PHARMACOLOGY AND TOXICOLOGY

Effect of Melatonin, Ascorbic Acid, and Succinic Acid on the Cumulative Toxic Effect of Repeated Treatment with Gammafos (Amifostine)

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Daily treatment of outbred albino mice with gammafos in radioprotective doses of 300 and 500 mg/kg for 4 days produced a cumulative toxic effect. This effect was not observed after decreasing the dose of gammafos to 100 mg/kg. Repeated peroral administration of melatonin and ascorbic acid in a dose of 200 mg/kg 30 min before treatment with gammafos reduced its cumulative toxic effect. Succinic acid in a dose of 100 mg/kg was ineffective under these conditions. The cumulative death time for 50% animals receiving gammafos alone or in combination with melatonin, ascorbic acid, and succinic acid was 3.08, 4.29, 4.06, and 2.97 days, respectively.

Key Words: *amifostine; melatonin; ascorbic acid; succinic acid; toxicity*

Extensive clinical trials are performed to evaluate the effectiveness of amifostine as a cytoprotective and radioprotective drug during chemotherapy and radiotherapy of patients with tumors [10]. Published data show that elimination of radioprotective drugs of the aminothiol family occurs (by their toxic effects) over a 2-fold longer period than realization of their radioprotective activity [1]. Repeated treatment with cysteamine and amifostine leads to cumulative toxic effect [3,12].

Ionizing radiation and chemotherapy can potentiate the cumulative toxic effect of aminothiols [1]. These procedures decrease the tolerance to medicinal drugs, reduce their protective activity [2], and affect the efficiency of therapy.

One of the approaches to prevent the cumulative toxic effect of chemotherapy in patients with tumors is the use of natural antioxidants ascorbic acid (AA), bioflavonoids, and selenium preparations that reduce cytotoxic activity of aminothiols [4,7].

Here we studied the effects of antioxidant compounds melatonin, AA, and succinic acid (SA) on the cumulative toxic effect of repeated daily treatment with gammafos (amifostine).

MATERIALS AND METHODS

Experiments were performed on 340 female outbred albino mice weighing 23-27 g. The animals were kept in a vivarium at normal temperature, fed mixed food, and received water from an automatic drinking bowl.

An aqueous solution of gammafos (amifostine, 0.25 ml) was injected intraperitoneally in daily doses of 100, 300, and 500 mg/kg for 4 days. AA (200 mg/kg), melatonin (200 mg/kg), or SA (100 mg/kg) were administered perorally through a tube (0.5 ml) 30 min before treatment with gammafos. The test preparations in specified doses produce the maximum antitoxic and

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antioxidant effect in mice [4,8,13]. Each group included 15 animals.

We studied acute toxicity of gammafos injected intraperitoneally in toxic doses of 600, 700, 800, 850, 1000, and 1200 mg/kg.

Toxic activity of gammafos was estimated by the mortality rate of animals over 4 days of poisoning and in the follow-up period (8 days). The cumulative toxic effect of gammafos was determined by the mean effective time (ET_{50}) to death during repeated treatment with the preparation. LD_{50}, ET_{50}, and confidence interval at p=0.05 were calculated by probit analysis (Litchfield test and Wilcoxon test).

Control mice received physiological saline. Correlation and regression analyses were performed to evaluate the dose- and time-dependence of the toxic effect produced by gammafos. The significance of differences between treated and control mice was estimated by Student's t test.

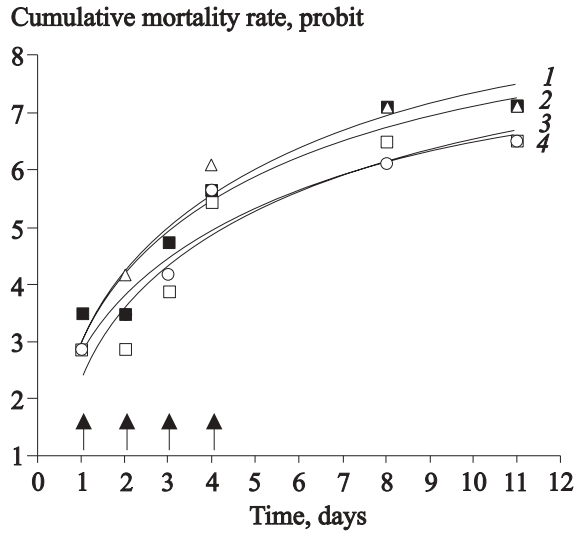

Fig. 1. Influence of melatonin, ascorbic acid, and succinic acid on the cumulative toxic effect of gammafos administered to mice in daily dose of 500 mg/kg for 4 days. Arrows: administration of gammafos. Succinic acid and gammafos (*1*), gammafos (*2*), melatonin and gammafos (*3*), and ascorbic acid and gammafos (*4*).

RESULTS

Acute toxicity of gammafos injected intraperitoneally to outbred albino mice was 701.5 mg/kg (LD_{50} 675.8-728.2 mg/kg). This value is within the limits of variation in mice of various strains [15]. Gammafos in single doses of 100, 300, and 500 mg/kg (325, 975, and 1625 mg/m^2) was well tolerated by mice. The mortality rate over 3 days after treatment was 0/80, 0/48, and 1/90, respectively.

Repeated administration of gammafos in daily doses of 100 and 300 mg/kg for 4 days did not cause toxic death of mice. However, repeated administration of gammafos in a daily dose of 500 mg/kg for 4 days was followed by toxic death of animals: 13.3, 40, and 73.3% animals died on days 2, 3, and 4 of treatment, respectively. The remaining animals died 4 days after the last administration of gammafos (Fig. 1).

The cumulative curve for the toxic mortality rate of gammafos-treated mice was described by a logarithmic function (Table 1). ET_{50} of gammafos estimated by its cumulative toxicity after administration in a daily dose of 500 mg/kg for 4 days was 3.08 days (2.63-3.60 days).

Peroral administration of AA and melatonin abolished the cumulative toxic effect of this radioprotective agent. AA and melatonin increased ET_{50} of gammafos by 1 day. This index was 4.06 (3.41-4.83 days) and 4.29 days (3.67-5.02 days), respectively. SA in a dose of 100 mg/kg was ineffective under these conditions (ET_{50} 2.97 days, 2.58-3.42 days).

When gammafos was administered repeatedly in a daily dose of 300 mg/kg for 4 days, the cumulative toxic effect was observed on day 5 after treatment with the preparation in toxic doses of 350-700 mg/kg (Fig. 2). LD_{50} of gammafos in these mice (565.3 mg/kg, 471.1-678.4 mg/kg) was much lower than in animals receiving physiological saline (763.8 mg/kg, 707.2-824.9 mg/kg, p<0.05, Fig. 2).

The acute toxic effect of aminothiols is related to the development of acute cerebral hypoxia. It results from the impairment of blood supply during acute hypotonia and cytotoxic action that alters tissue respiration. Acute death of mice was observed 22-125 min (mean 65.5±8.4 min) after intraperitoneal injection of gammafos in doses of 500-800 mg/kg. The

TABLE 1. Regression Equations for the Influence of Melatonin, AA, and SA on the Cumulative Toxic Effect of Gammafos (Changes in ET_{50}, p<0.01)

Group, n=15	Equation	m_y	m_a	m_b	r	ET_{50}
Gammafos	y=2.99+1.79 ln$x$	±0.51	±0.39	±0.26	0.96	3.08 (2.63-3.60)
AA+gammafos	y=2.71+1.63 ln$x$*	±0.42	±0.32	±0.21	0.97	4.06 (3.41-4.83)
Melatonin+gammafos	y=2.36+1.81 ln$x$*	±0.62	±0.47	±0.31	0.95	4.29 (3.67-5.02)$^+$
SA+gammafos	y=2.92+1.91 ln$x$	±0.38	±0.29	±0.19	0.98	2.97 (2.58-3.42)

Note. *p<0.05: pairwise t test. ^+p<0.05: probit analysis, compared to gammafos.

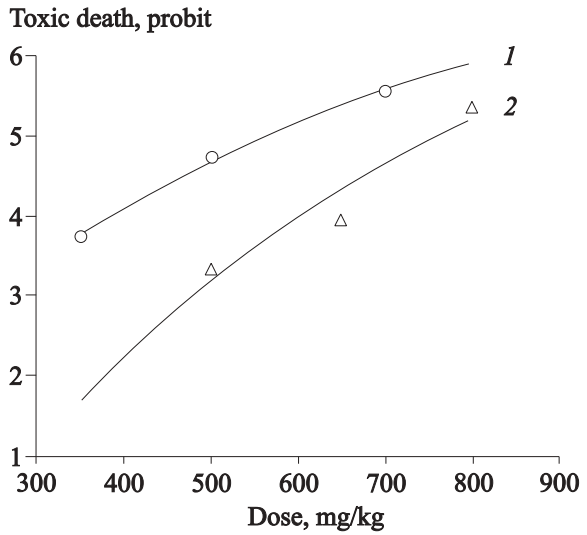

Fig. 2. Acute toxicity of repeated treatment with gammafos (*1*) and physiological saline (*2*) in a dose of 300 mg/kg for 4 days. Day 5 of the experiment.

mortality rate of treated and control animals over the first 5 h after administration of gammafos in toxic doses (measurement of LD_{50}) was 20.0 and 21.7%, respectively. We showed that 1-4 days after treatment with gammafos, the mortality rate of treated mice was 3-fold higher than that of control animals (33.3 and 9.3%, respectively, $p<0.05$). These results indicate that the development of cumulative toxic changes is not related to the acute toxic effect of gammafos. Most likely, they result from slowly developed toxic damage to the bone marrow and parenchymal organs (liver and kidneys).

The cumulative toxic effect of aminothiols was not observed after decreasing the dose of repeatedly administered gammafos to 100 mg/kg. Pretreatment with gammafos in a dose of 100 mg/kg for 4 days did not increase the sensitivity to toxic doses on day 5 of the experiment. LD_{50} of gammafos in treated mice and control animals receiving physiological saline was 782.3 (717.7-852.7 mg/kg) and 714.1 mg/kg (658.2-774.8 mg/kg), respectively.

The question arises: whether daily administration of amifostine possessing specific pharmacokinetic characteristics of radioprotective drugs can produce the cumulative effect of its metabolites? Experiments on mice showed that ET_{50} for elimination of cysteamine (product of amifostine conversion) and products of its degradation by labeled sulfur is 1.2 h [1]. This period is 1.5 times shorter than ET_{50} for elimination of the preparation estimated by its toxic effect. The radioprotective agent in active form was not detected in the urine of animals and humans.

The main toxic product of amifostine formed during oxidation with Cu^{2+}-dependent amine oxidase is H_2O_2 [9,11]. This highly reactive compound is not accumulated in the organism and initiate oxidation of organic substances. H_2O_2 produces damage to the kidneys, liver, and bone marrow, which is related to high concentration of amifostine in these organs.

The radio- and chemoprotective agent amifostine is repeatedly and daily administered during radiochemotherapy of patients with tumors. It is necessary to take into account that the cumulative toxic effect of this radioprotective drug can lead to the development of complications. For example, administration of amifostine in a daily dose of 300 mg/m^2 for 6 weeks increased the incidence of leukopenia in patients with inoperable cancer of the head receiving chemo- and radiotherapy (70.6 *vs*. 12.5% patients not receiving amifostine) [14].

Natural antioxidants melatonin and AA hold promise as medicinal preparations to reduce the cumulative toxic effect of amifostine. Toxicity of aminothiols is accompanied by generation of radicals in cells [9, 11]. Therefore, the use of antioxidants under these conditions has high pathogenetic significance. SA capable of reducing the severity of poisoning with ethyl alcohol or aspirin [5,6] was ineffective and did not abolish the toxic effect of amifostine.

REFERENCES

1. M. V. Vasin, B. I. Davydov, and V. V. Antipov, *Radiobiologiya*, 11, No. 4, 517-521 (1971).
2. M. V. Vasin, Yu. N. Chernov, and L. A. Semenova, *Ibid*., **31**, No. 2, 271-275 (1991).
3. B. I. Davydov, *Problemy Kosmicheskoi Meditsiny*, **14**, 137-158 (1971).
4. P. P. Saksonov, V. V. Antipov, and B. I. Davydov, *Problems of Space Medicine. Essays on Space Radiobiology* [in Russian], 1968, Vol. 9.
5. A. S. Saratikov, A. P. Bulatnikov, A. I. Vengerovskii, *et al*., *Eksper. Klin. Farmakol.*, **63**, No. 5, 56-58 (2000).
6. G. A. Sheveleva, V. G. Filimonov, V. A. Akin'shina, *et al*., *Ibid*., **59**, No. 6, 41-43 (1996).
7. A. Ganasoundari, *Mutat. Res*., **397**, No. 2, 303-312 (1998).
8. O. Gonchar, E. Klyuchko, M. Seredenko, and S. Oliynik, *Acta Physiol. Pharmacol. Bulg*., **27**, Nos. 2-3, 53-58 (2003).
9. K. D. Held, *Radiat. Res*., **139**, No. 3, 424-429 (1994).
10. M. Mabro, S. Faivre, and E. Raymond, *Drug. Saf*., **21**, No. 5, 367-387 (1999).
11. T. Meier and R. D. Issels, *Biochem. Pharmacol*., **50**, No. 4, 489-491 (1995).
12. F. A. Stewart and A. Rojas, *Br. J. Radiol*., **55**, No. 649, 42-47 (1982).
13. A. Teixeira, M. P. Morfim, C. A. de Cordova, *et al*., *J. Pineal Res*., **35**, No. 4, 262-268 (2003).
14. D. Trog, P. Bank, T. G. Wendt, *et al*., *Strahlenther. Onkol*., **175**, No. 9, 444-449 (1999).
15. J. M. Yuhas, *Radiat. Res*., **44**, 621-628 (1970).