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ORIGINAL ARTICLE

Acute toxicity and radioprotective effects of amifostine (WR-2721) or cystamine in single whole body fission neutrons irradiated rats

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Summary
The radioprotective substances amifostine (WR-2721) and cystamine were tested in rats following their parenteral administration (i.p., i.m., and i.v.). Cystamine is more toxic than amifostine in mice as well as in rats. Amifostine was less toxic after intravenous injection. The radioprotective effects of WR-2721 (160 $mg.kg^{-1}$) and cystamine (40 $mg.kg^{-1}$) were not significant when they were administered parenterally 15 – 20 mins before lethal doses of whole body fission neutron irradiation in the thermal column of reactor VVR-S and 30-days lethality served as an integral criterion of postradiation injury to the rat body. The fission neutrons spectrum was characterized by mean energy 0.9-1.0 MeV with 30-40% fluency participation of moderate (E=0.1 MeV) neutrons. The contamination with gamma rays was 22-30 %; the dose rate of whole irradiation was within 0.3 to 0.8 $Gy.min^{-1}$.

Keywords: amifostine – WR-2721 – cystamine – radioprotective effect – fission neutrons irradiation

INTRODUCTION

Both the radioprotective agents amifostine and cystamine, synthetized in Czechoslovakia, proved their radioprotective effects in whole body gamma irradiated mice and rats, when the radioprotective efficiency of both radioprotectors was evaluated using 30-days lethality and the comparison of lethal doses of irradiation in control and protected rats, administered intramuscularly 15 minutes before the

beginning of irradiation (Kuna and Krajčovič 1981, Kuna 1982, 1982a, 1983, 1983a, 1985, Kuna et al. 1983).

The reason for our further experiments were findings of the weak radioprotective effectiveness of sulphur containing radioprotectors administered parenterally, except amifostine (WR-2721), in mice exposed to whole body fission or fast neutron irradiations (Ferle-Vidovic et al. 1981, Kuna et al. 1988, Langendorff et al. 1971, Sedlmeier et al. l981, Sigdestad et al. 1976, 1986, 1992, Sverdlov 1974),. The aim of the experimental series was to discover if the tested agents, injected parenterally or given orally, are able to protect the animals of other species (rats, rabbits and dogs) against the initial neutron radiation injury, manifested as bone marrow or gastrointestinal syndromes.The degree of protective ability of amifostine and cystamine against single fission neutron irradiation in rats, evaluated by lethality studies, is presented in this paper.

The experiments were performed in the laboratories of the department of radiobiology of Purkyně Military Medical Academy (PMMA) in Hradec Králové. The animals were irradiated in the thermal column of the VVR-S reactor in the Institute of Nuclear Research in Řež near Prague.

Table 1. **The comparison of acute toxicity ($LD_{50/48\ h}$ in $mmol.kg^{-1}$) of WR-2721 and cystamine in mice following different methods of parenteral administration**

Administration	Sex	WR-2721	cystamine	WR2721/cystamine
intraperitoneal	male	3.688	1.267	2.911
Intraperitoneal	female	4.265	1.346	3.169
Intramuscular	female	4.171	1.674	2.492
Intravenous	female	6.186	1.024	6.041

Table 2. **The comparison of acute toxicity ($LD_{50/48\ h}$ in $mmol.kg^{-1}$) of WR-2721 and cystamine in rats following different methods of parenteral administration**

Administration	Sex	WR-2721	cystamine	WR2721/cystamine
intraperitoneal	male	–	0.637	–
intraperitoneal	female	2.162	0.657	3.391
intramuscular	female	2.096	0.624	3.359
intravenous	male	2.619	–	–
intravenous	female	–	0.788	(3.324)

METHODS

Animals. Adult male and female Wistar SPF rats with a body mass of 180–220 g were delivered from Velaz farm, Praha. The rats were housed 4–6 per cage with free access to a pelleted standard diet DOS-2b-St and to drinking water *ad libitum.*

Chemicals. Radioprotective agents amifostine,WR-2721 (S-2-(3-aminopropylamino)-ethyl-phosphorothioc acid, and cystamine (disulfide 2-mercaptoethylamine) dihydrochloride were synthetized by the chemical team of C. Krajčovič in VOZ 072 in Zemianské Kostoľany, Slovakia. For the synthesis of WR-2721 the procedure of Piper et. al. (1969) was utilized. The chemicals were disolved in saline (Imuna, Šarišské Michaľany, Slovakia). Solutions were prepared a few minutes before administration. Cystamine doses are given in the doses with a base of cystamine dihydrochoride salt.

Table 3. **Values of lethal doses of single whole body irradiated female rats by fission neutrons.** 20 min before irradiation, the rats received intramuscularly: saline, WR-2721 (200 $mg.kg^{-1}$) or cystamine (50 $mg.kg^{-1}$). Season: July – September, dose rate 0.320 $Gy.min^{-1}$.

Treatment	Irradiation (Gy)	Lethality	Lethal doses of fission neutrons (Gy) $LD_{5/30}$	$LD_{50/30}$	$LD_{95/30}$	DRF
Saline i.m.	1.5	0/15	2.14 (1.77–2.41)	3.34 (3.06–3.68)	5.23 (4.58–6.51)	1.00
	2	0/30				
	2.5	1/15				
	3.5	13/30				
	4	14/15				
	4.5	15/15				
	5	15/15				
WR-2721 i.m.	1.5	1/14	1.79 (1.20–1.94)	3.31 (3.01–3.89)	6.13 (5.60–10.48)	0.96 (0.84–1.09)
	2	3/30				
	2.5	0/15				
	3.5	12/30				
	4	12/15				
	4.5	15/15				
	5	15/15				
Cystamine i.m.	1.5	0/15	2.00 (1.63–2.27)	3.12 (2.95–3.52)	5.16 (4.52–6.43)	0.98 (0.81–1.18)
	2	3/30				
	2.5	3/15				
	3.5	15/30				
	4	13/15				
	4.5	15/15				
	5	15/15				

The acute toxicity of the protective substances WR-2721 and cystamine of Czechoslovak production were tested in rats after differential methods of parenteral (intraperitoneal, intramuscular or intravenous) administration. The lethality of non-irradiated rats was followed 48 hours after the tested protector injection. The values of $LD_{5/48\ h}$, $LD_{50/48\ h}$ and $LD_{95/48\ h}$ were calculated from the lethality data using the probit-logarithmic method (Roth et al. 1962).

Irradiation. Fission-spectrum neutrons were applied in the thermal column of the VVR-S reactor (USSR production) with mean energy 0.9–1.0 MeV with 30–40% fluency participation of moderate neutrons (E=0.1 MeV). The contamination with gamma rays was 22–30 %. The dose rate of the whole dose of irradiation was determined within 0.3 to 0.8 $Gy.min^{-1}$.

Table 4. **Values of lethal doses of single whole body irradiated female rats by fission neutrons**. 20 minutes before irradiation the rats received intravenously: saline, WR-2721 (160 mg.kg^{-1}) or cystamine (40 mg.kg^{-1}). Season: August-September, dose rate = 0.684 Gy.min^{-1}.

Treatment	Irradiation (Gy)	Lethality	Lethal doses od fission neutrons (Gy) $LD_{5/30}$	$LD_{50/30}$	$LD_{95/30}$	DRF
Saline	2.5	0/9	3.05	3.95	5.11	1.00
i.v.	3	0/10	(2.41–3.36)	(3.66–4.29)	(4.60–6.62)	
	3.5	3/10				
	4	3/10				
	4.5	9/10				
	5	10/10				
	5.5	5/5				
	6	5/5				
	6.5	5/5				
WR-2721	2.5	1/7	2.47	4.36	7.70	1.07
i.v.neutron	3	2/10	(1.37–3.00)	(3.82–5.58)	(5.87–18.94)	(0.90–1.29)
	3.5	0/10				
	4	2/9				
	4.5	4/10				
	5	9/10				
	5.5	5/5				
	6	5/5				
	6.5	5/5				
Cystamine	3	1/9	3.06	4.47	6.53	1.11
i.v.	3.5	1/10	(1.94–3.48)	(4.01–5.75)	(5.31–15.07)	(0.98–1.29)
	4	2/10				
	4.5	4/10				
	5	10/10				
	5.5	5/ 5				
	6	5/5				
	6.5	5/5				

Table 5. **The influence of WR-2721 (160 mg.kg^{-1}) or cystamine (40 mg.kg^{-1}) given intraperitoneally 15–20 min before single whole body fission neutrons irradiation (4 or 6 Gy) on mean survival time of female rats**. Season: June, dose rate 0.330 Gy.min^{-1}. Letter *a* indicates statistically significant difference from rats' group, which received saline only before irradiaton.

Treatment before Irradiation	Irradiation (Gy)	Mean survival time of irradiated rats (days)
Saline i.p.	4	12.1 (8.6 – 15.6)
WR-2721 i.p.	4	9.9 (6.8 – 13.1)
Cystamine i.p.	4	11.0 (7.9 – 14.1)
Saline i.p.	6	4.1 (3.9 – 4.3)
WR-2721 i.p.	6	4.7 (4.1 – 5.3)
Cystamine i.p.	6	5.2 (4.5 – 5.9)

The evaluation of the radioprotective effects of amifostine (WR-2721) or cystamine using 30-days postradiation lethality of irradiated animals. For the determination of lethal doses ($LD_{5/30}$, $LD_{50/30}$, $LD_{95/30}$) 5–7 rising doses of whole body exposure to fission neutrons were as a rule applied to different groups of rats, each containing the specified number of animals (see tables in the results section). Control rats in postradiation survival studies received the saline in the volume of 0.2–0.5 ml.100 g^{-1} of body weight. The doses of radioprotectors are mentioned in particular experiments. The intervals of from 15 to 20 minutes between the application of the protectors and the beginning of whole body irradiation were used in these experiments with the single whole body fission neutrons irradiation in the thermal column of reactor VVR-S. The number of animals per group and their sex , season of experiment (months) are mentioned in the results tables.The survival of exposed rats to radiation was followed up until the 30th day after irradiation.

From the lethality date, the lethal doses and relative radiation effect with 95% confidence limits were calculated by probit-logarithmic analysis (Roth et al. 1962) using the Hewlett-Packard 9830A calculator of the Laboratory of medical cybernetics of PMMA. The dose reduction factors (DRF) of parenterally applied radioprotectors for 30-days mortality are reverse values of the relative radiation effect in rats pretreated with protective agents.

RESULTS

The acute toxic effects of WR-2721 and cystamine of Czechoslovak origin after different kind of administration in non-irradiated mice and rats were described in many our experiments (Kuna 1985). Comparisons of the acute toxicity of WR-2721 and cystamine in mmol.kg^{-1} of the body weight in mice and rats are presented in tables 1 and 2.

From a comparison of lethal doses of WR-2721 and cystamine in non-irradiated mice and rats, it is clearly evident that cystamine is a more toxic agent than amifostine (WR-2721), when both protectors were administered parenterally. The best method of WR-2721 administration in mice is intravenous injection (tab. 1) as well as in rats (Table 2). I.v. application of cystamine was preferable in rats only.

Radioprotective effectiveness of WR-2721 and cystamine against single whole body irradiation of rats by fission neutrons in the thermal column of the reactor VVR-S.

The radioprotective ability of both protectors following i.m. and i.v. injections was compared in two experiments on female rats. The results are presented in the tables 3 and 4.

No significant radioprotective effect of WR-2721 and cystamine was observed in rats after single whole body irradiation by fission neutrons, when radioprotectors were administered parenterally (intramuscularly or intraveneously) and 30-days lethality served as an integral criterion of postradiation injury of the mammalian organism. The DRF values of 1.07 for amifostine and 1.11 for cystamine in the case of i.v. injections were not significant, but intravenous injection seems to be better in rats in comparison with i.m. administration.

In additional experiments on female rats, we followed mean survival times = MST (days) after single whole body fission neutron irradition following saline, WR-2721 and cystamine intraperitoneal administrations (table 5). The mean survival time of rats indirectly depends on the dose of fission neutrons. Following a whole body dose of 4 Gy MST the value of 12.1 days was attained, after 6 Gy, only 4.1 days. The duration of postradiation survival of rats was not significantly modified by the radioprotectors used; in the second experiment with a fission neutrons dose of 6 Gy, cystamine prolonged the survival time from 4.1 to 5.2 days.

DISCUSSION

The radioprotective effectiveness of WR-2721 and cystamine against single whole body gamma irradiation in rats was summarised in our monograph (Kuna 1985). Already the cystamine dose of 30 mg.kg^{-1} i.p. was effective (DRF = 1.16). As a rule, in rats the dose of 50 mg.kg^{-1} was administered intramuscularly in our experiments with DRF 1.18–1.39. This cystamine dose represents approximately 50 % of its mean toxic dose ($LD_{50/48\ h}$). The radioprotective doses of WR-2721, 160 and 200 mg.kg^{-1} for i.m. applications, represent 34.6 and 45.4% of the amifostine toxic value ($LD_{50/48\ h}$) for the same method of administration in non-irradiated rats. The DRF values of WR-2721 for the lethal doses of whole body ^{60}Co gamma irradiation (dose rate 0.74–0.34 Gy.min^{-1}), were estimated to be within 1.27–1.71 when the 30-days survival of exposed rats was observed..

Administration of chemical radioprotective drugs could be useful in two main circumstances (Pospíšil 1999). Firstly in clinical conditions in oncological radiotherapy with the aim of protecting normal tissues against the undesirable effects of irradiation and to elevate the radiation dose in

tumor cells. The best method of protector application may be chosen according to the individual situation. Repeated administration is without complications. The most recent recommendations for amifostine use in clinical oncology involve intravenous infusion only (Schuchter et al. 2002).

Secondly, an important occasion for radioprotector treatment is to manage the risk of whole body gamma-neutron or gamma only external irradiation in the case of nuclear weapons attack or nuclear power station accident. Fission weapons with a very small calibre of up to 1 kt, or neutron weapons, produce an initial gamma-neutron irradiation with the greatest portion (70 %) of energy released during the explosion and therefore are most suitable for terrorist attacks (Kuna et al. 2003). The duration of the radioprotective effect of amifostine after i.m. injection did not exceed 1 hour in whole body gamma irradiated rats (Kuna et al. 1983) We cannot assume long term increased radioresistance in humans after parenteral radioprotector administration and we may not have the opportunity to predict the time of a terrorist attack. Chemical radioprotection against gamma-neutron whole body irradiation with different type of physical shielding of the body has an important role in battlefield conditions, when the possibility of nuclear weapons attack is not excluded or even expected .

The most important use of chemical radioprotective drugs remains for the situation when we know that rescuers are going to help in an area with high levels of radiation dose rate following the sedimentation of radioactive materials after a nuclear weapons attack or after a nuclear power station accident. In such case the external gamma rays exposure is most significant. Internal contamination will contribute to external radiation injury, when personnel ingest, inhale, or are wounded by radioactive material.

Pharmacologists and patients prefer easy oral drug administration. Intramuscular . injection is also accepted using an autoinjector, when active molecules of the protector are inactivated in the stomach. Therefore such a practical easy application remains of great interest to us in our futher experiments on different mammals.

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