ONCOLOGY

Effect of Mesna on Lethal Effect and Hematological Toxicity of Taxol and Vepeside in Mice

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Mesna, an SH-containing uroprotector, attenuates the lethal effect and hematological toxicity of vepeside and taxol, but did not reduce specific activities of the studied cytostatics in mice with transplanted tumors. This selective antitoxic effect of mesna towards vepeside and taxol allows to intensify the anticancer chemotherapy with these highly effective but extremely toxic cytostatics and to improve the efficiency of anticancer therapy.

Key Words: *vepeside; taxol; mesna; toxicity; modification*

One of the main approaches to development of drug therapy, including anticancer therapy, is optimization of the effects of the known drugs. The majority of cytostatics are highly toxic compounds, and their maximum therapeutic efficiency sometimes cannot be attained because of pronounced side effects; in some cases the treatment has to be discontinued despite pronounced tumor sensitivity. Therefore selective protection of normal tissues from the damaging action of cytostatics is a way to improve the results of anticancer therapy.

We investigated the antitoxic activity of mesna, a thiol preparation, representative of a large group of SH-containing antitoxic modifiers. Unlike many other thiols, the drug is not toxic in the studied doses and administration shedules and is therefore widely used for reducing the toxicity of oxasaphosphorines not only in adults but also in children [3,11]. This modifier is effective upon intravenous and subcutaneous injection and can be used orally, and therefore it can used in outpatient settings [7,8]. Anticarcinogenic [10, 14], antiteratogenic [12], and antigenotoxic [9] effects of mesna were reported. The drug demonstrates anticancer activity towards superficial bladder cancer [6] and *in vitro* cytotoxic activity towards some tumor cells [1].

Modern protocols of high-dose polychemotherapy in combination with ifosfamide, high doses of cyclophosphamide, and mesna include many anticancer drugs. The majority of authors reported the absence of oxazaphosphorine urotoxicity against the background of mesna treatment and moderate manifestations of toxicity of other anticancer drugs. We assumed that this effect was due to mesna injected for a long time, so that its effect overlapped the action of all cytostatics included in polychemotherapy protocols.

This hypothesis prompted us to evaluate the possible antitoxic effect of mesna towards anticancer drugs other than oxazaphosphorines. We investigated the effect of mesna on lethal effect and hematological toxicity of vepeside and taxol, two effective modern anticancer drugs, whose toxic effects strongly limit their adequate use.

MATERIALS AND METHODS

Experiments were performed on 2-4-month-old male CBA mice weighing 22-28 g (Stolbovaya Breeding Center, Russian Academy of Medical Sciences). Ani-

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mal groups (control, anticancer drug±modifier) consisted of animals differing by no more than 2 g body weight.

Anticancer drugs vepeside and taxol (Bristol-Myers Squibb) were injected intraperitoneally in a single dose. Mesna (Uromithexane, ASTA Medica, 200 mg/kg) was injected intraperitoneally 30 and 5 min before and 15 and 30 min after vepeside or taxol.

The drugs were dissolved in 0.9% NaCl immediately before injection (0.1 ml/mouse).

The lethal effect of anticancer drugs was evaluated by animal mortality and the mean life-span over 30 days after injection of highly toxic doses of vepeside and taxol. Hematological toxicity of the cytostatics was evaluated by the number of leukocytes in peripheral blood and bone marrow cells in various terms after injection of anticancer drugs. Blood was drawn from the caudal vein, leukocytes were counted routinely in a Goryaev chamber after erythrocyte lysis with 3% acetic acid. Bone marrow cells were washed out of the femur with 0.9% NaCl and counted in a Goryaev chamber.

The results were statistically processed by Fisher—Student test, the differences were considered significant at $p<0.05$.

RESULTS

The choice of the dose and schedule of mesna administration was based on the data of our previous studies with unithiol. This drug is similar to unithiol by chemical structure, but antitoxic effect of unithiol towards doxorubicin was observed only after repeated injections before and after cytostatic treatment [4]. Clinically confirmed antitoxic efficiency of mesna towards oxasaphosphorine urotoxicity was observed only after repeated injections of the modifier before and after cytostatics, and therefore we injected mesna 4 times intraperitoneally. LD_{50} of mesna in mice for single intraperitoneal injection is 2000 mg/kg [5]. Previous experiments showed that antitoxic activity of 200 mg/kg mesna towards the lethal effect of vepeside was more expressed than at lower doses of the modifier, and we therefore selected this dose of the drug.

Injection of mesna protected the animals from the lethal effects of 70 mg/kg vepeside and 20 mg/kg taxol (LD_{100}) (Fig. 1). The modifier almost 1.5 times prolonged animal life-span: from 9.8±0.6 to 14.3±0.8 days after injection of vepeside and from 4.3±0,5 to 6.0±0.0 days after injection of taxol. One more evidence of pronounced antitoxic effect of mesna is an essential decrease in animal mortality: 50 and 30% over 30 days after injections of vepeside and taxol in LD_{100} in combination with mesna, respectively.

The effect of mesna on hematological toxicity of vepeside was evaluated by the number of bone marrow cells. This parameter was chosen because in our previous experiments no clear-cut relationship between this type of toxicity and vepeside dose was found on the basis of peripheral blood leukocyte count. Moreover, in some experiments blood leukocyte count after high doses of vepeside surpassed that after lower doses. Visually the blood looked darker and it was difficult to take it into a melanger, presumably because of changed blood rheology (condensation) under the effect of the cytostatic.

The count of bone marrow cells decreased after injection of vepeside. This decrease was more pronounced after administration of a high dose of the cytostatic (Fig. 2). After injection of vepeside in a dose of 10 mg/kg the number of bone marrow cells was about 50% of that in intact animals and remained at this level for 2 days after treatment. On day 3 the cell count increased and approached the lower boun-

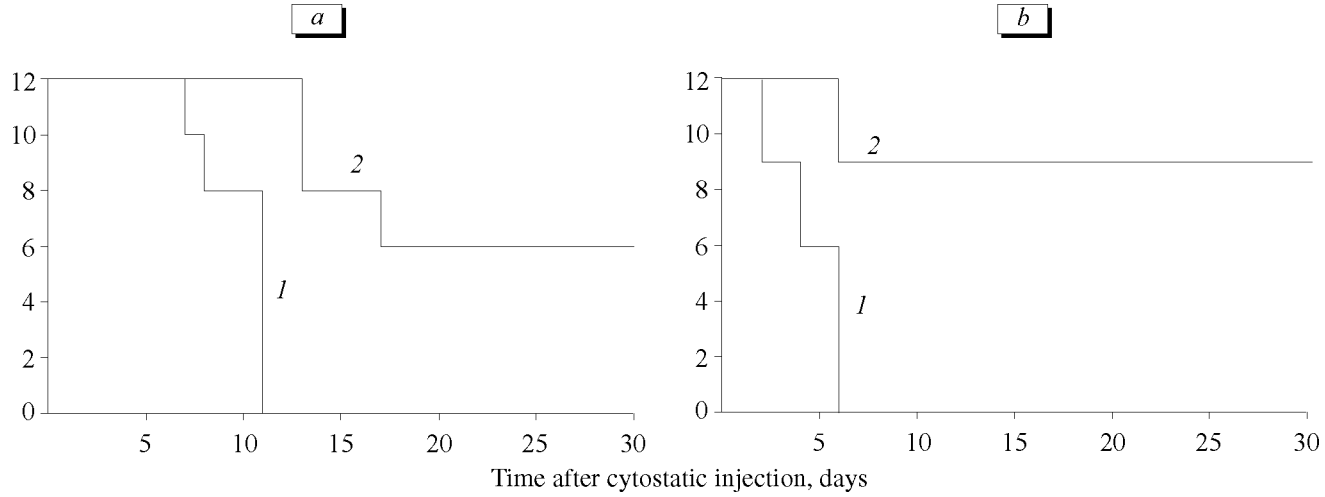

Fig. 1. Effect of mesna on mouse life-span after injections of vepeside (*a*) and taxol (*b*). Ordinates: number of survivors. *1*) cytostatic; *2*) cytostatic+mesna, all $p<0.05$ compared to mice receiving cytostatic without correction.

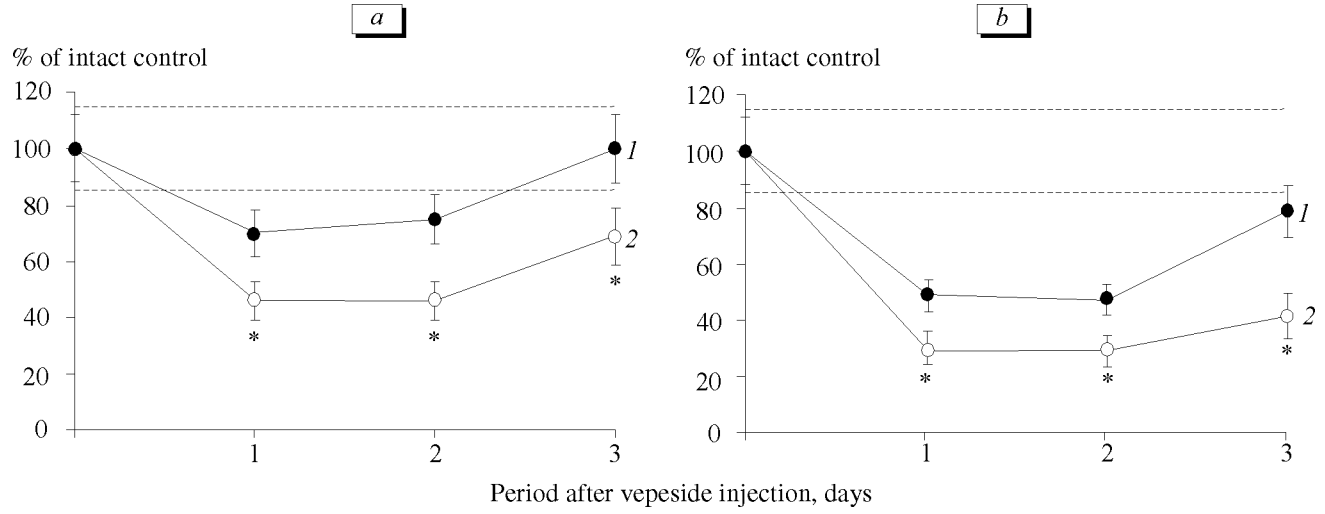

Fig. 2. Effect of mesna on bone marrow cell count in mice after injections of vepeside in doses of 10 (*a*) and 45 mg/kg (*b*). Ordinates: bone marrow cell count. *1*) vepeside; *2*) vepeside+mesna. Here and in Fig. 3: **p*<0.05 compared to mice receiving cytostatic without correction. Dotted line shows physiological fluctuations of this parameters in intact mice.

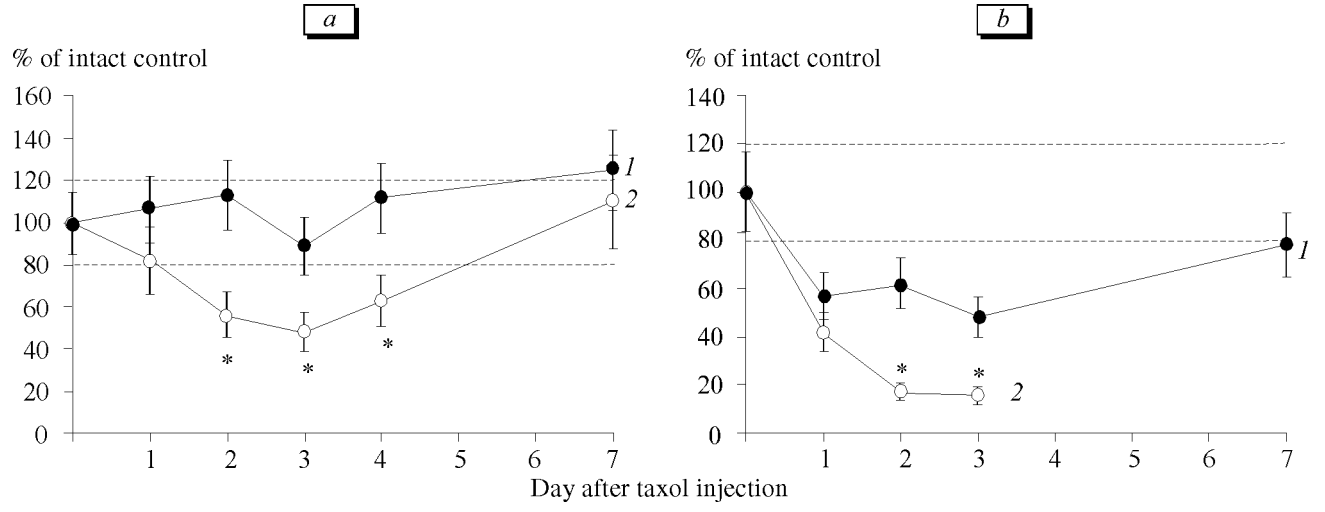

Fig. 3. Effect of mesna on leukocyte count in peripheral blood of mice after injections of taxol in doses of 15 (*a*) and 20 mg/kg (*b*). *1*) taxol; *2*) taxol+mesna. Ordinates: peripheral blood leukocyte count.

dary of the normal. After administration of a higher dose of vepeside (30 mg/kg) the bone marrow cell count decreased more drastically (to 70%) and remained at this level for at least 3 days. In both cases injections of the cytostatic in combination with mesna resulted in a less pronounced and shorter decrease in the number of bone marrow cells. On day 3 after injection vepeside in doses of 10 and 30 mg/kg in combination with mesna the count of bone marrow cells returned to normal (Fig. 2, *a*) or approached it (Fig. 2, *b*), respectively.

After injection of 15 mg/kg taxol, the leukocyte count decreased starting from day 2 postinjection and remained low for 3 days, the maximum decrease was about 50% (Fig. 3, *a*). Mesna abolished the hematotoxic effect of 15 mg/kg taxol: peripheral blood leukocyte count in mice receiving the cytostatic in a combination with the modifier was virtually normal at all terms. The antitoxic effect of mesna manifested after injection of a lethal dose of taxol (20 mg/kg). Progressive leukopenia appeared on day 1 after the cytostatic treatment, on days 2-3 the leukocyte count in the peripheral blood decreased by 80% compared to that in intact controls (Fig. 3, *b*). At later terms the leukocytes were not counted in this group, because 50% animals died on day 3 and 100% on day 4. Injection of taxol in this highly toxic dose against the background of mesna produced a less pronounced decrease in peripheral blood leukocyte count (no more than 50% *vs.* intact animals), and on day 7 after cytostatic treatment this parameter reached the lower boundary of normal.

The following mechanisms of the protective effect of mesna towards vepeside and taxol toxicity can be

hypothesized. It is known that mesna reduces oxasaphosphorine urotoxicity by decreasing degradation of their 4-hydroxymetabolites yielding toxic acrolein and/or by inactivation of acrolein in the reaction with mesna yielding stable nontoxic thioester. A similar metabolic antitoxic effect of mesna seems possible for vepeside and taxol. In animals and humans these drugs are oxidized by cytochrome P-450-dependent liver monooxigenases and form numerous reactive metabolites with different toxic and anticancer activities [13].

On the other hand, it is well known that apart from reactive cytostatic metabolites, peroxides, hydroperoxides, superoxide and hydroxide radicals formed during cytostatic action on cells contribute to toxicity. These highly toxic compounds can be inactivated by agents with antioxidant activity such as SH-containing compounds, *e.g.* mesna. This characteristic of the modifier is probably essential for the realization of the antitoxic effect of mesna towards vepeside and taxol.

Experiments on animals with transplanted tumors demonstrated that mesna did not modify the therapeutic effects of vepeside and taxol (data not presented). In other words, antitoxic effect of mesna for these cytostatics, similarly as for oxasaphosphorines, is selective for normal tissues, while the toxic effect on tumor cells (specific anticancer activity of cytostatics) remained unchanged when these drugs are administered in combination with mesna, which is in line with previous reports about selective protection of normal tissues by thiol compounds [4] and the absence of mesna effect on the transport of doxorubicin (anthracycline antibiotic) and its interaction with tumor cell DNA [2].

We should like to emphasize the significance of the detected antitoxic activity of mesna towards vepeside and taxol, because this will help to intensify the anticancer chemotherapy with these highly effective but extremely toxic cytostatics and to improve the efficiency of anticancer treatment.

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