	Group 1 08.00 h (9 rats)	p values	Group 1 17.00 h (9 rats)	Group 2 08.00 h (9 rats)	p values	Group 2 17.00 h (9 rats)
Body weight (g)	229 ± 23	NS	218 ± 15	209 ± 17	NS	220 ± 21
Stomach weight (g)	9.6 ± 2.2	$p<0.001$	4.0 ± 1.6	3.2 ± 1.7	$p<0.001$	10.0 ± 2.0
Liver weight (g)	10.01 ± 1.48	$p<0.02$	8.76 ± 1.48	8.46 ± 1.22	$p<0.001$	10.89 ± 1.51
Liver weight/body weight	0.043 ± 0.004	NS	0.040 ± 0.006	0.041 ± 0.006	$p<0.01$	0.049 ± 0.007
Bile flow µl min^{-1} 100 g $b.wt^{-1}$	9.0 ± 1.5	$p<0.01$	7.5 ± 1.2	8.6 ± 1.0	$p<0.01$	9.6 ± 1.1
Bile flow µl min^{-1} g $l.wt^{-1}$	2.2 ± 0.5	$p<0.01$	1.8 ± 0.3	2.1 ± 0.4	NS	1.9 ± 0.3
Erythritol B/P ratio	1.14 ± 0.20	NS	1.18 ± 0.13	1.13 ± 0.22	NS	1.10 ± 0.14

* Values are means ± 1 SD.

Discussion. Normally rats eat during the night and sleep during the day[7]. When the food schedule is reversed they are perfectly able to eat during the light period, as shown in group 2 by the increase in s.wt, l.wt and l.wt/b.wt ratio at the end of the light period.

As shown before, bile flow, in the normal food and light schedule, is higher in the morning than in the evening whether expressed per 100 g $b.wt^{-1}$ or g $l.wt^{-1}$ [6]. When food is only available during the light period, bile flow is higher at the end of the feeding period but only when expressed per 100 g $b.wt^{-1}$. Therefore food seems to be a major stimulus for bile secretion. Rats do not have a gallbladder; therefore bile cannot be stored. The increment in bile flow was of canalicular origin as suggested by the constant B/P erythritol ratio. In these experiments we have not measured the bile salt secretory rate, therefore it is difficult to speculate whether the bile salt dependent or non-dependent fraction is responsible for the increment. The bile salt secretory rate follows a circadian rhythm[5] with a peak at midnight. The bile salt non-dependent fraction is also increased during the feeding period compared to the fasting period[6]. The reasons for the bile flow increment in relation to food are unknown. During digestion, hepatic blood flow increases. In the isolated perfused rat liver, bile flow was shown to be largely independent of hepatic blood flow[8]. Blood levels of several hormones rise during digestion. Insulin and glucagon increase the bile acid independent fraction[9,10]. Secretin increases mainly canalar bile flow when injected at very high dose via the portal vein or the hepatic artery[11]. Cholectystokinin seems to increase both canalicular and ductular bile flow[12]. Gastrin does not increase bile flow[12]. It is difficult to know whether those hormones play a physiological role.

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The influence of a new antitumor agent on hemopoietic colony forming cells

W.D. Gassel, H. Laukel, R. Braun and G. Wolf

Medizinische Poliklinik, Robert-Koch-Strasse, D-3550 Marburg a.d. Lahn, Institute of Pharmacology and Toxicology, Lahnberge, D-3550 Marburg a.d. Lahn (Federal Republic of Germany), 29 November 1977

Summary. The cytostatic and immunsuppressive agent N′-methyl-N′-β-chloroethylbenzaldehyde hydrazone (B1) in in-vitro experiments has a stimulating effect on colony-forming culture (CFUc) of bone marrow from C57BL mice. This unusual behaviour, which is in contrast to other cytostatics, could also be observed in vitro with CFUc obtained from mice treated with therapeutic doses of B1 for 2 weeks. This stimulation is not a particular effect of B1 alone but seems to depend on a synergistic effect of the combination of B1 and the colony-stimulating activity (CSA) present in the serum from endotoxin-treated mice (MP) in the testing system. The results suggest that the described effect of B1 is due to an interference at the cell membrane of CFUc or their precursor cells.

In previous papers we have reported that N′-methyl-N′-β-chloroethylbenzaldehyde hydrazone (B1), whose structure is similar to that of cytostatic mustards and methylhydrazine derivatives, has cytostatic[1,2] fungistatic[3,4] and powerful immunosuppressive[5] qualities. In mice (NMRI) a high dose of B1 (150 mg/kg) caused a depression of leucocytes which affected lymphocytes more strongly than the granulocytes. After 2 weeks with a daily dose of 65 mg/kg of B1, there was an unexpected increase in the number of neutrophils[6]. For this reason we studied the influence of B1 on the colony-forming cells (CFUc) of the bone marrow from C57BL mice in vitro and from mice which were treated with a daily dose of 15 mg/kg and 75 mg/kg for 2 weeks. Furthermore, the colony-forming capacity of bone marrow from mice treated with a single dose of 400 mg/kg B1 was tested.

Methods. In the in-vitro experiments, colony-forming cells (CFUc) were tested in an agar system described by Pluznik

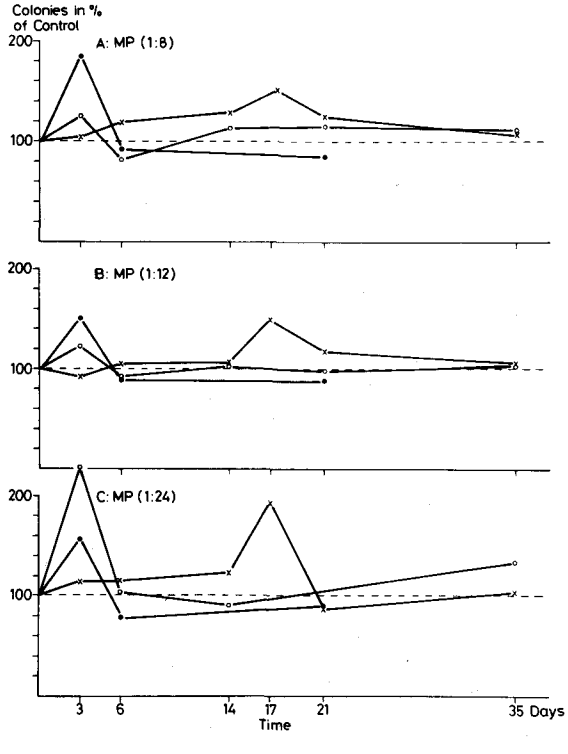
Table 1. Effect of B1 on femoral CFUc from C57BL mice in vitro (mean values ± SE; n = 3)

Molar concentration of B1	Dilution of MP-serum 1:4 CFUc per 7.5×10^4 cells	Percent	1:8 CFUc per 7.5×10^4 cells	Percent	1:16 CFUc per 7.5×10^4 cells	Percent
Control	158 ± 6.2	100	86 ± 4.2	100	60 ± 3.6	100
2×10^{-5}	132 ± 4.3	84	63 ± 3.2	73	51 ± 4.1	85
10^{-5}	155 ± 1.8	98	127 ± 2.9	148	71 ± 3.8	135
2×10^{-6}	155 ± 5.2	98	109 ± 4.9	127	131 ± 2.2	218
10^{-6}	186 ± 3.8	118	195 ± 3.6	227	133 ± 5.4	222
4×10^{-7}	181 ± 6.7	115	124 ± 5.2	144	75 ± 3.0	125
2×10^{-7}	224 ± 5.8	142	129 ± 4.0	150	62 ± 3.1	103

Table 2. Colony forming capacity per 7.5×10^4 nucleated marrow cells from C57B1 mice after preincubation with B1 for 30 or 60 min (mean values ± SE; n = 3)

Molar concentration of B1	Dilution of MP-serum 1:4 30 min Colonies Plate	Percent	60 min Colonies Plate	Percent	1:8 30 min Colonies Plate	Percent	60 min Colonies Plate	Percent
Control	63 ± 2.8	100	63 ± 3.6	100	40 ± 2.4	100	40 ± 4.2	100
10^{-5}	94 ± 3.5	148	67 ± 2.4	106	36 ± 3.2	90	32 ± 3.8	79
5×10^{-6}	111 ± 6.3	176	67 ± 4.1	106	59 ± 2.7	146	35 ± 5.1	86
2×10^{-6}	115 ± 4.2	184	75 ± 5.1	118	61 ± 3.8	152	37 ± 4.3	91
10^{-6}	112 ± 5.8	177	79 ± 3.9	125	70 ± 4.2	174	33 ± 2.1	81
4×10^{-7}	101 ± 4.6	160	80 ± 2.7	126	56 ± 3.1	131	42 ± 2.8	105
2×10^{-7}	126 ± 3.2	199	56 ± 3.4	88	64 ± 4.2	158	43 ± 4.0	107
10^{-7}	122 ± 3.6	193	59 ± 2.8	93	59 ± 3.2	146	41 ± 2.2	102

and Sachs[7] and Bradley and Metcalf[8]. The femoral bone marrow cells of at least 2 male mice (C57BL, Hannover) were pooled and adjusted to a final concentration of 7.5×10^4 cells/ml culture. Various dilutions of heat inactivated (40 min, 56 °C) pooled serum (MP) from mice treated with an endotoxin (*Salmonella abortus equium*, Difco) was used as source of colony-stimulating activity (CSA). B1 was diluted in 0.1 ml Eagle's medium in various concentrations (see table 1) and pipetted into the Petri dishes. Colonies were counted on the 7th day of culture.



Effect of a single dose (400 mg/kg, ● —— ●) and a treatment for 2 weeks with daily 15 mg/kg (× —— ×) or 75 mg/kg (○ —— ○) of B1 femoral CFUc from C57BL mice (MP-serum: *A* 1:8, *B* 1:12, *C* 1:24; mean values±SE; n = 3).

For the in-vivo experiments B1 was injected i.p. either as a single dose (400 mg/kg) or as a daily injection (15 or 75 mg/kg) for 14 days. At days 3, 6, 14, 17, 21 and 35 after the beginning of the therapy, 2 animals were sacrified and the bone marrow contents of CFUc was examined as described above.

Results. In-vitro experiments. As shown in table 1, a clear increase in colony formation was found when CFUc from untreated animals were plated with B1. The effect of B1 seems to be dose-dependent and dependent on the concentration of the stimulus (CSA). At lower concentrations of CSA, the maximum colony formation is found at higher concentrations of B1. Furthermore, it is conspicuous that lower concentrations of B1 are more effective than higher ones. It seems there is a synergism between B1 and CSA.

Table 2 presents comparable results of a preincubation experiment. Here bone marrow cells were preincubated with the same concentration of B1, but, in contrast to the previous experiment, the stimulation by CSA began after 30 or 60 min. In both cases no cytostatic or cytotoxic effect could be observed. Marrow cells incubated for 30 min with B1 showed a clear increase in colony formation as compared with the control. Prolongation of the incubation period caused a minor increase (MP 1:4) or no alteration compared to the controls (MP 1:8).

This new effect of B1 is also maintained when it is given as an i.p. injection. The data of this experiment are presented in the figure. 3 days after a single B1 dose (400 mg/kg), an increase in colony formation was found. On days 6 and 21 after the treatment, CFUc showed a nearly equal decrease of about 10–15% of the control animals.

2 other experiments were performed with a daily injection of 15 and 75 mg/kg of B1 for 2 weeks. At the lower dose, a slight increase of the colony formation during the therapy could be observed. 3 days after discontinuation of the therapy, the colony formation reached a maximum and

then normalized by day 35. In contrast to these results, at the higher dose the colony formation resembles the data obtained after the single dose of 400 mg/kg B1. The maximum was found on day 3. From day 6 the values are comparable to the control. In these in-vivo experiments, there is no recognizable dependence of dilution of MP.

This is to our knowledge the first time that a CFUc stimulating effect by a cytostatic drug has been observed. All previous publications employing cytostatics in vivo or in vitro in this agar culture system showed a decrease in the number of leucopoietic stem cells[9]. This finding is in particular described for nitrogen mustard and the hydrazine derivative procarbazin[10]. In some cases the decrease is followed by a later rebound increase. The effect of B1 seems to depend on a direct action on the CFUc or its precursor(s), as the increase in colonies not only occurred during the treatment of the animal, but also when the bone marrow was incubated with this substance in vitro. The occurrence of the synergism between B1 and CSA, as shown in the in vitro experiments, may be explained by a greater sensitivity of CFUc to CSA under the influence of B1.

In Ehrlich ascites tumor cells (EAT), we could show that B1 is not able to penetrate the cell but affects the cell membrane. However, a penetration is probable when dimethylsulfoxid (DMSO 0.5% v/v) is present in the culture medium. Under this condition, the glycolysis of tumor cells was reduced vigorously by B1 in contrast to a strong stimulation in absence of DMSO[12]. Therefore we investigated in an additional experiment the influence on CFUc after preincubation with B1 for 5 and 30 min in the presence of DMSO (0,5% v/v) in the agar culture. As shown in table 3, the colony-forming capacity decreased strongly in contrast to the control when DMSO was present. Prolongation of the incubation time and lower concentrations of MP enhanced the inhibition.

As an indication of the membrane alteration caused by B1, we found an increase in SH-groups at the cell surface. The increase was 180% as compared with the control. This similar alterations at the surface of CFUc might be responsible for the higher sensitivity to CSA.

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Reconsideration of a test for dopaminergic stimulation: Inability of apomorphine to induce mouse jumping

M.J. Bannon and C.H. Jarboe

Department of Pharmacology and Toxicology, School of Medicine, University of Louisville, Louisville (Kentucky 40232, USA), 8 November 1977

Summary. Apomorphine was administered parenterally to mice in an unsuccessful attempt to induce amphetamine and L-3,4-dihydroxyphenylalanine-like elicited jumping. The efficacy of jumping behavior as an indicator of dopaminergic overstimulation is criticized in light of the results.

In recent years, many experimental models have been proposed for the study of dopamine systems in the brain[1,2] in an effort to elucidate the role they may play in such disorders as Parkinson's disease and schizophrenia. Several articles[3-5] have attributed amphetamine and L-3,4-dihydroxyphenylalanine (L-DOPA) induced mouse jumping to dopaminergic overstimulation similar to that yielding stereotypic behavior in rodents treated with amphetamine, apomorphine, or other drugs. If dopaminergic overstimulation were in fact responsible for the observed behavior then dopaminergic antagonists should effectively attenuate the effect. Lal et al.[5] showed dose-dependent antagonism of the jumping with neuroleptics, but not with other psychoactive agents, including apomorphine. It is reasonable that apomorphine, a dopamine like agonist[6], should induce or even potentiate behavior believed due to dopaminergic stimulation when administered subsequent to amphetamine and L-DOPA. In view of this anomaly, attempts were made to elicit mouse jumping with various doses of apomorphine and under the conditions reported[5].

Methods. Random bred, male Swiss Webster albino mice (Laboratory Supply Co.) were housed in air conditioned quarters with no light from 11.00–23.00 h, and were allowed food and water ad libitum. All animals weighed between 24–31 g. They were treated with i.p. apomorphine HCl dissolved in 0.9% saline. The mice were immediately placed in separate glass jars (15 × 24 cm) for observation. Control animals, as well as those treated with 5 mg/kg apomorphine or less were observed for 30 min. Mice treated with larger doses were observed for 1 h. As in previous reports, a jump response was defined as simultaneous lifting of all paws from the floor.

Results. Control animals averaged 1 jump during the observation period (table). Confirming earlier results[5], 64% of the controls did not jump, while 36% jumped 1–10 times. The mouse jumping caused specifically by combined amphetamine and L-DOPA treatment was not induced with apomorphine over a wide range of doses (table). If jumping were due to dopaminergic overstimulation, it is difficult to explain why apomorphine, an acknowledged direct dopaminergic agonist, failed to stimulate this behavior. It should be noted that stereotypy, a well recognized and much studied attribute of dopaminergic overstimulation[2], was seen with all but the lowest dose of apomorphine. While the duration and intensity of the stereotypic behaviors (gnawing, licking and sniffing) appeared to be dose dependent, experimental conditions did not lend themselves to accurate quantitation.

Discussion. The discrepancy between the present report and the results presented and conclusions drawn in previous papers[3-5] may be attributable to several factors. In contrast to the site specificity of apomorphine action, amphetamine