ONCOLOGY

The Ability of Neoplastic Tissue to Produce 12- and 15-Hydroxyeicosatetraenic Acids as a Test for Metastatic Activity of Human Lung Tumors

I. A. Kudryavtsev, N. V. Myasishcheva, B. E. Polotskii, Z. O. Machaladze, and M. I. Davydov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 126, No. 9, pp. 338-341, September, 1998
Original article submitted July 16, 1997

The metabolism of ^{14}C-arachidonic acid in cell-free homogenates of 65 primary, primary multiple, and metastatic tumors of human lungs is studied. The biosynthesis of arachidonic acid lipoxygenase metabolites 12- and 15-hydroxyeicosatetraenic acids is suppressed in all metastatic tumors.

Key Words: *primary and metastatic human lung tumors; metastases; arachidonic acid metabolism; biosynthesis of 12- and 15-hydroxyeicosatetraenic acids*

The important role of eicosanoids of the arachidonic acid (AA) cascade in the pathogenesis of malignant tumors is well established [1,2]. The role of eicosanoids in the metastatic process is not quite clear; they act as its negative or positive modifyers. Some cycloxygenase AA metabolites, prostanoids (prostacyclin, prostaglandins PGD_2 and $PGF_{2\alpha}$) exert strong antimetastatic effect, while others (PGE_2 and thromboxane A_2) stimulate the formation of metastases [3,6,8,9]. The best studied lipoxygenase AA metabolite is 12-hydroxyeicosatetraenic acid (12-HETE). It modulates tumor cell reactions with the extracellular matrix and their migration [4,10,11], but its chemokinetic properties are typical of leukotriene B_4, 5-, and 15-HETE [5]. Reactions between eicosanoids during metastases cannot be disclosed without comparative analysis of the profiles of produced eicosanoids in tumors with different metastatic potential, which was never carried out. Our purpose was to study the metabolism of ^{14}C-AA in metastatic and nonmetastatic tumors of human lungs.

MATERIALS AND METHODS

Profiles of ^{14}C-AA metabolites produced in neoplastic tissues were studied in 65 operated patients with primary, primary multiple, and metastatic tumors of the lungs. For analysis of eicosanoids, tissue fragments (100-200 mg) were rapidly dissected and frozen in liquid nitrogen, after which they were homogenized at 4°C in 10 volumes of 0.05 M Tris-HCl buffer (pH 7.4). Cell-free homogenates (0.5 ml) were incubated with 0.05 μCi ^{14}C-AA (Amersham, specific activity 50-60 μCi/mmol) at 37°C for 30 min. ^{14}C-AA metabolites extracted by ethyl acetate separated by thin-layer chromatography on Kieselgel-60 plates (Merck) using the ethyl acetate:isooctane:acetic acid:water (110:50:20:100) system [7]. Autoradiochromatograms on x-ray films X-OMAT AR (Kodak) and HS-11 (ORWO) were ana-

Department of Endogenous Carcinogenesis Modifyers, Institute of Carcinogenesis; Department of Thoracal Oncology, Institute of Clinical Oncology, N. N. Blokhin Cancer Research Center, Russian Academy of Medical Sciences, Moscow

0007-4888/98/0009-0943$20.00

lyzed in a KS-3 densiscan (Kipp and Zonen). In some cases repeatedly extracted eicosanoids were analyzed by radiometry. Results were statistically processed using Student's *t* test.

RESULTS

Although all prostanoids are characterized by pronounced modulating effect on metastatic processes, we failed to detect a relationship between the level of their production and metastatic activity of the studied tumors. On the other hand, our results point to a considerable decrease in the production of the main lipoxygenase ^{14}C-AA metabolites 12- and 15-HETE in tumors metastasizing into lymph nodes or other organs. Unlike a benign tumor, carcinoid (Fig. 1, *a*), the ability of all metastatic primary tumors of the lungs, particularly small-cell carcinoma, to produce these metabolites was very low (Table 1). Such a suppression of biosynthesis of 12- and 15-HETE was characteristic of metastatic tumors of different histogenesis (Fig. 1, *b*) and of primary multiple tumors.

The differences in the ability to produce 12- and 15-HETE by metastatic and nonmetastatic ma-

TABLE 1. Production of 12- and 15-HETE from Exogenous ^{14}C-AA in Cell-Free Homogenates of Metastatic and Nonmetastatic Tumors of Human Lung (*M±m*)

Histological type of tumor	Metastases	Number of patients	% label in HETE
Primary tumors			
carcinoid	—	3	13.07±2.16
squamous-cell carcinoma:			
nonkeratosic	—	2	0.60±0.40
	+	4	0.11±0.03*
keratosic	—	10	2.76±0.49
	+	6	0.88±0.15**
adenocarcinoma	—	4	2.58±0.56
	+	7	0.66±0.12**
mixed cancer (squamous-cell and adenocarcinoma)	—	—	
	+	3	0.60±0.06*
large-cell carcinoma	+	1	0.6
small-cell carcinoma	+	3	0.12±0.04*
Primary multiple tumors			
squamous-cell carcinoma		4	0.75±0.28*
Metastatic tumors			
giant-cell tumor		1	
dermatofibrosarcoma		1	
malignant thymoma		1	
uterine leiomyosarcoma		1	
melanoma		1	
rabdomyosarcoma		1	
breast cancer		2	
colonic cancer		1	0.63±0.16*
nasal cancer		1	
rectal cancer		2	
cancer of the corpus uteri		1	
thyroid cancer		1	
synovial sarcoma		2	
chondrosarcoma		1	
embryonal ovarian cancer		1	

Note. *$p<0.001$ *vs.* mean values in the carcinoid group; *$p<0.01$ *vs.* tumors without metastases. [1]All metastasizing tumors.

lignant tumors of the lung were the most pronounced in squamous-cell keratosic carcinoma (Fig. 1, *c, d*) and in adenocarcinoma. There was a tendency to a decrease in the production of the above HETE in neoplastic tissue in cases with more pronounced metastatic involvement of lymph nodes and other organs. On the other hand, the difference between these parameters in squamous-cell nonkeratosic carcinoma was negligible. The decrease in conversion of ^{14}C-AA into 12- and 15-HETE was maximum in this malignant tumor and in small-cell carcinoma, a tumor with a high metastatic potential. In general, conversion of ^{14}C-AA into 12- and 15-HETE in cases with foci of tumor tissue beyond one organ did not surpass 1-1.5%. Such a level of their biosynthesis from labeled precursor in tumor tissue can be regarded as a criterion of metastatic activity of lung tumors.

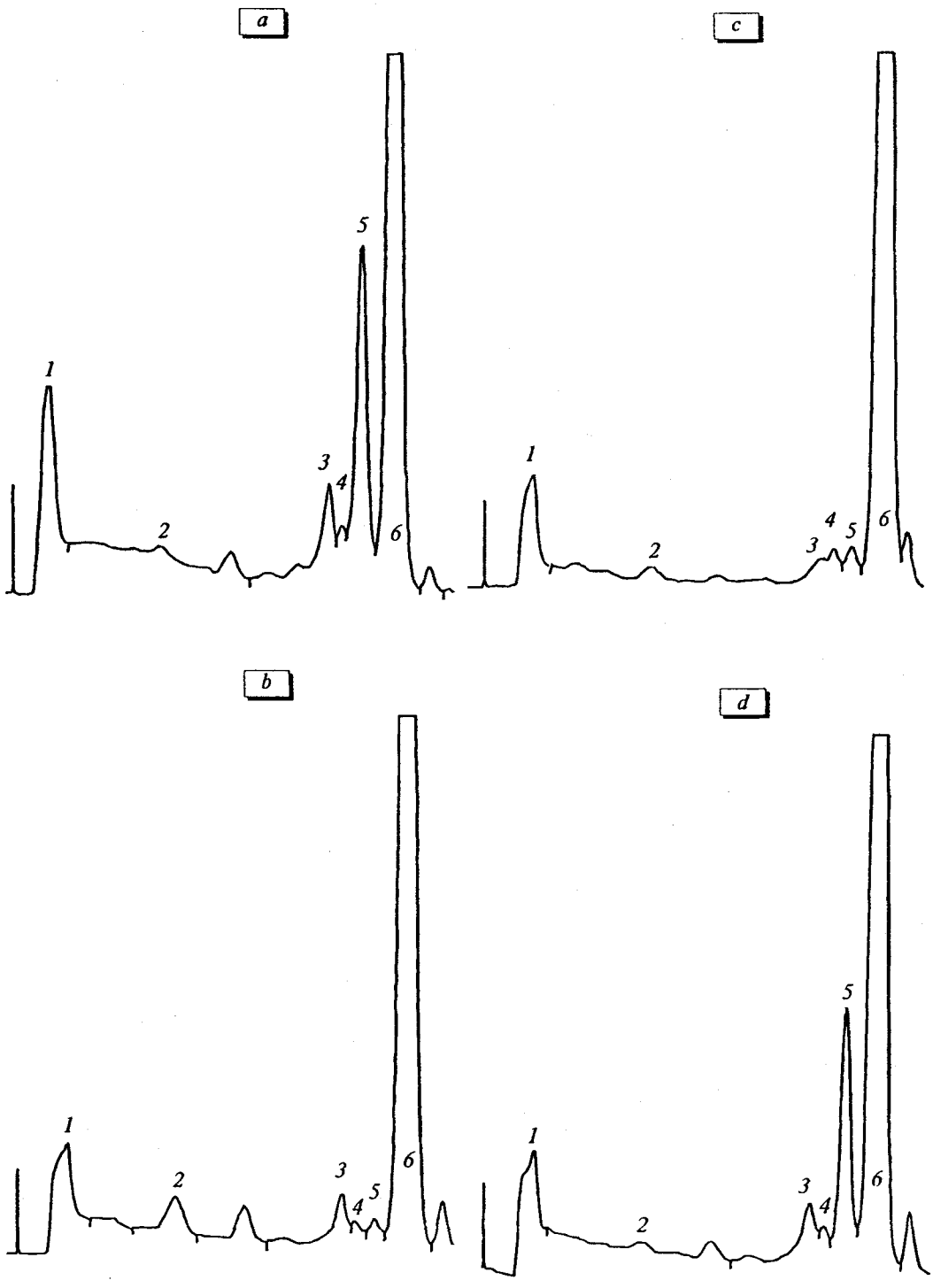

Fig. 1. Profiles of eicosanoids metabolized from ^{14}C-arachidonic acid in cell-free homogenates of metastatic and nonmetastatic tumors of human lung. Densitometried radioautographs of thin-layer chromatograms. *a*) carcinoid; *b*) metastatic tumor of the lung (giant-cell tumor of the bone); *c*) squamous-cell keratosic carcinoma with metastases to regional lymph nodes; *d*) squamous-cell keratosic carcinoma without metastases. *1*) start; *2*) prostaglandin E_2; *3*) 5-HETE; *4*) 12-hydroxyeicosatetraenic acid; *5*) 12- and 15-HETE; 6) arachidonic acid.

Therefore, decreased ability to produce the main lipoxygenase AA metabolites 12- and 15-HETE is a characteristic feature of metastatic tumors of human lungs. The mechanism of so strong suppression of production of these eicosanoids in tumors with a high metastatic potential requires further studies.

REFERENCES

1. L. S. Bassalyk, Z. G. Kadagidze, and N. E. Kushlinskii, *Prostaglandins and Cancer* [in Russian], Vsesoyuzn. Inst. Meditsinsk. Informatsii, Medicine and Public Health, Ser. Oncology, Moscow (1988), No. 1.
2. I. A. Kudryavtsev, *Eksp. Onkol.*, **10**, No. 6, 3-8 (1988).
3. Yu. P. Shmal'ko and S. N. Grinzhevskaya, *Ibid.*, **9**, No. 5, 70-72 (1987).
4. Y. G. Chen, Z. M. Duniec, W. Hagman, *et al.*, *Cancer Res.*, **54**, No. 6, 1574-1579 (1994).
5. F. M. Cunningham and P. M. Woolard, *Prostaglandins*, **34**, No. 1, 71-78 (1987).
6. F. A. Fitzpatrick and D. A. Stringfellow, in: *Essential Fatty Acids and Prostaglandins*, Oxford (1982), pp. 705-708.
7. C. J. Hawkey, N. K. Boughton-Smith, and B. J. R. Whittle, *Dig. Dis. Sci.*, **30**, No. 12, 1161-1165 (1985).
8. K. V. Honn, in: *Prostaglandins and Cancer*, New York (1982), pp. 733-752.
9. K. V. Honn, D. G. Tang, I. Grossi, *et al.*, *Cancer Res.*, **54**, No. 2, 565-574 (1994).
10. D. G. Tang, C. A. Diglio, K. V. Honn, *et al.*, *Ibid.*, No. 4, 1119-1129.
11. M. R. Young, M. Newby, and J. Meunier, *Ibid.*, **45**, 3918-3923 (1985).