CONCERNING THE DISTURBANCE OF METABOLISM OF THYMIDINE AND DESOXYURIDINE IN IRRADIATED RATS

(UDC 616-001.28-07 : 616.633.1)

V. K. Mazurik

Moscow

(Presented by Member of the Academy of Sciences USSR, P. D. Gorizontov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 59, No. 2, pp. 59-61, February, 1965
Original article submitted June 24, 1964

In 1958 a group of Czechoslovakian investigators [14] reported the appearance of increased amounts of desoxycytidine—one of the components of DNA—in the urine of rats subjected to total x-ray irradiation.

In the opinion of these authors [15], as a result of a block in the synthesis of DNA or a decomposition of this compound under the influence of radiation, desoxyribosides, phosphorolytically degraded to the final products, with the exception of desoxycytidine, accumulate in the tissues. The peculiar situation of desoxycytidine was explained by the absence of a specific phosphorylase in the rat tissues, essential for the subsequent conversions of this nucleoside to the ultimate compounds. However, it was soon clearly demonstrated [1, 3] that in the urine of irradiated rats there is an increased concentration of another DNA component besides desoxycytidine—thymidine, although to a lesser degree. P. D. Gorizontov, T. A. Fedorova, Yu. A. Zharkov, et al. [1, 3], studying the dependence of the level of excretion of this compound on the dose of irradiation, and also the dynamics of the excretion of thymidine as a function of the development of radiation sickness, established that the excretion is directly dependent upon the dose, and the maximum excretion took place on the first day. This is in excellent agreement with the data obtained by other authors for desoxycytidine [7, 14] and pseudouridine [10].

The abovementioned facts contradict the representation of the specific condition of desoxycytidine in metabolism [16] and are evidence of the possibility of the presence of some general mechanisms leading to hyperexcretion of both desoxycytidine and thymidine.

The purpose of this investigation was to study the role of the thymidine-desoxyuridine system in the mechanism of the increase in nucleoside excretions. Moreover, we proceeded from the fact that: 1) desoxyuridine may be an effective precursor of thymidine [17]; 2) during irradiation, the influence of desoxycytidine on free desoxyuridine becomes minimal as a result of the defectiveness of the system of deamination of desoxycytidine monophosphate [20].

PROCEDURE

The work was conducted with 23 white mongrel male rats, weighing 150-250 g; 18 of them were kept in pairs in metabolic cages and five singly. They were fed a normal diet. The rats were totally irradiated on the RUM-3 x-ray setup at a dose of 650 R under conditions: amperage: 15 mA, voltage: 180 kV, focal length: 40 cm, filters: 0.5 mm Cu and 1 mm Al, dose rate: 30 R/min.

The urine was collected during the day before and after irradiation, filtered and kept in a refrigerator at -20°. The animals were sacrificed.

The determination of thymidine and desoxyuridine was carried out using a method we developed, based on the semipreparative isolation of the thymidine-desoxyuridine fraction on a Dowex-1 anion exchange resin, with the subsequent quantitive spectrophotometric determination of these compounds after paper chromatography.

Dowex resin 1 x 8, 100-200 mesh in a $HCOO^-$-cycle was used. The treatment of the exchange resin was carried out according to the method of Hurlbert and coauthors [11] after preliminary washing with 0.5 N NaOH and 1 N HCl. 50-ml burettes with stopcocks, with internal diameter of 11 mm, were used as a columns; the height of the

Thymidine and Desoxyuridine Excretion in Urine (in micromoles/day) in Normal Rats and During the First Day after Total X-ray Irradiation at the Minimum Absolute Lethal Dose (650 R)

Index	Thymidine		Desoxyuridine		Thymidine desoxyuridine	
	normal	irradiated	normal	irradiated	normal	irradiated
Number of animals	23	11	23	11	22	11
M	0,23	0,92*	0,58	0,99*	0,40	0,98
m±	0,04	0,20	0,05	0,15	0,07	0,33
p	0,001		0,001		0,01	
Multiplicity factor for the increase in relation to the normal	3,99 Fold		1,72 Fold		2,32 Fold	

*Differences are statistically insignificant.

layer was 30 cm. The rate at which the solutions were passed through was 0.5 ml/min. The urine was unfrozen and subjected to a partial desalinization by the addition of 2-3 ml of concentrated ammonia. The volume of the urine was brought to 100 ml with distilled water, and to a pH of 10.5 with ammonia. The urine solution was absorbed after washing the column with ammonia water. Elution was carried out with a 0.1 M solution of ammonium formate by gradually changing the pH. The pH 9.5 fraction of the eluate was discarded. The pH 8.5 eluate was collected, evaporated to dryness under vacuum at a temperature no higher than 37°, and dissolved in 3 ml of distilled water. The solution was subjected to further fractionation using paper chromatography (descending system of n-butanol-water, for a duration of 18 h of partitioning). The spots absorbing in the ultraviolet spectrum and having the chromatographic mobility of thymidine and desoxyuridine were eluted with a 0.1 N HCl solution.

The spectra were taken in the wavelength range 225-290 mμ. Substances were isolated from the urine which had the characteristic thymidine and desoxyuridine spectra and spectrophotometric characteristics and, moreover, reacted positively with cysteine and sulfuric acid (Dische reaction for 2-desoxyribose) [19]. The amounts of the desoxyribosides, calculated from the absorption spectra by the method of differential extinction and according to the Dische reaction were in good agreement.

RESULTS OF THE EXPERIMENTS

The table cites average values for the excretion of thymidine and desoxyuridine in a normal rat urine and during the first day after total X-ray irradiation at a dose of 650 R.

As it follows from the data cited in the table, the average level of thymidine excretion in the normal rat was 2.5 times lower than the level of desoxyuridine excretion. After irradiation, the concentrations of thymidine and desoxyuridine become almost equal, since the thymidine content in the urine rises 4-fold and the desoxyuridine content rises only 1.7-fold.

Hyperexcretion of thymidine into the urine, as we have already noted, is not a new fact. It has been observed by many authors [1, 4, 9, 10, 15].

Among the causes of the increased excretion of the desoxynucleosides in the urine, those which in our opinion require special consideration are the following:

1. An increased decomposition of DNA in rapidly regenerating tissues, preceeding or accompanying destructive processes. T. A. Fedorova and Yu. A. Zharkov link the increase in excretion of thymidine in the first day after irradiation with this [3]. This hypothesis coincides with the data of the morphological works in which a rapid decrease is detected in the number of cellular elements of the tissues with a high mitotic index in the first hours after total irradiation [8]. This is also good in agreement with the data of biochemical investigations in which a decrease in the total DNA content was observed in the radiosensitive organ [4] and the appearance of various DNA decomposition products was noted in the early period after irradiation: from free polymeric DNA [2] to desoxynucleosides.

2. The inhibition of DNA synthesis in the stages preceeding the formation and polymerization of triphosphates [13, 21]. The probable role of this factor is insignificant, since during a possible increase in the concentration of

metabolites due to a block in the final stages of synthesis, mechanisms regulating the metabolism, acting on the principle of negative feedback, which inhibit the synthesis of DNA de novo at the earliest stages [5] and prevent the accumulation of nucleic acid precursors, are instantaneously developed.

3. Relative insufficiency of the systems leading to the decomposition of thymidine derivatives to the final products. As a result of the lowering of the concentration of nicotine adenine dinucleotide (NAD) and adenosine triphosphate (ATP) after irradiation [6], a relative insufficiency of conversion of bases to the dehydrobases, catalyzed by reduced NAD phosphate, arises [12].

An increase in the thymidine concentration partially blocks thymidine phosphorylase and, consequently, leads to a slowing of its decomposition [15] and an accumulation of the latter.

As a result, the reaction of methylation of desoxyuridine decreases, and the concentration of desoxyuridine increases in the tissues. The surplus desoxynucleosides we excreted with the urine.

LITERATURE CITED

1. P. D. Gorizontov, T. A. Fedorova, Yu. A. Zharkov, et al., Radiobiologiya, No. 4, (1963), p. 514.
2. N. V. Ermolaeva, Radiobiologiya, No. 5, (1961), p. 670.
3. Yu. A. Zharkov, T. A. Fedorova, and L. F. Mikhailova, Radiobiologiya, No. 6, (1964).
4. I. I. Ivanov, O. N. Borovikova, V. G. Vladimirov, et al., Dokl. AN SSSR, 155, No. 3, (1964), p. 683
5. K. A. Kafiani, Zh. Vsesoyuzn. Khim. Obshch. im. D. I. Mendeleeva, No. 1, (1963), p. 23.
6. A. M. Kuzin, Radiation Biochemistry [in Russian], Moscow, (1962), p. 300.
7. M. M. Skurikhina and O. Ya. Tereshchenko, Radiobiologiya, No. 6, (1963), p. 829.
8. I. B. Smirnova and I. M. Shapiro, Radiobiologiya, No. 6, (1962), p. 897.
9. H. K. Berry, et al. Science, 142, (1963), p. 396.
10. D. Dranovsky, A. Winkler, and J. Skoda, Nature, 201, (1964), p. 411.
11. R. B. Hurlbert, et al., J. biol. Chem., 209, (1954), p. 23.
12. J. C. Marsh and S. Perry, Arch. Biochem., 104, (1964), p. 146.
13. O. F. Nygaard and R. L. Potter, Radiat. Res., 10, (1959), p. 462.
14. J. Parizek, M. Arient, Z. Dienstbier, et al., Nature, 182, (1958), p. 721.
15. J. Parizek (1960) Cited by Y. Sugino, E. P. Frenkel, R. L. Potter.
16. J. Parizek, In the Book: Abstracts of the Section Reports of the Fifth International Biochemical Congress [Russian translation] Moscow, 1, (1962), p. 184.
17. E. A. Phear and D. M. Greenberg, J. Am. Chem. Soc., 79, (1957), p. 3737.
18. J. Rotherham and W. C. Schneider, Biochim. biophys. Acta, 41, (1960), p. 19.
19. P. K. Stumpf, J. biol. Chem., 169, (1947), p. 367.
20. V. Sugino, E. P. Frenkel, and R. L. Potter, Radiat. Res., 19, (1963), p. 682.
21. J. L. Van Lancker, Biochim. biophys. Acta, 45, (1960), p. 63.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
