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Review

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Bladder urotoxicity pathophysiology induced by the oxazaphosphorine alkylating agents and its chemoprevention

Patofizjologia jatrogennego uszkodzenia pęcherza po lekach alkilujących z grupy oksazafosforan i jego chemioprewencja

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Summary

The use of oxazaphosphorines (cyclophosphamide, ifosfamide) in the treatment of numerous neoplastic disorders is associated with their essential adverse effect in the form of hemorrhagic cystitis, which considerably limits the safety and efficacy of their pharmacotherapy. HC is a complex inflammatory response, induced by toxic oxazaphosphorines metabolite – acrolein with subsequent immunocompetetive cells activation and release of many proinflammatory agents. However, there are some chemoprotectant agents which help reduce the HC exacerbation.

The article briefly discuses the mechanism of action of oxazaphosphorines, the pathophysiology of the hemorrhagic cystitis development and currently accepted chemopreventive agents, applied to the objective of urotoxicity amelioration. Moreover, the rationale for some phytopharmaceuticals administration as novel bladder protective compounds accompanying cyclophosphamide or ifosfamide therapy was also mentioned.

Key words: **oxazaphosphorines • cyclophosphamide • ifosfamide • hemorrhagic cystitis • chemopreventive agents**

Streszczenie

Leki cytostatyczne z grupy oksazafosforyn (cyklofosfamid, ifosfamid) stosuje się w chemioterapii wielu typów nowotworów, jednak ich podawanie jest związane z ich zasadniczym działaniem niepożądanym, ograniczającym bezpieczeństwo i skuteczność tych cytostatyków – rozwojem krwotocznego zapalenia pęcherza (hemorrhagic cystitis – HC). Patogeneza HC jest złożona, inicjowana toksycznym metabolitem oksazafosforyn – akroleiną, co powoduje aktywację komórek immunokompetentnych i uwolnienie wielu prozapalnych mediatorów w pęcherzu. Istnieje możliwość zmniejszania lub zapobiegania uszkodzeniom pęcherza podczas terapii oksazafosforynami przez stosowanie związków chemioprewencyjnych.

W pracy omówiono cytostatyczny mechanizm działania oksazofosforyn, patofizjologię krwotocznego zapalenia pęcherza oraz obecnie stosowane środki chemioprewencyjne, łagodzące HC). Omówiono ponadto wybrane fitofarmaceutyki, jako potencjalne, nowe związki o aktywności chemioprewencyjnej, stosowane podczas terapii cyklofosfamidem lub ifosfamidem.

Słowa kluczowe: **oksazafosforyny • cyklofosfamid • ifosfamid • krwotoczne zapalenie pęcherza moczowego • leki chemioprewencyjne**

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Abbreviations: **AP-1** – activator protein-1; **BSO** – L-buthionine-sulfoximine; **CAT** – catalase; **CNS** – central nervous system; **CP** – cyclophosphamide; **GSH-Px** – glutathione peroxidase; **HC** – hemorrhagic cystitis; **IF** – ifosfamide; **Il-1** – interleukin-1; **NF-κB** – nuclear factor –κB; **NO** – nitric oxide; **NOS** – nitric oxide synthase; **ONOO$^-$** – peroxynitrite; **ORG 2766** – an analogue of adenocorticotropin hormone (ACTH) fragment; **PARP** – polyadenosine-diphosphatase-ribose polymerase; **ROS** – reactive oxygen species; **SOD** – superoxide dismutase; **TNF-α** – tumor necrosis factor α; **WR-1065** – amifostine thiol metabolite; **WR-33278** – amifostine disufide metabolite.

Introduction. The oxazaphosphorines structure, mechanism of action and position in oncology

Oxazaphosphorines belong to a group of alkylating agents with a broad spectrum of antitumor activity in man. Cyclophosphamide (CP) is a key substance, that possesses high activity against both many experimental tumors and in clinical conditions (lymphoproliferative disorders, Hodgkin disease, small cells lung carcinoma, breast cancer). Thus, CP has been chosen as a model compound in many scientific fields: molecular biology, biochemistry, pharmacology and toxicology in the areas of teratogenesis, mutagenesis, carcinogenesis and immunology, as well as preclinical and clinical research. As a drug, CP is also mostly used in non-neoplastic disorders such as nephrotic syndrome, systemic lupus erythematosus and rheumatoid arthritis. Oxazaphosphorines group contains also other agents: ifosfamide, trofosfamide, sufosfamide and mafosfamide, however, from all the compounds mentioned above, ifosfamide (IF) is most commonly administrated in clinical practice [13,14].

In the past, open-chain phosphoramide structures were derived. They were chemically stable, but unsuitable as substrates for enzymatic activation and their cytotoxic activity was insufficient, resulting in high and narrow therapeutic range [22]. The modern general approach to oxazaphosphorines synthesis is aimed at obtaining cyclic N-phosphorylation products of nor-nitrogen mustard compounds as prodrugs with reduced high reactivity and toxicity while retaining the antitumour efficacy. These novel forms are synthesised by reacting N,N-bis(2-chloroethyl)phosphoramide dichloride with α,ω-alkanolamines of various chain lengths. In the same manner other cyclic variants were synthesised with appropriate bifunctional alkanes (diamines, glycols, thioglycols) that lead to the formation of monoamides, diamides and triamides. From the pharmacological point of view, the diamides seem to be of the utmost importance, especially those from the group of N-mustard phosphamide esters. These prodrugs forms are regarded to be chemically and pharmacologically inactive transitory transport forms, which undergo a metabolic conversion to active chemotherapeutics *in vivo* [13,14]. All oxazaphosphorines differ by the specific formations that are bound to the heteroatoms and one neighbouring carbon. Cyclophosphamide is characterised as possessing two N,N-2-chloroethyloamine substituents and its chemical constitution became a model structure for the other oxazaphosphorines, as it was mentioned above. Ifosfamide is a single N-2-chloroethyloamino substituted derivative while the second of the 2-chloroethyl group is moved to the nitrogen heteroatom of the oxazaphosphorine ring. The trofosfamide structure is similar to the cyclophosphamide one with additional third chloroethylo fragment bound to the cyclic nitrogen atom (similar to ifosfamide). Sufosfamide possesses a unique N-methylosulfonoethyloamino substitutive group with one 2-chloroethyl linked to heterocyclic nitrogen (similar to CP and IF) [12,13,14, 31].

It has been revealed, that cytotoxic action of all oxazaphosphorines is closely related to the reactivity of the 2-chloroethyl substituent group, which in turn is linked with the basicity of the central nitrogen atom, however, *in vivo*, these chemotherapeutics are subject to complex biotransformation processes. To sum up, the metabolic pathway consists of three major steps; activation, toxification and deactivation. The oxazaphosphorine chemotherapeutics must undergo biotransformation before they can exert their alkylating cytotoxic properties. The initial activation of a non-active iso-oxazaphosphorine transport form entails a hydroxilation of the C-4 atom, which is effectuated by the mixed system of liver oxidases. At a further stage of toxification the formed 4-hydroxyoxazaphosphorines get metabolised with a spontaneous elution of acrolein and a formation of diamine derivatives of phosphoric acid of alkylating properties. Simultaneously, because of the fact that toxification reaction is a rate-limiting enzymatic step, a reversible or an irreversible deactivation may take place. Due to the saturation of enzymes synthesising alkylating metabolites, reactions of dehydrogenation (oxidation) to aldo-oxazaphosphorine forms may occur alternatively, which formations are then further converted into 4-keto or carboxy derivatives that are excreted in the end. This deactivation pathway hyperactivity may explain the presence of oxazaphosphorine-resistant tumours.[12,13,14,31]. However, during metabolic pathways, 4-mercaptooxazaphosphorines are also

produced which are regarded to be a transport form of higher reactivity. These indirect compounds can be produced in a non-enzymatic reaction of 4-hydroxyoxazaphosphorines with sulfhydryl compounds. Thus, the proteins that have free sulfhydryl groups may also react in this way and temporarily carry oxazaphosphorines in the blood, contributing to the delay in their final hepatic toxification until they enter the tumour cells. Hohorst et al. [28] showed in the process of their investigations that toxification processes may also take place inside cancer cells which would at least explain the specificity and selectivity of oxazaphosphorines cytostatics. The current elucidation of oxazaphosphorines cytotoxic action involves the possibility of cancer intercellular toxification. There are various 3' and 5' exonucleases and phosphodiesterases catalysing the intracellular release of alkylating metabolites from the 4-hydroxyoxazaphosphorines (they are restored again from their thiol protein transporter features). The active enzymes mentioned above are associated with DNA polymerases; hence they are considered to be the specific targets for 4-hydroxyoxazaphosphorines. According to Ross et al. [56], who formulated their concept over sixty years ago, the cytotoxic action of alkylating agents is based on the alkylation of nucleophilic centers of biomolecules (such as the N7 position of guanine in DNA). Thus, the intracellular release of alkylating agents leads to the DNA polymerases inhibition and specific DNA alkylation (by the covalent bonding) with subsequent DNA polymerisation depletion ("suicidal inactivation") [12,13,14,22].

Pathophysiological aspects of oxazaphosphorines-induced haemorrhagic cystitis

As it was mentioned above, oxazaphosphorine alkylating compounds are still widely used cytostatic agents. Cyclophosphamide (CP) and ifosfamide (IF) are most frequently applied representatives of this group. The chronic side effects of both CP and IF pharmacotherapy include mild or moderate disturbances (transient irritative voiding symptoms – dysuria, urgency, suprapubic discomfort, strangury with microhematuria) [40,41]. They have already been reported by Coggins et al. in 1960 [18]. However, the most important acute complication, associated with their use is severe urotoxicity, resulted in hemorrhagic cystitis (HC). The prevalence of HC development as a result of CP/IF treatment was reported to be as high as about 70%. Life-threatening HC with uncontrolled haemorrhage is estimated at about 4% [24,36].

The pathophysiological mechanism of HC development is complex, based on oxazaphosphorines-derived metabolites inflammatory response activation. As it has already been mentioned, both CP and IF are metabolised with liver microsomal enzymes, with the simultaneous formation of active so-called yperite mustard derivatives (particularly 4-hydroxy derivatives) (especially 4-hydroxy metabolites) and co-additional acrolein synthesis. Final metabolic compounds together with acrolein are subsequently excreted intact into the urinary bladder and initiate HC. Acrolein is a reactive unsaturated aldehyde, that can react with various structures (proteins, DNA nucleophilic sites, glutathione and other thiols compounds causing their cellular depletion). Moreover, acrolein may also produce other biological effects. It has the ability to activate both nuclear factor –κB (NF-κB) and activator protein-1 (AP-1), as well as to initiate lipids peroxidation and to exaggerate oxidative stress [36].

The proposed pathomechanism of acrolein-induced hemorrhagic cystitis involves several steps. After entering the urothelium, acrolein directly and indirectly increases the reactive oxygen species (ROS) overproduction in the bladder epithelium. Acrolein seems to react with gluthatione to form glutathione adduct – glutathionylpropionaldehyde. This compound interacts with several enzymes, including xanthine oxidase and aldehyde dehydrogenase that produce acroleinyl and superoxide radicals. Toxic acrolein metabolite – glutathione propionaldehyde is co-responsible for the overproduction of free oxygen radicals by urotelium previously damaged by the same acrolein [1,36]. Moreover, it has also been demonstrated that inflammatory reaction evoked by oxazaphosphorines involves increasing nitric oxide (NO) and its secondary derivatives production, being an important factor of HC pathogenesis [36]. An experimental study carried out by Oter et al. [50] revealed that administration of S-methylisothiourea – an inhibitor of NO synthase (NOS) produced marked inhibition of CP induced bladder damage, while L-arginine (NO donor) treatment augmented cystitis severity. Similar conclusions about endogenous NO participation in the inflammatory-based bladder disturbations were reported by Souza-Fiho et al. [60] whose experimental research demonstrated that CP increased the level of inducible NOS with constitutive NOS activity decrease. The other NOS inhibitors – L-NG-nitroarginine methyl ester and L-NG-nitroarginine significantly reduced CP-induced urothelial damage. This reduction was completely reversed again after L-arginine [60].

It is also known that reactive oxygen and nitrogen species may react and give secondary toxic metabolites. When NO and superoxide O_2^- react especially in equimolar concentrations, peroxynitrite ONOO- is formed, that is in pH-dependent equilibrium with peroxynitrous acid (ONOOH). Its homolysis gives highly reactive OH^- molecule. The evidence of peroxynitrite formation during CP-induced HP in rats gave Korkmaz et al. [35] who studied the influence of selective NOS inhibitor – aminoguanidine and peroxynitrite scavenger – ebselen on bladder damage. They revealed that ebselen administration gave similar results to those observed after aminoguanidine – both agents protected the bladder histologically against CP damage and decreased NOS induction [35]. Thus, the study by Korkmaz et al. [35] suggested that not only nitric oxide but also peroxynitrite is involved in the pathogenesis of CP induced cystitis. It is regarded that $ONOO^-$ together with OH^- augment tissue damages. Higher $ONOO^-$ and OH^- levels may result in unwanted oxidation and covalently modification of all major biomolecules, leading to destruction of many cellular constituents, with special attention to DNA cellular injury. Peroxynitrite oxidant modifies tyrosine in various proteins to nitrotyrosine and these nitration of structural proteins, including neurofilaments and actin, can disrupt filament assembly with major pathophysiological consequences [5,36]. Additionally, peroxynitrite causes increase in DNA strand breakage – that triggers the overactivity of polyadenosine-diphosphatase-ribose polymerase (PARP) – a DNA repairing enzyme. As a consequence, the depletion of oxidised nicotinamide-adenine dinucleotide (NAD) and

adenosine triphosphate (ATP) predisposing to cellular necrosis takes place. The problem of PARP activation pathway and its involvement in the pathogenesis of CP-induced bladder ulceration was investigated again by Korkmaz et al. in another study [33]. They compared the influence of selective NOS inhibitor (labelled as 1400W), peroxynitrite scavenger (ebselen) and PARP inhibitor (3-aminobenzamide) on the morphological bladder estimation after experimental HC development with CP. Both selective NOS inhibitor and ebselen produced lesser macroscopic hemorrhage, oedema, inflammation and ulceration together with decreased bladder NOS activation, as compared to those changes observed in pure CP-induced HC model. These beneficial effects were also noted after PARP inhibitor, which suggests that PARP activation involves pathogenesis of CP-induced bladder impaired uroepithelial cellular integrity resulting in ulceration [33].

The significance of the pathophysiological changes caused by these oxidants has served as the basis for the "devile triangle" theory ($NO - O_2^- - ONOO^-$). On the other hand, there are intracellular antioxidant defence mechanisms against ROS. They consist in superoxide dismutase (SOD) – converting radical superoxide to H_2O_2, catalase (CAT) and glutathione peroxidase (GSH-Px), which inactivate H_2O_2. Under physiological conditions, oxidants and antioxidant defense mechanisms maintain a redox equilibrium. An excessive ROS production and increased NOS activity (both evoked in bladder by acrolein) disturb the normal equilibrium and NO excess can outcompete SOD for O_2^-, secondarily resulting in a peroxynitrite formation, with subsequent protein and lipids oxidation, DNA damage, PARP activation and finally cellular energy crisis, which has been already mentioned above [36].

Several cytokines and other regulatory proteins are also implicated in the pathogenesis of oxazaphosphorines – induced hemorrhagic cystitis. Among them, nuclear factor κB (NF-κB) plays the pivotal role. This factor normally resides in the cytoplasm as a connection with inhibitory protein (inhibitor-κB; I-κB). Several agents, including ROS and proinflammatory cytokine – tumour necrosis factor α (TNF-α) or interleukin -1 (Il-1) degrade I-κB that allow NF-κB enter the nucleus and derepress transcription of genes associated with a variety of stress conditions. In the course of oxazaphosphorines-induced HC, acrolein itself together with ROS and proinflammatory cytokines mentioned above activate NF-κB dependent pathways finally resulting in exacerbation and intensification of the harmful bladder effects of acrolein [36]. The involvement of TNF-α and Il-1 in CP-induced HC in mice was studied by Gomes et al. [23]. They demonstrated that administration of anti TNF-α serum and anti Il-1 one diminished mucosal erosion, hemorrhage, edema, leukocyte migration and ulceration when compared to control. These results suggest that these proinflammatory cytokines are crucial mediators involved in inflammatory events occurring in CP-induced hemorrhagic cystitis [23]. The similar conclusions obtained Ribeiro et al. [54] who repeated Gomes et al. experiment but using ifosfamide-induced HC model and estimating NOS reactivity in bladder epithelial cells. The pretreated with anti TNF-α and anti Il-1 serums groups displayed inhibited histological abnormalities that were evoked by ifosfamide. Moreover, they observed similar improvement in mice that were administered thalidomide (TNF-α synthesis inhibitor) or pentoxyfilline (Il-1β synthesis inhibitor). These treatments also inhibited the NOS expression in the urothelium. Thus, they concluded that the urothelial damage observed in ifosfamide induced HC is due to NOS increased activity that appears to be dependent on TNF-α and Il-1 action [54].

In summary, according to Korkmaz, Topal and Oter [36], the most likely pathomechanism of oxazaphosphorines (more exactly: acrolein-induced) hemorrhagic cystitis, can be described using several details. In the first step, acrolein enters into the uroepithelium and initiates its injury by itself, that is deepened by acrolein-evoked both ROS and NO overproduction. Moreover, acrolein is responsible for further uroepithelial damage intensification by some intracellular factors such as NF-κB and AP-1 inducement. These compounds cause the overexpression of genes encoding proinflammatory cytokines (TNF-α and IL-1) and a further increase in the synthesis of ROS and NO. At a later stage, cytokines leave the uroepithelium and spread to detrusor smooth muscle and bloodstream. It seems that the key point in HC development plays peroxynitrite synthesis that is formed from ROS and NO. Peroxynitrite attracts cellular macromolecules (lipids, proteins, DNA), further exaggerates bladder damage. As a result, damage to the integrity of cells and tissues occurs, manifesting itself in the form of histological and morphological abnormalities, such as swelling, bleeding and ulcerations. Thus, our current knowledge about pathogenesis of oxazaphosphorines-induced hemorrhagic cystitis is evolved. There is no doubt that this is a complex inflammatory process with several cytokines, free radicals and non-radical molecules and that all these components of a pathophysiological description must be taken into consideration when looking for more effective chemopreventive compounds against CP/IF-induced cystitis [36].

Cytoprotection treatment as an adiuvant treatment to antineoplastic chemiotherapy. Current haemorrhagic cystitis chemoprevention

Any kind of antineoplastic chemiotherapy is associated with both mild or moderate and serious life-threatening side effects. They develop due to the inability of cytotoxic drugs to differentiate between their target – malignant cells and normal ones. Antineoplastic chemotherapeutics mostly affect fast-dividing cells, such as blood immature progenitors, the epithelial cells lining the mouth, stomach, intestines, respiratory and urinary tracts, skin and mucosal layers resulting in myelosuppression (anaemia, depression of the immune system, tendency to bleed easily), malnutrition, progressive body weight loss, hair loss, infertility. These antineoplastic agents also produce nausea and vomiting which is often reported by patients as an especially unpleasant and severe chemotherapeutic adverse effect. Moreover, damage of the specific organs may also occur, with resultant cardiotoxicity, hepatotoxicity, nephrotoxicity, ototoxicity or even encephalopathy [43].

Taking into consideration the inevitable cytostatics toxicity, numerous researches are being carried out to find methods to abolish, or at least ameliorate the toxicity of the antineoplastic chemiotherapy. General developments include

the synthesis of analogues of established cytotoxic agents with improved toxicity profiles of comparable antitumour efficacy, enhancement in control of chemiotherapy-associated emesis (5HT3 antagonists – setrons; in combinations with corticosteroids) or alternations in schedules of cytotoxic drug administration [42].

However, the challenge of cytoprotection became the most pharmacologically attractive method of diminishing the cytostatic agents toxicity. The theory of cytoprotection phenomenon during antineoplastic chemiotherapy is related to the design of chemoprotectants development. Chemoprotectants are defined as compounds providing tissue-specific cytoprotection, without compromising the desired antitumour efficacy and affecting the additional own toxicity that might jeopardise the effects of adequate chemotherapy [42].

Since most cytotoxic drugs are myelosuppressive, the bone marrow is a major target for chemoprotective agents and hematopoietic colony-stimulating factors, used to alleviate treatment-related myelosuppression and its consequences, may be considered as one of the first chemoprotectants to have been introduced. These agents entered a wide clinical practice enabling recovery of leukocytes. Erythropoietin use that escalates bone marrow erythrocytes restoration is a next example of alleviating of special toxicities [42,48].

However, reference books suggest that the term of "chemoprotectans" pertains to several compounds displaying their protective role in relation to various extra-haematologic tissues.

The first used agent administered as chemoprotectant was folinic acid (calcium folinate; leukovorin), designed to overcome methotrexate-associated toxicity. However, the meaningful advance in cytoprotection associated with cytostatics was the development of dexrazoxane use as a cardioprotector during the anthracycline cytotoxic antibiotics treatment. As it was mentioned above, anthracyclines (doxorubicin – adriamycin, daunorubicin, epirubicin) produce specific adverse affect, manifested as a chronic, cumulative dose-related toxic cardiomyopathy with cardiac insufficiency. Dexrazoxane is a cyclic derivative of EDTA acid that provides cardiac protection from anthracyclines primarily by means of a metal-chelating action (especially free iron and iron bound in anthracycline complexes), therefore preventing the formation of cardiotoxic reactive oxygen radicals [19]. Iron is regarded to be of central importance for radical formation because of its catalytic role for the synthesis of hydroxyl radicals by the – so called – Haber-Weiss reaction. The similar effect is observed after desferrioxamine and other iron chelators which are being currently investigated in terms of cancer chemiotherapy treatment (heterocyclic carboxaldehyde thiosemicarbazones, pyridoxal isonicotinoyl hydrazone, tachpyridine, O-trensox, desferrithiocin) [16]. However, in contrast to desferrioxamine, dexrazoxane is a more potent agent which is also able to upregulate transferrin receptor expression, leading to enhancement of the iron uptake into different cells and, therefore, to the removal of metabolically available iron from the circulation. Additionally, iron sequestration by macrophages has recently been detected as a powerful mechanism to reduce extracellular hydroxyl radicals synthesis and increased uptake of iron by macrophages upon exposure to dexrazoxane is the next element of the anthracyclines-induced cardiomyopathy preventing mechanism of this drug [66]. There are also reports that dexrazoxane exerts reno-protective effects in rats receiving anthracyclines, which in turn might suggest that this agent is also active in other tissues, apart from the heart ones [63].

Numerous studies have demonstrated that nucleophilic sulfur containing compounds such as glutathione can antagonise some harmful effects of the alkylating agents. At present, two agents in this class have drawn the attention of the scientists: amifostine and reduced glutathione. In contrast to dexrazoxane, amifostine is the first broad-spectrum chemoprotectant (bone marrow, peripheral nerve, heart and kidney) [42]. Amifostine was originally developed as radioprotective agent and was later on evaluated as a cytoprotective one against cis-platin [42,58]. Amifostine is dephosphorylated *in vivo* by alkaline phosphatase to the active metabolite, free form of thiol (labelled as WR-1065) which is further metabolised into a disulfide form (WR-33278). It has been demonstrated that amifostine reduces the incidence of xerostomia and oral mucositis which is a significant long-term repercussion arising in patients undergoing irridation of head and neck cancers [7,25]. Several studies revealed the cytoprotective role of amifostine against radiation-induced esophagitis and pneumonitis in the treatment of thoracic cancers as well as pelvic irradiation [37]. The amifostine cytoprotective mechanism is suspected to be complex and the selective wide protection of normal tissue is the result of a greater accumulation of WR-1065 in normal than in tumour cells (most neoplastic cells are characterised by a lesser alkaline phosphatase expression). Once inside the cells, the free thiol provides an alternative target to DNA, RNA and other macromolecules for ROS and WR-1065 scavenges free radicals (similar to metal-chelating chemoprotectants), however, oxidation of WR-1065 to WR-33278 is followed by a rapid consumption of oxygen and a transient cellular anoxia may be responsible for radioprotection [37,58]. Preclinical studies have revealed that the amifostine administration protected against a variety of antineoplastic chemiotherapy-associated toxicities, including cis-platin nephrotoxicity and neurotoxicity, cyclophosphamide and bleomicin – induced pulmonary toxicity and cytotoxicity evoked by doxorubicin [58]. The broad cytoprotective effects of amifostine seem beneficial and, therefore, the use of this chemoprotectant should be encouraged [37,42].

The second nucleophilic sulfur compound – glutathione is also being investigated in view of its potential use especially in cis-platin induced nephrotoxicity. However, its role in determining the cytoprotection remains controversial – extracellular glutathione is not normally taken up by cells except those expressing high level of γ-glutamyltranspeptidase activity. This compound is still in preclinical studies [42].

Another agent worth remembering is an analogue of adenocorticotropin hormone (ACTH) fragment – an agent labelled in experimental studies as ORG 2766 that has been shown to reduce peripheral nerve damage after cis-platin and paclitaxel (taxol) treatment. It is likely that ORG 2766 facilitates nerve repair, although the exact mechanism is still unclear. The confirmation of the clinical usefulness of such an effect has not yet been forthcoming [42,48].

The haemorrhagic cystitis, which is the adverse effect of oxazaphosphorines chemiotherapy, can also be prevented by co-administration of thiol-containing compound – mesna (sodium 2-mercaptoethane sulfonate). This agent is regarded to be a specific chemoprotectant against acrolein-induced bladder toxicity. After *per os* or *intravenous* administration and entering the circulation, mesna is rapidly oxidised into the inactive dimer dimesna that does not inhibit the antineoplastic action of oxazaphosphorines. Dimesna is then filtered in the kidneys and about 30-50% of glomerularly filtered dimesna undergoes tubular reabsorption and is reduced back to mesna in the renal tubular epithelium by glutathione reductase and thiol transferase. In such a form it is then delivered into the bladder, and the resulting free sulfhydryl groups of mesna can combine directly with the double acrolein bonds and with other oxazaphosphorine metabolites to create nontoxic compounds. As mesna activity is restricted to the urinary tract, the systemic action (and non-urological adverse effects of the oxazaphosphorines) is not affected and, therefore, it is possible to apply mesna and these alkylating agents simultaneously [21,42,59]. At present, mesna has been widely accepted as chemoprotectant with established clinical position in cyclophosphamide or ifosfamide induced HC. Moreover, it has been shown that it is as effective in bladder inflammatory alternations blockage as dexamethasone [47]. However, in about 20% of oxazaphosphorines treated patients receiving mesna and hydration as present standard against HC, the development of cystitis is observed. Hence, there are further attempts to introduce other methods of HC prevention. There are also reports that amifostine exerts uroprotective propriety in CP induced HC in rats, although the clinical data is still inconclusive [62]. An experimental study has also revealed that the combination of mesna and hyperbaric oxygen supplementation provides almost complete protection of HC, although it is not practical for the majority of patients undergoing antineoplastic chemiotherapy [34]. Thus, nowadays mesna still remains the common administered chemoprotective agent against HC during CP or IF treatment.

The summary of the chemoprotectants currently used as agents alleviating cytostatic toxicity is given in the table 1 below.

The possible future options for haemorrhagic cystitis chemoprevention

As it was shown in the second chapter of this work, haemorrhagic cystitis pathophysiology is now accepted as a non-microbial inflammation with the high level of ROS (peroxynitrite!) and proinflammatory cytokines and other proteins production. Thus, based on the HC pathogenesis, there are efforts of other chemopreventive agents development, targeted at inflammatory background mentioned above. Among them, numerous phytopharmacological derivatives are being studied, with flavonoids and polyphenoles, being of special interest. The rationales for the investigation of these compounds were the reports about their significant antioxidant and anti-inflammatory properties [51].

Flavonoids were shown to inhibit enzymes responsible for superoxide production (xanthine oxidase, protein kinase C) as well as other enzymes involved in ROS synthesis and inflammatory process initiation and maintenance: cyclooxygenase, lipooxygenase, microsomal monooxygenase, mitochondrial succinoxidase and NADH oxidase [15,27]. Some of them also diminish the activity of immunoglobulin proteins of cell adhesion molecule class (CAM), especially intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) as well as integrins (CD11b or MAC-1) and selectines (E-selectin and P-selectin) families of CAMs [61]. Moreover, flavonoids are regarded to be non-enzyme antioxidants reducing molecular damage by oxygen and nitrogen species. Beneficial role of the flavonoids has been confirmed in many various experimental models of inflammation, and, therefore, they are applied in many clinical inflammatory disturbances [57]. Moreover, apart from anti-inflammatory properties, flavonoids also exert hepatoprotective activity in experimental cirrhosis and antibiabetic one by means of insulin release stimulation. These compounds affect the cardiovascular system, preventing endothelial dysfunction by enhancing the vasorelaxant process, exerting the antiatherosclerotic effect (protecting LDL against oxidative stress) and antithrombotic one (flavonoids have been shown to be effective inhibitors of platelet adhesion and aggregation and because of their ability to maintain proper

Table 1. Currently used chemoprotectans alleviating cytostatic toxicity [48]

Drug	Mechanism of action	Toxic cytostatic agent	Protected tissue
Leukovorin	Folic acid supplementation	Methotrexate	Bone marrow
Dexrazoxane	Chelation ↑ plasma iron reuptake ↓ free radicals	Anthracyclines (doxorubicin and analogues)	Cardiac muscle Kidneys (?)
Amifostine	free radicals scavenger	Cis-platin Cyclophosphamide Bleomicin Doxorubicin	*the widest spectrum:* Kidneys Peripheral nerves Heart Lungs Bone marrow
Mesna	acrolein detoxification	Oxazaphosphorines – cyclophosphamide, ifosfamide	Urinary bladder
ORG 2766	nerve repairs facilitation (?)	Cis-platin Paclitaxel	Peripheral nerves

concentration of endothelial prostacycline and nitric oxide). To sum up, the therapeutical potential of flavonoids is fairly obvious and it should be expected that these agents might be applied in bladder inflammatory disturbances [64].

There are two flavonoids being currently evaluated in cyclophosphamide-induced toxicity in rats: morin and ternatin. Morin is a pentahydroxyflavone derivative which is isolated from the *Moracae* family (ex. *Chlorophora tinctoria* and *Prunus dulcis*). This flavonoid is reported to have marked cytoprotective properties against oxidative stress by means of its antioxidative and anti-inflammatory abilities that may stem from not only its scavenging activity, but also the anti-NF-κB pathways activation [32]. Morin was also proven to protect cells against radiation-induced oxidative stress, causing reduced Bax, phospho Bcl-2 and active caspases expression together with exertion of anti-apoptotic effects [70]. Based on these data, it seems that morin anti-inflammatory and antioxidative properties may have potential therapeutic application in the treatment and prevention of ROS-induced inflammatory processes, including HC. In one study, this compound partly improved haematological parameters and restored to normal the plasma protein level values in CP treated rats. However, the morin impact on bladder motility and morphology has not yet been studied and, thus, it is preliminary to predict its role in HC chemoprevention and it is necessary to further clarify its potential application [39].

The other flavonoid initially investigated as a HC chemoprotectant is ternatin, which is isolated from *Egletes viscosa (Compositae)*. This compound is attributed to possess antiproliferative effect together with anti-inflammatory, and antithrombotic properties [53]. The interesting ternatin feature was the revealing in experimental research that it inhibits fat accumulation and reduces fat mass by affecting differentiation stages of adipocyes and decreasing lipogenic enzyme activity. Consistent with these findings, ternatin was demonstrated to diminish triglyceride synthesis, and hence, it might provide a new therapeutic approach to metabolic disorders treatment [29]. Regarding ternatin applicability and haemorrhagic cystitis, some very interesting results have been reported by Vieira et al. [65]. Their excellent study revealed that ternatin, when combined with classical HC chemoprotectant – mesna, could ensure both CP and IF – induced HC. These researchers compared the effects after the ternatin replacement of 1 or 2 doses of mesna to the threefold mesna administration in oxazaphosphorine-induced bladder damage. They found evidence indicating that a replacement of 2 doses of mesna with ternatin was even more efficient in preventing CP-induced HC than the treatment with 3 doses of mesna and as efficient as 3 mesna doses in the prevention of IF-induced HC. This could be explained in light of the anti-inflammatory ternatin properties. Moreover, what was also interesting, is that replacing all mesna doses with this flavonoid did not prevent both CP and IF – induced HC, suggesting that mesna seems to be necessary for initial uroprotection via its neutralising effect on urothelial damage initiated by acrolein, while ternatin seemed to inhibit the inflammatory mediators that follow the acrolein action [65]. In our opinion, the Vieira et al. work [65] strongly supports the potential flavonoid clinical applicability as adjuvant chemoprotective agents in HC after oxazaphosphorines.

The other phytopharmacological derivative that has also been the subject of considerable attention in view of its varied therapeutic potential is a class of constituents together known as withanolides (steroidal lactones with ergostane skeleton). These agents were isolated from *Withania somnifera (Solanacae)*, popularly known as Ashwagandha or Winter Cherry. Since the withanolides have a structural resemblance to the active compounds present in the plant *Panax ginseng* known as ginsenosides, *Withania somnifera* is also named as "Indian Ginseng". The withanolides group consists of alcaloids (withanine, somniferine, somnine, somniferinine, withananine, tropine, choline, cuscohygrine, isopelletierine, anaferine, anahydrine and others), glycowithanoloids (sitoindoside IX and X) and other sitoindosides. Withaferin A was one of the first isolated member of withanolides family. Considered in general terms, multiple biological properties of withanolides have been described – anti-inflammatory, immunomodulatory, antineoplastic, antistress and adaptogenic; they also exerted some cardiovascular effects [38,68]. The antioxidant activity of *Withania somnifera* active principles was displayed as similar to the one demonstrated by flavonoids. Withanolides (especially withaferin A and sitoindosides VII-X) modulate the oxidative stress, significantly reducing the lipid peroxidation while increasing the antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase) activity, thus possessing free radicals scavenging properties. Withanolides have also been found to inhibit complement activity, lymphocyte proliferation and delayed-type hypersensitivity. These findings may explain at least in part the reported anti-inflammatory, immunomodulatory, antistress and anti-aging effects produced by these compounds in experimental studies and observed in clinical conditions [11,30,45]. These features are also closely connected to possible antineoplastic withanolides activity. These compounds may mitigate unregulated cell growth and proliferation via the potent tumour suppressor gene p53 by sustaining its control activity, affecting NF-κB apoptic pathways and tumour neoangiogenesis inhibition [46,67]. Withanolides are believed to strongly affect the functioning of the central nervous system (CNS), displaying anxiolitic and antidepressant actions in rats. These constituents influence the cholinergic and GABA-ergic neurotransmission in the cortical and basal forebrain, accounting for various central nervous system related disorders. Thus, withanolides are being studied as potential agents in epilepsy and anxiety treatment. Moreover, withanolides displayed inhibitory potential against butyrocholinesterase and acetylocholinesterase and enhance the cortical muscarinic acetylocholine receptor capacity, and thereby, are suspected to be potentially useful in Alzheimer disease [17,38,45]. These mechanisms may also constitute the rationale for the use of withanolides use in senile dementias as nootropic, cognition and memory-improving agents. There are reports that extracts of *Withania somnifera* had the ability to induce neurite extension and causing the neuritic regeneration with synaptic reconstruction, thus probably being potential agents valuable in neurodegenerative disorders, such as in Parkinson disease [38,45]. Apart from CNS effects, withanolides were experimentally demonstrated to show antihyperglycemic and antidyslipidemic properties [45] while in excretory and cardiopulmonary system prolonged hypotensive, respiratory-stimulant [45] and diuretic action [6] was observed.

Withanolides were also studied in cyclophosphamide-induced urotoxicity rats by Davis et al. [20] Their research

demonstrated that administration of *Withania somnifera* extract along with cyclophosphamide could normalise bladder morphological pathology. CP treated rats, after 24 hours of CP application, showed severe bladder inflammatory changes with uroepithelium completely replaced by metaphasic squamos epithelium with high mitotic activity, whereas, 48 hours after CP administration the lining epithelium was completely replaced by necrotic cells and numerous inflammatory cells. In the presence of Withania compounds bladder histology was normalised, showing almost normal architecture. Moreover, blood urea nitrogen level was significantly reduced in Withania extract-treated group while the glutathione content both in bladder and liver was enhanced. Therefore, according to Davis et al. [20] morphological, histopathological and biochemical analysis showed that *Withania somnifera* could alleviate the urotoxicity induced by cyclophosphamide and further that withanolides seem to be promising chemoprotectant agents.

Several other natural compounds and herbal extracts have been investigated as modulators of oxazaphosphorines urotoxicity. One of them is *Juglans regia (Juglandacae)*. The walnut bark has been widely used in folk medicine for treatment of venous insufficiency and haemorrhoidal symptomatology and for its antidiarrheic, astringent and antihelmintic properties. This plant was also shown to have antioxidant, antiproliferative, blood purifying, and depurative activities by exhibiting high phenolic content [2,49,52]. This plant is considered to be a source of many phytochemicals also decreasing the risk of oxidative stress and macromolecular oxidation, such as: juglone (naphtoquinone derivative), hydroxycinnamic acids (3-caffeoylquinic, 3-p-coumaroylquinic, 4-p-coumaroylquinic) and flavonoids (quercetin, kaempferol) [2,52]. The walnut extract and its immunomulatory effect have also been studied in CP-induced HC in mice. This treatment resulted in protective restoration of decreased antioxidants in CP-treated animals and lowered the lipid peroxidation in the bladder, thus the promising activity of this plant warrants possible clinical investigations [10].

The next plant studied in bladder toxicity is *Trigonella foenum-graecum (Leguminosae)*. This plant has already been extensively evaluated as a source of antibiabetic and antihyperlipidemic compounds. One of the active constituent of *Trigonella foenum-graecum (Fenugreek)* is an aminoacid – 4-hydroxyisoleucine, which has been shown to exhibit insulinotropic activity related to the immolation of the glucose concentration. Thus, it might be considered as a novel secretagogue with a potential interest for the treatment of type II diabetes [4]. Moreover, this plant contains steroidal saponins, such as diosgenine and flavonoids (witexin and its derivatives). It has been found that Trigonella extract is characterised to have immunomodulatory property that might be an advantageous alternative to prevent oxazaphosphorine-induced HC. Bhatia et al. [9] examined restorative effect of Trigonella extract on reduced glutathione (GSH), antioxidant enzymes activity and lipid peroxidation level in CP-treated mice, additionally exposed to a GSH reducing agent – L-buthionine-sulfoximine (BSO) which inhibits γ-glutamylcysteine synthetase. The researchers revealed that Fenugreek extract pretreatment not only showed protective effect on CP urotoxicity but that it was also effective in protecting the animals treated with the CP + BSO combination, with depleted GSH and other antioxidants restoration and bladder lipid peroxidation reduction. Thus, anti-inflammatory and immunomodulatory herbal extract like Fenugreek holds again great promise in the reducing the adverse effects in cyclophosphamide treated patients [9].

Other interesting plant investigated for its possible applicability in urotoxicity amelioration is *Ipomoea obscura (Convolvulaceae)*. This plant has already been shown to have analgesic and antimicrobial features and is externally used in phytomedicine for pain relief, treatment of sunburns, burns, boils, insect bites and small wounds. Internally, it is suggested to be administered to cure stomach ulcers and in neoplastic tumours. Several indolizidine alkaloids were isolated from extract of Ipomoea: ipomine, ipalbidine, ipalbine and ipalbinium. This plant also contains muricatin and other lipooligosaccharides derivatives – so called resin glycosides [69]. The protective role of *Ipomoea obscura* against CP-induced uro- and nephrotoxicities in mice was investigated by Hamsa and Kuttan. [26]. The toxicities caused by cyclophosphamide were reversed by Ipomoea extract administration as evident from the decrease in blood urea nitrogen, serum creatinine levels, as well as an increase in body weight. Histopathological assessment of urinary bladder indicated that CP-induced tissue damage was significantly reduced in extract-treated animals. Moreover, the level of proinflammatory cytokine – TNF-α, which was elevated during CP administration, was significantly reduced by extract application. Thus, Hamsa and Kuttan study demonstrated that Ipomoea obscura can ameliorate CP-induced bladder and renal toxicities by modulating antioxidant status and proinflammatory cytokines levels [26].

Incidentally, it should be also mentioned, that naturally occurring sulphur compounds are also regarded to prevent bladder damage after oxazaphosphorines. S-allylcysteine – an organosulphur compound of garlic (*Allium sativum*; *Amaryllidaceae*) extract regulates the thiol status of the cells and scavenges free radicals. Because depletion of thiols along with an excessive oxidative stress are implicated in CP/IF-induced HC, S-allylcysteine is the another phytopharmaceutical agent with potential anti-urotoxicity action. Bhatia et al. study [8] revealed, that S-allylcysteine showed protection in bladder tissue histology and also improved the decreased activities of antioxidant enzymes in mice with CP-evoked HC. This compound increased GSH level. Although S-allylcysteine treatment did not ensure full recovery, the marked improvement in bladder histology and antioxidants suggest that it has a significant modulatory effect on CP-induced urotoxicity [8]. Similar results were obtained by Manesh and Kuttan, who studied diallyl sulphide and diallyl disulphide in the same experimental model [44]. Moreover, there are also reports that sulphur containing aminoacids – seleno L-methionine [3] and L-cysteine [55] administration in CP-induced animals resulted in GSH elevation. All these findings certainly deserve a further exploration involving both experimental and clinical conditions.

To sum up, there are lot of naturally occurring phytopharmacological agents which bring large hopes on the findings of new chemoprotectant agents providing the if not the complete reduction of oxazaphosphorines-evoked haemorrhagic cystitis. Some of them were mentioned in this mini-review and they are listed in the table 2 below.

Table 2. Phytopharmacological compounds studied as alleviating cytostatic toxicity agents

Source (plant family)	Phytopharmacological group	Major compounds with potential uroprotective activity	The major references number
Chlorophora tinctoria *Prunus dulcis* (Moracae) *Egletes viscosa* (Compositae)	flavonoids	morin ternatin	32,39 65
Withania somnifera (Solanacae)	withanolides	withaferin A sitoindosides VII–X	20
Juglans regia (Juglandacae)	phenoles flavonoids	juglone hydroxycinnamic acids quercetin, kaempferol	10
Trigonella foenum-graecum (Leguminosae)	steroidal saponins flavonoids	diosgenine witexin	9
Ipomoea obscura (Convolvulaceae)	indolizidine alkaloids lipooligosaccharides derivatives – resin glycosides	ipomine, ipalbidine, ipalbine, ipalbinium muricatin	26
Allium sativum (Amaryllidaceae)	organosulphur compounds	S-allylcysteine	8

Conclusions

The HC development is a serious adverse effect which considerably limits the use of alkylating antineoplastic agents from the oxazaphosphorine group. The HC pathogenesis is complex, induced by toxic influence of the acrolein on the uroepithelium, with the consequent cascade of cellular inflammatory response and releasing numerous proinflammatory and cytotoxic mediators, heightening bladder tissues injury.

The HC pathophysiology description progress allows for new anti-inflammatory chemopreventive agents searching, which are expected to affect the determinated pathophysiological elements mentioned above. At present, several studies are carried out with some plant derivatives, mainly flavonoids, polyphenoles, alkaloids and sulphur containing consistents.

The preliminary, promising results of HC alleviating activities of some phytopharmacological agents allow for the expectation, that several new compounds, co-applied during oxazaphosphorines therapy, will be introduced in close future to the group of cytoprotective agents (currently containing mainly mesna and amifostine). The modern chemoprotection will contribute to the better tolerance and efficacy of the oxazaphosphorines antineoplastic treatment.

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