COMBINED ACTION OF A SINGLE APPLICATION OF 7,12-DIMETHYLBENZ(A)ANTHRACENE AND REPEATED APPLICATIONS OF BENZ(A)PYRENE ON THE SKIN

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UDC 616.5-006-092.9

During prolonged (for 52 weeks) application of a 0.001% solution of benz(a)pyrene (BP) in benzene after a single application of 30 or 50 μg 7,12-dimethylbenz(a) anthracene (DMBA) a synergic effect was observed: 79.4% of animals with tumors and 2.48 tumors per mouse after the combination with 30 μg DMBA and BP compared with 42.8% and 0.86 when 30 μg DMBA was given alone. After a combination of 100 μg DMBA and BP the corresponding figures were 100% and 3.32, and after application of 100 μg DMBA alone they were 76% and 2.24. It is concluded that the difference between the effects of a combination of two true carcinogens, on the one hand, and the effects of a combination of carcinogen and cocarcinogen, on the other hand, are quantitative and not qualitative in character. The role of dose of the agents in the effects of their combined action is discussed.

It has often been shown that the process of tumor development, when "started" by one carcinogen, can be "finished" by another [5, 14]. It has also been suggested [4] that the character of the effects of a combination of two carcinogenic agents is largely dependent on their dose. With rare exceptions [3], the doses used in combinations of true carcinogens were very large.

The object of the present investigation was to study the action of a combination of two carcinogenic polycyclic hydrocarbons given in the classical 2-stage scheme; a single application of one of them, used in the ordinary doses for "carcinogen–croton oil" experiments, followed by repeated application of the other carcinogen, in a subcarcinogenic dose [2].

EXPERIMENTAL METHOD

Male F_1 (C57BL × CBA) mice weighing 25-30 g were used. Six drops of a solution of 7,12-dimethylbenz(a)anthracene (BMBA), containing 30 or 100μg of the carcinogen, were applied once to the skin on the animal's back in the resting phase of the hair cycle. Daily application (two drops) of 0.001% of a solution of benz(a)pyrene (BP) in benzene was started 1.5 months later and continued for 1 year. Different combinations were used (five groups of animals): 1) 30 μg DMBA; 2) 30 μg DMBA + BP; 3) 100 μg DMBA; 4) 100 μg DMBA + BP; 50 0.001% benz(a)pyrene for 2 years. The mice in the first four groups were sacrificed 52 weeks after the beginning of BP application.

EXPERIMENTAL RESULTS

The first papillomas appeared in groups 1 and 2, 27 weeks, in group 3, 22 weeks, and in group 4, 19 weeks after the beginning of BP application. The frequency of the tumors and their latent periods are given in Table 1.

Laboratory of Prophylaxis of Carcinogenic Action, Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 73, No. 3, pp. 80–83, March, 1972. Original article submitted January 15, 1971.

TABLE 1. Frequency of Tumors and Their Latent Period after Separate and Combined Application of DMBA and BP

Group	Treatment	Number of animals	Number of animals with tumors			Number of papillomas				Mean latent period (in weeks)	
			abs.	%	P	total	per mouse	P	regression	three primary papillomas	all papillomas
1	30 μg DMBA	28	12	42,8	—	24	0,86±0,26		1	29,0	41,6±1,82
2	30 μg DMBA + BP	29	23	79,4	0,01*	72	2,48±0,42	0,01[1]	3	28,0	46,7±1,80
3	100 μg DMBA	25	19	76,0		56	2,24±0,41		4	23,3	41,8±1,41
4	100 μg DMBA + BP	25	25	100,0	0,025†	83	3,32±0,56	0,02[2]	7	21,6	41,6±1,18
5	0.001% BP for 2 years	30	—			—					

*Value of P compared with group 1.
†Value of P compared with group 3.

By the end of the period of observation, after application of 30 μg DMBA alone (group 1) tumors were present in 42.8% of animals, but when a single application of DMBA was followed by repeated applications of BP (group 2) tumors were present in 79.4% of mice. The mean number of papillomas per mouse also was increased: 0.86 in group 1 and 2.48 in group 2. After a single application of 100μg DMBA (group 3) tumors were found in 76% of mice, and their mean number per mouse was 2.24. With a combination of 100 μgDMBA and BP (group 4) the corresponding figures were 100% and 3.32.

Regression of the papillomas was more marked in the groups in which a higher dose of DMBA was used. In these groups regression of about 10% of all the developing papillmoas was observed.

The number of malignant tumors was small: in group 2 one keratoacanthoma with conversion into carcinoma was found, and in group 4 there were one carcinoma and two keratoacanthomas; in groups 1 and 3 there were only papillomas measuring 1-3 mm or, occasionally, 4 mm.

Repeated application of BP without preliminary application of DMBA (group 5) did not induce tumors for 1 year after the beginning of their application. Not until the end of the 2nd year (after 24 months) did a keratoacanthoma appear in one of the 16 mice which still remained alive.

During the period of observation five mice in group 1 and four mice in group 2 died from intercurrent infections (between the 48th and 56th weeks after application of DMBA); one mouse died in each of groups 3 and 4.

The results show that the process of carcinogenesis was definitely stimulated when the two agents were used in conjunction. The effect observed in these experiments can evidently be interpreted as synergic.

Comparison of the results of this experiment with the effect of a "carcinogen–croton oil" combination reveals similarities and differences. The similarity lies in the obtaining of an effect which is much greater than purely additive:this applies both to the number of animals with tumors and the mean number of tumors per animal. However, quantitative differences were present in this case: the mean number of papillomas per mouse after the combination of DMBA + BP was 2-3, whereas after the "carcinogen–croton oil" combination multiple papillomas are frequently observed (7-10 or even more per animal).

So far as the frequency of conversion of tumors into the malignant form is concerned, this was not significantly altered by the combination of the two carcinogens by comparison with the frequency of malignant tumors induced by DMBA alone. This largely resembles the typical experiments with the "carcinogen–croton oil (or Tween-60)" combination, when the papillomas in fact remained benign or even regressed, and evidence of malignancy developed only in isolated cases. However, this index is very relative even for the "carcinogen–croton oil" combination. When croton oil was used in sufficiently high concentrations and for

long enough [9, 10, 12, 13] a clear increase in the incidence of malignant tumors was observed. This was demonstrated most clearly by Roe and Clark [11]. The same result was observed when high doses of another weak carcinogen, Tween-60, was used [8].

Using highly active fractions of croton oil in combination with true carcinogens, Van Duuren [14] observed the appearance of malignant tumors in 40–60% of cases.

Frequent regression of papillomas (up to 60%) is characteristic of weakly active fractions of croton oil [7]; strongly active fractions induce regression much less frequently (0-8% according to Van Duuren [14]).

These results, in conjunction with those of the present investigation, suggest that the character of the combined effect of two carcinogens is largely dependent on the dose of the agents used. If the doses of the two agents are high enough, an additive effect or even the absence of effect of the weaker carcinogen under the particular experimental conditions used is more probable. If the doses of the agents are small, the probability of a synergic effect increases. This must be borne in mind when the carcinogenic risk of pollution of the external enviroment is assessed.

As regards the mechanisms of the synergic effect of a combination of two carcinogens nothing more subtantial than an hypothesis is possible at present. A single application of DMBA may perhaps induce changes in the skin as the result of which each subsequent dose of BP applied is retained for longer, as was demonstrated by Bock [6] who applied BP several days after DMBA or who applied BP to the previously irradiated skin [1].

The effect may be produced by both morphological and biochemical changes at the site of application of the carcinogen, when the character of the changes depend on the dose. Large doses give rise to a marked toxic effect followed by gross structural changes, while small doses merely change the character or intensity of metabolism of the active compound, thereby affecting sensitivity to the subsequent application of carcinogens.

LITERATURE CITED

1. L. A. Andrianov and V. S. Turusov, Vopr. Onkol., No. 10, 74 (1966).
2. L. A. Andrianov, The Time Factor and The Role of Depot Formation in Chemical Carcinogenesis in Mouse Skin. Candidate's Dissertation, Moscow (1968).
3. M. M. Baigusheva, Pat. Fiziol., No. 3, 30 (1967).
4. I. M. Neiman, Fundamentals of Theoretical Onocology [in Russian], Moscow (1961).
5. V. S. Turusov, Vopr. Onkol., No. 6, 108 (1969).
6. F. Bock, Nat. Cancer Inst. Monogr., 10, 361 (1963).
7. J. V. Frei, Cancer Res., 28, 947 (1968).
8. H. Rappaport and U. Saffiotti, Arch. Path., 61, 305 (1956).
9. F. J. C. Roe, Brit, J. Cancer, 10, 61 (1956).
10. F. J. C. Roe, Brit. J. Cancer, 10, 72 (1956).
11. F. J. C. Roe and J. Clark, Brit. J. Cancer, 17, 596 (1963).
12. M. H. Salaman and F. J. C. Roe, Brit. J. Cancer, 7, 472 (1953).
13. M. H. Salaman and F. J. C. Roe, Brit. Med. Bull., 20, 139 (1964).
14. B. Van Duuren, Progress in Experimental Tumor Research, 11, 31 (1969).