[CANCER RESEARCH 35, 2762 2765, October 1975]

Elevation of Histaminase and Its Concurrence with Regan Isoenzyme in Ovarian Cancer[1]

Chi-Wei Lin, Mary Lou Orcutt, and Leo L. Stolbach

Tufts Cancer Research Center and The Department of Pathology, Tufts University School of Medicine, Boston 02111 [C-W. L., M. L. O.], and Massachusetts Department of Public Health and Department of Medicine, Pondville Hospital, Walpole, Massachusetts 02081 [L. L. S.]

SUMMARY

Histaminase has been shown to be associated with several types of human cancer. In the present study, we examined the activity of histaminase and its relationship with Regan isoenzyme of alkaline phosphatase in ascitic fluids obtained from patients with ovarian and several other types of cancer. We have found that about 44% of the ovarian cancer patients had elevated levels of histaminase in the ascitic fluid, whereas a less frequent incidence was observed in fluids obtained from other types of cancer. There was concurrence in the elevation of histaminase activity with the appearance of Regan isoenzyme in most of the samples examined. Of the 10 patients who showed elevated histaminase, 9 had high Regan isoenzyme activity; whereas in 9 patients with normal levels of histaminase, all except 1 had low or moderate levels of Regan isoenzyme activity. These results, therefore, confirm the observation of an association of histaminase with human cancer and suggest the possibility for the utilization of histaminase, in conjunction with Regan isoenzyme and cancer-associated proteins, for cancer diagnosis and clinical evaluation of tumor progression and regression during therapy.

INTRODUCTION

The activity of histaminase has been reported to be associated with several types of cancer. Benda and Miravet (6) observed the increase of serum histaminase in some cases of bronchial carcinoma. Borglin and Willert (7) later showed that the enzyme activity was elevated in plasma of patients with endometrial adenocarcinoma, uterine myosarcoma, and granulosa cell carcinoma. More recently, Baylin *et al.* (4, 5) reported a significant elevation of the enzyme activity in sera of patients with medullary carcinoma of the thyroid gland. In this study, 70% of the patients with metastatic tumor were found to have high serum histaminase activity. Subsequent study by Atkins *et al.* (3) showed that the thyroid tumor contained 15 to 1500 times more histaminase activity than the adjacent thyroid tissue, thus suggesting that the elevated histaminase in serum had originated from the tumor.

In the present paper, we report our findings of increased histaminase activity in ascites fluids obtained from patients with ovarian cancer. The incidence of the elevation of this enzyme is also found to concur with the presence of Regan isoenzyme of alkaline phosphatase, which is known to be associated with a number of human tumors (9, 13, 14, 17).

MATERIALS AND METHODS

Histaminase activity was determined by a radioassay procedure modified from that of Roscoe and Kupfer (15). The incubation mixture contained 0.5 ml of sample solution, 0.4 ml of 0.25 M phosphate buffer (pH 7.5), 0.1 ml of 0.81 mM histamine containing 0.25 μCi of [^{14}C]histamine ([*ring*-2-^{14}C]; Amersham/Searle Corp., Arlington Heights, Ill.), and water to make up a total volume of 2.6 ml. The mixture was incubated at 37° for 5 hr and the enzyme reaction was stopped by addition of 0.5 ml of 12% trichloroacetic acid. After centrifugation, 2 ml of the supernatant were removed, adjusted to pH 6.0 with 0.1 N NaOH and diluted to 7.5 ml with H_2O before the supernatant was applied to a phosphocellulose (Cellex P; Bio-Rad Laboratories, Richmond, Calif.) column (0.5 x 7 cm) at pH 6.0 to separate the deaminated reaction product from the unreacted histamine. The column was eluted with 10 mM phosphate buffer (pH 6.0) until a total of 10 ml of eluent was collected. The radioactivity of the eluent was then measured by liquid scintillation counting. The enzyme activity is expressed as μg of histamine hydrolyzed per hr per ml of sample. Our preliminary experiments with placental homogenate have shown that a linear relationship exists between the amount of histamine hydrolyzed and time of incubation, up to 6 hr, when 60% of the substrate had been consumed. The sensitivity of the method is about 0.01 μg of histamine hydrolyzed per hr.

The activity of Regan isoenzyme of alkaline phosphatase is measured by an automated technique described by Anstiss *et al.* (2). The enzyme samples were heated at 65° for 5 min to inactivate the heat-labile non-Regan alkaline phosphatase before the activity was assayed at pH 10.7 in 5 mM carbonate-bicarbonate buffer with phenyl phosphate (72 mM) as substrate. The enzyme activity is expressed as mg of phenol released per 15 min of incubation per 100 ml of sample. Recently, a heat-stable activity of 0.30 placental isoenzyme unit has been established as the 95th percentile in serum of normal population (9).

[1] This work was supported by Grant-in-aid CA-12924 from the National Cancer Institute.

Received June 17, 1974; accepted July 1, 1975.

RESULTS

As shown in Chart 1, the levels of histaminase in normal sera are very low, below 0.02 unit/ml. This is also the upper limit of normal serum histaminase found by Ahlmark (1) using a biological assay method which has been shown later to correlate well with the radiochemical method of Tryding and Willert (18). Since the limit of sensitivity of the method used in the present study is 0.01 enzyme unit, values below this level are not meaningful. Pregnancy sera, collected at term or in the 3rd trimester, exhibit histaminase activities more than 100 times that of the normal sera. Of the ascitic fluids of 25 ovarian cancer patients examined, 11 have histaminase activity above 0.02 unit/ml, ranging fom 0.024 to 0.684 unit/ml.

Elevation of histaminase is also observed in ascitic and pleural fluids obtained from other types of cancer. Of the 17 nonovarian cancer patients examined (cancer of the breast, 9; lung, 2; stomach, 1; colon, 2; endometrium, 1; and unknown primary, 2), 4 have levels above 0.02 unit of histaminase activity per ml (breast, 2; stomach, 1; and lung, 1). The highest histaminase activity (1.19 units/ml) was observed in the pleural fluid of a patient with lung cancer; this fluid was also rich in Regan isoenzyme of alkaline phosphatase (over 200 placental isoenzyme units). Of 8 ascitic fluids obtained from patients with noncancerous diseases (alcoholic, 6; and liver cirrhosis, 2) none showed elevated activity of histaminase (Chart 1).

Sera from 28 patients with cancer of various origins were also assayed for the enzyme activity. Only 1 showed a significant increase of histaminase. However, analysis of the Regan isoenzyme on these samples showed that 7 were above the normal level, set at 0.3 unit/ml.

Concurrence of histaminase and Regan isoenzyme in ovarian cancer fluids is shown in Table 1. Of the 10 patients who showed elevated histaminase, 9 have high Regan isoenzyme activity. In the 9 patients with levels of histaminase equivalent to normal serum, all except 1 have low or moderate levels of Regan isoenzyme activity.

The concurrence between the activities of histaminase and Regan isoenzyme in ovarian cancer fluids is further shown in Chart 2, where activities of these 2 enzymes were followed in a series of samples obtained from 2 patients during the course of their disease. In 1 patient, the histaminase increases with time in a nonexponential fashion while the Regan isoenzyme activity is elevated exponentially. In the other patient, both histaminase and Regan isoenzyme show no change over 5 months of study. In both cases, an apparent association between these enzymes was observed in all the samples obtained during the course of the disease.

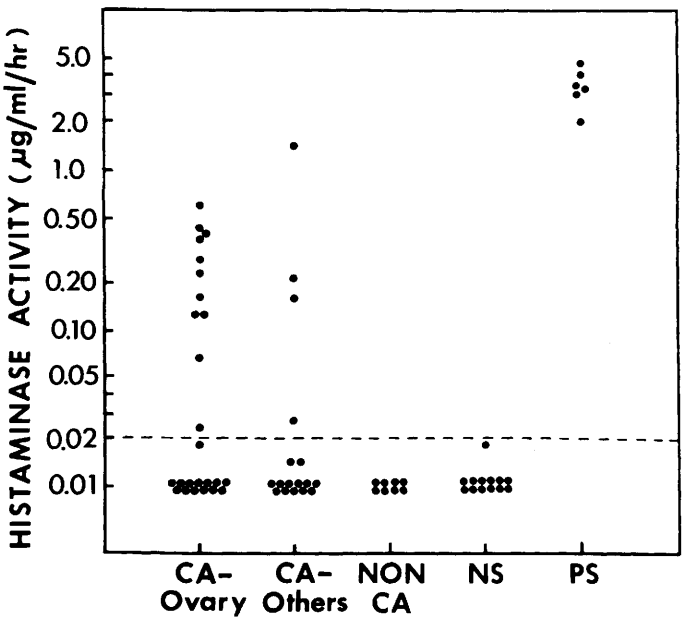

Chart 1. Histaminase activity in fluids obtained from cancer of the ovary (*CA-Ovary*), cancers of other sites (*CA-Others*), noncancerous patients (*Non-CA*), and in sera of normal (*NS*) and pregnancy (*PS*) subjects. The enzyme activity is expressed as μg histamine hydrolyzed per hr per ml of sample and is plotted on log scale. The limit of sensitivity of the method in the present study is about 0.01 unit; values below this are plotted at 0.01.

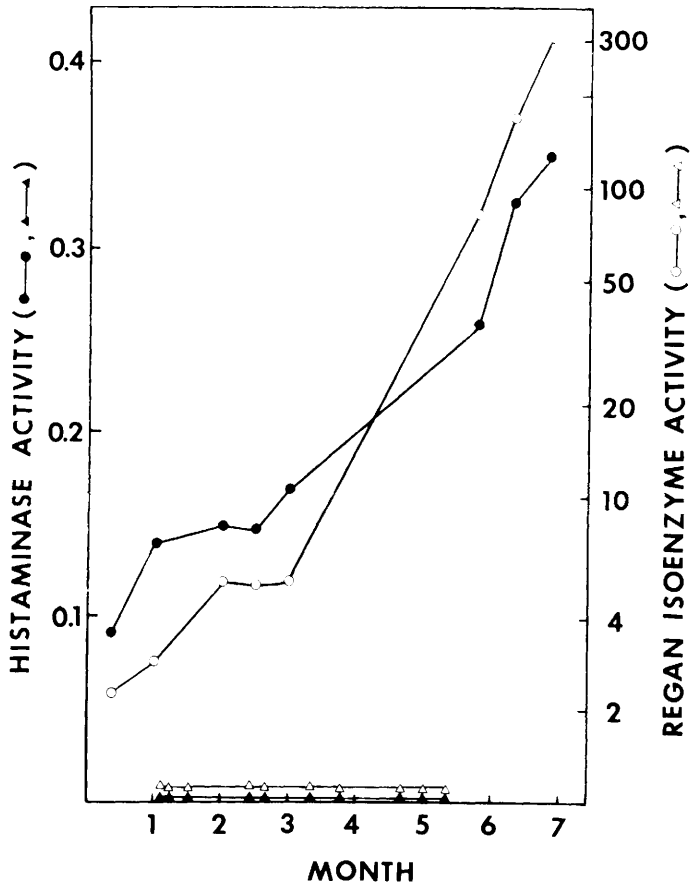

Chart 2. Activities of histaminase and Regan isoenzyme in ovarian cancer fluids obtained from 2 cases during the course of disease. The activity of histaminase is plotted on a linear scale and the Regan isoenzyme is plotted on log scale. One of these cases (○, ●) showed continual increase of these 2 enzymes during the 7-month period; the other (△, ▲) maintained low levels of both enzymes throughout the 5.5-month period. The histaminase activity is expressed as μg of histamine hydrolyzed per hour per ml, and the Regan isoenzyme is expressed as mg of phenol released per 15 min per 100 ml.

Table 1
Activities of histaminase and Regan isoenzyme in ascitic and pleural fluids

Patient	Site of cancer	Histaminase		Regan isoenzyme (PI units)[a]	
		μg/hr/ml	X normal[b]	mg/15 min/100 ml	X normal
		Ovarian cancer			
4		0.011	0.5	11.50	36.7
p-17		0.072	3.6	5.33	17.8
p-1		0.138	6.9	2.80	9.3
p-54		0.187	9.4	2.95	9.8
n-3		0.019	0.9	0.17	0.6
p-46		0.405	20.3	11.20	37.3
p-56		0.139	6.9	0.70	2.3
p-0		0.259	12.9	2.23	7.4
p-53		0	0	0.34	1.1
s-5		0.225	11.2	0.22	0.7
p-61		0	0	0.14	0.5
p-33		0.340	19.5	0.77	2.6
p-30		0.024	1.2	0.06	0.2
p-106		0.002	0.1	0.16	0.5
p-99		0.003	0.1	0.15	0.5
LK		0.001	0	0.15	0.5
p-4		0	0	0.19	0.6
p-107		0	0	0.13	0.4
N-68		0.684	34.2	14.10	47.0
		Other types of cancer[c]			
s-11	Lung	0	0	0.06	0.2
LS-2	Lung	1.187	59.3	200	666.6
p-104	Breast	0	0	0.09	0.3
p-81	Breast	0.170	8.5	0.14	0.5
p-80	Breast	0.027	1.3	0.06	0.2
p-16	Breast	0.004	0.2	0.19	0.6
p-45	Breast	0	0	0.38	1.3
p-92	Breast	0	0	0.43	1.4
s-9	Breast	0.015	0.7	0.27	0.9
p-65	Breast	0	0	0	0
p-52	Endometrium	0	0	0.05	0.2
s-41	Stomach	0.207	10.3	0.15	0.5
p-58	Colon	0.015	0.7	0.46	1.5
p-66	Colon	0	0	0.06	0.2
p-93	Lymphoid tissue	0	0	0.08	0.3

[a] PI, placental isoenzyme.
[b] The "normal" limit of histaminase is arbitrarily set at the serum level, 0.02 unit/ml, and that of Regan isoenzyme at 0.3 PI unit.
[c] Pleural fluids were obtained from cancer of the lung and breast, and ascitic fluids were from cancer of endometrium, stomach, colon, and lympha.

DISCUSSION

Our results on the analyses of histaminase on effusion fluids showed that elevation of histaminase is associated with a number of human cancers, including that of the ovary, breast, stomach, and lung. This confirms earlier findings of Benda and Miravet (6) and Borglin and Willert (7), who showed that elevation of this enzyme occurred in various types of cancer. These results, collectively, do not support the suggestion of Baylin *et al.* (5) that increase in serum histaminase is a specific marker for medullary thyroid carcinoma.

We have also observed in a significant number of samples a concurrent increase of histaminase and Regan isoenzyme of alkaline phosphatase. Although no explanation for this concurrence is available at the present time, this observation may have potential significance in the utilization of multiple enzyme analysis for cancer diagnosis or clinical evaluation of tumor progression and regression during therapy.

One of the significant features of Regan isoenzyme in relation to human cancer is that the isoenzyme is identical to the placental type of alkaline phosphatase in its enzymatic, physical, and immunological characteristics (10–12). Because of the abundant availability of placenta relative to

tumor tissue, greater application of the Regan isoenzyme can be made to the study of cancer biology as well as to the diagnosis of cancer (9, 13, 14). Incidentally, placenta has the highest level of histaminase among all human tissues (8, 16, 19). Investigation is currently being undertaken to investigate the possibility that tumor histaminase may be identical to the placental enzyme.

ACKNOWLEDGEMENTS

We thank Dr. W. H. Fishman, Director of the Tufts Cancer Research Center, for his interest in and support of, this investigation. Grateful thanks are also due to C. L. Anstiss for the determination of Regan alkaline phosphatase values in this study.

REFERENCES

1. Ahlmark, A. Studies on the Histaminolytic Power of Plasma with Special Reference to Pregnancy. Acta Physiol. Scadn., *9*(28): 1–197, 1944.
2. Anstiss, C. L., Green, S., and Fishman, W. H. An Automated Technique for Segregating Populations with a High Incidence of Regan Isoenzyme in Serum. Clin. Chim. Acta, *33:* 279–286, 1971.
3. Atkins, F. L., Beaven, M. A., and Keiser, H. R. Dopa Decarboxylase in Medullary Carcinoma of the Thyroid. New Engl. J. Med., *289:* 545–548, 1973.
4. Baylin, S. B., Beaven, M. A., Engelman, K., and Sjoerdsma, A. Elevated Histaminase Activity in Medullary Carcinoma of the Thyroid Gland. New Engl. J. Med., *283:* 1239–1244, 1970.
5. Baylin, S. B., Beaven, M. A., Keiser, H. R., Tashjian, A. H., Jr., and Melvin, K. E. W. Serum Histaminase and Calcitonin Levels in Medullary Carcinoma of the Thyroid. Lancet, *1:* 455–458, 1972.
6. Benda, M. R., and Miravet, L. F. Histaminase Serique et Cancer. Bull. Mem. Soc. Med. Hop. Paris, *76:* 184–188, 1960.
7. Borglin, N. E., and Willert, B. Increased Histaminolytic Power of Plasma in Endometrial Adenocarcinoma. Cancer, *15:* 271–275, 1962.
8. Buffoni, F. Histaminase and Related Amine Oxidases. Pharmacol. Rev., *18:* 1163–1199, 1966.
9. Cadeau, B. J., Blackstein, M. E., and Malkin, A. Increased Incidence of Placental-like Alkaline Phosphatase Activity in Breast and Genitourinary Cancer. Cancer Res., *34:* 729–732, 1974.
10. Fishman, W. H. Immunologic and Biochemical Approaches to Alkaline Phosphatase Isoenzyme Analysis: The Regan Isoenzyme. Ann. N. Y. Acad. Sci., *166:* 745–759, 1969.
11. Fishman, W. H. Carcinoplacental Isoenzyme Antigens. Advan. Enzyme Regulation, *11:* 293–321, 1973.
12. Fishman, W. H., Inglis, N. R., and Green, S. Regan Isoenzyme: A Carcinoplacental Antigen. Cancer Res., *31:* 1054–1057, 1971.
13. Fishman, W. H., Inglis, N. R., Stolbach, L., and Krant, M. J. A Serum Alkaline Phosphatase Isoenzyme of Human Neoplastic Cell Origin. Cancer Res., *28:* 150–154, 1968.
14. Nathanson, L., and Fishman, W. H. New Observations on the Regan Isoenzyme of Alkaline Phosphatase in Cancer Patients. Cancer, *27:* 1388–1397, 1971.
15. Roscoe, H. G., and Kupfer, D. A New Histaminase Assay. Anal. Biochem., *47:* 418–425, 1972.
16. Southern, A. L., Kobayashi, Y., Brenner, P., and Weingold, A. B. Diamine Oxidase Activity in Human Maternal and Fetal Plasma and Tissues at Parturition. J. Appl. Physiol., *20:* 1048–1051, 1965.
17. Stolbach, L. L., Krant, M., and Fishman, W. H. Ectopic Production of an Alkaline Phosphatase Isoenzyme in Patients with Cancer. New Engl. J. Med., *281:* 757–762, 1969.
18. Tryding, N., and Willert, B. Determination of Plasma Diamine Oxidase (Histaminase) in Clinical Practice. Scand. J. Clin. Lab. Invest., *22:* 29–32, 1968.
19. Weingold, A. B. Enzymatic Indices of Fetal Environment. Clin. Obstet. Gynecol., *11:* 1081–1105, 1968.