Potentiating Effect of Thalidomide on Methylcholanthrene Oncogenesis in Mice

The teratogenic and neurotoxic effect of thalidomide in man are well known, but except for recently demonstrated teratogenesis in monkeys and rabbits, thalidomide has practically no toxicity in experimental animals[1]. An immunosuppressive effect was reported on the basis of a slight delay in rejection of skin homografts and cytologic changes in lymph nodes in thalidomide-treated mice[2,3]. A hormone-like effect was suggested by inhibition of hormone-dependent mammary tumors in thalidomide-treated rats[4]. Only 2 reports suggest that thalidomide may be oncogenic. Sarcomas developed at the site of injection of thalidomide in oil in 3 of 23 mice[5]. One armadillo developed a choriocarcinoma after receiving large doses of thalidomide in teratogenesis studies[6]. However, many drugs which are teratogenic are also oncogenic[7].

Previous studies in this laboratory[8] showed that mice treated concurrently with methylcholanthrene (MC) and certain antimetabolites such as iododeoxyuridine (IUDR) and chloramphenicol developed skin papillomas earlier and more often than controls given MC only. Possible mechanisms include direct effects on cells which increase the frequency of neoplastic transformation, or immunosuppression which impairs host surveillance mechanisms allowing more transformed cells to develop into tumors. In this paper we report studies in mice of the effect of thalidomide on antibody production and on skin papilloma response to MC.

Materials and methods. Four-month-old mice were non-inbred Swiss white females. Hair was removed from the back by mechanical clippers before MC was applied. Thalidomide (mp 272–273 °C) was suspended at 125 mg/ml in 0.5% type 20 high viscosity carboxymethyl cellulose (CMC) in water. It was prepared fresh daily by homogenizing in the CMC emulsion and was injected i.p., or given by gastric intubation (p.o.) using a curved ball-pointed needle, at a dose of 25 mg (0.2 ml) for 5 days in each of 4 consecutive weeks. Control groups which did not get thalidomide were given CMC solution or distilled water by the same routes and schedule. Both distilled water and CMC controls were used because it cannot be assumed that i.p. CMC is biologically inert[9].

A 1% solution of MC (Mann Research Laboratories New York City) in benzene was applied by dropping 0.2 ml onto the lower dorsal area for 5 consecutive days starting 1 week after the first dose of thalidomide. In one experiment this course was repeated for another 5 consecutive days after a 2-day interval. Thus the thalidomide was started 1 week before MC and was continued until 1 or 2 weeks after the MC was stopped.

Each mouse was inspected weekly, and the number and size of skin papillomas was recorded. Papilloma incidence was calculated as the number of mice bearing papillomas divided by the total number of mice at the start of the experiments.

For antibody studies, 4×10^9 sheep erythrocytes were injected i.p. and serums and spleens were collected 4 days later, when antibody production is near maximum in normal mice. After heating sera for 30 min at 56 °C, serial two-fold dilutions were prepared and 0.3 ml of diluted serum was added to 0.3 ml of 2% washed sheep erythrocytes, and 0.1 ml of 20% guinea-pig serum as a source of complement. 50% hemolysis titers were read visually after 2 h at 37 °C. Spleen cells were tested by the Jerne technique[10], in which spleen cells are mixed with sheep erythrocytes in agar, overlaid with guinea-pig complement and incubated. Production of hemolysin by individual spleen cells is revealed by small hemolytic plaques.

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Table I. Skin papillomas in mice following concurrent administration of methylcholanthrene and thalidomide

Treatment				No. and % of mice with papillomas at								
MC to skin	Thalidomide	Other	No. of mice[a]	4 weeks			8 weeks			12 weeks		
× 10	× 20 p.o.	-	31	6	19		8	26		9	29	
× 10	–	CMC × 20 p.o.	29	3	10		6	21		7	24	
Ratio, chi square, *p* values for above pairs				1.9	0.4	0.5	1.3	0.1	0.3	1.3	0.1	0.3
× 10	× 20 i.p.	–	30	7	23		10	33		12	40	
× 10	–	CMC × 20 i.p.	30	2	7		5	17		5	17	
Ratio, chi square, *p* values for above pairs				3.3	2.1	0.15	2.0	1.6	0.20	2.4	4.2	0.04
× 5	× 20 i.p.	–	60	25	42		35	59		–		
× 5	–	CMC × 20 i.p.	60	11	18		20	33		–		
× 5	–	Water × 20 i.p.	60	16	27		20	33		–		
Ratio, chi square, *p* values VS CMC controls				2.3	7.8	0.005	1.8	8.1	0.004	–		
Ratio, chi square, *p* values VS all controls				1.9	7.5	0.006	1.8	11.3	0.001	–		

MC, methylcholanthrene dissolved in benzene; thalidomide suspended in CMC; × 5, × 10, × 20, number of doses, one dose per day; CMC, 0.5% carboxymethyl cellulose in distilled water; p.o., per os, administered by gastric intubation; i.p., intraperitoneal; ratio, % papillomas after MC plus thalidomide divided by % papillomas after MC only; chi-square, computed as $\frac{(ab-cd-N/2)^2 N}{(a+b)(c+d)(a+c)(b+d)}$. [a] Mortality from all causes was only 5/300 and not over 1/30 in any group. For the calculations these dead mice were considered as having no papillomas.

Table II. Immune response of thalidomide treated mice and parallel controls 4 days after i.p. injection of 4×10^9 sheep erythrocytes

Day of immunization	Treatment	No. of mice	Serum hemolysin titer (tube No.) [c] Range	Median	Plaques per 10^6 spleen cells Range	Mean	Spleen weight (% of body weight) Range	Mean	Total spleen cells ($\times 10^6$) Range	Mean
—4 [a]	Thalidomide i.p. × 20	5	2–4	3	280–2310	1050	1.08–1.92	1.33	108– 368	170
	CMC i.p. × 20	6	4–6	5	850–3140	1600	0.80–1.14	0.92	63– 198	116
	Water i.p. × 20	6	4–8	5	590–2000	990	0.68–0.84	0.84	63– 149	103
2	Thalidomide i.p. × 20	4	5–6	6	3100–8200	5130	0.76–1.14	0.96	45– 86	66
	CMC i.p. × 20	6	4–6	4	1200–4200	2270	0.68–0.93	0.78	49– 63	58
	Water i.p. × 20	6	4–6	5	1140–3790	2780	0.57–0.74	0.74	45– 117	61
2	Thalidomide i.p. × 15	3	2–3	3	353– 750	580	–	–	219– 281	240
	CMC i.p. × 15	3	2–4	3	510– 680	620	–	–	162– 250	198
8	Thalidomide i.p. × 20	3	1–5	5	280–1020	770	–	–	–	–
	CMC i.p. × 20	3	2–4	4	310– 890	570	–	–	–	–
35 [b]	Thalidomide i.p. × 20	6	–	–	570–1160	760	–	–	170–1040	380
	CMC i.p. × 20	5	–	–	380– 870	640	–	–	141–352	211
	Water i.p. × 20	4	–	–	150– 615	320	–	–	185– 458	267
2	Thalidomide p.o. × 15	3	<1–4	2	120–1050	460	–	–	159– 178	166
	CMC p.o. × 15	3	2–4	2	430– 630	560	–	–	37– 134	88
8	Thalidomide p.o. × 20	3	3–4	4	380–1020	670	–	–	–	–
	CMC p.o. × 20	3	2–4	3	320– 590	430	–	–	–	–

For abbreviations and symbols see footnote to Table I. For dose schedules and techniques see text. [a] Day of immunization is relative to *last* dose of thalidomide (or CMC or water in control mice). That is '—4' is 4 days *before* the last dose of thalidomide, '2' is 2 days *after*. [b] The groups of mice which were immunized 35 days after the last dose of thalidomide, had also received 5 skin applications of MC during the second week of thalidomide administration. In each of the 3 groups (thalidomide, CMC or water) 2 or 3 mice had papillomas at the time of immunization. There was no difference between tumor-bearing and tumor-free mice in the spleen cell studies in any of the 3 treatment groups or in the combined data. [c] Tube No. 1 was 1:30 dilution of serum. Each successive tube was a further 2-fold dilution. That is tube 2 is 1:60, tube 3 is 1:120, etc.

Results. These massive doses of thalidomide had no discernable toxicity. Mice became somnolent after 4 weeks of thalidomide i.p. and their average body weight was 5% (1.5 g) less that the i.p. CMC controls, but weight returned to normal by 1 week after thalidomide was stopped. Thalidomide p.o. caused no somnolence or weight loss, which suggests that absorption of the drug was incomplete.

Mice given thalidomide i.p. had approximately twice as great a papilloma response to MC as did the controls. In the large experiment this difference was statistically significant. Mice given thalidomide p.o. also had a slightly greater papilloma response, but the difference from controls given only MC was not statistically significant. CMC alone did not affect the papilloma response to MC (Table I).

During i.p. thalidomide treatment, the mean hemolysin response was 1:120 and the average number of hemolytic plaques was 1050 per million spleen cells, as compared with 1:480 and 1600 respectively for the CMC-treated controls. This slight difference is probably not significant. Antibody production was not diminished in mice tested after thalidomide treatment was stopped. Spleen weights and total yield of nucleated cells per spleen were slightly greater in thalidomide treated mice than in the CMC or water controls (Table II).

Discussion. Although the local oncogenic effect of MC on the skin was significantly increased by i.p. thalidomide, these experiments did not reveal the mechanism responsible for the effect. Immunosuppression has been evoked as a plausible explanation of the 'joint oncogenic action' of other 'non-oncogenic' drugs such as IUDR and chloramphenicol[11]. In these studies we found no convincing evidence that thalidomide suppressed serum antibody, but the possibility that it suppressed immune reactions of the cell-mediated type, was not investigated, so an immunologic mechanism cannot be excluded. On the other hand, we cannot exclude a direct effect of thalidomide on skin cells such that the probability of neoplastic transformation was increased, even if thalidomide alone is not oncogenic[12].

Zusammenfassung. Durch Methylcholanthren-Bepinselung i.p. mit Thalidomid behandelter Mäuse entstanden mehr Hautpapillome als bei nicht mit Thalidamid behandelten Tieren. Der verantwortliche Mechanismus ist nicht bekannt. Ein analoges Phänomen wurde früher mit Immundepressiva festgestellt; eine Immundepression konnte jedoch für Thalidomid nicht nachgewiesen werden.

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