Table III. Aniline hydroxylase, Na-K-Mg-dependent ATPase and Mg-dependent ATPase activities in liver

Group	Additives		Aniline hydroxylase		Na-K-Mg-ATPase		Mg-ATPase	
	Diet	Water	3 months (nmoles *p*-aminophenol/min/mg protein)	7 months	3 months nmoles Pi/min/mg protein	7 months	3 months nmoles Pi/min/mg protein)	7 months
I	none	none	1.16 ± 0.06^{a}	1.48 ± 0.21^{a}	140 ± 7^{a}	155 ± 5^{a}	401 ± 34^{a}	328 ± 47^{a}
II	none	ABS	1.42 ± 0.12^{a}	1.22 ± 0.14^{a}	122 ± 21^{ab}	130 ± 7^{a}	424 ± 32^{a}	329 ± 56^{a}
III	PCBs	none	3.94 ± 0.38^{b}	4.19 ± 0.46^{b}	78 ± 8^{b}	76 ± 6^{b}	387 ± 31^{a}	342 ± 35^{a}
IV	PCBs	ABS	6.72 ± 0.57^{c}	6.90 ± 0.66^{c}	45 ± 12^{bc}	44 ± 10^{c}	343 ± 29^{a}	399 ± 85^{a}

Values are represent Mean $\pm$ SEM of 4 rats. Different letter superscripts (a, b, c) denote significant difference ($P < 0.05$) in each enzyme.

Discussion. Reports have confirmed that PCBs increased liver cholesterol levels[9,10] as well as microsomal drug metabolizing enzyme activity[11,12]. YAP et al.[13] have reported that PCBs inhibits Na-K-Mg-dependent ATPase activity in fish. The present study not only confirmed these results but clarified that the effect of PCBs on enzymes or cholesterol levels in the liver increased when PCBs and ABS were simultaneously administered.

Further investigation is expected to clarify the probability that ABS potentiates an increase in the toxicity of PCBs.

Zusammenfassung. Nachweis, dass in PCB vergifteten Lebern von Ratten die Quantität des Cholesterins und die Aktivität des Anilin-Hydroxylase-Enzyms zunahm, während sich die Na-K-Mg-abhängige ATPase verminderte. Gleichzeitige Verabreichung von ABS bewirkte eine signifikante Steigerung der PCB-Vergiftung.

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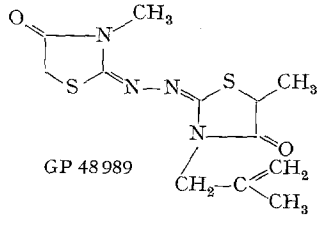
Carcinostatic Activity of a Thiazolidinonylidene-Hydrazonothiazolidinone Derivative Against DMBA-Induced Mammary Carcinomata in Female Sprague-Dawley Rats

The cytostatic agents currently in clinical use were originally selected on account of their inhibitory activity against transplantable tumours and leukaemias. With a view to finding new agents with different chemotherapeutic properties, we began to employ tests based primarily on chemically induced carcinomata. One such method which proved satisfactory in this respect was the technique of inducing mammary carcinomata in female Sprague-Dawley rats by administering DMBA (7,12-dimethyl-benz[a] anthracene), originally introduced by HUGGINS[1] for studies of hormonal dependence and sensitivity. In the present studies, some derivatives of a newly synthesized series of 2-[(4-oxo-2-thiazolidinylidene)hydrazono]-4-thiazolidinones were tested by this method. Among these, GP 48989, 5-methyl-3-(2-methylallyl)-2-[(3-methyl-4-oxo-2-thiazolidinylidene)hydrazono]-4-thiazolidinone, showed very promising carcinostatic activity and has been subjected to extensive investigations.



GP 48989

GP 48989 was prepared by reacting 1-acetyl-4-methyl-3-thiosemicarbazide with chloracetic acid, to form 3-methyl-2,4-thiazolidinedione-2-(2-acetylhydrazone). Treatment of the deacetylated hydrazone intermediate with 2-methylallyl isothiocyanate then yielded 3-methyl-2,4-thiazolidinedione-2-[4-(2-methylallyl)-3-thiosemicarbazone], which, after being reacted with 2-bromo-propionic acid, gave GP 48989, a white crystaline substance with an m.p. of 155–156 °C.

The carcinostatic activity of GP 48989 was assessed in the following way. The administration of a single oral dose of 15 mg DMBA in 1 ml sesame oil by stomach tube to 50-days-old female Sprague-Dawley rats induced mammary tumours in 90% of the animals. Histologically, 85% of the tumours were carcinomata, mostly adenocarcinomata. The growth of the tumours was estimated at intervals by comparing them with plastic balls of graded sizes. Between the 7th and the 20th week after the administration of DMBA, rats with 1–2 tumours of 8–12 mm in diameter were allotted at random to several groups of at least 7 animals each.

Various doses of GP 48 989 were administered by stomach tube on 5 days a week for 3, 6 or 12 weeks. In

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Table I. The effect of various doses of GP 48989 on regression of DMBA-induced mammary tumours in rats

Dose (mg/kg p.o.)	Number of animals		Number of tumours				Tumour regression (%)				New tumours	
	T[a]	C[a]	initial		final[b]		complete		partial		T	C
			T	C	T	C	T	C	T	C		
15×400	10	10	10	12	7	29	40	0	20	0	1	17
15×200	11	15	12	19	14	23	17	0	33	16	4	4
15×100	13	13	16	14	6	26	62	6	31	0	0	13
15× 50	14	14	16	16	17	44	13	0	44	0	3	28
30× 50	7	6	8	6	6	18	50	0	25	0	2	12
15× 25	9	10	13	13	15	22	8	0	39	23	3	9
30× 25	10	10	15	13	4	22	80	0	13	23	1	9
60× 25	7	9	7	10	10	40	86	0	0	10	9	30
15× 12.5	10	10	10	11	21	33	0	0	20	0	11	22
30× 12.5	9	10	9	11	7	35	56	0	33	0	3	24
60× 12.5	9	9	10	10	14	40	50	0	40	10	9	30
15× 6.25	10	10	10	11	26	33	0	0	30	0	16	22
30× 6.25	10	10	10	11	24	35	10	0	10	0	15	24
60× 6.25	9	9	9	10	16	40	22	0	11	10	9	30

[a] Treated/controls; [b] 10 days after treatment.

comparison with the incidence in untreated controls[2], the greatest reduction in the number of tumours visible upon gross inspection was noted in the animals treated with 30 or 60×25 mg/kg, in which 80 or 86% of the tumours had completely regressed (Table I). The administration of 15 consecutive doses of 100 mg/kg resulted in a decrease of 62%, and a 50% or greater regression was also observed after doses of 12.5 mg/kg (×30 or ×60) and 50 mg/kg (×30). Interestingly enough, doses in excess of 100 mg/kg were less effective and doses lower than 12,5 mg/kg seemed to be below the activity threshold.

As is shown in Table II, in a series of 6 tests carried out on 53 animals with altogether 57 tumours, treatment with 100 mg/kg p.o. (×15) resulted in the complete regression of 32 tumours (56%) and partial regression of 18 (31%); 13% of the tumours remained resistant. The development of new tumours during the treatment period was almost entirely prevented.

The duration of the remission of regressed mammary tumours following treatment with 15 consecutive doses of 100 mg/kg varied from 2 to 16 weeks. 30% of the tumours that had disappeared after treatment had still not reappeared 19 weeks after the withdrawal of the drug. The majority of recurrent tumours responded to a second course of treatment.

In another group of 48 rats, treatment with 100 mg/kg p.o. daily was started 6 weeks after the administration of DMBA and continued for 6 weeks (5 doses per week). In comparison with the findings made in 48 untreated controls, the appearance of tumours was almost completely suppressed during treatment. After treatment was stopped, the development of tumours was no longer inhibited (Figure 1).

The growth of Walker CaSa 256 was inhibited by treatment with GP 48 989 in oral doses of 50 mg/kg daily for 4 consecutive days, and the compound also showed some activity against the Ehrlich ascites carcinoma. Its effect on a series of transplantable tumours

[2] No placebo-treated control group was included in these experiments, since the vehicle used for the suspension of GP 48 989 had previously been shown to be indifferent.

Table II. Regression of DMBA-induced mammary carcinomata and inhibition of the appearance of new tumours under treatment with GP 48989

	GP 48989 (100 mg/kg/die)[a]	Untreated controls
Number of rats	53	58
Total number of tumours at start of experiment	57	60
Total number of tumours at end of experiment	27	120
Total number of tumours showing regression	50	2
a) complete	32	0
b) partial	18	2
Number of tumours showing no regression	7	58
New tumours during experiment[b]	2	60

[a] 15 oral administrations. [b] Included in the total number of tumours at end of experiment.

or leukaemias in mice and rats, however, was rather limited. DMBA-induced skin carcinomata in mice regressed in response to treatment with 250 mg/kg p.o. daily for 10 days.

Rats with DMBA-induced tumours tolerated the compound well. From the second week of treatment onwards, slight weightloss concurrent with reduced food consumption was noted. Four and 7 days, respectively, after treatment was with-drawn, food intake and weight-gain reverted to normal. Apart from a reduction in the size of the uterus, no abnormalities were observed upon macroscopic examination of the treated rats. Microscopically, increased lymphopoiesis was noted in the spleen. There were no changes in the white blood cell counts.

The median lethal oral and subcutaneous doses (LD_{50}) of GP 48 989 in rats and mice were greater than 5000 mg/kg. In view of its promising carcinostatic activity, GP 48 989 appears to merit thorough clinical investigation.

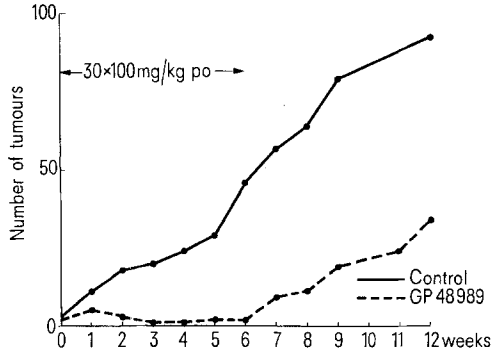

Fig. 1. Inhibition of the appearance of DMBA-induced mammary tumours in rats treated with GP 48 989.

Zusammenfassung. 5-Methyl-3-(2-methylallyl)-2-[(3-methyl-4-oxo-2-thiazolidinyliden)-hydrazono]-4-thiazolidinon erwies sich bei guter therapeutischer Breite als stark karzinostatisch wirksam bei DMBA-induzierten Mamma-Karzinomen weiblicher Sprague-Dawley Ratten. Eine geringere chemotherapeutische Wirkung fand sich auch bei Walker CaSa 256, DMBA-induzierten Hautkarzinomen und Ehrlich Ascites Karzinom.

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Cannabidiol and Cannabinol in Man

Evidence is rapidly accumulating that the major source of pharmacological activity of cannabis is due to Δ-9-tetrahydrocannabinol (THC) or its metabolites[1-3]. Although the Δ-8-THC isomer, normally a minor component of cannabis, is also active, its effects in man are qualitatively identical to those of the Δ-9-THC and only two-thirds as potent[4]. 2 other major cannabis constituents, cannabidiol (CBD) and cannabinol (CBN), have been considered to be inactive on the basis of animal studies. (Figure 1) Both these cannabinoids, as well as cannabichromene, cannabigerol and cannabicyclol were found to be inactive in monkeys, and did not alter the response of these animals to doses of Δ-9-THC given concurrently[5]. CBN in doses up to 180 mg/kg had no effect on key pecking of pigeons trained in an operant schedule[6]. As the possibility always exists that compounds may be metabolized differently in man than in other species, and that some found to be inactive in the latter may be active in man, both CBD and CBN were tested in volunteer subjects.

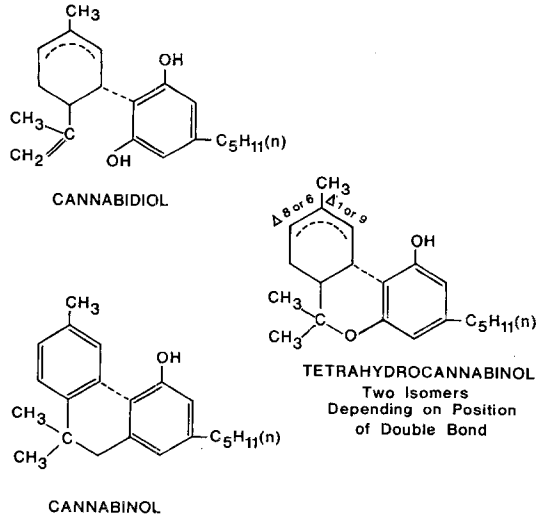

Fig. 1. Structural relationships between 3 major cannabinoids. The biosynthetic pathway is presumed to be from cannabidiol to tetrahydrocannabinol to cannabinol.

Methods and materials. The volunteer subjects were men, mostly in the 3rd and 4th decades of life. All had some prior experience with marihuana, and some with other pure cannabinoids, but none were more than casual social users of the drug. CBN and CBD were administered orally by laying an ethanolic solution of the drugs on chocolate cookies and then evaporating the solvent under nitrogen. This technique has been successfully employed in administering oral doses of THC. 6 subjects received oral doses of CBN ranging from 20 to 400 mg. 5 subjects received oral doses of CBD, ranging from 20 to 100 mg. CBD was given i.v. as well, the ethanolic solution being injected into a rapidly flowing stream of saline. 4 subjects received i.v. doses of CBD ranging from 5 to 30 mg. This technique has allowed i.v. injection of pharmacologically

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