THE EFFECT OF TOAD SKIN GLAND SECRETION ON THE PERMEABILITY OF TISSUES TO VITAL STAINS AFTER THEIR EXPOSURE TO EXPERIMENTAL ACUTE RADIATION

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The generally tonic or therapeutic action of small doses of various venoms on the human organism is well known, particularly the secretion from skin glands of the toad [3–7]. Recently, there has been evidence that administration of toad venom following irradiation of experimental animals with roentgen rays improves the hematological indices [8], normalizes tissue respiration in the bone marrow and spleen [11], and subdues the symptoms of radiation sickness [2].

The purpose of this investigation was to study the effect of toad venom on the penetration of vital stains into tissues of experimental animals subjected to acute radiation.

EXPERIMENTAL METHOD

For the experimental animals we used white rats of both sexes, weighing from 150 to 210 grams.

In the first and second series of experiments we studied the effect of ionizing radiation on the permeability of tissues to vital stains: neutral red and chrysoidin. For this purpose, the animals were subjected to a single whole-body exposure to γ-rays from radioactive cobalt in the radiotherapeutic apparatus GUT-Co^{60}400, using doses of 400 and 800 r. The white rats were sacrificed by decapitation 6, 24, 48, 72 and 120 hours after irradiation. The liver, kidneys, small intestine, spleen, lungs, brain, adrenals and heart were quickly removed and placed in Ringer's solution for 30 minutes. Then the material being studied was immersed for staining in a 0.01% solution of neutral red and chrysoidin, prepared in Ringer's solution in which the sodium carbonate had been excluded.

The staining lasted 30 minutes, after which the tissue was removed from the staining solution, rinsed in Ringer's solution free of sodium carbonate, and, for extraction of the stains, placed in a test tube containing 70° alcohol, acidified with sulfuric acid.

Following extraction, which lasted 24 hours, the alcohol extract was measured colorimetrically, using the concentration colorimeter KOL-1M, and the amount of absorbed stain was calculated per gram of organ. Trials with the control, unirradiated rats were set up under the same conditions. Then, a comparison was carried out between the absorption of the stains in the irradiated tissues and in the control animals; staining in the controls was taken to be equal to 100%

In the experiments designed to study the effect of toad venom on the absorption properties of the tissues from irradiated animals (third and fourth series), the toad toxin was injected into the rats subcutaneously in a dose of 0.001 grams per rat, one hour and 24 hours after exposure to γ-radiation in doses of 400 and 800 r. The preparation of toad venom was obtained from the para-auricular glands of the green toad (Bufo viridis) by extruding it with forceps. The toad venom was dried at 15-18° C, and then ground into a powder. Immediately before injection the powder was dissolved (1:1000) in physiological saline and injected into the white rats in a total volume of 1 ml. It is known [5] that white rats are very resistant to toad venom. Doses of 0.008, 0.01, and 0.012 g/kg do not cause manifest pathological changes. The lethal dose for white rats is 0.014 g/kg. Doses of 0.001-0.005 are easily tolerated by the organism, and do not cause functional or organic disturbances. In these doses the toad venom deepens respiration, elevates the arterial pressure and increases the force of the cardiac contractions, demonstrating a stimulatory effect on the organism.

TABLE 1. The Effect of Toad Skin Gland Secretion on the Permeability of Tissues from White Rats to Vital Stains Following Irradiation with γ-Rays in a Dose of 400 r (in percents)

Stain	Tissue studied	Control	48 hours after irradiation				120 hours after irradiation			
			irradiation		irradiation + toad venom		irradiation		irradiation + toad venom	
		M	*M*	*t*	*M*	*t*	*M*	*t*	*M*	*t*
Neutral red	Liver	100	92,6	1,2	105,7	0,9	105,7	0,7	98,9	0,2
	Kidney	100	124,9	3,2	90,5	1,8	127,0	4,4	86,2	2,0
	Small intestine	100	142,2	6,6	94,2	1,3	131,6	5,0	86,5	2,4
	Spleen	100	147,3	4,7	120,0	2,5	135,3	3,8	88,8	1,9
	Lung	100	132,6	5,0	114,5	2,5	129,4	4,3	113,1	1,9
	Brain	100	100,1	1,3	97,7	0,3	125,6	2,2	91,5	1,2
	Adrenal	100	85,4	2,7	98,6	0,3	118,4	2,8	83,5	3,1
	Heart	100	97,5	0,4	112,2	2,7	116,7	2,0	108,6	1,4
Chrysoidin	Liver	100	97,3	0,5	94,7	1,0	110,6	1,3	96,7	0,5
	Kidney	100	120,0	3,7	94,0	0,8	123,2	3,1	81,1	2,7
	Small intestine	100	135,1	4,2	87,0	1,6	126,8	4,0	79,5	2,7
	Spleen	100	138,3	4,8	126,4	3,4	129,3	3,8	82,6	2,5
	Lung	100	128,3	3,8	118,1	2,7	125,6	4,3	105,5	0,7
	Brain	100	90,8	0,7	108,0	0,9	116,1	1,0	88,4	1,3
	Adrenal	100	86,2	2,8	96,8	0,4	111,7	2,0	77,4	4,0
	Heart	100	92,1	1,2	116,5	2,0	116,5	1,7	82,7	2,1
	Number of animals	25	12		12		12		12	

Note. t) Criterion for significance in the differences between results (differences are significant when t is equal to, or greater than ,3). The figures for the significance indices (t) pertain to the difference between the experimental and control values.

TABLE 2. The Effect of Toad Skin Gland Secretion on the Permeability of Tissues from White Rats to Vital Stains Following Irradiation with γ-Rays in a dose of 800 r (in percents)

Stain	Tissue studied	Control	72 hours after irradiation			
			irradiated		irradiated	toad venom
		M	*M*	*t*	*M*	*t*
Neutral red	Liver	100	134,6	3,6	94,3	0,9
	Kidney	100	131,3	3,7	101,3	0,2
	Small intestine	100	150,5	5,6	112,7	2,5
	Spleen	100	166,1	6,6	141,9	4,6
	Lung	100	136,9	4,8	125,2	4,1
	Brain	100	118,6	2,0	107,8	1,0
	Adrenal	100	113,4	2,2	103,6	0,6
	Heart	100	107,6	1,1	116,7	2,6
Chrysoidin	Liver	100	128,5	3,0	106,6	1,0
	Kidney	100	126,5	3,0	92,4	1,2
	Small intestine	100	140,1	3,8	106,3	1,1
	Spleen	100	152,2	4,9	131,8	3,5
	Lung	100	129,5	3,5	131,1	4,2
	Brain	100	110,3	0,9	115,1	1,6
	Adrenal	100	106,4	0,9	105,8	0,9
	Heart	100	102,1	0,3	120,1	2,1
	Number of animals	25	12		12	

Designations are the same as in Table 1.

In experiments that we expecially set up, it was observed that the injection of toad venom into the white rats in the dose indicated above was not accompanied by the development of pathological changes.

Investigation of the absorption properties of tissues from the irradiated animals that received the toad venom was carried out 48 and 120 hours after irradiation with a dose of 400 r, and 72 hours after exposure to radiation in a dose of 800 r, i. e., at the time that disturbances in the tissue permeability to the vital stains are most strongly manifested. All the data obtained in this investigation were subjected to variation statistics analysis.

EXPERIMENTAL RESULTS

Irradiation of the white rats with γ-rays in a dose of 800 r led to the death of 95% of the animals within 30 days after the exposure. The average life duration was 12.4 days. Beginning with the 3rd day after the irradiation the express picture of radiation sickness developed (refusal of food, sluggishness, diarrhea, disheveled pelt). The number of leukocytes in the peripheral blood decreased to 1.1 thousand per mm^3 by the 5th day.

Injection of the toad venom for therapeutic purposes increased the survival rate of the irradiated white rats by 16% The average duration of life was 14.2 days. The number of leukocytes on the 5th day after irradiation was equal to 2.9 thousand per mm^3. Use of the toad venom led to a certain weakening of the clinical symptoms of radiation sickness.

Data on the changes in the absorption properties of the tissues from the irradiated white rats, and on the effect of the toad skin gland secretion on these indices, are presented in Tables 1 and 2.

Exposure to ionizing radiation led to a regular increase in the permeability of certain tissues to the vital stains.

Table 1 shows that in the rats, irradiated with 400 r of γ-rays, an increase in the permeability to the neutral red and chrysoidin stains took place in the kidneys, small intestine, spleen, and lungs, 48 and 120 hours after irradiation. In other tissues investigated (liver, brain, heart) the difference between the experimental and control series was insignificant. The difference in staining of the adrenals was just on the border of statistical significance. ($t = 2.8$).

When the experimental animals were irradiated with a dose of 800 r (Table 2) a statistically significant increase in the absorption of the vital stains was observed, 72 hours after the exposure, in the liver, kidneys, small intestine, spleen, and lungs. At this time we did not note significant changes in the staining of the brain, adrenals or myocardium.

Conforming with the data in the literature [1, 2, 9, 10], analysis of the results obtained in the first and second series of experiments justifies concluding that the changes noted in the absorption properties of the tissues is apparently related to denaturation changes in the protein structures of cells from the irradiated animals.

Use of the toad venom following irradiation of the experimental animals with γ-radiation in a dose of 400 r (see Table 1) was seen to have a normalizing effect on the changes in the absorption properties of the tissues from the irradiated white rats. In this case, permeability to the neutral red and chrysoidin stains in the majority of investigated tissues from animals, sacrificed 48 and 120 hours after the irradiation, did not differ from the controls. However, there were certain exceptions. For example, 48 hours after exposure to ionizing radiation we did not observe a statistically significant reduction in the absorption of chrysoidin by the splenic tissue of the rats that received the toad toxin. A reduction in the permeability of the adrenal tissue to the vital stains took place 120 hours after the irradiation.

All these data were subjected to variation statistics analysis.

When the white rats were irradiated with a dose of 800 r of γ- rays (see Table 2) the therapeutic effect of the toad skin gland secretion was more weakly manifested. Under these conditions, injection of the irradiated animals with the toad venom was accompanied by a regular normalization of the tissue permeability in the liver, kidneys, and small intestine, relative to neutral red and chrysoidin.

The injection of the toad toxin after irradiation did not lead to an essential reduction in the absorption of the employed vital stains on the part of the spleen and lung tissues.

Thus, the results of this investigation indicate that the use of toad skin gland secretion under conditions of acute radiation injury brings about a beneficial effect on the course of some of the radiation reactions; this is more expressly seen when the experimental animals are irradiated with a sublethal dose.

Considering all this, as well as the large range in the biological activity of toad venom, it would be expeditious to further study the effect of toad skin gland secretion on the course and outcome of radiation injuries, so as to elucidate the potentials of its effective use in the therapy of radiation sickness.

In conclusion, we feel obligated to express our gratitude to Professor Doctor of Medical Sciences S.B. Balmukhanov for providing us with the toad venom preparation, and for his recommendations on its use.

SUMMARY

A study was made of the sorption properties of various albino rat tissues with respect to neutral red at various time periods after gamma irradiation in doses of 400 and 800 r.

After the irradiation there occurs a regular increase of the staining in some tissues (kidney, small intestine, spleen, lung), caused evidently by the appearance of denaturing changes in the protein structures of cells of the irradiated animals.

The use of secretion of the toad skin gland after the irradiation prevents changes of the tissue sorption properties in the irradiated animals. This effect is more marked with a dose of 400 r.

LITERATURE CITED

1. P. D. Gorizontov, in the book: Radiobiology and Radiation Medicine [in Russian] 5 (Moscow, 1959) p. 43.
2. E. Ya. Graevskii, and L. I. Korchak, in the book: Investigations on the Action of Ionizing Radiation on the Animal Organism [in Russian] (Moscow, 1959) p. 28.
3. V. I. Zakharov, Tezisy Dokl. 15-i Nauchnoi Sessii Kishinevsk Med. Inst. (1957) p. 8.
4. V. I. Zakharov, Nauchnye Izvestiya Kazakhsk. Med. Inst., No. 12 (1955) p. 105.
5. V. I. Zakharov, Toad Venom [in Russian] (Kishinev, 1960).
6. V. I. Zakharov, et al., Byull. Éksper. Biol. i Med., No. 2 (1960) p. 85.
7. V. I. Zakharov, and I. P. Krichevskaya, in the book: Questions in Experimental Biology and Medicine [in Russian] No. 2 (Moscow, 1952) p. 197.
8. N. V. Kolpikov, et al., Zdravookhranenie, No. 3 (Kishinev, 1958) p. 42.
9. L. I. Korchak, in the book: Radiobiology [in Russian] (Moscow, 1958) p. 130.
10. A. M. Kuzin, in the book: Radiobiology [in Russian] (Moscow, 1958) p. 3.
11. A. I. Robu, Tezisy Dokl. 17-i Ocherednoi Nauchnoi Sessii Kishinevsk. Med. Inst. (1959) p. 64.
12. M. V. Tikhomirova, in the book: Collection of References on Radiation Medicine for the year 1957 [in Russian] 2 (Moscow, 1959) p. 48.
