The influence of N-methyl-N-β-chloroethyl hydrazines on the mitotic index of Ehrlich ascites tumor cells and the leukopoiesis of the mouse

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Summary. The cytostatic activity of N'-methyl-N'-β-chloroethylbenzaldehyd hydrazone (B1) is at least equal to that of procarbazine when its effect is tested with the Ehrlich ascites tumor cells of the mouse and the Yoshida sarcoma of the rat. B1 causes a slighter decrease of mitotic cells and no shift from prophase to metaphase. These results suggest that the cytostatic effect of B1 is due to interference with cell metabolism or an effect at the cell membrane and not to an effect on cell proliferation. This assumption is supported by a considerable depression of lymphocytes and a minor effect on granulopoiesis, which is especially sensitive towards proliferation toxins. All these findings suggest a different mechanism of action of B1 and procarbazine.

Previous experiments[1,2] have shown the fungistatic qualities of N-methyl-N-β-chloroethylhydrazine (A1) and its benzaldehydhydrazone (B1). Comparing their cytostatic activity with that of equimolar doses of procarbazine, a monomethyl hydrazine derivate, we could achieve a prolongation of the survival time of mice with Ehrlich ascites tumor (EAT) and rats with Yoshida sarcoma[3]. In contrast to procarbazine, both substances exhibited an excellent in vitro activity: A1 interferes with DNA and RNA synthesis, the less toxic B1, in addition, inhibits the cellular uptake and the phosphorylation of nucleosides[4]. We now investigated the importance of these different mechanisms during the cell cycle in vivo and determined the mitotic index of the Ehrlich ascites tumor cells of the mouse under the influence of the β-chloroethylhydrazines. The influence on leukopoisis was studied in mice treated with therapeutic doses of B1 because of the well-known depression of leukocytes which is a side-effect of most radiomimetic agents. Control experiments were performed with procarbazine.

Methods. Mitotic index. 6×10^6 cells of a hyperdiploid strain of Ehrlich ascites tumor cells were injected into the peritoneal cavity of male albino mice (NMRI, 23 g). 7 days later, groups of ten animals were formed and the substances were applied intraperitoneally as a single injection dissolved in 0.2 ml phosphate buffer. Because of its higher toxicity, A1 was used in a concentration half of that of B1 and procarbazine which were equimolar. A control group received 0.9% saline. At the time indicated in table 1, 0.2 ml ascites were aspirated under sterile conditions and an air-dried smear was stained with orcein[5]; 500 cells were counted per slide.

Leukopoiesis. A1, B1 and procarbazin were injected intraperitoneally (see above) either as a single dose or as a daily injection for 14 days. (For details see table 3.) The leukocyte count was determinated and a smear was stained by the Pappenheim procedure[6].

Results. Mitotic index. All 3 hydrazine derivates inhibit mitosis in a dose-dependent correlation (table 1). The results for procarbazine are in accordance with those described in the literature[7,8]. Both procarbazine and A1 cause a considerable decrease of the mitotic index to 4–6‰ after 8 or 4 h of high dose treatment, respectively. The effect of B1 reaches its maximum with 9.6‰ after 2 h.

If only half the dose is used (which is approximately the therapeutic dose for a treatment of the EAT of the mouse). A1 and procarbazine again cause a significant depression of the mitotic index after the same periods of time ($p < 0.01$). In contrast to these results, no significant effect was observed 2 h after application of B1. Table 2 shows the distribution of the different mitotic phases under the high-dose-treatment. Procarbazine

Table 1. Mitotic index of Ehrlich ascites tumor cells in ‰ after treatment with A1, B1 and procarbazine

Substances mg/kg		Time in h 0	2	4	8	12	24	48	72
Control		26.2	26.6	28.9	31.1	37.7	31.5	26.3	31.4
62,5	A1	27.3	29.4	14.9	22.0	33,1	34.9	30.9	34.3
125	A1	26.6	18.4	6.0	7.3	12.5	21.3	29.3	19.6
225	B1	29.8	22.4	26.6	32.7	34.0	37.8	29.7	40.3
450	B1	32.0	9.6	12.0	13.2	14.5	26.4	31.2	25.2
250	Procarbazine	34.4	37.8	20.3	14.0	14.9	28.3	32.0	34.0
500	Procarbazine	37.2	21.7	5.6	4.3	8.5	28.0	26.0	28.9

Table 2. Phase ratio of mitosis at Ehrlich ascites tumor cells after single injection of B1 and procarbazine

Substances mg/kg	Time after single injection in h	Phase ratio in % Prophase	Metaphase	Ana- and telophase
Control	8	40.1	46.0	13.9
	24	32.1	41.6	26.4
	48	35.6	42.7	21.7
	8	21.7	59.4	18.9
A1	24	34.2	50.1	15.8
125	48	30.0	47.7	22.3
	8	25.5	49.6	24.9
B1	24	32.7	45.6	21.7
450	48	35.5	47.7	16.8
Pro-	8	10.9	60.0	29.1
carbazine	24	19.6	62.3	18.2
500	48	21.7	58.3	20.0

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Table 3. Effect of B1 and procarbazine on the leukopoiesis in mice

Substances mg/kg		Control	%	Day 3	%	5	%	Weeks 1	%	2	%	3	4	%	6	%
B1	Leukocytes	8900	100	5955	100	5545	100	7215	100	5715	100	6500			10330	
1×150	Lymphocytes	7085	79.6	4465	75.0	3620	65.3	4850	67.2	4380	76.6					
	Neutrophils	1750	19.7	1400	23.5	1845	33.3	2380	33.0	1325	23.2					
Procarbazine	Leukocytes	8900	100	3255	100	2835	100	3000	100	2400	100	1845			5965	
	Lymphocytes	7085	79.6	2590	80.3	2220	78.0	2315	77.1	1630	67.8					
1×165	Neutrophils	1750	19.7	620	19.2	600	21.3	665	22.9	760	32.0					
B1	Leukocytes	8900	100							9310	100		8300	100	6380	100
14×65	Lymphocytes	7085	79.6							5690	61.1		6690	80.6	4940	74.4
	Neutrophils	1750	19.7							3575	38.4		1585	19.1	1360	21.3
Procarbazine	Leukocytes	8900	100							3630	100		3080	100	2150	100
	Lymphocytes	7085	79.6							2350	64.8		2030	65.9	1150	53.6
14×50	Neutrophils	1750	19.7							1260	34.7		1020	33.1	975	45.3

causes a significant, long lasting shift from prophase to metaphase, an effect also found by Rutishauser and Bollag[9] in the case of 1-methyl-2-benzyl hydrazine. A similar but shorter shift is found for A1 after 8 h, whereas the distribution pattern of B1 does not differ from control. Thus, the prolongation of the interphase for B1 is not due to a direct influence on mitosis.

Leukopoiesis. We investigated the leukopoiesis after treatment with a single high equimolar dose of B1 and procarbazine and after 2 weeks of daily application of 1/10 DL_{50} (*mole B1/mole procarbazine* ≈ 1.5). Both drugs cause a depression of leukocytes, which is more intensive and lasts longer with procarbazine. The number of leukocytes reaches its minimum after 3 weeks of procarbazine treatment and is still below control values after 6 weeks, whereas B1 causes its maximal depression already after 5 days, but in this case the leukocytes recover at least after 6 weeks. Furthermore, the depression under B1 mainly concerns the differentiation of lymphocytes and barely affects the granuopoiesis. Compared with these effects, procarbazine causes a severe granulopenia. The lower toxicity of B1 becomes more distinct when the analysis is done after the 2 weeks' treatment. After application we could not determine any change of the leukocyte numbers. Under these conditions, procarbazine causes a long-lasting reduction of the myeloic and even a greater decrease of the lymphatic cells. The results are compatible with those obtained by Bollag in rats[10].

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Folic acid and the inhibition of brain L-glutamic decarboxylase

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Summary. Folic acid competitively inhibited brain L-glutamic decarboxylase ($K_i = 1.62 \times 10^{-3}$ *M*). This inhibition could possibly be associated with epilepsy.

Introduction. There is a possible link between epilepsy and folic acid metabolism. The administration of folate to rats induces convulsions[2,3]. Moreover, it has been observed that the folic acid content is increased in experimental epileptic cobalt foci[4].

In epileptic patients undergoing anticonvulsant drug therapy a reduction in serum folate levels has been measured[5]. Indeed, the development of megaloblastic anaemia has been noted in such patients[6]. Folate administration, intended to counteract this deficiency, has been reported to result in an increased frequency of seizures[7,8].

An explanation of the biochemical basis for the convulsant action of folic acid has not yet been forthcoming. However, Roberts[9] has demonstrated that glutamate uptake by nervous tissue is competitively inhibited by folate. This is of interest since glutamate has been proposed as an excitatory transmitter in the brain[10]. Several drugs that can induce convulsions have been shown to inhibit brain L-glutamate decarboxylase (GAD)[11–14].

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