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Radiation induced peripheral blood changes in mice and its modification by MPG

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Summary

The effect of anti-radiation compound MPG (2-mercaptopropionyl glycine) on the peripheral blood has been studied in Swiss albino mice irradiated (1 and 3 Gy) at the age of two weeks. It was observed that 1.0 Gy did not produce any noticeable changes in the erythrocyte count and hemoglobin level in both drug treated and non drug treated groups. Whereas after 3.0 Gy exposure a significant depletion in both the parameters was noticed on 3 and 4 weeks old animals. As compared to normal total leucocyte and lymphocyte counts were depleted significantly in two weeks old animals in both the exposure groups. MPG alone did not produce any noticeable changes in different blood parameters of two weeks old animals after 6 hours exposure. After one week exposure MPG afforded significant elevation in different blood components of three weeks old animals and compared with control it caused earlier and faster recovery.

Key words: 2-mercaptopropionylglycine Erythrocyte, Lymphocyte, Leucocyte, Chemical protection

Резюме

Модификация вызванных облучением изменений периферической крови мышей под действием МПГ

Действие радиозащитного вещества МПГ (2-меркаптопропионилглицина) на периферическую кровь исследовалось на мышах-альбиносах швейцарской линии, которые в возрасте 2 недель подтвергались облучению с дозой 1 и 3 Гй. Доза в 1 Гй не вызывала никаких заметных изменений числа эритроцитов и уровня гемоглобина как в группе, получившей химическую противолучевую защиту, так и в группе животных, не получавших МПГ. После дозы в 3 Гй наступает, однако, значительное понижение обоих параметров у животных в возрасте 3—4 недель. Число лимфоцитов и число лейкоцитов в обеих облученных группах животных в возрасте 2 недель также лежат отчетливо ниже нормальных значений. МПГ сам по себе не не вызывал никаких заметных изменений названных параметров крови у двухнедельных животных после шестичасового облучения. После длящегося одну неделю облучения удалось достичь очевидного повышения различных составляющих крови у животных в возрасте трех недель, а также более быстрого и раннего восстановления, чем в контрольный группе.

Ключевые слова: 2-меркаптопропионилглицин, эритроциты, лимфоциты, лейкоциты, радиозащитные вещества.

Zusammenfassung

Modifikation strahleninduzierter Veränderungen des peripheren Blutes von Mäusen durch MPG

Die Wirkung des Strahlenschutzstoffes MPG (2-Mercaptopropionylglycine) auf das peripheré Blut wurde an Schweizer Albinomäusen studiert, die im Alter von 2 Wochen mit 1 und 3 Gy bestrahlt wurden. 1 Gy ergab keine merklichen Veränderungen von Erythrozytenzahl und Hämoglobinspiegel sowohl bei der chemisch behandelten als auch bei der nicht behandelten Gruppe.

Nach 3 Gy trat jedoch eine signifikante Abnahme beider Parameter bei den 3 und 4 Wochen alten Tieren auf. Die Lymphozyten- und Leukozytenzahlen lagen bei beiden bestrahlten Gruppen für 2 Wochen alte Tiere 6 h nach der Bestrahlung demgegenüber deutlich unter den Normalwerten. Eine Woche nach der Bestrahlung konnte durch MPG eine deutliche Anhebung der verschiedenen Blutbestandteile erreicht und damit eine schnellere und frühere Erholung gegenüber den unbehandelten Tieren beobachtet werden.

Schlüsselwörter: 2-Mercaptopropionylglycin, Erythrozyten, Lymphozyten, Leukozyten, Strahlenschutzstoffe

Introduction

More than three decades have passed since the detection of protective resulting chemical substances against the acute lethal effect of radiation in animals. The intensive research, however, has failed to produce any clinically applicable radio protector. *Uma Devi* and *Kumar* [14] reported that MPG (2-mercaptopropionyl glycine), a synthetic-SH compound, exerts protection against radiation induced depletion in erythrocyte count of adult Swiss albino mice, if given intraperitoneally as little as 20 mg/kg b.wt. before irradiation. This study prompted the authors to evaluate the role of this compound on the peripheral blood of developing mice irradiated at early post-natal age.

Material and methods

Two weeks old Swiss albino mice, maintained on standard mice feed and water *ad libitum*, were selected from an inbred colony. The animals were divided into two groups, experimental and control. The animals of experimental group were injected 20 mg/kg b.wt. of MPG, intraperitoneally (MPG dissolved in double distilled water and PH of the solution adjusted 6.4 with 1 N NaOH). The control group received an equal amount of double distilled water in the similar fashion. 15—30 min after in injection all the animals were exposed to 1.0 and 3.0 Gy of gamma rays from a Co-60 source. A minimum: of five animals were sacrificed at different post-irradiation times i.e. from week 2 (just 6 hours post-irradiation) to week 6. Peripheral blood studies, like total leukocyte, erythrocyte, lymphocyte counts and hemoglobin levels were determined by routine procedure after exposure.

Results and discussion

No mortality was observed in the 1.0 Gy exposure groups during the study whereas a few animals were died in the 3.0 Gy exposure groups during the study (6.66 % and 3.44 % in control and experimental groups respectively) (Table 1).

Table 1

Design of experiment and mortality during the study

Groups	Treatment	Mortality during the study (%)
Normal (N)	Sham-irradiation	—
Control (C)	Only irrad. (1.0 Gy)	—
Experimental (E)	MPG + irrad. (1.0 Gy)	—
Control (C)	Only irrad. (3.0 Gy)	6.66
Experimental (E)	MPG + irrad. (3.0 Gy)	3.44

Table 2

Total leucocyte (/cmm) and lymphocyte (%) changes in mouse peripheral blood after gamma radiation in presence or absence of MPG (mean ± SE)

Doses of irradiation	Groups	Age in weeks							
		2		3		4		6	
		TLC	Lym	TLC	Lym.	TLC	Lym.	TLC	Lym.
Sham-irradiation	N	2.880.00 ± 178.83	61.88 ± 2.33	2.824.44 ± 150.50	64.23 ± 3.33	2.622.22 ± 212.12	62.52 ± 4.25	3.125.25 ± 136.66	64.66 ± 2.42
1.0 Gy	C	2,388.88 ± 124.53	54.83 ± 2.44	2,325.25 ± 110.00	50.28 ± 3.02	2,466.66 ± 133.33	58.88 ± 2.58	3,075.50 ± 178.28	64.52 ± 3.58
	E	2,550.50 ± 110.10	56.45 ± 3.32	2,680.00 ± 140.00	57.66 ± 2.18	2,550.50 ± 142.42	60.92 ± 2.84	3,166.66 ± 172.22	65.12 ± 3.22
		N.S.	N.S.	P 0.02	P 0.05	N.S.	N.S.	N.S.	N.S.
3.0 Gy	C	2,250.20 ± 120.20	48.10 ± 3.42	1,850.50 ± 160.00	46.52 ± 3.12	2,400.00 ± 125.50	57.50 ± 2.50	2,933.33 ± 153.33	63.80 ± 3.26
	E	2,333.33 ± 142.42	51.12 ± 2.88	2,280.00 ± 120.00	54.60 ± 2.22	2,520.00 ± 140.00	60.52 ± 3.22	3,080.00 ± 130.00	64.72 ± 2.92
		N.S.	N.S.	P 0.05	P 0.05	N.S.	N.S.	N.S.	N.S.

C — only irradiation
E = Irradiation + MPG

Table 3

Erythrocyte count (× 106 cmm) and hemoglobin level (gm/100 ml) changes in mouse peripheral blood after gamma radiation in presence or absence of MPG

Doses of irradiation	Groups	Age in weeks							
		2		3		4		6	
		Ery.	Hb.	Ery.	Hb.	Ery.	Hb.	Ery.	Hb.
Sham-irradiation	N	8.70 ± 0.42	13.68 ± 0.48	8.58 ± 0.32	13.82 ± 0.61	9.52 ± 0.42	14.22 ± 0.48	10.92 ± 0.51	14.46 ± 0.57
	C	8.48 ± 0.32	13.42 ± 0.52	8.32 ± 0.31	13.12 ± 6.73	9.45 ± 0.53	13.78 ± 0.71	10.70 ± 0.62	14.20 ± 0.53
1.0 Gy	E	8.62 ± 0.37	13.66 ± 0.47	8.48 ± 0.39	13.76 ± 0.62	9.55 ± 0.48	14.21 ± 0.62	10.98 ± 0.41	14.38 ± 0.78
		N.S.							
	C	8.47 ± 0.42	13.20 ± 0.73	7.41 ± 0.28	11.25 ± 043	7.78 ± 0.35	11.25 ± 0.43	10.52 ± 0.37	13.76 ± 0.33
3.0 Gy	E	8.72 ± 0.44	13.52 ± 0.56	8.35 ± 0.37	11.66 ± 0.49	8.82 ± 0.37	12.98 ± 0.63	10.79 ± 0.24	14.35 ± 0.66
		N.S.	N.S.	0.05	0.05	P 0.05	P 0.05	N.S.	N.S.

C — Only irradiation
E — Irradiation + MPG

The total leucocyte and lymphocyte counts depleted significantly in two weeks old animals at the very first autopsy time i.e. 6 hours after exposure weeks. A significant depletion in values was also observed in 3 weeks old animals. Thereafter, recovery was evident in both exposure groups. Normal values regined earlier in MPG treated groups in comparison to control groups. A significant difference between control and experimental groups was observed in 3 weeks old animals (Table 2).

The present finding on the peripheral blood leucocyte and lymphocyte depletion after gamma radiation in two weeks old animals (6 hours after exposure) supports the earlier findings of *Spangler* and *Cassen* [13], who observed a rapid fall in the lymphocytes, with a minimum at 1 day. *Rubin* and *Cassarett* [11] also reported that 100 R X-irradiation may produce twenty five percent reduction in the lymphocyte counts after whole body exposure. In the present study a significant depletion in lymphocyte (77 % of normal) as well as in leucocyte counts (78 % of normal) was observed in two weeks old animals after 3.0 Gy gamma radiation. The depletion in counts in the present study was dose dependent. i.e. decrease was higher with the increase in radiation dose (Table 2). A sharp decline in leucocyte and lymphocyte counts 6 hours after exposure (two weeks old animals) may be due to the direct cell killing by radiation in the peripheral blood as suggested by *Kumar* [5]. *Bond* and co-workers [1] also reported that a number of blood cells are damaged severely after irradiation.

After 1.0 Gy gamma rays the erythrocyte and hemoglobin value did not change significantly in both control and experimental groups of all ages except a slight depletion in 4 weeks old animals. In 3.0 Gy exposure groups the values depleted significantly in 3 and 4 weeks old animals as compared to normal. A significant elevation in values was observed in 3 and 4 weeks old animals in comparison to their respective control animals (Table 3). The erythrocyte and hemoglobin values run parallel with each other i.e. changes were similar in nature.

In the present study the erythrocyte and hemoglobin level did not change significantly after 1.0 Gy gamma rays. *Goyal* et al. [3, 4] irradiated animals *in uterus* and reported that erythrocyte count decreased at early post-natal age with 50 and 150 R gamma rays. They also reported that the depression in counts was dose dependent. In the present study, erythrocyte count did not show any noticeable changes after 1.0 Gy gamma rays. This may be due to animals irradiated *in utero* are more sensitive to irradiation than two weeks old animals.

A significant depletion in erythrocyte count was observed on weeks 3 and 4. *Norris* et al. [10] noted that after exposure to gamma radiation erythrocyte counts in dogs reached a minimum level at day 22. Recently *Uma Devi* and *Kumar* [14] reported that the erythrocyte count depleted continuously after exposures and depletion was more pronounced with higher radiation doses. MPG-pretreatment was not able to bring about any significant changes in the blood picture of two weeks old animals i.a. after (6 hours) exposure but the values were slightly higher in MPG treated group in comparison to control. One week after-irradiation a significant elevation in various blood components was observed in the MPG protected animals as compared to unprotected ones. A significant elevation in the blood components may be due to the protective action of MPG on the blood forming organs, by which replenishment of peripheral blood is effected. Thus protection of the functional compartment is a consequence of the protection of the proliferative compartment as suggested by *Kumar* [5]. Protection of hematopoietic stemcells of bone marrow and thymus in adult mice has been reported earlier by *Saini* and *Uma Devi* [12] and *Kumar* et al. [6]. The mechanism by which the drug MPG protects the peripheral blood cells is not yet celarly understood. But following assumptions may be relevant in the present case: formation of mixed disulphide as suggested by *Modi* and *Revesz* or by forming a bridge between two consecutive phosphate groups on the DNA in cells of the back bone as reported by *Brouch* et al. [2] for cysteamine. Further the NPSH (non protein sulphydryl) released in the process of mixed disulphide formation may also did in

scavenging the free raideals produced by ionizing radiation. Autoradiographic studies indicate that the drug inhibits mitosis temporarily during early hours [7]. This temporary inhibition of mitosis may allow time for repair processes to act before radiation induced structural defects are replicated. The lower number of degenerating cells in the MPG treated animals was observed by *Kumar* [5], *Kumar* and *Uma Devi* [8]. The protection after one week of irradiation may be dur to the repair process being faster in drug treated groups as compared to normal.
Further studies with the aim to understand the mechanism of drug protection as well as the application of this drug in clinical field are in progress.

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Literature

1. *Bond, V. P., T. M. Fliedner* and *J. O. Archambeau:* In: Mammalian Radiation Lethality. Academic Press, New York 1965. — 2. *Broch, H., D. Cabrol* and *D. Vsilesiu:* Quantum mechanical simulation of the interaction between the radioprotector cysteamine and DNA. Internat. J. Quantum-Chem. Biol. **7** (1980), 283. — 3. *Goyal, P. K., S. Kumar* and *P. K. Dev:* Radioresponse of erythrocytes and its modification by MPG (2-mercaptopropionyl glycine) during post natal development of mice exposed to gamma radiation in utero. AMPI bulletin India **5** (1981), 215.
4. *Goyal, P. K., S. Kumar* and *P. K. Dev:* Modification of radiation induced changes by MPG (2-mercaptopropionyl glycine) in the post-natal erythrocyte count of the Swiss albino mice against prenatal exposure. Strahlentherapie (in press). — 5. *Kumar, S.:* Modification of the radiation induced peripheral blood changes in Swiss albino mice by MPG (2-mercaptopropionyl glycine). A Ph.D. thesis Univ. of Rajasthan (India) 1981. — 6. *Kumar, S., A. Kumar, G. C. Jagetia* and *P. Uma Devi:* Radiation induced changes in thymus and oesophagus of Swiss albino mice by MPG. J. nucl. Med. & Allied Sciences 1983 (Accepted). — 7. *Kumar, S., A. Kumar* and *P. Uma Devi:* unpublished. — 8. *Kumar, S.,* and *P. Uma Devi:* Effect of MPG on the radiation induced peripheral blood changes in mice (submitted for publication). — 9. *Modig, H. G.,* and *L. Revesz:* Non protein sulphydryl and glutathione content of Ehrlich ascites tumor cells after treatment with the radioprotectors, AET, cysteamine and glutathione. Internat. J. Radiat. Biol. **13** (1967), 469. — 10. *Norris, W. Z., T. E. Fritz, C. E. Rehfeld* and *C. M. Polle:* The response of the Beagle dog to cobalt-60 gamma radiation: Determination of the LD 50/30 and description of assicoated changes. Radiat. Res. **35** (1968), 681. — 11. *Rubin, P.,* and *G. W. Cassarett:* In: Clinical Radiation Pathology. W. B. Saunders, Philadelphia 1968. — 12. *Saini, M. R., P. Uma Devi* and *S. S. Yadav:* Radiation protection of bone marrow lymphocytes by 2-mercaptopropionyl glycine (MPG). Experientia **34** (1978), 128. — 13. *Spangler, G.,* and *B. Cassen:* Electrophoretic mobility size distribution and electromicrograph response of lymphocytes to radiation. Radiat. Res. **30** (1967), 22. — 14. *Uma Devi, P.,* and *S. Kumar:* Radioresponse of peripheral blood and its modification by MPG (2-mercaptopropionyl glycine) in mice. I. Erythrocytes. Strahlentherapie **157** (1981), 63.

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