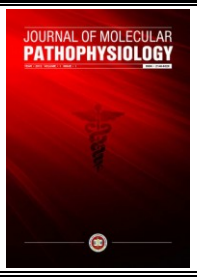
GESDAV

Journal of Molecular Pathophysiology

available at www.scopemed.org



Review Article

Prevention and treatment of cyclophosphamide and ifosfamide-induced hemorrhagic cystitis

Ertan Altayli[1], Ercan Malkoc[2], Bilal Firat Alp[3], Ahmet Korkmaz[4]

[1]Center for Research and Development, Division of Medical and Cancer Research, GMMA, Ankara, Turkey
[2]Department of Urology Çorlu Military Hospital, Edirne, Turkey
[3]Department of Urology School of Medicine, GMMA, Ankara, Turkey
[4]Department of Physiology School of Medicine, GMMA, Ankara, Turkey

Received: March 15, 2012

Accepted: March 21, 2012

Published Online: June 5, 2012

DOI: 10.5455/jmp.20120321060902

Corresponding Author:
Ahmet Korkmaz,
Department of Physiology School of Medicine, GMMA
korkmaza@gata.edu.tr

Keywords:
Cyclophosphamide, ifosfamide, cystitis, mesna, antioxidants, hyperbaric oxygen

Abstract
Free radicals and non-radical reactive molecules as well as several cytokines (e.g. tumor necrosis factor-α and interleukin family) and transcription factors (e.g. nuclear factor-κB and activator protein-1) are now known to take part in the pathogenesis of cyclophosphamide (CP) and ifosfamide (IF) induced hemorrhagic cystitis (HC). When these molecular factors are taken into account pathogenesis of bladder toxicity can be summarized in three steps: (1) acrolein rapidly enters into the uroepithelial cells, (2) activates intracellular ROS and NO production (directly or through transcription factors) leading to peroxynitrite production, and (3) finally the elevated peroxynitrite level basically damages lipids (lipid peroxidation), proteins (protein oxidation) and DNA (strand breaks) leading to PARP activation, a DNA repair enzyme. DNA damage causes PARP overactivation, resulting in the depletion of oxidized nicotinamide adenine dinucleotide (NAD+) and adenosine triphosphate (ATP), and consequently in necrotic cell death. There is no doubt that for an effective prevention against CP- and IF-cystitis all pathophysiological mechanisms must be taken into consideration. Experimental works reporting beneficial effects of antioxidants, iNOS inhibitors, cytokine blockers or hyperbaric oxygen (HBO) treatment, against CP- and/or IF-induced HC exist in literature. In this article, we discussed the possible mechanisms and effectiveness of agents used in addition to mesna to prevent CP- and IF-cystitis. In conclusion, antioxidants, iNOS inhibitors, peroxynitrite scavengers, anti-inflammatory agents, as well as HBO therapy may be added to mesna administration in clinical trials in order to obtain the best protocol to improve quality of patients comfort

OVERVIEW

Beside their military use, mustard group agents found their way into medical therapy. Several mustards such as nitrogen mustards indeed had been improved for chemical warfare agent. Soon after Second World War, their strong cytotoxic effects found to be useful in the therapy of cancer. Several mustard agents such as cyclophosphamide (CP), a synthetic analogue of CP; ifosfamide (IF), melphalan and chlorambucil have been widely used as antineoplastic agents. All mustards act mainly through alkylating of DNA, and make cells unable to divide. This mechanism is true for both therapeutic and crucial side effects causing dose limitations.

Hemorrhagic cystitis (HC) is the main side-effect of CP and IF and still encountered as an important dose-limiting problem. Acrolein is the main responsible molecule of CP- and IF-induced cystitis and mesna (2-mercaptoethane sulfonate) is the commonly used agent to protect against this side-effect [1]. Although mesna has been widely used as an effective agent against CP-induced cystitis, significant HC, defined as an episode of symptoms (e.g., burning, frequency and dysuria), microscopic or macroscopic hematuria have still been

encountered clinically [2, 3].

Our current knowledge lets us to get more information about the pathophysiological mechanism of HC in detail: Oxygen and nitrogen-based free radicals and non-radical reactive molecules, as well as poly(adenosine diphospate-ribose) polymerase (PARP) activation are now known to take part in pathogenesis of CP- and IF-induced HC [4]. Furthermore, there is evidence that HC is an inflammatory process and several cytokines play role in its pathogenesis such as tumor necrosis factor (TNF)-α and interleukin (IL) family. Cytokines may trigger activation of transcription factors such as nuclear factor (NF)-κB and activator protein (AP)-1 leading to further events associated with a variety of stress conditions. Briefly, pathophysiologic mechanisms of CP-induced bladder toxicity could be summarized in three steps: ***(1)*** acrolein rapidly enters into the uroepithelial cells, ***(2)*** then activates intracellular reactive oxygen species (ROS) and nitric oxide (NO) production (directly or through NF-κB and AP-1) leading to peroxynitrite ($ONOO^-$) production, and ***(3)*** finally the increased $ONOO^-$ level basically damages lipids (lipid peroxidation), proteins (protein oxidation) and DNA (strand breaks) leading to activation of poly ADP ribose polymerase (PARP). DNA damage causes PARP over-activation, resulting in the depletion of oxidized nicotinamide adenine dinucleotide (NAD^+) and adenosine triphosphate (ATP), and consequently in necrotic cell death [5].

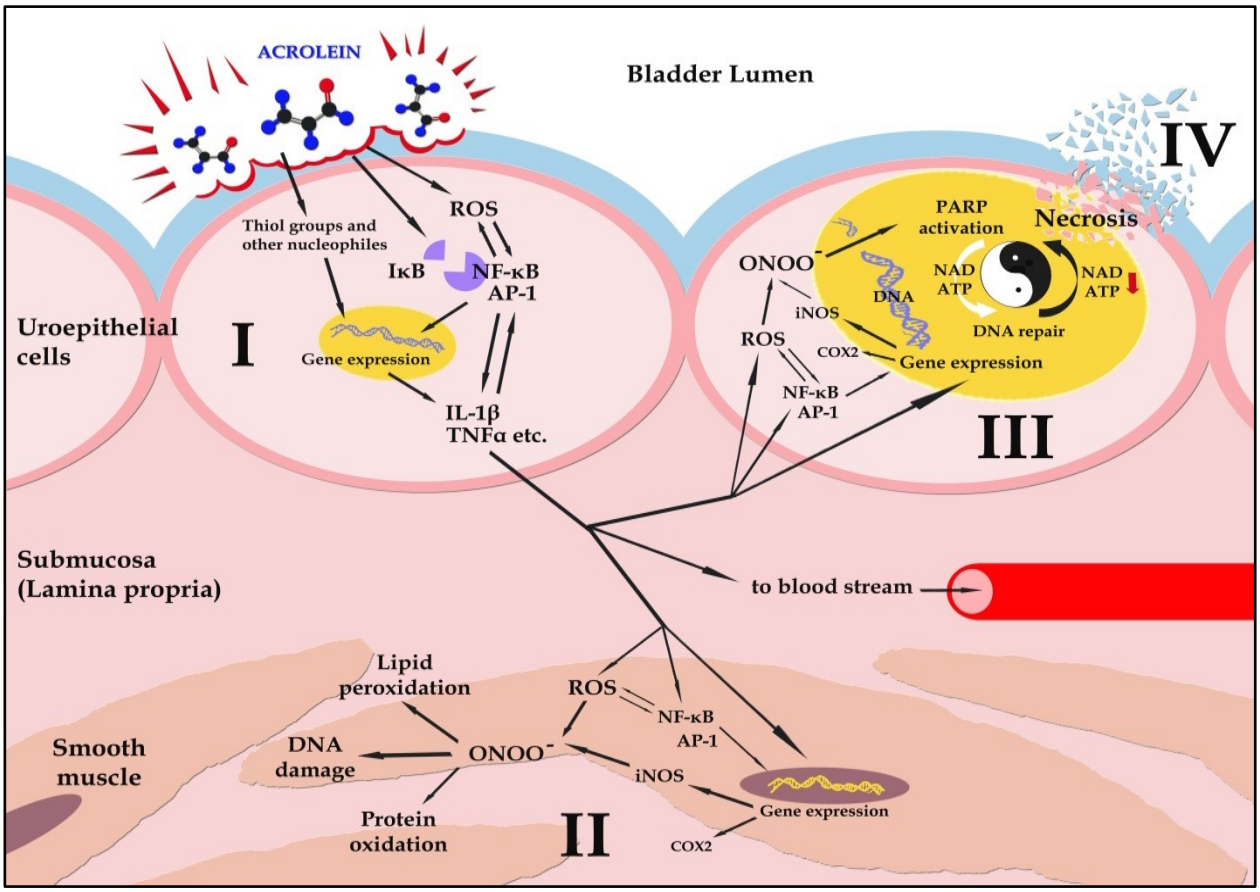


Figure. Acrolein-induced pathophysiological cascade resulting in clinical hemorrhagic cystitis: (I) Acrolein passes through the uroepithelial membrane and causes intracellular "redox imbalance". This steps initiates ROS production, activation of transcription factors, gene expression of pro-inflammatory enzymes (e.g., iNOS, COX-2) and cytokines (e.g., TNF-α, IL-1β). Several steps seen in neighbor uroepithelial cells (e.g., III) also occur in (I) simultaneously. (II–III) Cytokines produced in uroepithelial cells spread out into other uroepithelial cells, the bloodstream and the detrusor smooth muscle cells. ROS and NO form peroxynitrite in both uroepithelium and detrusor, leading to lipid peroxidation, protein oxidation and DNA damage (I-III). DNA damage causes PARP activation and energy crisis and eventually cellular necrosis (IV). This non-infectious inflammation also recruits leukocyte into bladder wall and strengths the inflammation. During necrotic cell death, the cellular content is released into the tissue, exposing neighboring cells to potentially harmful attack by intracellular proteases and other released factors. The overall mechanism results in clinical signs (e.g., microscopic and macroscopic hematuria, burning, frequency and dysuria) and pathologically in edema, inflammation and ulceration (modified from reference [4] with authors permission).

Indeed, when examined closely, it seems possible that nitric oxide synthase (NOS) induction and/or activation could explain the initiation of toxic effects of CP. Under oxidative stress conditions like HC, many of side effects seem to have parallels with the biological effects and functions of abundant NO including DNA damage, NAD^+ depletion, cell and tissue toxicity and inflammation through peroxynitrite production [6, 7]. Activated PARP may act in extremely different way called the Yin-Yang of PARP activation. The death-promoting and the cytoprotective effects of poly(ADP-ribosyl)ation represent two seemingly opposing faces of PARP. Oxidative and nitrosative stress induced DNA breakage causes a high level of PARP activation, leading to the depletion of NAD^+ and ATP and consequently to necrotic cell death. On the other hand, poly(ADP-ribosyl)ation facilitates DNA repair in cells. The overall mechanism has been illustrated in figure.

PREVENTIVE METHODS

The first step against acrolein toxicity is to block the acrolein entering into the uroepithelial cells. Mesna is the only already known drug that binds acrolein in the urinary system and block acrolein entering into the uroepithelial cells. Both experimental and clinical studies have clearly demonstrated that mesna is effective against CP-induced cystitis [8]. However, 5% of patients who get CP or IF treatment may still suffer from HC during or after the treatment. The reason of this may be suggested that several other compounds apart from acrolein such as chloroethylaziridine and phosphoramide mustard may involve HC pathogenesis. Mesna may not block these agents that causing HC symptoms. Therefore, hyperhydration is considered as another important option. Through this way, toxic compounds cannot accumulate in urinary system and rapidly removed from urinary bladder via frequent micturition. Although these two precautions are essential to prevent HC symptoms, mesna administration may cause several hypersensitivity-like cutaneous and systemic reactions in adult patients and hyperhydration may not be useful for some patients those severely ill or immobile. Thus some additional preventive precautions may be needed to cure the HC symptoms if not be cured by mesna and hyperhydration. In this review, preventive suggestions will be limited only to the known pathophysiologic mechanisms of HC. Many other preventive modalities such as conjugated estrogen, intravesical adrenaline are out of interest.

COMPLEMENTARY PREVENTIVE METHODS

CP and IF are metabolized in liver and appear several compounds those all lipophilic nature. Thus they readily enter into virtually all cells (antineoplastic effects) including bladder epithelium (side effects). All compounds act through several mechanisms in bladder epithelium and treatment/prevention modalities are summarized in table. According to pathophysiological mechanism, several preventive steps are as follows.

SCAVENGING REACTIVE OXYGEN SPECIES

Several antioxidants including β-carotene and α-tocopherol have been shown to diminish the HC symptoms [9, 10]. Furthermore, popular flavonoid antioxidants such as quercetin and epigallocatechin 3-gallate [11, 12] and curcumin [13] have also been shown beneficial. Ternatin, another flavonoid antioxidant, is a popular remedy in Brasilian folk medicine. Vieira et al. [14] showed that ternatin has beneficial effects against CP and IF induced HC. Another chemical compound with antioxidant properties, amifostine is a well-established uroprotector against alkylating agents-induced bladder damage [15-17]. This beneficial effect may be attributed to ROS scavenging property of antioxidants. However, it is important to take into consideration that scavenging ROS may block peroxynitrite production. Since NO itself is not toxic and has short life-time, the potent harmful compound, peroxynitrite may not be highly produced and HC symptoms are alleviated in this way.

It is interesting that another antioxidant compound is mesna itself [18, 19]. Thus, apart from acrolein binding property, antioxidant feature of mesna may contribute to beneficial effects against cystitis. Although it is not possible to separate the beneficial effects of mesna in case of cystitis, several inflammatory processes have been alleviated using mesna as an antioxidant [20, 21].

ANTI-INFLAMMATORY CONSIDERETIONS

NOS inhibitors both non-selective (e.g., L-NAME) and selective (e.g., S-methylthiourea, 1400W) have therapeutic potential in experimental works; however there is no any drug available for clinical use. Same is true for peroxynitrite scavengers such as metalloporphyrins, desferrioxamine, mercaptoalkylguanidines, amidine derivatives. Several marketed drugs including cabergoline, nebivolol, and acetaminophen with RNS scavenging/decomposition properties may have clinical interest in near future [6].

Table 1. Acrolein-initiated pathophysiological mechanisms and preventive/treatment modalities of hemorrhagic cystitis (step by step).

Pathophysiological Mechanisms	Preventive/Treatment modalities
Acrolein and others rapidly bind to and deplete cellular nucleophiles such as glutathione; proteins (enzymatic and structural) those reach of sulfur containing amino acids	Mesna Hyperhydration
Acrolein causes increased ROS production in the bladder epithelium	Antioxidants Melatonin
Acrolein causes both directly and/or indirectly (through transcription factors) iNOS induction leading to NO overproduction	iNOS inhibitors Melatonin
Acrolein induces several intracellular transcription factors such as NF-κB and AP-1	Steroids Non-Steroid anti-inflammatory drugs Melatonin
Activated NF-κB and AP-1 cause cytokine (e.g., TNF-α, IL-1β, IL-4, IL-6) gene expression, iNOS induction, and again ROS production	Antioxidants iNOS inhibitors Steroids Cytokine inhibitors Melatonin
The production of inflammatory molecules and enzymes (e.g., cytokines, ROS, RNS, COX-2 and iNOS) increases dramatically	Antioxidants iNOS inhibitors Steroids Cytokine inhibitors Melatonin
Cytokines leave the uroepithelium and spread to other uroepithelial cells, detrussor smooth muscle and into bloodstream	Cytokine inhibitors
Reactive nitrogen species, in particular $ONOO^-$, attacks cellular macromolecules (lipids, proteins, DNA) and causes further damage	Peroxynitrite scavengers Melatonin
ROS and RNS cause damage in both uroepithelium and detrussor smooth muscle	Peroxynitrite scavengers Melatonin
DNA damage induces PARP activation leading either recovery or cell necrosis via energy crisis	PARP inhibitors
Broken cellular and tissue integrity appear cystitis symptoms such as edema, hemorrhage, inflammation and ulceration	HBO

Since HC has been accepted as non-infectious inflammatory process, glucocorticoids, well known anti-inflammatory agents, might consider as a therapeutic modality in HC. Vieira and coworkers [22] observed that replacement of one or two doses of mesna with dexamethasone reduced the bladder wet weight induced by IF, showing no significant difference compared to the effect of three doses of mesna. Replacement of one or two doses of mesna with dexamethasone also abolished the mucosal erosion, inflammatory cell infiltration and ulcerations observed in microscopic analysis of the bladders. This finding is consistent with a previous report that showed dexamethasone substituting mesna treatment (second and third doses) inhibited CP-induced HC in rats [23]. Moreover, it is important to consider that although the two alkylating agents are known to induce HC, the incidence of this side-effect is greater with IF treatment, since this drug is usually administrated for a longer period and in a higher dose than that CP. It was reported that patients using glucocorticoids as an immunosuppressor combined with CP, for the treatment of systemic lupus erythematosus, have a low incidence of HC [24].

Glucocorticoids are known to inhibit the synthesis and the activity of transcription factors, a variety of cytokines including TNF-α, and IL-1β and the expression of pro-inflammatory enzymes such as iNOS and COX-2 [25]. As mentioned previously, studies have demonstrated that all chemical compounds (e.g., cytokines and proinflammatory enzymes) those are inhibited and/or suppressed by glucocorticoids are crucial mediators involved in inflammatory events of HC as well as urothelial damage and hemorrhage. Thus, the results demonstrating that dexamethasone inhibits IF- and CP-induced HC could be explained by a potent efficacy of the corticoid as an

inhibitor/suppressor of the cytokines, transcription factors and pro-inflammatory enzymes. However, the replacement of all mesna doses with dexamethasone did not prevent HC. It has been proposed that urothelial damage occurs by direct contact with acrolein. Therefore, the data presented in Vieira's study [22] could be explained by the fact that mesna seems to be necessary for the initial uroprotection through its neutralizing effect on urothelial damage initiated by acrolein, while dexamethasone may inhibit the mediators of the inflammatory process that follow.

In addition, a recent study has demonstrated an important role for neutrophils in the pathogenesis of HC. This study showed that glucose-mannose binding plant lectins reduce urothelial damage and the inflammatory events present in HC induced by CP, probably by the blockage of neutrophil migration to inflamed bladders [26]. As dexamethasone is a potent inhibitor of neutrophil migration [27] and leads to inhibiting excessive leukocyte egress and subsequent free radical-mediated damage caused by leukocyte components may attenuate or eliminate tissue damage. It is likely that this inhibitory effect on neutrophil migration of dexamethasone could also contribute to the prevention of HC.

Thalidomide (TNF-α inhibitor), pentoxyfylline (IL-1β inhibitor), steroids (glucocorticoids) and many non-steroidal anti-inflammatory drugs including aspirin, indomethacin are all non-specific TNF-α and several other cytokines inhibitors, which modulate TNF-α dependent and independent pathways. Experimental works have been indicated that several cytokines including TNF-α, interferon-γ (IF-γ), and IL-1β involve the pathogenesis of HC [28-30]. Recent evidence suggest that apart from well-known cytokines (e.g., TNF-α, IL-1β), IL-4 [31], IL-6 [32] among others [33] also involve HC pathogenesis.

Xu and coworkers [34] reported that exposure to TNF-α and interferon-γ (IF-γ) produced a marked increase in the expression of iNOS and NO production in culture of rat bladder smooth muscle cells. However, exposure to CP or its metabolite acrolein did not increase iNOS or NO metabolite levels in this culture medium. On the other hand; incubation of primary cell cultures with plasma from rats treated with CP produced a marked increase in iNOS expression and NO production. The authors have decided that NO has an important role in the pathogenesis of CP-induced cystitis in rats and some factors may be released in CP-treated rat plasma that stimulates iNOS expression. Their findings suggest that an increased production of NO, through increased expression of iNOS, is an important factor in the production of CP-induced inflammatory changes in the rat bladder. Initial increases in NO levels may represent physiological adaptive mechanisms in response to an irritant substance (acrolein); however, excessive and sustained production of NO may directly or indirectly (via production of cytokines) lead to inflammatory damages (HC). Part of the increased expression of iNOS in the bladder tissues, may be triggered by circulating factors; as evidenced by increased iNOS expression in bladder smooth muscle cells when exposed to plasma from rats treated with CP. Employing a primary culture of bladder smooth muscle cells, they demonstrated that the iNOS expression is highly responsive to cytokines and to plasma of CP-treated rats, but failed to respond to direct exposure to CP or acrolein.

Johansson et al [35] used cytokines to show iNOS activation in cultured bladder smooth muscle cell. They reported that, there was no iNOS activation in unstimulated isolated bladder smooth muscle cells. Then, different combinations of cytokines were investigated for iNOS induction in cultured bladder smooth muscle cells. The results clearly demonstrated that cytokines induced gene and protein expression of iNOS in these cells. It was also confirmed that induced protein was active and produced NO, as evidenced by production of the metabolite nitrite. The cytokine combination TNF-α plus IL-1β but not TNF-α or IL-1β alone caused iNOS protein expression and nitrite accumulation in rat bladder smooth muscle cells. Mainly iNOS is regulated at the transcriptional level and various transcription factors such as NF-κB and AP-1 are involved in regulating iNOS induction. Thus, activation of multiple regions of the rat iNOS promoter through different pathways by iNOS inducing agents may explain the synergistic effects of TNF-α or IL-1β in this study.

These two studies indicate that when bladder smooth muscle cells are induced by cytokines, it causes iNOS gene and protein expression. Thus, bladder muscle cells are able to induce iNOS when the cytokines are available in the medium. It is clear that uroepithelial cells are not present in primary culture of bladder smooth muscle cells. So, in Xu's work [34], the initial step (contact between acrolein with uroepithelium) was absent. Therefore, acrolein could not enter into the epithelial cell and activate the mechanism explained above. Neither TNF-α, nor IL-1β can be produced by uroepithelium. However, if these cytokines were put into the medium bladder muscle cells produce iNOS. As result, we speculate that contact with acrolein and uroepithelium (but not bladder smooth muscle cell) may be the trigger step to produce cytokines leading to iNOS induction.

As previously described, interacting acrolein with uroepithelium act as a trigger point. Further, not only HC but also in many of inflammatory processes including rheumatoid arthritis, NF-κB and AP-1 seems

the junction leading to gene expression such as TNF-α, IL-1β, IF-γ, iNOS and COX-2. Furthermore there is strong evidence that iNOS induction is almost always together with COX-2 activation. COX-2 may therefore involve the HC pathogenesis. Thus COX inhibitory effects of several agents in particular steroids may contribute the healing bladder. Natural antioxidants used against HC such as catechin (tea), quercetin (onion), ternatine are also COX-2 inhibitors [36, 37] and this feature may serve additional benefit apart from ROS scavenging properties. At this point, resveratrol (grape), curcumin (turmeric) and many other tea flavonoids may also be effective antioxidants with NF-κB and several pro-inflammatory cytokines inhibition properties against HC symptoms.

HYPERBARIC OXYGEN

HBO is a treatment modality which is sometimes life-saving in cases such as carbon monoxide poisoning and decompression sickness. Moreover it is crucial in many other disease treatments such as osteomyelitis, diabetic foot, and impaired wound healing. Undersea and Hyperbaric Medical Society (UHMS) has been approved a list of clinical conditions including air or gas embolism, carbon monoxide poisoning, clostridial myositis and myonecrosis (gas gangrene), crush injury, compartment syndrome and other acute traumatic ischemias, decompression sickness, enhancement of healing in selected problem wounds, exceptional blood loss, necrotizing soft tissue infections, refractory osteomyelitis, osteoradionecrosis, compromised skin grafts and flaps, thermal burns, and intracranial abscess [38, 39]. Nevertheless, plenty of experimental studies, most of them have been fulfilled in our laboratory, are available in literature which suggest many successful cures obtained with HBO treatment such as colitis [40], myositis [41], pancreatitis [42, 43] and sepsis [44] which are not in approved indication list of UHMS.

Several works have been suggested that HBO attenuates bladder damage, such as edema, hemorrhage, inflammation and even ulceration. Hader et al [45] treated rats with HBO before and after exposure to acrolein, and noted a significant decrease in tissue damage and enhanced urothelial regeneration compared to control animals. Our laboratory has shown that HBO treatment has alleviated experimental cystitis [46, 47]. It is also documented that HBO cannot prevent acrolein-dependent cystitis rather it accelerates the healing of damaged bladder [48].

Hughes et al [49] reported a case of HC occurring after autologous peripheral stem cell transplantation for multiple myeloma. The patient had received CP and busulfan, and the clinical course was complicated by viral infections. After antiviral therapy and conventional urological management for intractable hematuria and bladder spasms, the patient was referred for HBO and completed 37 treatments with complete resolution of bleeding. At 14-month follow-up he was free of hematuria. Davis et al [50] has recently reported that six patients, with life-threatening hemorrhage at the time of referral for HBO weeks or months after initial presentation with HC in New Zealand. Cessation of bleeding occurred in all six patients after 14 to 40 HBO session without complications. All patients remained clear of hematuria at 11 to 36 months follow-up. Authors have recommended the use of HBO in the management of intractable CP-induced HC as an effective and low-risk therapy. Bevers et al [51] also reported similar effects of HBO treatments in radiation-induced HC. Beneficial effects of HBO on painful bladder syndrome and interstitial cystitis have also documented [52-54].

A SPECIAL CONSIDERATION REGARDING MELATONIN

There is abundance of both experimental and clinical studies regarding to melatonin possess antioxidant and free radical scavenging properties [55, 56]. This feature of melatonin appears in both administrations of physiologic and pharmacologic doses [57, 58]. Antioxidant properties of melatonin have been shown in many tissues including kidney, brain, pancreatic islet, retina and others [59].

Melatonin directly neutralizes a number of toxic oxygen and nitrogen based reactans, including OH•, H_2O_2, hipochlorous acid (HOCl), singlet oxygen and several nitrogen-based compounds including peroxynitrite anion or peroxynitrous acid [59-61]. Furthermore, it also has indirect antioxidative actions, including stimulating the synthesis of important intracellular antioxidant, i.e. glutathione as well as promoting its enzymatic recycling in cells to ensure it remains primarily in its reduced form [55]. Melatonin preserves the functional integrity of other antioxidative enzymes, including superoxide dismutase and catalase. Melatonin may also reduce free radical generation in mitochondria by improving oxidative phosphorylation, thereby lowering electron leakage, and increasing ATP generation [62]. Moreover, melatonin's chief hepatic metabolite, 6-sulfatoxymelatonin, as well as several other by-products of melatonin is effective free-radical scavengers [59]. Recent studies performed in our laboratory and others have also shown that melatonin can directly scavenge the peroxynitrite [63-65]. Apart from these beneficial effects, melatonin regulates gene expression of several immunomodulatory cytokines and attenuates NF-κB binding to DNA [66-70]. These results indicate that melatonin is a widely acting antioxidant including direct (antioxidant effect),

indirect (enzymatic support) and nitrogen-based compounds scavenging properties. Apart from this collective efficacy against both oxidative and nitrosative stress, the same indole has relatively strong anti-inflammatory efficacy. These features make the melatonin a very good add-on therapy along with mesna. We tested melatonin for alleviation of CP-induced cystitis and it exerted beneficial effects [10, 71]. Recently, Zupancic et al [72, 73] and Tripathi et al [74] have documented similar beneficial effects of melatonin with detailed mechanism against CP-induced HC. The implications of the accumulated observations suggest that it is time to consider clinical trials using melatonin for inhibition of HC symptoms in conjunction with mesna, hyperhydration and other therapeutic applications. Note that melatonin has very few side effects and no toxicity or teratogenicity at any given physiological or pharmacological doses [75, 76].

FINAL CONSIDERATIONS

It is far known that acrolein is the main molecule responsible for CP- and IF-induced cystitis. Nevertheless, now we know more molecular mechanisms which take part in the pathogenesis of HC including cytokines, ROS, RNS leading to transcription factors and/or PARP activation and resulting in cell damage. Mesna is a widely used and relatively successful agent in order to detoxify acrolein however; CP-induced HC still remains a clinical problem. Thus, we suggest that clinicians may be encouraged to add alternative drugs or methods to standard protocol which are known to have no important side-effects, such as antioxidants, HBO and in particular melatonin in clinical trials. This will support the a number of above mentioned experimental studies and improvement of comfort of patients such as in the case reported by Kalayoglu et al [77] in which a patient was saved from cystectomy by HBO administration.

REFERENCES

1. Gray KJ, Engelmann UH, Johnson EH, Fishman IJ. Evaluation of misoprostol cytoprotection of the bladder with cyclophosphamide (Cytoxan) therapy. J Urol 1986;136:497-500.
2. Brock N, Pohl J, Stekar J. Studies on the urotoxicity of oxazaphosphorine cytostatics and its prevention. 2. Comparative study on the uroprotective efficacy of thiols and other sulfur compounds. Eur J Cancer Clin Oncol 1981;17:1155-63.
3. Brock N, Pohl J, Stekar J. Studies on the urotoxicity of oxazaphosphorine cytostatics and its prevention--I. Experimental studies on the urotoxicity of alkylating compounds. Eur J Cancer 1981;17:595-607.
4. Korkmaz A, Topal T, Oter S. Pathophysiological aspects of cyclophosphamide and ifosfamide induced hemorrhagic cystitis; implication of reactive oxygen and nitrogen species as well as PARP activation. Cell Biol Toxicol 2007;23:303-12.
5. Pacher P, Szabo C. Role of the peroxynitrite-poly(ADP-ribose) polymerase pathway in human disease. Am J Pathol 2008;173:2-13.
6. Szabo C, Ischiropoulos H, Radi R. Peroxynitrite: biochemistry, pathophysiology and development of therapeutics. Nat Rev Drug Discov 2007;6:662-80.
7. Korkmaz A, Oter S, Seyrek M, Topal T. Molecular, genetic and epigenetic pathways of peroxynitrite-induced cellular toxicity. Interdiscip Toxicol 2009;2:219-28.
8. Haselberger MB, Schwinghammer TL. Efficacy of mesna for prevention of hemorrhagic cystitis after high-dose cyclophosphamide therapy. Ann Pharmacother 1995;29:918-21.
9. Yildirim I, Korkmaz A, Oter S, Ozcan A, Oztas E. Contribution of antioxidants to preventive effect of mesna in cyclophosphamide-induced hemorrhagic cystitis in rats. Cancer Chemother Pharmacol 2004;54:469-73.
10. Sadir S, Deveci S, Korkmaz A, Oter S. Alpha-tocopherol, beta-carotene and melatonin administration protects cyclophosphamide-induced oxidative damage to bladder tissue in rats. Cell Biochem Funct 2007;25:521-6.
11. Ozcan A, Korkmaz A, Oter S, Coskun O. Contribution of flavonoid antioxidants to the preventive effect of mesna in cyclophosphamide-induced cystitis in rats. Arch Toxicol 2005;79:461-5.
12. Boeira VT, Leite CE, Santos AA, Jr., Edelweiss MI, Calixto JB, Campos MM, Morrone FB. Effects of the hydroalcoholic extract of Phyllanthus niruri and its isolated compounds on cyclophosphamide-induced hemorrhagic cystitis in mouse. Naunyn Schmiedebergs Arch Pharmacol 2011;384:265-75.
13. Arafa HM. Uroprotective effects of curcumin in cyclophosphamide-induced haemorrhagic cystitis paradigm. Basic Clin Pharmacol Toxicol 2009;104:393-9.
14. Vieira MM, Macedo FY, Filho JN, Costa AC, Cunha AN, Silveira ER, Brito GA, Ribeiro RA. Ternatin, a flavonoid, prevents cyclophosphamide and ifosfamide-induced hemorrhagic cystitis in rats. Phytother Res 2004;18:135-41.
15. Kanat O, Kurt E, Yalcinkaya U, Evrensel T, Manavoglu O. Comparison of uroprotective efficacy of mesna and amifostine in Cyclophosphamide- induced hemorrhagic cystitis in rats. Indian J Cancer 2006;43:12-5.
16. Batista CK, Mota JM, Souza ML, Leitao BT, Souza MH, Brito GA, Cunha FQ, Ribeiro RA. Amifostine and glutathione prevent ifosfamide- and acrolein-induced hemorrhagic cystitis. Cancer Chemother Pharmacol 2007;59:71-7.
17. Srivastava A, Nair SC, Srivastava VM, Balamurugan AN, Jeyaseelan L, Chandy M, Gunasekaran S. Evaluation of uroprotective efficacy of amifostine against cyclophosphamide induced hemorrhagic cystitis. Bone Marrow Transplant 1999;23:463-7.

18. Sener G, Sehirli O, Cetinel S, Yegen BG, Gedik N, Ayanoglu-Dulger G. Protective effects of MESNA (2-mercaptoethane sulphonate) against acetaminophen-induced hepatorenal oxidative damage in mice. J Appl Toxicol 2005;25:20-9.

19. Kabasakal L, Sehirli AO, Cetinel S, Cikler E, Gedik N, Sener G. Mesna (2-mercaptoethane sulfonate) prevents ischemia/reperfusion induced renal oxidative damage in rats. Life Sci 2004;75:2329-40.

20. Ypsilantis P, Tentes I, Lambropoulou M, Anagnostopoulos K, Papadopoulos N, Kortsaris A, Simopoulos C. Prophylaxis with mesna prevents oxidative stress induced by ischemia reperfusion in the intestine via inhibition of nuclear factor-kappaB activation. J Gastroenterol Hepatol 2008;23:328-35.

21. Ypsilantis P, Lambropoulou M, Anagnostopoulos C, Tentes I, Tsigalou C, Pitiakoudis M, Kortsaris A, Papadopoulos N, Simopoulos C. Mesna preserves hepatocyte regenerating capacity following liver radiofrequency ablation under Pringle maneuver. J Surg Res 2011;169:44-50.

22. Vieira MM, Brito GA, Belarmino-Filho JN, Macedo FY, Nery EA, Cunha FQ, Ribeiro RA. Use of dexamethasone with mesna for the prevention of ifosfamide-induced hemorrhagic cystitis. Int J Urol 2003;10:595-602.

23. Morais MM, Belarmino-Filho JN, Brito GA, Ribeiro RA. Pharmacological and histopathological study of cyclophosphamide-induced hemorrhagic cystitis - comparison of the effects of dexamethasone and Mesna. Braz J Med Biol Res 1999;32:1211-5.

24. Austin HA, 3rd, Klippel JH, Balow JE, le Riche NG, Steinberg AD, Plotz PH, Decker JL. Therapy of lupus nephritis. Controlled trial of prednisone and cytotoxic drugs. N Engl J Med 1986;314:614-9.

25. Brattsand R, Linden M. Cytokine modulation by glucocorticoids: mechanisms and actions in cellular studies. Aliment Pharmacol Ther 1996;10 Suppl 2:81-90; discussion 91-2.

26. Assreuy AM, Martins GJ, Moreira ME, Brito GA, Cavada BS, Ribeiro RA, Flores CA. Prevention of cyclophosphamide-induced hemorrhagic cystitis by glucose-mannose binding plant lectins. J Urol 1999;161:1988-93.

27. Radi ZA, Kehrli ME, Jr., Ackermann MR. Cell adhesion molecules, leukocyte trafficking, and strategies to reduce leukocyte infiltration. J Vet Intern Med 2001;15:516-29.

28. Macedo FY, Baltazar F, Almeida PR, Tavora F, Ferreira FV, Schmitt FC, Brito GA, Ribeiro RA. Cyclooxygenase-2 expression on ifosfamide-induced hemorrhagic cystitis in rats. J Cancer Res Clin Oncol 2008;134:19-27.

29. Ribeiro RA, Freitas HC, Campos MC, Santos CC, Figueiredo FC, Brito GA, Cunha FQ. Tumor necrosis factor-alpha and interleukin-1beta mediate the production of nitric oxide involved in the pathogenesis of ifosfamide induced hemorrhagic cystitis in mice. J Urol 2002;167:2229-34.

30. Gomes TN, Santos CC, Souza-Filho MV, Cunha FQ, Ribeiro RA. Participation of TNF-alpha and IL-1 in the pathogenesis of cyclophosphamide-induced hemorrhagic cystitis. Braz J Med Biol Res 1995;28:1103-8.

31. Macedo FY, Mourao LT, Freitas HC, Lima-Junior RC, Wong DV, Oria RB, Vale ML, Brito GA, Cunha FQ, Ribeiro RA. Interleukin-4 Modulates the Inflammatory Response in Ifosfamide-Induced Hemorrhagic Cystitis. Inflammation 2011.

32. Dantas AC, Batista-Junior FF, Macedo LF, Mendes MN, Azevedo IM, Medeiros AC. Protective effect of simvastatin in the cyclophosphamide-induced hemorrhagic cystitis in rats. Acta Cir Bras 2010;25:43-6.

33. Malley SE, Vizzard MA. Changes in urinary bladder cytokine mRNA and protein after cyclophosphamide-induced cystitis. Physiol Genomics 2002;9:5-13.

34. Xu X, Cubeddu LX, Malave A. Expression of inducible nitric oxide synthase in primary culture of rat bladder smooth muscle cells by plasma from cyclophosphamide-treated rats. Eur J Pharmacol 2001;416:1-9.

35. Johansson RK, Poljakovic M, Andersson KE, Persson K. Expression of nitric oxide synthase in bladder smooth muscle cells: regulation by cytokines and L-arginine. J Urol 2002;168:2280-5.

36. Garcia-Mediavilla V, Crespo I, Collado PS, Esteller A, Sanchez-Campos S, Tunon MJ, Gonzalez-Gallego J. The anti-inflammatory flavones quercetin and kaempferol cause inhibition of inducible nitric oxide synthase, cyclooxygenase-2 and reactive C-protein, and down-regulation of the nuclear factor kappaB pathway in Chang Liver cells. Eur J Pharmacol 2007;557:221-9.

37. Xiao X, Shi D, Liu L, Wang J, Xie X, Kang T, Deng W. Quercetin suppresses cyclooxygenase-2 expression and angiogenesis through inactivation of P300 signaling. PLoS One 2011;6:e22934.

38. Ozler M, Akay C, Oter S, Ay H, Korkmaz A. Similarities and differences of hyperbaric oxygen and medical ozone applications. Free Radic Res 2011.

39. Le P-NJ, Thom SR. Mechanistic perspective for experimental and accepted indications of hyperbaric oxygen therapy. J Exp Integr Med 2011;1:207-214.

40. Gulec B, Yasar M, Yildiz S, Oter S, Akay C, Deveci S, Sen D. Effect of hyperbaric oxygen on experimental acute distal colitis. Physiol Res 2004;53:493-9.

41. Oztas E, Kilic A, Ozyurt M, Korkmaz A, Basustaoglu A. Effect of hyperbaric oxygen and penicillin in a murine model of streptococcal myositis. Undersea Hyperb Med 2001;28:181-6.

42. Uysal B, Yasar M, Ersoz N, Coskun O, Kilic A, Cayc T, Kurt B, Oter S, Korkmaz A, Guven A. Efficacy of hyperbaric oxygen therapy and medical ozone therapy in experimental acute necrotizing pancreatitis. Pancreas 2010;39:9-15.

43. Mas N, Isik AT, Mas MR, Comert B, Tasci I, Deveci S, Ozyurt M, Ates Y, Yamanel L, Doruk H, Yener N. Hyperbaric oxygen-induced changes in bacterial translocation and acinar ultrastructure in rat acute necrotizing pancreatitis. J Gastroenterol 2005;40:980-6.

44. Thom SR, Lauermann MW, Hart GB. Intermittent hyperbaric oxygen therapy for reduction of mortality in experimental polymicrobial sepsis. J Infect Dis 1986;154:504-10.
45. Hader JE, Marzella L, Myers RA, Jacobs SC, Naslund MJ. Hyperbaric oxygen treatment for experimental cyclophosphamide-induced hemorrhagic cystitis. J Urol 1993;149:1617-21.
46. Korkmaz A, Oter S, Deveci S, Ozgurtas T, Topal T, Sadir S, Bilgic H. Involvement of nitric oxide and hyperbaric oxygen in the pathogenesis of cyclophosphamide induced hemorrhagic cystitis in rats. J Urol 2003;170:2498-502.
47. Korkmaz A, Oter S, Deveci S, Goksoy C, Bilgic H. Prevention of further cyclophosphamide induced hemorrhagic cystitis by hyperbaric oxygen and mesna in guinea pigs. J Urol 2001;166:1119-23.
48. Oztas E, Korkmaz A, Oter S, Topal T. Hyperbaric oxygen treatment time for cyclophosphamide induced cystitis in rats. Undersea Hyperb Med 2004;31:211-6.
49. Hughes AJ, Schwarer AP, Millar IL. Hyperbaric oxygen in the treatment of refractory haemorrhagic cystitis. Bone Marrow Transplant 1998;22:585-6.
50. Davis M, Macdonald H, Sames C, Nand K. Severe cyclophosphamide-induced haemorrhagic cystitis treated with hyperbaric oxygen. N Z Med J 2011;124:48-54.
51. Bevers RF, Bakker DJ, Kurth KH. Hyperbaric oxygen treatment for haemorrhagic radiation cystitis. Lancet 1995;346:803-5.
52. Tanaka T, Nitta Y, Morimoto K, Nishikawa N, Nishihara C, Tamada S, Kawashima H, Nakatani T. Hyperbaric oxygen therapy for painful bladder syndrome/interstitial cystitis resistant to conventional treatments: long-term results of a case series in Japan. BMC Urol 2011;11:11.
53. Tanaka T, Kawashima H, Makino T, Kamikawa S, Kato N, Nakatani T. Hyperbaric oxygen therapy for interstitial cystitis resistant to conventional treatments. Int J Urol 2007;14:563-5.
54. van Ophoven A, Rossbach G, Oberpenning F, Hertle L. Hyperbaric oxygen for the treatment of interstitial cystitis: long-term results of a prospective pilot study. Eur Urol 2004;46:108-13.
55. Korkmaz A, Reiter RJ, Topal T, Manchester LC, Oter S, Tan DX. Melatonin: an established antioxidant worthy of use in clinical trials. Mol Med 2009;15:43-50.
56. Reiter RJ, Tan DX, Mayo JC, Sainz RM, Leon J, Czarnocki Z. Melatonin as an antioxidant: biochemical mechanisms and pathophysiological implications in humans. Acta Biochim Pol 2003;50:1129-46.
57. Reiter RJ, Tan DX, Maldonado MD. Melatonin as an antioxidant: physiology versus pharmacology. J Pineal Res 2005;39:215-6.
58. Reiter RJ, Tan DX, Sanchez-Barcelo E, Mediavilla MD, Gitto E, Korkmaz A. Circadian mechanisms in the regulation of melatonin synthesis: disruption with light at night and the pathophysiological consequences. J Exp Integr Med 2011;1:13-22.
59. Tan DX, Manchester LC, Terron MP, Flores LJ, Reiter RJ. One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? J Pineal Res 2007;42:28-42.
60. Korkmaz A, Manchester LC. Reactive nitrogen species; devastating intracellular players and melatonin as a defender. J Exp Integr Med 2011;1:63-65.
61. Hardel R. Melatonin and its metabolites as anti-nitrosating and anti-nitrating agents. J Exp Integr Med 2011;1:67-81.
62. Reiter RJ, Paredes SD, Korkmaz A, Jou MJ, Tan DX. Melatonin combats molecular terrorism at the mitochondrial level. Interdiscip Toxicol 2008;1:137-49.
63. Ucar M, Korkmaz A, Reiter RJ, Yaren H, Oter S, Kurt B, Topal T. Melatonin alleviates lung damage induced by the chemical warfare agent nitrogen mustard. Toxicol Lett 2007;173:124-31.
64. Gocgeldi E, Uysal B, Korkmaz A, Ogur R, Reiter RJ, Kurt B, Oter S, Topal T, Hasde M. Establishing the use of melatonin as an adjuvant therapeutic against paraquat-induced lung toxicity in rats. Exp Biol Med (Maywood) 2008;233:1133-41.
65. Ersoz N, Guven A, Cayci T, Uysal B, Turk E, Oztas E, Akgul EO, Korkmaz A, Cetiner S. Comparison of the efficacy of melatonin and 1400W on renal ischemia/reperfusion injury: a role for inhibiting iNOS. Ren Fail 2009;31:704-10.
66. Kang JW, Koh EJ, Lee SM. Melatonin protects liver against ischemia and reperfusion injury through inhibition of toll-like receptor signaling pathway. J Pineal Res 2011;50:403-11.
67. Negi G, Kumar A, Sharma SS. Melatonin modulates neuroinflammation and oxidative stress in experimental diabetic neuropathy: effects on NF-kappaB and Nrf2 cascades. J Pineal Res 2011;50:124-31.
68. Shang Y, Xu SP, Wu Y, Jiang YX, Wu ZY, Yuan SY, Yao SL. Melatonin reduces acute lung injury in endotoxemic rats. Chin Med J (Engl) 2009;122:1388-93.
69. Mazzon E, Esposito E, Crisafulli C, Riccardi L, Muia C, Di Bella P, Meli R, Cuzzocrea S. Melatonin modulates signal transduction pathways and apoptosis in experimental colitis. J Pineal Res 2006;41:363-73.
70. Wang H, Wei W, Wang NP, Gui SY, Wu L, Sun WY, Xu SY. Melatonin ameliorates carbon tetrachloride-induced hepatic fibrogenesis in rats via inhibition of oxidative stress. Life Sci 2005;77:1902-15.
71. Topal T, Oztas Y, Korkmaz A, Sadir S, Oter S, Coskun O, Bilgic H. Melatonin ameliorates bladder damage induced by cyclophosphamide in rats. J Pineal Res 2005;38:272-7.
72. Zupancic D, Jezernik K, Vidmar G. Effect of melatonin on apoptosis, proliferation and differentiation of urothelial cells after cyclophosphamide treatment. J Pineal Res 2008;44:299-306.
73. Zupancic D, Vidmar G, Jezernik K. Melatonin prevents the development of hyperplastic urothelium induced by

repeated doses of cyclophosphamide. Virchows Arch 2009;454:657-66.

74. Tripathi DN, Jena GB. Effect of melatonin on the expression of Nrf2 and NF-kappaB during cyclophosphamide-induced urinary bladder injury in rat. J Pineal Res 2010;48:324-31.
75. Reiter RJ, Gultekin F, Flores LJ, Terron MP, Tan DX. Melatonin: Potential utility for improving public health. TAF Prev Med Bull 2006;5:131-158.
76. Jahnke G, Marr M, Myers C, Wilson R, Travlos G, Price C. Maternal and developmental toxicity evaluation of melatonin administered orally to pregnant Sprague-Dawley rats. Toxicol Sci 1999;50:271-9.
77. Kalayoglu-Besisik S, Abdul-Rahman IS, Erer B, Yenerel MN, Oguz FS, Tunc M, Sargin D. Outcome after hyperbaric oxygen treatment for cyclophosphamide-induced refractory hemorrhagic cystitis. J Urol 2003;170:922.