THE SEARCH FOR ANTI-TUMOR PREPARATIONS AMONG THE DERIVATIVES OF BENZIMIDAZOLE

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Benzimidazole is an analogue of purine and differs from the latter in that instead of a pyrimidine ring it contains a benzene ring.

The benzimidazole ring plays an important part in metabolism (enters into the composition of vitamin B_{12}, participates in the synthesis of thymine nucleosides).

On the other hand Woolley [1] has shown that benzimidazole added to the nutrient medium inhibits the growth of yeast and of some bacteria. The inhibitory action of benzimidazole can be abolished by adenine or guanine.

Taking these data into consideration with respect to the intensive synthesis of nucleic acids in malignant tumors, L. F. Larionov and L. L Malyugina [2] undertook the study of the effect of benzimidazole and some of its derivatives on tumors in animals.

The compounds were synthesized by O. F. Ginzburg and L. S. Efros (1952-1956) in the Laboratory of Organic Dye-stuffs (Director B. A. Poral-Koshits) of the Lensovet Technological Institute, Leningrad.

However, none of the derivatives of benzimidazole obtained (with substituents in position 2, which included methyl, phenyl, benzyl and pyridyl) showed any activity against transplanted tumors in rats and mice.

The problem in the present work was to investigate the possibility of using benzimidazole as a carrier of an anti-tumor active chemical group and as its "conductor" into the tumor.

METHODS AND RESULTS

Six derivatives of benzimidazole, prepared by O. F. Ginzburg, were studied; these were called "dimezoles" and included dimezole-7 (2-chloro-methylbenzimidazole), dimezole-8 (1-β-hydroxyethylbenzimidazole), dimezole-9 (1-β-chloroethyl-2-chloromethylbenzimidazole), dimezole-12 (1-β-chloroethyl-2-benzylbenzimidazole), dimezole-13 [1-β-chloroethyl-2-bis-(β-chloroethyl)-aminomethylbenzimidazole] and dimezole-14 [2-bis-(β-chloroethylaminomethylbenzimidazole)].

Investigation of the benzimidazole derivatives was started with determination of their toxicity. Experiments with single administration of the substances to rats (80) showed that dimezole- 11, 12 and 13 were non-toxic, since doses of 2.5-2 and 0.5 g per 1 kg body weight did not cause death of the animals. Dimezole-7 is slightly toxic – 30% of the rats succumbed to a dose of 1.5 g/kg body weight. Dimezole-9 is rather more toxic; 80% of rats succumbed to a dose of 0.5 g/kg. According to the data of L. F. Larionov and L. L. Malyutina [2] the lethal dose of dimezole-8 (for mice) is 1.5 g per 1 kg. Dimezole-14 is the most toxic, the 100% LD for it being 0.5 g/kg and the 50% LD about 0.1 g/kg.

The anti-tumor properties of the preparations were studied on transplanted tumors: Ehrlich's, sarcoma 45 and Gueren's carcinoma. A total of 370 mice and 500 rats was used.

Experiments were always performed on measurable tumors. The tumors were measured with callpers in three directions. The arithmetic mean was calculated from the figures obtained and this served as the mean diameter of the tumor. The results were evaluated by percentage inhibition of tumor growth. The data were treated statistically by the modified method of Student as described by V. I. Romanovsky [3] (the difference was considered significant if $t = 0.955$).

Experimental results are summarized in the table.

The Effect of Benzimidazole Derivatives on Transplanted Tumors

Preparation	Tumor Strain	Dose in mg/kg	No. of animals	% Inhibition	Criterion of signif.
Dimezole-7	Ehrlich's tumor	275	20	—12	0.397
	Sarcoma 45	275	25	21	0.730
Dimezole-8	Ehrlich's tumor	300	15	20	0.661
Dimezole-9	Ehrlich's tumor	50	120	40	0.987
	Sarcoma 45	50—60	140	18	0.379
Dimezole-12	Sarcoma 45	300	30	30	0.979
		250	30	—30	0.723
		300	—	—20	0.838
Dimezole-13	Sarcoma 45	400	25	45	0.927
Dimezole-14	Ehrlich's tumor	5—7	145	40	0.998
	Sarcoma 45	3.5	60	95.1	0.997
	Guerin's carcinoma	3.5	30	70	0.979

Note. All preparations were administered per os except dimezole-8 which was given intraperitoneally.

The table shows that neither dimezole-7 nor dimezole-8 exerted any appreciable effect on the growth of Ehrlich's tumor. Nor does dimezole-7 inhibit the growth of sarcoma 45.

Dimezole-9 produces noticeable inhibition of the growth of Ehrlich's tumor but not of sarcoma 45.

Dimezole-12 inhibited the growth of sarcoma 45 in one experiment by 30% but in other experiments produced statistically unreliable stimulation of the growth of the same tumor.

Dimezole-13 failed to show anti-tumor activity since the inhibition obtained in 45% proved to be non-significant.

Definite anti-tumor activity was found in dimezole-14. In daily doses of 3.5 mg/kg or 15-20 mg/kg given every 72 hours, this preparation not only produced strong inhibition of the growth of sarcoma 45 (by 95%) but also caused resolution of this tumor in 34% of the rats if the tumor weighed about 1 g at the beginning of treatment. No such resolution was observed on treating rats with large tumors (about 4 g) even when toxic doses were used, but tumor growth was inhibited by 90%.

Anti-tumor activity of dimezole-14 is also seen in the case of other transplanted tumors. It inhibits the growth of Ehrlich's tumor in mice by 40% and of Gueren's rat carcinoma by 70%.

Rats treated with dimezole-14 showed a $1^1/_2$-2-fold decrease in the weight of the spleen as compared to control animals.

Investigation of the effect of dimezole-14 on hemopoiesis was carried out on 10 rabbits; dimezole-14 was introduced into the stomach for 8 days. Daily leucocyte counts and determinations of the leucocyte formula were done. In addition, at the beginning and end of the experiment examination of bone marrow (puncture of the tibia) was performed, and when the animals were sacrificed the hemopoietic organs were examined microscopically (bone marrow, spleen, thymus and lymph nodes). The results of these experiments showed that dimezole-14 under these conditions had a weak depressing effect on hemopoiesis.

Of the 6 derivatives of benzimidazole investigated considerable anti-tumor activity was thus found in one preparation – dimezole-14 – which has one bis-(β-chloroethyl) -amine group linked with the ring by a CH_2 group.

The results of this investigation suggest that further search for anti-tumor preparations in this group of compounds is promising.

SUMMARY

The authors studied the effect of C derivatives of benzimidazole on the transplanted tumors of mice and rats. Pronounced anti-tumor activity was revealed in dimezole-14 [2-bis-(β chlorethylaminomethylbenzimidazole)]. Dimezole-14 not only inhibits the growth of sarcoma 45, Gueren's carcinoma and Ehrlich's tumor, but even causes resolution of sarcoma 45 in certain rats. This shows that the search of anti-tumor preparations among derivatives of benzimidazole has important prospects.

LITERATURE CITED

[1] D. W. Woolley. J. Biochem., 1944, 152, 225.

[2] L. F. Larionov. In the book: 30 Years of the Institute of Oncology,* 1956, pp. 37-42.

[3] V. I. Romanovsky. The Use of Mathematical Statistics in Experimental Work,* 1947.

* In Russian.