SEX DIFFERENCES IN THE EXCRETION OF O-AMINOAZOTOLUENE BY MICE

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Primary malignant tumors of the liver affect males from 3 to 6 times more frequently than females [14, 16, 18]. In experimental conditions sex differences in the development of hepatomas are also observed [1, 2, 7-10, 12, 13, 15, 19]. The reasons for this phenomenon have not so far been studied.

Arising from the suggestion that the action of a carcinogen is connected with the character of its excretion, we studied the sex differences in the excretion of O-aminoazotoluene (OAAT) by mice.

EXPERIMENTAL METHOD

Experiments were conducted on mice of two lines: CC57 brown (184 animals) and O20 (65 animals). Spontaneous hepatomas are not found in mice of these lines [3, 22].

Mice aged 2-3 months and weighing 20-25 g received OAAT, moistened with glycerol (3 drops to 100 mg), by subcutaneous implantation, in a dose of 500 μg of the crystalline substance/1 g body weight, or by a daily subcutaneous injection of a 0.5% solution in sunflower oil. Control animals received diethylaminoazobenzene (DEAB) subcutaneously; this compound is similar in structure to OAAT but, according to observations in the literature [27], it is not carcinogenic. Other control animals received indigocarmine and trypan blue (Table 1).

In groups of 3 or 4, mice of the first, second, seventh, and eighth series of experiments were placed for 24 h in a cage for collection of urine, where they received bread and milk ad lib. The volume of urine excreted and the concentration of dye in the urine were investigated every 4 h for 20 days. The excretion of dye was expressed in μg/g body weight. Urine was collected from the mice of the third to the sixth series of experiments by pressure on the bladder once every 2 days throughout the period of excretion of one dose of dye.

The concentration of dye in the urine was determined by means of an Autenrit colorimeter, making use of the ability of OAAT and DEAB to exhibit tautomerism of the keto-enol type [4, 6]. Samples of urine from mice receiving OAAT and DEAB were examined in the colorimeter after dilution with 20% ethanol sulfate in a proportion of 1:17.5, while the urine from the mice receiving indigocarmine and trypan blue was diluted with unacidified ethanol.

On the 20th day of the experiment all the mice of the first series were sacrificed, and the OAAT remaining under the skin was dissolved in ethanol and estimated colorimetrically. The capsules forming around the residue of carcinogen were examined histologically. In the remaining series of experiments the animals were sacrificed for this purpose in groups of 3 or 4 after the concentration of OAAT in their urine had fallen so low that it could not be determined quantitatively.

EXPERIMENTAL RESULTS

In the course of 24 h, 6 samples of urine were tested from the mice of the first series of experiments. This allowed the pattern of excretion of OAAT to be determined not only in the course of one experiment, but for the whole 24 hours.

TABLE 1. Sex Differences in the Excretion of Various Dyes in the Urine of Mice of Lines CC57 Brown and O20

Series of experiments	Line of mice	Sex	No. of mice	Mean wt of mouse at beginning of experiment (in mg)	Dye	Method of administration	Sessional dose of dye (in mg)	Maximal duration of excretion of free dye (in days)	Mean excess of concentration of free dye in female urine over male urine (in %)
First	CC57 Brown	♀ ♂	14 13	21 24	OAAT	Subcutaneously	10,5 12	— —	60±4,47
Second	The same	♀ ♂	10 10	22 25	The same	Per os	0,5 0,5	3 3	46±11,13
Third	" "	♀ ♂	20 20	20 23	" "	Subcutaneously	10 10	47 43	55±10,86
Fourth	" "	♀ ♂	24 31	23 25	" "	The same	11,5 12,5	49 45	45±10,58
Fifth	O20	♀ ♂	20 20	20,5 24	OAAT	" "	10,2 12	45 41	49±11,18
Sixth	O20	♀ ♂	14 11	18,5 23,5	DEAB	" "	9,1 11,7	51 45	49±10,63
Seventh	CC57 Brown	♀ ♂	10 11	24 27	Trypan blue	" "	12 13,5	11 11	—7±10,34
Eighth	The same	♀ ♂	9 12	23,5 26,5	Indigo-carmine	" "	11,7 13,2	5 5	0

TABLE 2. Sex Differences in the Excretion of OAAT by Mice of Line CC57 Brown

OAAT (in μg/g)	Females	Males
Administered	500 ± 20	500 ± 20
Excreted	317 ± 16	155 ± 8
Remaining under the skin	155 ± 13.4	175 ± 14.7
Deficit	28	188

From the first to the last day of the experiment, at any time of day, the urine of the females contained much higher concentrations of OAAT than the urine of the males. During the first day, the OAAT concentration in the urine of the males rose from 21 to 33.2 mg%, whereas in the urine of the females during this period it rose from 35 to 94.5 mg%. Subsequently this difference in the concentrations invariably amounted to 40-80% for the females.

Measurements of the voluume of urine excreted in 24 h showed that after administration of OAAT the diuresis of the females fell by 10-12% (calculated per gram body weight) and that the excretion of urine by the animals of both sexes was irregular. These factors affected the amount of carcinogen actually excreted by the males and females, and also the pattern of its excretion (see figure). Throughout the 20 days of the experiment the females excreted in their urine an average of 317 ± 16 μg/g body weight of free OAAT, i.e., twice as much as the males, which excreted 155 ± 8 μg/g of free OAAT (Table 2).

After oral administration of the carcinogen (second series of experiments), equally marked sex differences in its excretion were observed.

In the next three series we studied the excretion of carcinogen until it could no longer be determined quantitatively. urine samples were collected during the 12-hour period when the free OAAT concentration was maximal. Quantitative estimation of the dye after implantation was possible in the females until the 45th-49th day, and in the males until the 41st-45th day. Throughout this period the concentration of free OAAT in the urine of the females of the third and fourth series of experiments was on the average 50-55% higher than in the urine of the males.

The sex differences in the excretion of OAAT by the mice of line O20 (fifth series of experiments) were clearly defined throughout the experiment, amounting to an average of 49 11.8% for the excess of the concentration of free carcinogen in the female urine over that in the male urine.

At the end of the experiment the residual carcinogen under the skin in the first series of experiments amounted to 155 ± 13.4 μg/g in the females and 157 ± 14.7 μg/g for the males. The carcinogen remaining under the skin of the females in the third, fourth, and fifth series of experiments weighed on the average of 1.9 mg, and of the males 1.0 mg. The capsules around the residual OAAT were thin and consisted of several layers of young connective tissue cells, infiltrated in places with lymphocytes. No sex differences in the structure of the capsule were found.

Apparently no free OAAT was excreted in the feces, for the qualitative tests for OAAT performed on homogenates and alcoholic extracts of the feces were invariably negative.

A clear difference was observed between the excretion of DEAB by the females and males of the sixth series of experiments. The females always excreted the carcinogen in a concentration exceeding that in the males by 40-60% throughout the 45 days of the experiment. No sex differences in the excretion of these dyes were observed in the animals of the seventh and eighth series of experiments, receiving trypan blue and indigocarmine.

The incidence of hepatoma is known to depend on the dose of carcinogen: other conditions being equal, with an increase in the dose more tumors are produced and the latent period is shortened [14, 27]. In the light of this fact, at first glance the results of our experiments appear to be paradoxical.

In fact, on the one hand the same doses of OAAT induced substantially more hepatomas in females than in males [1, 2, 7-10, 12, 13, 15, 19], and on the other hand the females in our experiments excreted in their urine almost twice as much free carcinogen as the males. This apparently indicates that larger doses of OAAT remained and acted in the body of the males than in that of the females. Yet this was not so. Despite the marked difference in excretion of the carcinogen, its residue under the skin at the end of the experiment was approximately the same in the animals of both sexes. For the females of the first series of experiments this residue amounted to 155 ± 13.4 μg/g, and for the males 157 ± 14.7 μg/g. If it is remembered that initially the animals of both sexes received 500 μg/g of OAAT, the deficit of carcinogen for the females was actually 28 μg/g, and that for the males 188 μg/g, i.e., 5 times more.

The fate of this portion of the carcinogen is not quite clear. Undoubtedly, some of it remained in the animal body, for it has been shown that carcinogenic azo compounds are closely bound to the liver proteins [20, 21].

It is possible that the sex differences which were observed were to some extent dependent on differences in the permeability of the capsules formed around the implanted dye, but the role of this factor cannot be important, for when OAAT was given by mouth, the sex differences in its excretion were just as pronounced.

Another possibility is that the liver of the male animal can destroy the dye more intensively, although evidence has been obtained showing that liver slices and homogenates from animals of both sexes can destroy azo compounds to an equal extent [24].

Meanwhile, it may be postulated that the results we obtained are partly explicable by sex differences in the kidney function of the mice. In contrast to females, male mice are known to excrete larger amounts of protein in their urine [23, 28], and this protein contains nucleoproteins [26]. In our experiments the male mice of lines O20 and CC57 brown constantly excreted from 6.6 to 3.3 ‰ of protein in their urine, and the females less than 0.033 ‰.

It may be assumed that azo compounds or their metabolites are bound with some form of protein and are excreted in the urine in this bound state. That this binding may occur has been demonstrated in principle by several workers [5, 20, 21]. If this assumption is correct, it will demonstrate that much less active carcinogen circulates in the male body than in the female. This may explain our results.

Our studies of the excretion of noncarcinogenic dyes showed that indigocarmine and trypan blue were excreted equally by the animals of both sexes, whereas in the case of the noncarcinogenic azo compound DEAB the sex differences were just the same as were observed in the excretion of OAAT. This may be evidence that male mice are capable of inactivating larger doses of the series of azo dyes than females, irrespective of their carcinogenicity.

SUMMARY

Following administration of O-aminoazotoluene and diethylaminoazobenzene subcutaneously to mice of the CC57 brown and O20 strains, marked sexual differences were revealed in the urinary excretion of these preparations. Female excretion of free carcinogen and of diethylaminoazobenzene is nearly twice as high as in males. No sexual differences were revealed in excretion of trypan blue and indigocarmine.

LITERATURE CITED

1. V. I. Gel'shtein. Arkh. pat., 5, 58 (1953).
2. V. I. Gel'shtein. Byull. éksper. biol., 2, 49 (1955).
3. V. I. Gel'shtein, and A. M. Dyad'kova. Vopr. onkol., 2, 32 (1955).
4. T. A. Korosteleva. In the book: Problems in Oncology [in Russian], Vol. 1, p. 255. Moscow, 1949.
5. T. A. Korosteleva. Uspekhi sovr. biol. 41, 3, 284 (1956).
6. L. M. Shabad. Essays in Experimental Oncology [in Russian], p. 330. Moscow, 1947.
7. H. B. Andervont, Publ. Hlth Rep. (Wash.), 1939, Vol. 54, p. 1986.
8. H. B. Andervont and J. E. Edwards, J. nat. Cancer Inst., 1941, Vol. 2, p. 139.
9. H. B. Andervont, H. G. Grady, and J. E. Edwards, J. nat. Cancer Inst., 1942, Vol. 3, p. 131.
10. H. B. Andervont and J. E. Edwards, J. nat. Cancer Inst., 1943, Vol. 3, p. 349.
11. H. B. Andervont and J. E. Edwards, J. nat. Cancer Inst., 1943, Vol. 3, p. 355.
12. H. B. Andervont, J. White, and J. E. Edwards, J. nat. Cancer Inst.,1944, Vol. 4, p. 583.
13. H. B. Andervont and T. B. Dunn, Cancer Res., 1947, Vol. 7, p. 730.
14. H. B. Andervont and T. B. Dunn, J. nat. Cancer Inst., 1947, Vol. 7, p. 455.
15. H. B. Andervont, J. nat. Cancer Inst., 1958, Vol. 20, p. 431.
16. I. Berman. In the book: Advances in the Study of Cancer [Russian translation], Vol. 5, p. 142, Moscow, 1960.
17. J. Grinstein. The Biochemistry of Cancer [Russian translation], p. 77. Moscow, 1951.
18. E. Cowdrey. Cancer Cells [Russian translation], pp. 358, 440. Moscow, 1958.
19. A. H. M. Kirby, Nature, 1944, Vol. 154, p. 668.
20. G. A. Miller and E. C. Miller. In the book: Advances in the Study of Cancer [Russian Translation], Vol. 1, p. 7. Moscow, 1955.
21. E. C. Miller and G. A. Miller, Cancer Res., 1947, Vol. 7, p. 468.
22. O. Mühlbock and T. G. Von Rijssel, J. nat. Cancer Inst., 1954, Vol. 15, p. 73.
23. I. A. Parfentjev and W. A. Perlzweig, J. biol. Chem., 1933, Vol. 100, p. 551.
24. H. W. Rumsfeld, Jr., W. L. Miller, Jr., and C. A. Baumann, Cancer Res., 1951, Vol. 11, p. 814.
25. T. Sasaki and T. Yoshida, Arch. path. Ant., 1935, Bd. 295, S. 175.
26. H. Selye, J. Urol. (Baltimore), 1939, Vol. 42, p. 637.
27. K. Sugiuira, C. R. Halfer, C. J. Kensler, et al., Cancer Res., 1945, Vol. 5, p. 235.
28. L. F. Wicks, Proc. Soc. exp. Biol. (N. Y.), 1941, Vol. 48, p. 395.

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