protective levels could be attained in the urothelium with these doses. Pharmacokinetic studies in mice show that peak blood levels are achieved 5 min after intra-

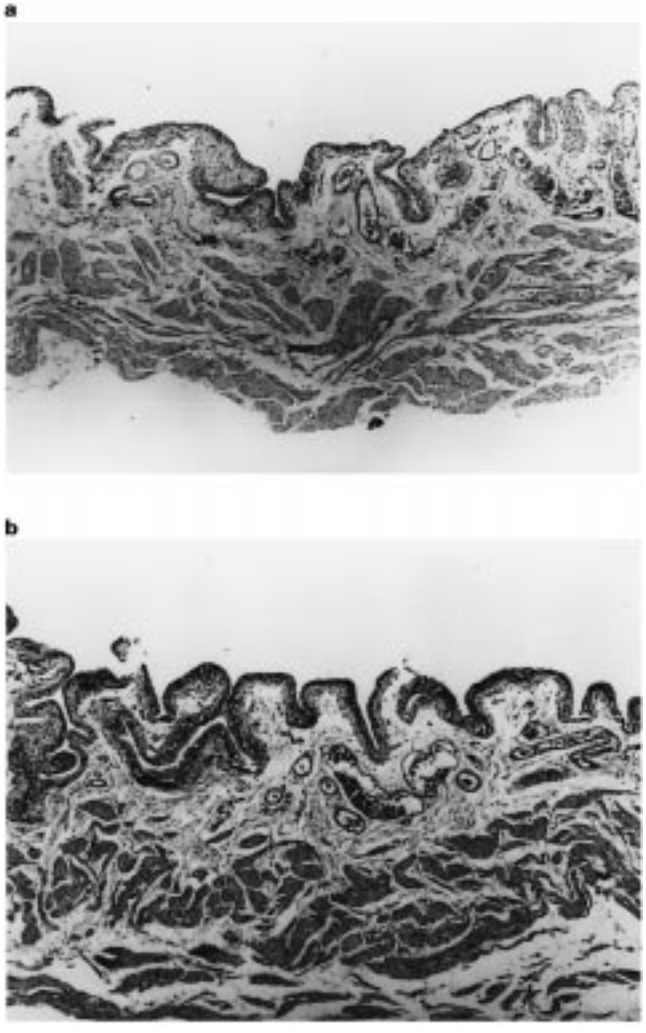

Figure 4 (**a**) Cyclophosphamide and amifostine (200 mg/kg) treated bladders – day 1. Mild edema and congestion. H&E ×40. (**b**) Cyclophosphamide and amifostine (200 mg/kg) treated bladders – day 7. Minimal edema and congestion. H&E ×40.

peritoneal administration of amifostine followed by a 10-fold reduction in 30 min.[16] Peak tissue concentrations were observed 10–30 min after administration. This is similar to the pharmacokinetics of amifostine observed after intravenous administration.[14] Therefore, clinical trials to assess urothelial cytoprotection against oxazaphosphorine drugs should be possible using amifostine at standard dosage, watching carefully for the frequent side-effects of mild transient hypotension and emesis.[17] The fact that amifostine protects all normal cells from chemotherapy and radiation, precludes its use in allogeneic BMT.

Apart from preventing HC, there could be another advantage of using amifostine in this situation. The use of oxazaphosphorine drugs is associated with mutagenesis and bladder cancers, particularly in those patients who develop cystitis.[18,19] Amifostine has been shown to prevent drug-induced mutagenesis.[20] It is therefore possible that when used in conjunction with cyclophosphamide and ifosfamide, it may not only provide immediate protection from HC but also prevent mutagenesis and evolution of secondary malignancies.

In conclusion, this study demonstrates the uroprotective effects of amifostine against cyclophosphamide-induced HC in rats. This will require confirmation in clinical trials. It could then be used for uroprotection in patients receiving high-dose cyclophosphamide and ifosfamide as part of their chemotherapy or conditioning regimen for autologous stem cell transplantation.

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