EFFECT OF METABOLITES OF 7,12-DIMETHYLBENZ(a)ANTHRACENE ON THE DYNAMICS OF ITS FLUORESCENCE IN THE SKIN OF HAIRLESS MICE

A. Kh. Lopp and G. A. Belitskii

UDC 577.158:547.674:578.001

The effect of two metabolites of 7,12-dimethylbenz(a)anthracene (DMBA) — 7-hydroxymethyl-12-methylbenz(a)anthracene and 7,12-dihydroxymethylbenz(a)anthracene — on the dynamics of its fluorescence in the skin of living mice was studied. The first metabolite did not change the dynamics of DMBA fluorescence whereas the second, if applied to the skin in equimolar proportions with DMBA, delayed its fluorescence. A similar effect was obtained with 7,8-benzoflavone — an inhibitor of DMBA metabolism.

KEY WORDS: carcinogenic hydrocarbons; metabolites; fluorescence; hairless mice; skin.

The study of photoactivated co-oxidation of polycyclic hydrocarbons has shown that under these conditions one hydrocarbon may stimulate or inhibit oxidation of the other [5]. A similar phenomenon is also observed in biological systems [9, 14, 19]. Meanwhile the problem of whether its derivatives can influence the metabolism of a carcinogenic hydrocarbon has not been studied.

It was therefore decided to study the dynamics of oxidation of 7,12-dimethylbenz(a)-anthracene (DMBA) in the presence of its hydroxymethyl derivatives: 7-hydroxymethyl-12-methylbenz(a)anthracene (7-OHM-12-MBA) and 7,12-dihydroxymethylbenz(a)anthracene (7,12-di-OHMBA). This paper gives the results of an investigation of the effect of hydroxymethyl metabolites of DMBA on the dynamics of its fluorescence after application to the skin of hairless mice.

EXPERIMENTAL METHOD

The following reagents were used: DMBA (Flucka), 7-OHM-12-MBA synthesized by the method of Bronovitskaya et al. [3], 7,12-di-OHMBA synthesized by the method of Badger and Cook [7], 7,8-benzoflavone (7,8-BF) (Chamapol), analytically pure distilled benzene.

Experiments were carried out on hairless female mice aged 3-4 months [10-12]. About 5 μg DMBA in benzene or a solution of DMBA mixed with one of its metabolites in molar proportions of 1:0.5 or 1:1 was applied to the skin of the animals in the interscapular region.

The dynamics of fluorescence of the polycyclic hydrocarbons in the skin was investigated with an apparatus consisting of a luminescence microscope, a photoelectric multiplier, and a recorder [2]. Measurements were made for 2 h after application, at intervals of 30 min, at 430 nm, corresponding to the principal maximum in the fluorescence spectrum of DMBA and of its above-mentioned metabolites at 20°C. At each time the intensity of fluorescence of the applied hydrocarbon was measured at nine points of each mouse after which the mean

---

Department for the Study of Carcinogenic Agents, Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR, Moscow. Sector of Destructive Processes, Institute of Chemistry, Academy of Sciences of the Estonian SSR, Tallin. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 80, No. 12, pp. 78-81, December, 1975. Original article submitted December 4, 1974.

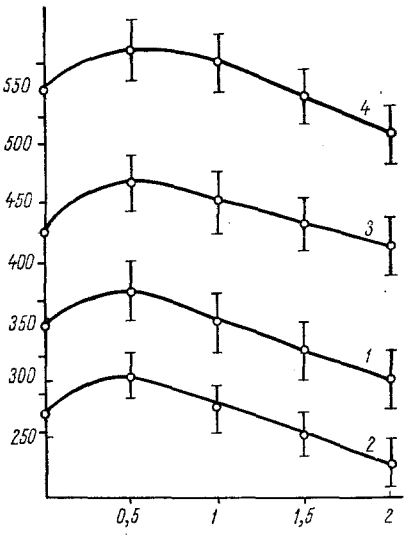

Fig. 1

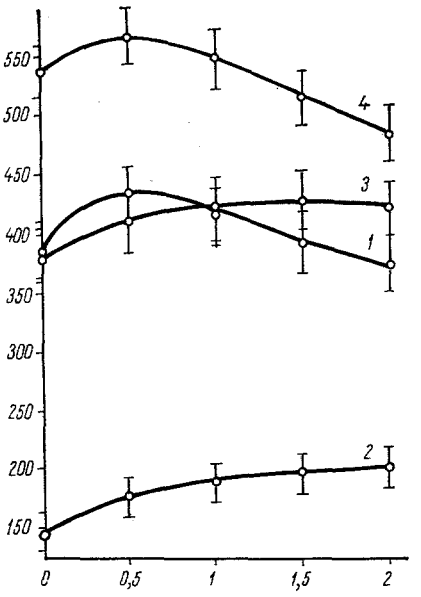

Fig. 2

Fig. 1. Dynamics of changes in intensity of fluorescence of DMBA and 7-OHM-12 MBA when applied separately and together to the skin: 1) DMBA; 2) 7-OHM-12-MBA; 3) DMBA + 7-OHM-12-MBA in the ratio of 1:1; 4) DMBA + 7-OHM-12-MBA in the ratio 1:0.5. Here and in Figs. 2 and 3: abscissa, time after application of carcinogen to skin (in h); ordinate, intensity of fluorescence in relative units (in μA).

Fig. 2. Changes in intensity of fluorescence of DMBA and 7,12-di-OHMBA when applied separately and together to the skin: 1) DMBA; 2) 7,12-di-OHMBA; 3) DMBA + 7,12-di-OHMBA in the ratio of 1:1; 4) DMBA + 7,12-di-OHMBA in the ratio of 1:0.5.

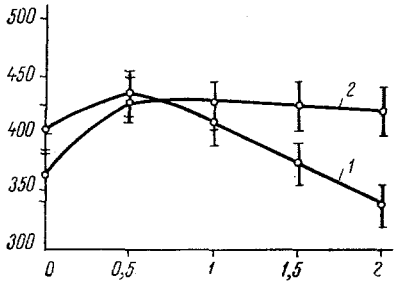

Fig. 3. Changes in intensity of fluorescence of DMBA when applied to the skin together with 7,8-BF: 1) DMBA; 2) DMBA + 7,8-BF in the ratio 1:0.5.

value was calculated [2]. The experimental groups each consisted of 6-9 mice and each experiment was repeated twice or three times. The curves were plotted from the mean value for a given group at a given time.

EXPERIMENTAL RESULTS AND DISCUSSION

Observations on fluorescence of the carcinogen enabled the course of its penetration into the skin and the dynamics of its elimination to be studied. The curve of DMBA fluorescence has ascending and descending parts. According to data in the literature [1, 4] the ascending part of the curve reflects the process of solution of DMBA or its metabolites in tissue lipids (free and intracellular), whereas the descending part of the curve reflects metabolism of the hydrocarbons and their diffusion into the deeper layers of the skin and the bloodstream.

Compared with DMBA, its metabolite 7-OHM-12-MBA possessed weaker fluorescence but the dynamics of its elimination from the skin completely coincided with the elimination curve of DMBA (Fig. 1). The fluorescence of 7,12-di-OHMBA was weaker still, but it remained at the same level for 2 h after application (Fig. 2).

When DMBA was applied to the skin together with 7-OHM-12-MBA in the ratio of 1:1 or 1:0.5 (Fig. 1) or together with 7,12-di-OHMBA (1:0.5) (Fig. 2), the rate of decrease of recordable fluorescence was unchanged and the elimination curve was indistinguishable in form

from that of DMBA. Meanwhile the total intensity of fluorescence of the applied hydrocarbons was never additive. When DMBA and its derivatives were applied to the skin in the ratio of 1:1 their combined fluorescence was less than additive, whereas if applied in the ratio of 1:0.5 it was greater than additive (Figs. 1 and 2). When DMBA and 7,12-di-OHMBA were applied to the skin in equimolar proportions the character of the fluorescence curve was modified: Fluorescence remained constant in intensity during the period from 30 min to 2 h after application of the hydrocarbons (Fig. 2).

If DMBA was applied to the skin together with 7,8-BF, one of the most powerful inhibitors of the enzyme system which metabolizes DMBA and other hydrocarbons [11], the fall of the curve also was almost completely prevented (Fig. 3). This observation shows that the character of the second half of the fluorescence curve of DMBA in the mouse skin is chiefly due to metabolic conversions of the carcinogen. It must be emphasized that 7,8-BF has no local irritant action, it is nontoxic in the concentrations used, and it does not prevent penetration of the carcinogen into the cells.

In the character of its elimination from the skin 7-OHM-12-MBA was indistinguishable from DMBA. This evidently reflects further conversions of the metabolite, which may undergo further oxidation in the system of microsomal oxidases to 7,12-di-OHMBA, phenols, and dihydrodiols [16].

Although DMBA and 7-OHM-12-MBA are metabolized by the same enzyme system [8, 15, 16], 7-OHM-12-MBA did not change the character of elimination of DMBA when the two were applied together. It would therefore be interesting to compare the ability of the carcinogen and metabolite to compete for the enzyme in a simpler object.

7,12-di-OHMBA is eliminated from the skin much more slowly. The reason for this may be that the metabolite penetrates to a lesser degree into the skin because of its weaker hydrophobic properties or its inability to induce increased enzyme activity in the cells [17]. The elimination curve observed during application of DMBA together with 7,12-di-OHMBA in equimolar proportions (Fig. 2) resembles the pattern of fluorescence of DMBA in the presence of 7,8-BF (Fig. 3), but further experiments in tissue culture and with isolated microsomes are necessary to confirm that this metabolite can inhibit oxidation of DMBA in the same way as 7,8-BF. Further investigations also are required to explain why the metabolites of the carcinogen alter the intensity of the combined fluorescence differently when applied in different molar proportions.

Experiments in this field would help to show whether competitive relationships exist between DMBA and some of its metabolites which play a role in the regulation of oxidation of the carcinogen, or whether the intensity of metabolism of polycyclic hydrocarbons is determined entirely by the concentration of the original compound.

LITERATURE CITED

1. L. A. Andrianov, G. A. Belitskii, S. P. Zavadina, et al., Vopr. Onkol., No. 1, 54 (1972).
2. L. A. Andrianov and V. S. Turusov, Vopr. Onkol., No. 12, 64 (1969).
3. V. P. Bronovitskaya, A. A. Kizel'shtein, I. A. Oglobina, et al., Zh. Prikl. Khim., 43, 2116 (1970).
4. T. Karu, U. Kirso, L. A. Andrianov, et al., Izv. Akad. Nauk Est. SSR, Biologiya, 21, 255 (1972).
5. L. P. Paal'me and M. Ya. Gubergrits, in: Proceedings of the Fourth All-Union Symposium on Current Problems in Self-Purification and Quality Control of Water [in Russian], Tallin (1972).
6. L. M. Raikhman, B. Annaev, A. B. Shapiro, et al., Biokhimiya, 37, 548 (1972).
7. G. M. Badger and J. W. Cook, J. Chem. Soc., 802 (1939).
8. E. Boyland and P. Sims, Biochem. J., 104, 394 (1967).
9. N. P. Buu Hoi and S. S. Sung, Naturwissenschaften, 57, 135 (1970).
10. C. T. Crew and L. Mirskaia, J. Genet., 25, 17 (1932).
11. L. Diamond and H. V. Gelboin, Science, 166, 1023 (1969).
12. O. H. Iversen, Prog. Exp. Tumor Res. (Basel), 4, 169 (1964).
13. H. V. Gelboin, Advances Cancer Res., 10, 1 (1967).

14. H. Kunte, Z. Krebsforsch., 72, 57 (1969).
15. P. Sims, Biochem. Pharmacol., 19, 285 (1970).
16. P. Sims, Biochem. Pharmacol., 19, 2261 (1970).
17. K. L. Syndor and J. W. Flesher, Cancer Res., 29, 403 (1969).
18. J. Webb, Enzyme and Metabolic Inhibitors, New York (1963).
19. D. Williams, F. J. Wiebel, J. C. Leutz, et al., Biochem. Pharmacol., 20, 2130 (1971).