The Protective Role of Mesna in Renal Toxicity Associated with Radiotherapy in Rats

Ratlarda Radyoterapiye Bağlı Renal Toksisitede Mesnanın Koruyucu Rolü

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ABSTRACT

Objective: Sodium 2-mercaptoethanosulphinate (mesna) is a synthetic thiol known to prevent hemorrhagic cystitis and nephrotoxicity induced by alkylating anti- neoplastic drugs such as cyclophosphamide and iphosphamide. We proposed that mesna might be effective on protection of radiation induced renal injuries because of its low molecular weight, its ability to concentrate in the kidneys, its conversion to active -SH compounds in the tubular epithelium and its antioxidant potential.

Material and Methods: This experimental study was carried out on 24 8-week old Sprague-Dawley rats. The rats were allocated into 4 groups, 6 rats in each. Group I: Only saline solution was infused. Group II: Only mesna was infused. Group III: Mesna was infused, 20 minutes before radiotherapy, for 5 days. Group IV: Radiotherapy was given bilaterally to the kidneys at a dose of 20 Gy, in 400cGy fractions, using 6 MV photon in two parallel fields.

Results: There was no statistical difference between the radiotherapy and radiotherapy+mesna groups in terms of creatinine clearance ($p>0.05$). However; there was statistically significant difference between the control group and radiotherapy/mesna+radiotherapy in terms of blood BUN levels ($p<0.05$). Inability of mesna to prevent radiotherapy injuries was also confirmed by light microscopy and electron microscopy ($p>0.05$).

Conclusion: Our study may pioneer studies searching for other agents instead of mesna for preventing hemorrhagic cystitis induced by cyclophosphamide and iphosphamide because it showed that mesna cannot protect kidneys from radiation nephrotoxicity on histopathologic evaluation. This is also the first study demonstrating ineffectiveness of mesna as a radioprotectant.

Key Words: Mesna, Nephrotoxicity, Radioprotectant

ÖZ

Amaç: Sodyum 2-merkaptoethanosülfonat (mesna) alkilleyici antineoplastik ilaçlardan siklofosfamid ve ifosfomidin neden olduğu hemorajik sistiti ve nefrotoksisiteyi önlediği bilinen sentetik bir tiyoldür. Mesnanın küçük moleküler yapısı, böbrekte konsantre olma özelliği, tübüler epitelde aktif –SH bileşiklerine dönüşmesi ve antioksidan potansiyeli ile böbreklerdeki radyasyon hasarını engellemekte faydalı olabileceğini düşündük.

Gereç ve Yöntemler: Bu deneysel çalışma 8 haftalık 24 Sprague-Dawley ratları ile yapılmıştır. Her grupta 6 rat olmak üzere 4 grup oluşturuldu. Grup I: Sadece serum fizyolojik uygulandı. Grup II: Sadece mesna uygulandı. Grup III: Mesna, 5 gün boyunca radyoterapiden 20 dakika önce verildi. Grup IV: İki paralel alandan 6 MV foton ile, 400 cGy'lik fraksiyonlarla toplam 20 Gy doz bilateral böbreklere uygulandı.

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Bulgular: Radyoterapi ile radyoterapi + mesna grubu arasında kreatinin klirensi açısından istatistiksel fark saptanmadı (p>0,05). Ancak kontrol grubu ile radyoterapi / mesna + radyoterapi arasında kan BUN düzeyleri açısından istatistiksel olarak anlamlı fark vardı (p<0,05). Radyoterapiye bağlı nefrotoksitenin önlenmesinde mesnanın etkili olmadığı ışık mikroskobu ve elektron mikroskobisi bulguları ile doğrulandı (p>0,05).

Sonuç: Bizim çalışmamızda, mesnanın histopatolojik değerlendirmede radyasyon nefrotoksisitesinden böbrekleri koruyamadığı gösterildiğinden, siklofosfamid ve ifosfamid tarafından indüklenen hemorajik sistiti önlemek için mesna yerine başka ajanları araştıran çalışmalara öncülük edebilir. Bu aynı zamanda bir radyasyondan koruyucu olarak mesnanın etkisizliğini gösteren ilk çalışmadır.

Anahtar Sözcükler: Mesna, Nefrotoksisite, Radyasyon koruyucusu

INTRODUCTION

The radiation injury induced by radiotherapy in the kidneys within the radiotherapy field is mostly dose and volume dependent. The radiation nephropathy gains importance in cancer patients of all ages with prolonged life expectancy since time elapsed to the first symptoms and the nature of the symptoms varies. It is reported in many animal studies that the radiation nephropathy occurs earlier and in more severe form with higher radiotherapy doses (1). The higher nephropathy risk is reported in rats younger than 12 months old since they have higher proliferative activity (2). Both chemical and biological radioprotective agents are used to minimize the nephropathic effects of the radiotherapy (3).

Sodium 2-mercaptoethanosulphinate (mesna) is a synthetic thiol known to prevent hemorrhagic cystitis and nephrotoxicity induced by alkylating anti-neoplastic drugs such as cyclophosphamide and iphosphamide (4). Mesna is oxidized by ethylenediaminetetraacetic acid (EDTA) to its disulfide metabolite dimesna which is physiologically inert and excreted by the kidneys (5). Dimesna is partly reabsorbed in renal tubule where it is reduced to mesna (6). This reduction is carried out by thiol transferase in expense of glutathione (GSH) leading to depletion of intracellular (GSH). Both mesna and dimesna are cleared from the plasma by glomerular filtration. The plasma clearance halftime is 0.36 hours and it reaches its maximum kidney concentration 20 minutes after its administration (7).

Despite all these properties, mesna causes subclinical renal tubulopathy in 40% of children using iphosphamide. Despite all these properties, mesna causes subclinical renal tubulopathy in 40% of children using iphosphamide. In this subgroup of patients ,15% of them were suffering from De Conti-Debrecen-Fanconi syndrome characterized by renal tubular dysfunction (8). In in vivo studies mesna seems not to protect the renal tubules either (9).

The majority of studies concerning renal protective capacity of mesna were carried out on chemotherapeutic agent induced toxicity. The only mesna study with radiotherapy was the study of the Zheng et al. which concerned the protective effects of mesna on radiation induced defects on DNA of thymus cells. In this study, the protective effect of mesna has been shown but mesna was reported to be less radioprotective compared to amifostine, cysteamine and 2- mercaptoethanol (10). There is no study in the literature on the protective effect of mesna on radiation induced nephrotoxicity. Although we know that mesna is not protective against chemotherapeutic agents induced nephrotoxicity we proposed that mesna might be effective on protection of radiation induced renal injuries because of its low molecular weight, its ability to concentrate in the kidneys, its conversion to active -SH compounds in the tubular epithelium and its antioxidant potential.

MATERIALS and METHODS

This experimental study was carried out on 24 8-week old Sprague-Dawley rats. The rats were allocated into 4 groups, 6 rats in each. Group I: Only saline solution was infused through a jugular vein catheter for 5 days. Group II (Mesna Only): Mesna was infused through a jugular vein catheter at a dose of 180 mg/kg prepared at a concentration of 100 mg/ml, for 5 consecutive days. Group III (mesna+radiotherapy): Mesna was infused, 20 minutes before radiotherapy, through a jugular vein catheter at a dose of 180 mg/kg prepared at a concentration of 100 mg/ml, for 5 days. Group IV (radiotherapy only): Radiotherapy was given bilaterally to kidneys at a dose of 20 Gy, in 400 cGy fractions, in 5 consecutive days.

An approximately 1.5 cm dermal and epidermal incision was performed on the right external jugular vein, which is 1 cm of right lateral to the cervical midline, after regional cleaning was performed and sterile conditions were ensured for all rats. The jugular vein was retracted from its proximal and distal points with 4-0 silk suture after dissection. Proximal end was ligated. A silastic catheter with 1 mm of external diameter was advanced through the vein from an approximately 1 mm cut performed on the lateral wall of the vein. A distal retraction suture was tied in order to fix the catheter. The other end of catheter was fixed to the skin after it exited through the incision performed on the posterior section of the right ear by following the tunnel sized approximately 3 cm which was created on the neck. 1 mg/kg ketamine and 0.1 mg/kg xylazine were intraperitoneally used during the procedure for anesthesia. The surgical pro-

cedure was performed on a 8x magnifier microscope. The catheter was removed after radiotherapy was completed.

Prior to radiotherapy both kidneys were visualized after IV injection of 2 mg/kg iohexol (Omnipaque, Opakim-Istanbul) and radiation plan was carried out with a safety margin of 5 mm in the simulator.

Radiotherapy was administered to bilateral kidney locations at a total dose of 20 Gy, in 400 cGy per fraction 6 MV photons, using a linear accelerator. The rats were restrained on a straphore after ether inhalation anesthesia.

All rats were placed in metabolic cages before administration of any drugs or radiotherapy where urines of rats were collected for 24 hours. In these 24 hours, urine samples were obtained and BUN and creatinine determinations were carried out. After study protocols were completed for each group, the rats were followed up for 6 months. After 6 months of follow-up, the rats were placed in metabolic cages again to collect 24 hours urine samples for the purpose of determining BUN, creatinine values again. The rats were sacrificed with high dose of anesthetic substances and their abdomen was opened to take blood samples from the abdominal aorta. In these blood samples, BUN and creatinine determinations were carried out. The creatinine clearance (CrCl) was calculated for each rat as follows:

CrCl= Urine creatinine x Urine volume / Blood creatinine x 1440

After the collection of blood samples, both kidneys were taken out for histopathologic examinations. A total of 24 kidney specimens from 4 different groups were fixed in 10 % formaldehyde for 24 hours. Paraffin blocks were prepared and tissue samples of 4-5 micron thickness were prepared by cutting these blocks. The materials were prepared with hemotoxylin-eosin and Masson-trichrom dyes. All histopathologic materials were examined by a single pathologist under the Nikon 200 light microscope. The lesions of severe cystic formation, glomerular sclerosis, mesensiolysis, interstitial expansion (expansion due to interstitial edema, increase in vascularisation), tubuler atrophy (shrinking of proximal and distal tubular epithelial cells and resulting tubular diameter narrowing) and basal labyrinth expansion (basal cell membrane expansions) were scored.

All histopathologic materials were examined under the electron microscope. Kidney specimens of 1-2 mm^3 in size were fixed in 3 % glutaraldehyde solution for 24 hours. They were passed through alcohol series 1% osmium tetroxide for 2 hours and embedded in Epon 812. Thick slices were cut with an ultratom and stained toluidine blue. From these toluidine stained specimens, 60-70 mm thick fine slices were cut using the same microtome and stained with uranyl acetate and lead citrate. The specimens were evaluated under the electron microscope (JEOL 1010). Vacuole formation (increase of vacuole number, microvillus degeneration (tearing of the microvillus membrane, apical loss of microvillus), microvillus downsizing, electron dense cytoplasmic deposit formation, undulations of basal lamina and basal laminar thickness were scored with the electron microscope.

Comparisons among groups were conducted with the Mann-Whitney U test while the chi-square test was used for electron and light microscopy results. Results were given as mean ± standard deviation. $P<0.05$ was accepted as significant. This study protocol was approved by Karadeniz Technical University Ethical Council of Sciences (Approval date 13.03.2003, File no 2003/20)

RESULTS

In the control groups, BUN and creatinine clearance were significantly different than radiotherapy and mesna+radiotherapy group ($p<0.05$). There was no statistical difference between the radiotherapy and mesna+radiotherapy groups in terms of BUN and creatinine clearance ($p>0.05$). However; there was a statistically significant difference between the control group and radiotherapy/mesna+radiotherapy in terms of blood BUN levels ($p<0.05$). Addition of mesna to radiotherapy did not improve blood BUN levels ($p>0.05$). Rats receiving only radiotherapy and rats receiving mesna+radiotherapy gained less weight than group I and II ($p<0.05$) (Table I). In all groups there was no cyst formation, glomerular sclerosis, interstitial fibrosis and glomerular mesensiolysis under light microscopy. The outstanding findings under light microscopy were interstitial expansion, tubular cell atrophy and basal labyrinth expansion. There was statistically significant difference between

Table I: Weight and biochemical changes.

Group	BUN	CrCl	Weight (g)
I	9.4±1.2	0.01±0.02	235±24.3
II	9.2±2.3	0.01±0.02	220±17.6
III	20.16±4.0*	0.03±0.02*	191±17.2*
IV	20.16±5.2*	0.06±0.08*	185±20.8*

*p<0.05, **BUN:** Blood Urea Nitrogen, **CrCl:** Creatinine Clearance.

group I/II and group III/IV when compared under light microscopy but there was no statistically significant differences between group III and IV ($p<0.05$) (Table II). Inability of mesna to prevent radiotherapy injuries was also confirmed by light electron microscopy ($p>0.05$) (Table III, Figure 1).

DISCUSSION

Mesna is a thiol compound and after oral or intravenous administration, mesna undergoes rapid oxidation in the plasma to dimesna. Mesna which is a thiol compound, rapidly oxidized in plasma to dimesna after it is absorbed from intestinal tract or administered intravenously Both mesna and dimesna are very hydrophilic and are rapidly cleared by the kidneys. Mesna and dimesna both undergo rapid clearance as a result of their hydrophilic properties. The free sulfhydryl groups of mesna combine directly with a double bond of acrolein and with other urotoxic metabolites. Mesna binds acrolein and other urotoxic metabolites through its free sulfhydryl groups. Thus, mesna has been used as a systemic uroprotective agent (11). With these properties mesna has been used as a systemic uroprotectant (11) Before the use of mesna, hematuria was reported in 20-40% of patients, limiting iphosphamide use. After mesna being routinely introduced, this frequency dropped to about 5% (12). After routine use of mesna, the incidence of hematuria complicating the iphosphamide treatment dropped to 5% from 20-40% (12). Most studies use urinalysis as parameters for evaluating iphosphamide urotoxicity and mesna protective effect. Cystoscopy and bladder biopsy are not routinely performed. A detailed microscopic evaluation from bladder biopsy showed that mesna is not capable of providing complete uroprotection in Lima et al study (13). Most studies evaluate uroprotective effects of mesna with urinalysis. Bladder biopsy were not routinely performed in these studies. Lima et al showed in their study that mesna did not provide complete uroprotection in detailed microscopic evaluation of bladder biopsy (13).

In recent years, it has been demonstrated that hemorrhagic cystitis caused by cyclophosphamide is not only due to direct contact of acrolein with bladder mucosa. It has been also known that increased free oxygen radical levels are involved in pathogenesis of hemorrhagic cystitis (14). In the studies of Skinner et al., more prevalent protective effect of mesna was considered as its thiol providing effect. Auto oxidation process becomes saturated in high doses of mesna and high levels of free mesna occur in the renal tubule. Then mesna provides free thiol groups which cause detoxification of free oxygen radicals after tubular absorption of mesna (8). It has been thought of as a radioprotectant due to its presence in renal tubules in high concentrations. However, even its limited benefit on side effects of cyclophosphamide could not be acquired on injury of rat kidneys due to radiotherapy in our study. There was no difference between the group that only received radiotherapy and the kidneys that received mesna+radiotherapy in terms of electron microscopy.

Lord-Fontaine and Averill observed that cytotoxicity caused by hyperthermia was reduced and intracellular glutathione level was increased in Chinese hamster ovary cells after mesna administration (15). It has been also known

Table II: Kidney histology changes in rats under light microscopy.

Group	Interstitial expansion	Tubuler atrophy	Basal labyrinth expansion
I	-	-	-
II	-	-	_
III	+++	++	_
IV	+++	++	+

*** **+:** minor changes, **++:** moderate changes, **+++:** major changes

Table III: Kidney histology in rats; electron microscopy findings.

Group	Vacuole formation	Microvillus degeneration	Microvillus downsizing	Electron dense cytoplasmic deposit formation	Ondulations of basal lamina	Basal laminar thickness
I	-	-	-	-	-	-
II	-	-	-	-	-	-
III	+	++	+++	++	++	+++
IV	++	+++	+++	++	+++	+++

*** **+:** minor changes, **++:** moderate changes, **+++:** major changes

that mesna uses glutathione for turning into dimesna (7). Its glutathione usage may reduce its chemoprotective and radioprotective effects. Our study is the first to demonstrate its inability to prevent radiotherapy induced nephrotoxicity both in electron and light microscopic levels and therefore it is a preliminary study on this subject.

Yildirim et al. used β-carotene, melatonin and α-tocopherol in addition to mesna for preventing hemorrhagic cystitis in preclinical studies and they observed that melatonin and α-tocopherol that were added to mesna were better in protecting the kidneys than mesna alone (14). In this study, mesna was administered three times a day at a dose of 21.5 mg/kg. It has been known that the renoprotective effect of mesna at this dosage is lower and higher doses are necessary for a better protection (7). Kabasakal et al. demonstrated that the oxidating response to renal ischemia-reperfusion injury was reduced along with increased renal function and microscopic damage after mesna administration. In this study, mesna was used with a dosage of 150 mg/kg (16). These studies indicate that other factors except mesna dosage may play a role in the lack of an expected protectant effect. In our study, mesna could not provide adequate protection although it was used in high doses of up to 180 mg/kg.

Mesna has been intraperitoneally used in many preclinical studies (8, 14, 15). Ormstad et al. compared intravenous, intraperitoneal and oral administration in their study conducted for evaluating the pharmacokinetics and metabolism of mesna. Free thiol providing feature of mesna could not be demonstrated in oral administration. Although they could not find a statistically significant difference between intravenous and intraperitoneal administration, higher concentrations were obtained in the early hours of intravenous administration when graphics were evaluated. During the later periods, reducible disulfide concentrations were higher for longer time periods (17).

We also chose the intravenous route due to rapid auto oxidation and rapid renal uptake of dimesna after intravenous administration (7). However, renoprotection could not be achieved with mesna.

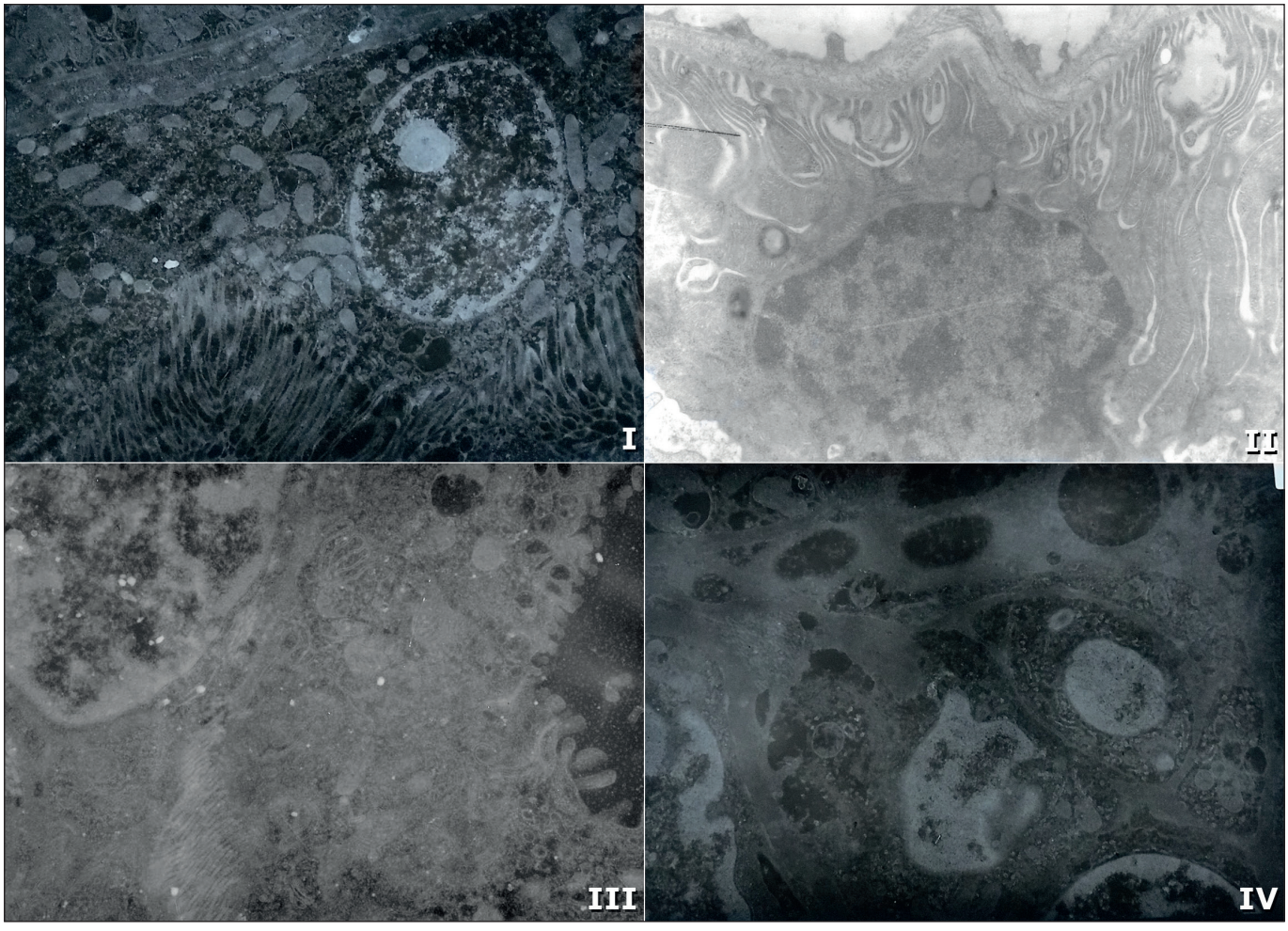

Figure 1: Tubule interstitial changes. I. Saline, II. Mesna only, III. Radiotherapy+Mesna, IV. Radiotherapy only. Electron microscopy x 5000.

Moulder et al. reported the threshold radiation dose for renal failure in rats 5.5 weeks, 7.5 weeks and 12 weeks old as 17.6 Gy, 20.5 Gy and 27.5 Gy respectively (18). We used 20 Gy as the renal failure dose in 8 weeks old rats in our study.

In our study, under light microscopy there was no cyst formation, glomerular sclerosis interstitial fibrosis or glomerular mesensiolysis, which are renal failure findings, but there were tubulointerstitial changes such as interstitial expansion, tubular cell atrophy and basal labyrinth expansion. In the radiotherapy and mesna +radiotherapy groups, on electron microscopic examination, we observed renal failure changes such as increased number of vacuoles, microvillus degeneration, electron dense cytoplasmic deposit formation, undulations of basal lamina and basal laminar thickening. These findings were in accordance with the literature (19).

The study of Zheng et al. on the radioprotective effects of Mesna in thymus DNA cells support our study, and they also demonstrated inferior radioprotectivity of mesna compared to amifostine, cysteamine and 2-mercaptoethanol (10).

Mesna is still an agent recommended as an uroprotectant for iphosphamide and cyclophosphamide in the American Society of Clinical Oncology 2008 Clinical Practice Guideline Update (20). Studies suggest that mesna cannot prevent hemorrhagic cystitis and therefore alternative agents must be investigated as a uroprotectant instead (21, 22). Our study may pioneer studies searching for other agents instead of mesna for preventing hemorrhagic cystitis induced by cyclophosphamide and iphosphomide because it showed that mesna cannot protect kidneys from radiation nephrotoxicity on histopathologic evaluation. This is also the first study demonstrating ineffectiveness of mesna as a radioprotectant.

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