ORIGINAL ARTICLE

RADIOPROTECTIVE EFFECTS OF AMIFOSTINE (WR-2721) OR CYSTAMINE ON RADIATION DAMAGE AND ITS REPAIR IN RATS WHOLE BODY EXPOSED TO FISSION NEUTRONS

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Summary: Sulphur containing radioprotective drugs amifostine (gammaphos, WR-2721) or cystamine (disulfide of meracaptoethylamine) of Czechoslovak production were examined in whole body fission neutrons irradiated rats in the thermal column of reactor VVR-S. Using the split-dose technic the first sublethal neutron dose in the range 1–2 Gy was followed by second lethal exposures in the two time intervals (3 or 6 days) using whole body fission neutrons irradiations (3 days interval) or whole body γ-irradiations (6 days interval) for $LD_{50/30}$ evaluation within next 30 days survival observation. In other experiments the mean survival time (MST) in days was estimated in different rats group, when animals were whole body fission neutrons irradiated twice with 3-days interval using the total lethal doses of 4 or 5 Gy. Protected rats received amifostine (160 $mg.kg^{-1}$ i.p. and 200 $mg.kg^{-1}$ i.m.) or cystamine (40 $mg.kg^{-1}$ i.p. and 50 $mg.kg^{-1}$ i.m.), control rats obtained saline 20 min before beginning of irradiation in the amount of 0.5 ml.100 g^{-1} of the rat·s body weight. Non-significant DRF value 1.13 for WR-2721 i.p. was calculated in survival studies in rats twice neutron irradiated with 3 days interval (DRF 1.04 for cystamine). Chemical protectors were administered before each neutron exposure. MST of twice neutron lethal iradiated rats was prolonged not regularly by radioprotectors tested. WR-2721 and cystamine i.m. were not able to increase 6 days reparation processes after sublethal 2 Gy fission neutrons whole body irradiated rats.

***Key words**: Fission neutrons; Radioprotective drugs; Amifostine; WR-2721; Cystamine; Rats; Reparation of radiation injury*

Introduction

Amifostine (WR-2721) and cystamine administered parenterally were ineffective in our studies with single whole body fission neutrons irradiation of rats, when integral postradiation injury as 7 or mostly 30 days lethality and mean survival time of died animals served for the assessement of radioprotective effectivneess after the comparison of control non-protected and protected rats using probit analysis method (11).

In earlier experiments WR-2721 was effective in mice exposed to the same fission-spectrum neutrons (12). DRF was 1.26 (1.12–1.41) when amifostine was i.m. injected 20 min before irradiation in the dose 200 $mg.kg^{-1.}$ Increased dose of WR-2721 to 300 $mg.kg^{-1}$ elevated the DRF value to 1.48 (1.33–1.66).

Both chemical radioprotectors synthetized in Czechoslovakia occured clear radioprotective effect in single whole body gamma irradiated small laboratory rodents as mice (3–8) and rats (13,15). WR-2721 was effective in the protection of bone morrow in case of repeated exposures of mice to gamma rays, in different regimen of fractionation of irradiation with 6-hours interval for a total treatment 4 times. WR-2721 was given 30 min before each radiation fraction at a dose 200 $mg.kg^{-1}$ i.p. (24). 24-hours interval for repeated whole body gamma irradiation during 5 days and the doses of WR-2721 50 or 300 $mg.kg^{-1}$ used Petýrek et al. (16) with very good protective effect on bone marrow in mice.

The results about radioprotective effectiveness of cystamine and WR-2721 against neutron whole body irradiation in other mammals than mice are not accessible with exceptions. Our atempt for the explanation of lack of radioprotective efficiency of both chemical compounds in the case of fission neutrons whole body irradiation of rats was divided in two orientations: an analysis of initial radiation injury in control and protected animals and the evaluation of the ability of protectors to improve the repair postradiation processes. Split-dose technic, used in our earlier experiments (26), was the base for a judgement of reparative processes in presented experiments. Fission neutrons were produced in thermal column of the reactor VVR-S in Institute of nuclear research in Řež. Gamma irradiation from ^{60}Co source Chisotron and the laboratories of the department of radiobiology of Purkyně Military medical academy (PMMA) in Hradec Králové served for these experiments.

Methods

Animals. Male and female rats of Wistar strain (180–230 g) were delivered by the breeding centre Velaz, Prague. The animals were kept in propylen boxes, five rats in one, fed with standard pellet diet DOS-2b-St and supplied with drinking water *ad libitum*. The animals were adapted on vivarium conditions during 10–14 days.

Irradiation. The rats were irradiated by gamma rays from ^{60}Co source Chisotron (Chirana). The distance between the source and the middle of the animal was 100 cm. Exposure was measured in roentgens by FRT clinical dosimeter "Otto Schön" No. 27012 in the air. The dose in Gy was calculated according to formula 1 Gy=95.7 R. Fission neutrons were produced and applied in thermal column of the reactor VVR-S (USSR) 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 whole irradiation was determined on 0.453 Gy.min^{-1}. Each rat was irradiated in own propylen cell, which was fixed on rotating panel, 15 rats in individual boxes for one radiation procedure. The rate of 4 cycles per 1 min was used. This turning was necessary for relatively uniform fission neutrons irradiation of the rat body.

Two irradiations with 3 days interval were applied for the evaluation of reparative ability of irradiated rats following the first exposure to fission neutrons.

In the initial experimental study the animals were treated i.p. with saline (control group) or with protectors (experimental groups) before the first non-lethal standard dose of 1 Gy of fission neutrons. After 3 days the same animals were irradiated using the increased doses of fission neutrons (1, 2 and 3.5 Gy) following the same i.p. pretreatment by saline, WR-2721 (160 mg.kg^{-1}) or cystamine (40 mg.kg^{-1}) and the lethal doses ($LD_{5/30}$, $LD_{50/30,}$ $LD_{95/30}$) of both irradiations together were estimated by probit-logarithmic method. In the case of higher reparation after the first irradiation injury of rat organism, the higher dose of second irradiation for the equieffective injury was presumed.

In the second experiment the 3 days interval between the first and the second fission neutron irradiations was used too. The first dose was 1 or 2 Gy, the second one 2–4 Gy and mean survival time (MST) in days served as criterion of lethal radiation injury. Before each neutron irradiation the rats were pretreated i.m. by saline, WR-2721 (200 mg.kg^{-1}) or cystamine (50 mg.kg^{-1}) 20 min before beginning of irradiation.

In the last (3rd) experiment the first fission neutrons irradiation by standard 2 Gy dose was performed 20 minutes following i.m. administration of saline, WR-2721 (200 mg.kg^{-1}) or cystamine (50 mg.kg^{-1}). The second whole body gamma irradiation using increased doses (3–8 Gy) was given 6 days latter without any pretreatment and the lethal gamma rays doses were calculated for 7 or 30 days postirradiation intervals.

Lethality studies used 30-days survival observation. From the lethaliy data the lethal doses $LD_{5/30}$, $LD_{50/30}$ and $LD_{50/30}$ and relative radiation effect with 95% confidence limits were calculated by the probit-logarthmic method (18) in the Laboratory of medical cybernetics of PMMA. The radiation dose reduction factors (DRF) of parenterally injected radioprotective substances for 30-days postirradiation lethality are reverse values of the relative radiation effect in rats premedicated with tested protective agents (amifostine and cystamine).

Chemicals used. Radioprotective drugs amifostine, WR-2721, chemicaly S-2-(aminopropylamino)-ethyl-phosphorothioic acid and cystamine (disulfide-2-mercaptoethylamine) dihydrochloride were synthetized by C. Krajčovič (13). The doses of cystamine.2HCl are given in the the dose of cystamine base. The chemicals were dissolved in saline (Imuna, Šarišské Michaľany, Slovakia) and administered without pH modification.

Results

Twice administrations of radioprotective drugs before repeated exposures of rats to fission neutrons were tested in two experimental series. In the first experiment the rats were irradiated by the 1st standard sublethal dose of 1 Gy of fission neutrons and following 3 days by the second exposure with increased doses 1, 2 and 3.5 Gy of fission neutrons (Tab. 1). The radioprotectors or saline were injected intraperitoneally 20 min before each whole body irradiation in the same group of female rats. No significant radioprotective effects of amifostine (WR-2721) or cystamine were demonstrated in described irradiation conditions and repeated protectors i.p.administration, but calculated lethal doses were regularly higher in protected rats in comparison with control saline treated rats. The DRF values were calculated in rats for WR-2721 on 1.13, for cystamine on 1.04 respectively.

In the second experiment of this experimental serie the female rats were irradiated twice by fission neutrons with

Tab. 1: Values of lethal doses of twice whole body fission neutrons irradiations with 3 days interval. 20 min before each irradiation the same rats groups received i.p. saline, WR-2721 (160 $mg.kg^{-1}$) or cystamine (40 $mg.kg^{-1}$). Season: August-September, dose rate 0.453 $Gy.min^{-1}$. Numbers in brackets are 95% confidence limits.

Treatment before each irradiation	1st dose of fission neutrons (Gy)	2nd dose of fission neutrons (Gy)	Lethality	Lethal doses of both fission neutrons irradiations (Gy)			DRF
				$LD_{5/30}$	$LD_{50/30}$	$LD_{95/30}$	
Saline	1	1	0/10	2.24	**3.37**	5.05	1.00
i.p.	1	2	1/8	(1.42-	(2.82-	(3.98-	
	1	3.5	10/10	4.48)	4.48)	10.37)	
WR-2721	1	1	0/10	2.27	**3.96**	6.91	**1.13**
i.p.	1	2	0/9	(1.04-	(3.20-	(5.04-	(0.83-
	1	3.5	7/10	2.87)	5.86)	23.35)	1.54)
Cystamine	1	1	0/10	2.29	**3.52**	5.41	**1.04**
i.p.	1	2	0/10	(1.43-	(2.91-	(4.25-	(0.78-
	1	3.5	9/10	2.79)	4.61)	10.59)	1.37)

Tab. 2: Mean survival times of female rats irradiated twice by whole body fission neutrons exposures with 3 days interval.
20 min before each irradiation the same groups of rats received i.m. saline (S), WR-2721 (W) in the dose of 200 $mg.kg^{-1}$ and cystamine (C) in the dose of 50 $mg.kg^{-1}$. Season: August-September, dose rate 0.453 $Gy.min^{-1}$. Numbers in brackets are 95% confidence limits.Letter *a* indicates statistically significant difference from rats group received before irradiation saline only, letter *b* from rats group received before irradiation amifostine (WR-2721). Numbers in brackets are 95% confidence limits.

Scheme of experiment	Sum of the doses (Gy)	Mean survival time (days)
S 1 Gy + S3 Gy	4	**11.09** (9.09 – 13.09)
W 1 Gy + W 3 Gy	4	**15.09** (10.67 – 19.51)
C 1 Gy + C 3 Gy	4	**12.73** (9.12 – 16.33)
S 1 Gy + S4 Gy	5	**5.53** (4.70 – 6.37)
W 1 Gy + W 4 Gy	5	**7.50** (5.94 – 9.07) **a**
C 1 Gy + C 4 Gy	5	**7.93** (5.90 – 9.97) **a**
S 2 Gy + S2 Gy	4	**14.11** (10.37 – 17.91)
W 2 Gy + W 2 Gy	4	**15.50** (6.29 – 24.71)
C 2 Gy + C 2 Gy	4	**12.58** (9.79 – 15.38)
S 2 Gy + S3 Gy	5	**7.64** (5.34 – 9.95)
W 2 Gy + W 3 Gy	5	**5.20** (4.24 – 6.16) **a**
C 2 Gy + C 3 Gy	5	**9.00** (6.73 –11.27) **b**

Tab. 3: Values of lethal doses of whole body gamma irradiation for 30-days observation period in male rats, exposed 6 days following the first whole body fission neutrons irradiation in the dose of 2 Gy. 20 min before the 1st gamma-neutron irradiation the rats received in the group of 15–20 animals saline, WR-2721 (200 $mg.kg^{-1}$) or cystamine (50 $mg.kg^{-1}$) i.m. Season: July – September, dose rate 0.453 $Gy.min^{-1}$). Numbers in brackets are 95% confidence limits.

Treatment before 1st neutrons 2 Gy irradiation	The doses of 2nd γ-irradiation (Gy)	Lethality till 30th day/ /number of irradiated rats	Lethal doses of γ-irradiation (Gy)		
			$LD_{5/30}$	$LD_{50/30}$	$LD_{95/30}$
Saline	3	4/15	1.86	**3.86**	8.02
i.p.	5	12/15	(0.86-	(2.86-	(6.78-
	6.5	12/15	2.60)	4.53)	11.10
	7	27/30			
	7.5	15/15			
	8	15/15			
	9	15/15			
WR-2721	3	7/15	0.97	**3.21**	10.67
i.p.	5	11/15	(0.05-	(1.22-	(7.78-
	6.5	14/15	1.93)	4.22)	35.74)
	7	22/29			
	7.5	15/15			
	8	15/15			
	9	15/15			
Cystamine	3	10/15	0.86	**2.54**	7.47
i.p.	5	9/13	(0.03-	(0.69-	(5.77-
	6.5	14/15	1.76)	3.52)	17.43)
	7	28/28			
	7.5	15/15			
	8	13/13			
	9	15/15			

the same time interval of 3 days. The rats received 20 min before each exposures in VVR-S reactor intramuscularly saline, WR-2721 (200 mg.kg^{-1}) or cystamine (50 mg.kg^{-1}). Mean survival time of rats following the second fission neutrons exposure served for evaluation of postradiation injury of control and protected rats. Altogether the rats were irradiated by the lethal doses of 4 or 5 Gy (Tab. 2). MST was not regularly prologed using amifostine or cystamine before each neutron irradiation, but significantly. For statistical comparison of the duration of survival time interval in days in examined rats groups the tables of Genes (1) were used.

In the last (3rd) experiment for the evaluation of the reparation processes the male rats were used. Whole body fission neutrons irradiation was performed in the standard sublethal dose of 2 Gy with i.m. praetreatment by saline, WR-2721 (200 mg.kg^{-1}) or cystamine (50 mg.kg^{-1}), 20 min before the start of exposure. Following 6 days the rats were exposed without any treatment by increased doses of whole body gamma irradiation (3–8 Gy). The lethality of gamma irradiated rats were followed untill the 30th day after γ rays exposure (Tab. 3).

Lethality values after the 2nd γ-irradiation in grays estimated following 30-days observation excluded any positive influence of both tested protectors on 6-days reparation processes in rats exposed to 2 Gy of fission neutrons whole body irradiation. Similarly the calculation of lethal doses during 7-days observation period post the 2nd γ-rays exposure, which explaines dominantly the intestinal neutrons postradiation injury and its reparation (Tab. 4), had the same conclusion: no effect of radioprotective drugs tested was occured.

Tab. 4: Values of lethal doses of whole body gamma irradiation for 7-days observation period in male rats, exposed 6 days following the first whole body fission neutrons irradiation in the dose of 2 Gy. 20 min before the 1st gamma-neutrons irradiation the rats received the same amount of WR-2721 or cystamine i.m. as mentioned in the Tab. 3 (the same animals used for the calculation).

The doses of 2nd γ-irradiation (Gy)	Lethality till 7 day/number of irradiated rats	Lethal doses of γ-irradiation (Gy)		
		$LD_{5/30}$	$LD_{50/30}$	$LD_{95/30}$
3 5 7 9	0/15 4/15 5/15 8/15	2.91 (0.29- 4.28)	**8.60** (6.75- 22.04)	25.45 (13.80- 1296)
3 5 7 9	1/15 1/15 4/15 10/15	3.42 (1.50- 4.53)	**8.41** (6.77- 13.47)	20.66 (13.08- 93.71)
3 5 7 9	0/15 0/13 2/15 11/15	4.30 (2.56- 5.34)	**8.39** (6.99- 11.71)	16.36 (11.72- 41.82)

Discussion

According to results presented in our experiments on rats, the radioprotective substances amifostine and cystamine were not able to improve reparative processes of initial radiation damage induced by sublethal doses of fission neutrons, when 3 or 6 days intervals of reaparation were chosen. The rats received the first dose of fission neutrons in the range of 1 to 2 Gy of whole body exposure with or without protectors praetreatment. The values of $LD_{50/30}$ of single whole body fission neutrons irradiation in reactor VVR-S for non-protected rats were determined on 3.34 and 3.95 Gy and the doses 1.5 or 2 Gy did not cuased lethal effect within 30 day postradion period (11). Following 3 days the second increased doses of fission neutrons also with or without protective drugs premedication were applied. In non-protected saline control rats the value of $LD_{50/30}$ remained simillar as in single neutron irradiated rats – 3.37 Gy. The division of the sum doses of neutrons into two fractions had not favorable effect nor on radiopotective effects of WR-2721 or cystamine in twice neutron irradiated rats with 3 dyas interval. Lethal doses of gamma rays were apllied without radioprotectors praetreatment 6 days latter following fission neutrons whole body irradiation. Presented $LD_{50/30}$ value of whole body gamma irradiadet rats 3.86 Gy was lower about 50 % in comparison with $LD_{50/30}$ value in single whole body gamma irradiated rats. They were determined on 6.71, 7.49 and 8.44 Gy (15). This finding indicates great remained postneutrons injury, which chemical radioprotectors are not able to decrease.

In our experiments on rats this split-dose method was used for an evaluation of the radioprotective effect of cystamine and its combination with calcium gluconate on the reparative processes following head X-rays irradiation (26,27). The intervals 80 min, 1 day, 3 days and 7 days were chosen for reparation of the first head irradiation injury. The repair of the radiation damage towards the 1st day had an exponential course and on the 7th day more than 50 % of the initial damage was repaired. In rats whole body irradiated by fission neutrons Rudakov and Taciy (18) demonstrated the half time of reparation of neutron radiation injury on 11.2 ± 0.4 days. It was 1.5 times more in comparison with the reparation following X-irradiation. Sverdlov (23) introduced in mice $T_{1/2}$ of reparation on 2 or 6 days, when lethal doses ($LD_{50/30}$) used as criterion of neutrons injury. An explanation for such differences could be found in different kind of neutrons spectrum used and the greatness of the dose of the first irradiation, from sublethal to 2/3 of $LD_{50/30}$, which reparation is tested with next irradiation(s).

Cystamine was ineffective in the prevention of the development of the early abscopal effects of the mice head gamma irradiation with the dose of 20 Gy (9). Cystamine lessed certain changes and it especially speeded up the onset of the reparation of the dry matter of brain and also certain distant nonirradiated radiosensitive structure as leucocyte

count in peripheral blood, cellularity of the femur bone marrow and small inestine and the dry matter of salivary glands, spleen and testes. WR-2721 was more effective than cystamine in combination with the head shielding of the gamma lethal irradiated mice as in the case of whole body exposure (10).

The positive results with chemical radioprotection in neutron irradiated mice (2,19–23) we also prooved with WR-2721 (11).The analysis of early changes following whole body fission neutrons and an evaluation of radioprotective ability of WR-2721 and cystamine on these events as well as on the course of postradiation changes in radiosensitive tissues could explain worse effectiveness of sulphur-containing chemical radioprotective substances in neutron irradiated rats.

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