NERVOUS COMPONENT IN THE MECHANISM OF ACTION OF RADIOPROTECTIVE SUBSTANCES

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The experimental evidence accumulating during recent years from the study of the mechanisms of action of radioprotective substances has come into conflict with the classical concept of the uniformity of action of all radioprotective agents at the molecular and cellular levels. The results obtained show that, unlike the sulfur-containing preparations, giving a protective action both in vivo and in vitro, amine preparations not containing SH groups, indolylalkylamines, catecholamines, etc.), do not exhibit anti-radiation properties in experiments on isolated cells and microorganisms [21, 26, 30]. This fact is regarded by both authors as the result of the "oxygen effect" – the creation of ischemia in radiosensitive organs following administration of amine preparations capable of producing spasm of the blood vessels [5, 6, 18, 20, 22].

Tissue hypoxia plays an important role in the mechanism of the protective action of the radioprotective amines. However, some pharmacological agents causing a significant fall in the level of pO_2 in radiosensitive tissues do not possess protective properties, and complete correlation does not exist between the degree of hypoxia and the degree of the protective effect [14, 27].

In the present investigation the action of radioprotective agents was studied on the cornea, in the epithelium of which the processes of division are extremely radiosensitive [3, 4, 7]. The high level of innervation and the absence of blood vessels in the cornea make it possible to study the part played by the nervous and vascular systems separately in the mechanism of the radioprotective action of the preparations.

EXPERIMENTAL METHOD

Experiments were carried out on 90 male albino mice weighing from 18 to 20 g. In the first variant of the experiment the radioprotective agents were instilled into the conjuctival sac 30 min before irradiation in the maximally tolerated concentration: mexamine – 0.007 M, β-mercaptoethylamine (BMEA) – 0.02 M. In the second variant of the experiments the preparations to be tested were injected subcutaneously in doses of: mexamine – 75 mg/kg, BMEA – 150 mg/kg. Irradiation was carried out from a cobalt source with a discharge of 2 kCi and a dose rate of 75 R/min. The dose of irradiation was 100 R.

The control and experimental mice were sacrificed 3 h after irradiation. Total preparations were made from the epithelium of the cornea after fixation in Carnoy's fluid and staining with Ehrlich's hematoxylin. The degree of protection of the cornea by the radioprotective agent was estimated from the changes in mitotic activity in the corneal epithelium. In each preparation from 100 to 350 fields of vision were examined and the stages of cell division recorded. The mitotic index was taken as the number of mitoses in 100 fields of vision. Since the mitotic activity of the corneal epithelium of the right and left eyes in the same animal, other conditions being equal, is identical [11], the cornea of only one eye was taken for investigation.

EXPERIMENTAL RESULTS

When applied locally, BMEA was a much more effective preparation than the amino-compound mexamine (see the table). The results of the statistical analysis of the material show that the difference between the values of the mitotic index in the group of animals protected by BMEA, on the one hand, and the group protected by mexamine, on the other hand, is significant. The difference between the mitotic index of the group of animals protected by mexamine and the control group (instillation of distilled water) is also statistically significant.

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Effect of Radioprotective Agent on Mitotic Activity of Corneal Epithelium of Albino Mice Irradiated in a Dose of 100 R (Mean Value of Mitotic Index with Confidence Limits at P = 0.05)

Preparation	No. of animals	Local action (instilled into conjunctival sacs)		No. of animals	Central action (subcutaneous injection)	
		Without irradiation	Irradiation		Without irradiation	Irradiation
Distilled water (control)	10	57.6 ± 3.6 (49.6-65.6)	4.9 ± 0.6 (3.46-6.34)	20	84.1 ± 9.3 (63.5-104.7)	10.6 ± 1.08 (8.2-13.0)
Mexamine	10	64.9 ± 3.1 (58.0-71.8)	7.7 ± 1.3 (5.3-10.1)	20	62.0 ± 7.3 (45.7-78.3)	21.0 ± 4.2 (11.6-30.4)
β-Mercapto-ethylamine	10	64.3 ± 4.0 (55.4-73.2)	32.0 ± 2.9 (25.5-38.5)	20	58.5 ± 4.7 (48.0-69.0)	7.3 ± 2.1 (2.8-12.4)

The subcutaneous injection of BMEA had no protective action on the corneal epithelium of the albino mice. In contrast to BMEA, mexamine, when injected subcutaneously, caused a statistically significant increase in the mitotic index in the irradiated animals by comparison with the controls receiving distilled water instead of the protective preparation. Bearing in mind the absence of a vascular system in the cornea and the impossibility of the tested agents having any direct action on the nerve centers because of the small doses of the preparations absorbed from the conjunctival sacs, the result of the experiments of series I can be interpreted as due strictly to the local action of the protective agents on the corneal epithelium. This conclusion is in agreement with data in the literature concerning the manifestation of the protective properties of radioprotective agents containing SH groups in experiments in vitro [2, 10, 16, 17, 21, 23, 25, 29]. The absence of a protective effect from the local application of mexamine may be explained by two largely opposite assumptions. On the one hand, the reason may be the exclusion of the vasoconstrictor action of mexamine in the tissue containing no blood vessels, and hence, the impossibility of the development of hypoxic conditions in it. On the other hand, it may be assumed that mexamine, having no effect on the nerve centers in this variant of the experiment (because of the insufficient dose of the substance absorbed), does not bring about the corresponding reorganization of the physiological processes in the animal body including in the corneal tissue, capable of playing an important role in reducing the severity of the radiation injury.

To test these hypotheses, the experiments of series II were carried out. Subcutaneous injection of mexamine and BMEA created the essential conditions for the central action of these preparations. It has been shown that BMEA passes readily through the blood-brain barrier [1], while the indolylalkylamines substituted in position 5 of the indole ring (which includes mexamine), when given parenterally, in comparatively small doses (15 mg/kg) cause marked depression of the cortical electrical activity of animals [6].

Analysis of the results obtained in the experiments with BMEA showed that this agent, the leading preparation from the class of sulfur-containing radioprotective substances, had no central effect in the experimental conditions described: the subcutaneous injection of the sulfhydryl compound did not protect the processes of cell division in the richly innervated corneal epithelium against the harmful action of ionizing radiation. However, it must be emphasized that in this variant of the experiment the humoral component of the possible central action of BMEA, which is mentioned in the literature [13], was not taken into account. This question requires special examination.

In contrast to BMEA, the indolylalkylamine mexamine (5-methoxytryptamine), when injected subcutaneously (providing conditions for development of the central action of this preparation) had a protective action on the corneal epithelium of the albino mice. The results obtained confirm existing suggestions [9, 10, 15, 24] that the nervous system may play the decisive role in the development of the protective properties of the radioprotective amines. In fact, it is difficult to imagine that in this variant of the experiment an "oxygen effect" was present, and that the protection obtained following the subcutaneous injection of mexamine was associated with the creation of hypoxia in the cornea. Even the spasm of the blood vessels carrying blood enriched with oxygen to the eye could not significantly change the level of pO_2 in the corneal epithelium, because in this case the constant concentration of oxygen in the surrounding air maintains its

concentration in the epithelium also. Proof of the absence of a hypoxic state of the corneal epithelium in this experiment was given by the fact that the creation of hypoxia in cells causes a sharp and prolonged depression of mitotic activity [12], which was not observed in these experiments following injection of mexamine without irradiation. Further confirmation of this hypothesis is provided by clinical evidence showing that prolonged (for more than 5-6 h) wearing of contact lenses, which prevent the access of oxygen from the external environment to the cornea (but do not prevent excretion of oxygen from the body) leads to the development of a Sattler's veil in the corneal epithelium [19, 28].

It may thus be concluded from these results that, besides a vasoconstrictor component, causing hypoxia in radiosensitive organs, a specific nervous component may also be present in the mechanism of action of the radioprotective amino-compound mexamine.

In the variant of the experiments carried out, no evidence was obtained of the presence of a nervous component in the mechanism of action of the sulfur-containing radioprotective agent β-mercaptoethylamine.

LITERATURE CITED

1. S. Ya. Arbuzov et al., Abstracts of Proceedings of Sections at the 9th Congress of the All-Union Society of Physiologists, Biochemists, and Pharmacologists [in Russian], 2, Moscow–Minsk (1959), p. 24.
2. É. A. Val'dshtein, In the book: The Reaction of Cells to Extreme Influences [in Russian], Moscow–Leningrad (1963), p. 126.
3. G. P. Gruzdev, Radiobiologiya, No. 1, 52 (1961).
4. N. P. Dubinin, Problems in Radiation Genetics [in Russian], Moscow (1961).
5. P. G. Zherebchenko and N. N. Suvorov, Radiobiologiya, No. 4, 595 (1963).
6. P. G. Zherebchenko, In the book: The Pathogenesis, Experimental Prophylaxis, and Treatment of Radiation Injury [in Russian], Moscow (1964), p. 193.
7. N. I. Nuzhdin and O. P. Domareva, Doklady Akad. Nauk SSSR, 125, No. 3, 650 (1959).
8. Z. G. Pershina and D. R. Kaulen, In the book: Textbook of the Microbiology, Clinical Features, and Epidemiology of Infectious Diseases in Several Volumes [in Russian], 2, p. 109, Moscow (1962).
9. L. F. Semenov, In the book: Current Problems in Hematology and Blood Transfusion [in Russian], No. 34, Moscow (1959), p. 269.
10. R. B. Strelkov, In the book: Problems in Physiology, Experimental Pathology, and Radiobiology [in Russian], Sukhumi (1965), p. 333.
11. I. A. Utkin, Relationship between the Patern of Cell Division and the Physiological State of the Organism. Author's abstract of candidate dissertation, Moscow (1952).
12. I. A. Utkin, Path. et Biol., 9 (1961), p. 519.
13. B. A. Fedorov, The State of the Testes of Rats in Acute Radiation Sickness. Candidate dissertation, Sukhumi (1962).
14. Z. M. Bacq, P. Ciccarone, and J. Renson, Experientia, Basel, 15 (1959), p. 175.
15. D. W. van Bekkum and J. B. de Groot, In: Progress in Radiobiology, Springfield (1956), p. 243.
16. E. H. Betz and G. Booz, C.R. Soc. Biol., 156 (1962), p. 1199.
17. C. Biagini, Nature, 172 (1953), p. 868.
18. H. A. S. van den Brenk and R. Moore, Ibid., 183 (1959), p. 1530.
19. J. Dallos, Brit. J. Ophthal., 30 (1946), p. 607.
20. R. F. Gautieri and H. P. Ciuchta, J. Pharm. Sci., 51 (1962), p. 55.
21. G. Grant and O. Vos, Int. J. Radiat. Biol., 5 (1962), p. 413.
22. L. H. Gray, Proceedings of the 4th International Conference on Radiobiology, Edinburgh (1956), p. 267.
23. A. Hollaender, G. E. Stapleton, and F. L. Martin, Nature, 167 (1951), p. 103.
24. H. Langendorff and R. Koch, Strahlentherapie, 102 (1957), p. 58.
25. H. Marcovich, Ann. Inst. Pasteur., 93 (1957), p. 456.
26. C. van der Meer, O. B. Zaalberg, et al., Int. J. Radiat., 4 (1962), p. 311.
27. C. van der Meer, P. Valkenburg, and M. Remmelts, Ibid., 6 (1963), p. 151.
28. E. Obrig, Arch. Ophthal., 19 (1938), p. 735.
29. H. M. Patt, M. E. Blackford, and R. L. Straube, Proc. Soc. exp. Biol., New York, 80 (1952), p. 92.
30. O. Vos, L. Budke, and A. Vergroesen, Int. J. Radiat. Biol., 5 (1962), p. 543.