J Cancer Res Clin Oncol (1988) 114:588–592

Journal of Cancer Research and Clinical Oncology

Blood histamine and solid malignant tumors

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Summary. A clinical study was performed to determine whether patients with a newly diagnosed solid malignant tumor manifest an alteration in whole-blood histamine levels. Our results indicate that such patients have blood histamine nearly three times greater than either normal, healthy individuals or noncancerous disease controls. Following surgical removal of the tumor, blood histamine levels remained high for 2 months and then dropped close to the normal range 3 months after surgery. Basophil counts did not change significantly in the presence of a malignant tumor. Patients receiving either chemotherapy or radiation therapy, and terminal cancer patients who were no longer receiving any therapy except for pain control had blood histamine within or below the normal range. By analogy with animals studies, we suggest that nascent histamine synthesis is increased in the presence of a developing tumor. The clinical usefullness of this observation remains to be determined.

Key words: Cancer – Histamine – Tumors

Introduction

Histamine has been implicated in a host of physiological and pathological phenomena including inflammation, allergic reactions, regulation of microcirculation, tissue growth, wound healing, gastric secretion and neuroregulation. Considerable evidence also exists to suggest a link between histamine and cancer. Included are reports that (a) tumor-bearing animals exhibit increased tissue histamine both in the tumor and at sites distant from the tumor (Bartholeyns and Bouclier 1984; Burtin et al. 1981; Maslinski et al. 1984; Matsuzaki et al. 1978); (b) both tumor promoters and the presence of developing tumors cause a marked rise in the activity of histidine decarboxylase, and thus the synthesis of nascent histamine, both in the tumor and at distant sites (Bartholeyns and Bouclier 1984; Bartholeyns and Fozard 1985; Nolte et al. 1987; Taguchi et al. 1982); (c) the actively growing region of a tumor displays elevated histamine levels, while the necrotic core is low in histamine (Burtin et al. 1981; Maslinski et al. 1984); and (d) simultaneous activation of H_1 and blockade of H_2 receptors frequently promotes tumor regression (Bloskma et al. 1984; Burtin et al. 1982). In addition, several studies have shown that tumor-bearing animals display increased levels of blood histamine, which revert to normal 2 weeks after tumor resection (Scheinmann et al. 1979), although a clinical study reported that levels of whole-blood histamine decreased in patients with a primary malignant tumor (Burtin et al. 1983).

We sought to carry out a clinical study to ascertain whether patients with solid malignant tumors manifest altered blood levels of histamine and, if so, whether such measurements might be clinically useful. Our results, in agreement with the above-mentioned animal studies, indicate that individuals with a newly diagnosed solid malignant tumor exhibited blood histamine levels three times greater than either noncancer disease controls or normal, healthy individuals. Within 3 months following tumor resection, the blood histamine levels returned close to the normal range.

Materials and methods

Consecutive, monthly blood samples were obtained from individuals in the following groups: normal, healthy controls; patients with a newly diagnosed, biopsy-verified, primary solid malignant tumor who were scheduled for surgical resection; patients with solid malignant tumors with or without metastases currently receiving chemotherapy or radiation therapy; terminal cancer patients no longer receiving any therapy except for pain control; and three disease control groups.

The normal, healthy controls were paid volunteers, primarily employees and students. The three disease control groups included one group of patients without known allergies but diagnosed as hav-

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Table 1. Characteristics of patients in study

Group	*n*	Male	Female	Primary affected organ						
				Breast	Heart	Reproductive organs	Prostate	Bone	Lung	Other
Normal	25	13	12	NA	NA	NA	NA	NA	NA	NA
Disease control	25	10	15	9	3	1	1	1	0	9
Allergy	25	10	15	NA	NA	NA	NA	NA	NA	NA
Steroid	10	9	1	0	0	0	0	0	10	0
Prim. cancer	30	12	18	7	0	5	2	0	2	14
Chemotherapy	20	5	15	8	0	3	2	0	0	7
Radiation	20	13	7	7	0	5	0	0	0	8
Terminal	17	11	6	1	0	0	1	0	9	6

ing noncancerous diseases, one group with extensive allergies but no history of cancer, and one group with no known allergies or history of cancer but who were receiving large doses (>10 mg/day) of corticosteroids, typically prednisone. This last group of patients was included to minimize the possibility that changes in blood histamine might be influenced by stress or drug-induced steroid elevations. Patient demographics are shown in Table 1.

The study was designed to sample each subject for 2 consecutive months. A subset of each group was followed for 2 additional months. The study was approved by both the University of Nebraska Institutional Review Board and the Veterans Administration Hospital Human Subjects Committee.

After identifying a suitable subject and obtaining informed consent, 10 ml blood was withdrawn into a heparinized vacutainer. Duplicate histological slides were immediately prepared as described below for a basophil count. The remaining blood was split into a whole-blood and a plasma fraction and stored at −70 °C. Each sample received a code number and all assays were done under "blind" conditions. Prior to histamine measurements, all blood samples were cycled through three freeze/thaw cycles to insure basophilic breakage.

The single-isotope, single-extraction procedure of Beaven et al. (1982) was used to measure blood histamine. Briefly, this involves the enzymatic conversion of histamine to methylhistamine, with *S*-[^{14}C]adenosylmethionine used as the methyl donor, through the action of methyl transferase isolated from rat kidney (Shaff and Beaven 1979). The labeled methylhistamine was isolated with an isoamyl alcohol/toluene extraction and the assay was readily quantified. Each unknown was run in triplicate and histamine standards in quadruplicate. Recovery was determined by adding known amounts of histamine to a second set of triplicates. Interassay variability had a coefficient of variation of 7%. Standard curves were highly linear ($r=0.99$) up to 300 ng/ml. Histamine contamination of the commercial heparin in the vacutainers was measured and found to be negligible.

The peripheral basophil count employed the technique of Hirsch et al. (1974). Briefly, 2 µl whole blood was placed on a clean, glass microscope slide and evenly distributed in a thick smear approximately 8 mm in diameter. The slide was air-dried, fixed in methanol, and stained in a filtered 0.2% toluidine blue, 2,5% aqueous solution of $Al_2(SO_4)_3$ for 2 min to yield a highly specific stain for sulfated mucopolysaccharides (Heath 1961). After clearing and adding a coverslip, the stained basophils were counted by a single individual using 200× magnification.

Statistical significance was determined using the Student's *t*-test. The Dixon gap test was used for statistical outliers (Dixon 1950). Data were examined for effects of medications or diseases known to affect either histamine or basophils.

Results

We intended this initial study to be neither tumor- nor organ-selective. However, we did exclude patients with either thyroid or gastrointestinal involvement. The former were excluded since elevated histamine in the thyroid has been shown not to correlate with the presence of a malignant tumor (Matsuzaki et al. 1978); the latter, since histamine and alterations in histamine metabolism are associated with both physiological and noncancerous pathological changes in the gastrointestinal tract (Baylin and Luk 1981).

For normal, healthy individuals, a total of 59 measurements from 25 individuals yielded an average blood histamine of 42.6 ± 1.9 ng/ml (mean ± SE). The best estimate for normal blood histamine is 40–60 ng/ml (Beaven et al. 1982), in good agreement with our values.

Figure 1 shows the longitudinal results in five patient groups. Patients receiving high doses of prednisone had significantly lower ($P<0.002$) levels of blood histamine at each time point, confirming reports that such steroids depress blood histamine (Bruce et al. 1976; Saavedra-Delgado et al. 1980). Aside from the first time, for which we have no explanation, there was no significant difference between values for the normal, healthy controls and for the disease control group. Blood histamine values were significantly greater ($P<0.04$) for the allergy patients than for the disease controls although, as previously reported (Bruce et al. 1976), not significantly greater than for normal, healthy controls.

We found a marked increase ($P<0.001$) in blood histamine from patients who presented with a newly diagnosed solid malignant tumor. Such patients were typically sampled 1–2 days prior to surgical removal of the tumor. Since histamine levels in peripheral blood are known to increase transiently following major abdominal surgery (Roher et al. 1982) before returning to normal within 3–5 days, we scheduled the

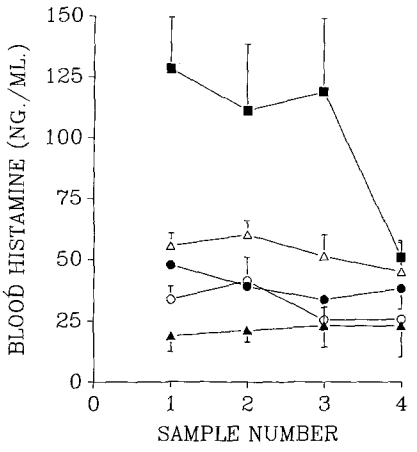

Fig. 1. Whole-blood histamine levels of five patient groups determined at monthly intervals. ■, Patients with newly diagnosed solid malignant tumor in which the tumor was surgically removed immediately after the first blood sample; △, patients with extensive allergies; ●, normal, healthy controls; ○, non-cancerous disease controls; ▲, patients receiving high levels of corticosteroids. *Error bars*, shown when they extend beyond the appropriate symbol, represent 1 SE

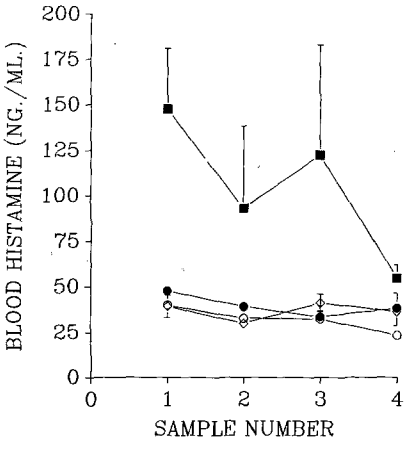

Fig. 2. Monthly whole-blood histamine values of female subjects in four groups: ■, with newly diagnosed breast cancer with mastectomy performed immediately after the first sample; ●, normal, healthy controls; ○, with non-cancerous diseases; ◇, with malignant tumors currently receiving chemotherapy. *Error bars*, shown when they extend beyond the appropriate symbol, represent 1 SE

second sample for 1 month after surgery and found no significant change in blood histamine until 3 months following resection. Even then, blood histamine in these patients remained slightly higher than in the disease controls, but not significantly different from the normal, healthy group. The decline from the 2nd to the 3rd month after resection was significant at the 94% confidence level.

The largest subclass of patients in the primary cancer group was that of females with diagnosed breast cancer. Blood histamine values of this group, together with those of female disease controls, normal, healthy females and females receiving chemotherapy are shown in Fig. 2. While the number of patients ($n=7$) in this subset is small, there is an indication of the same effect observed with the cancer population as a whole. The average blood histamine values at 3 months post-mastectomy, while still greater ($P<0.01$) than those in the disease controls group, did, however, decrease significantly ($P<0.035$) compared with pre-surgery values.

Patients with diagnosed solid malignant tumors, with or without metastases, and receiving either chemotherapy or radiation therapy had blood histamine values indistinguishable from either the disease controls or normal, healthy controls (data not shown). In addition, we tested 15 terminal cancer patients in whom therapy had been discontinued at least 6 months before except for pain medication. The average value of the 1st month's sample was the same for these patients as for the disease controls. However, in the ensuing months, each terminal cancer patient who survived showed a progressively decreasing blood histamine level. The only individual to survive three months after entering the study had a final blood histamine value of 10.5 ng/ml.

During the study we encountered one patient diagnosed with systemic mastocytosis and obtained monthly samples as a procedural control. Four separate blood histamine measurements from this patient averaged 132.0 ± 10.7 ng/ml, i.e., they were comparable to those recorded in patients with a newly diagnosed primary tumor and 2–3 times normal.

Hirsch et al. (1974), using the identical procedure, found the peripheral basophil count for normal, healthy controls to be 36.7 basophils/μl. In the present study, the average of 43 determinations in normal, healthy controls was 43.3 basophils/μl. We found no statistical difference in the average basophil concentration between any two groups except when patients receiving steroids or chemotherapy were considered, in which case basophil levels were less than one-half those in the healthy controls.

Discussion

The most striking observation in this study was the elevation of blood histamine in patients with a newly diagnosed, solid malignant tumor. Since the majority of blood histamine is typically found in circulating basophils (Graham et al. 1955; Ishizaka et al. 1972) and we found no significant increase in their basophil count, one might suspect an increase in the histamine content per basophil. However, given the variation in

basophil counts, the calculated histamine/basophil was not significantly greater in the primary cancer patients. Beyond this, a number of factors suggest that nascent histamine synthesis, and not cellular histamine stores, might be the critical factor. Both tumor-bearing animals and animals exposed to tumor promoters show increases in histidine decarboxylase activity resulting in an increased synthesis of nascent histamine, which appears to be involved in tumor growth and development (Bartholeyns and Bouclier 1984; Bartholeyns and Fozard 1985). Such increases in histamine, both in tissues and blood, occur in animals genetically deficient in mast cells and in mice lacking basophilic leukocytes. While irreversible inhibition of histidine decarboxylase decreases the histamine content of tumors and retards the growth of both tumor cells in culture and tumors induced in animals, extensive depletion of tumor histamine stores with the secretagogue 48/80 has no anti-tumor effect (Matsuzaki et al. 1978). These results collectively suggest that changes in tissue and blood histamine levels are not associated with intracellular histamine stores.

The observed increase in tumor histidine decarboxylase is also reflected at sites distant from the tumor suggesting involvement of a circulating factor (Bartholeyns and Fozard 1985). This conclusion is further supported by cross-circulation experiments (Ishikawa et al. 1970). In tumor and other cells typically lacking intracellular storage granules, the increased nascent histamine may leave the cell and enter the extracellular fluid. Indeed, when cultured tumor cells are exposed to a tumor promoter, histamine is released into the culture medium (Nolte et al. 1987).

The functional role played by nascent histamine, if any, is unknown. Of interest is the modulatory role histamine plays in the immune system with H_1 receptors participating in the activation of T effector or contrasuppressor cells and H_2 receptors in the activation of suppressor cells. The relative effectiveness of such opposing actions of histamine could influence the early host defense to a tumor.

In humans, an i.v. injection of 20–40 μg histamine typically evokes flushing and allergic-type symptoms (Curry 1946). If such histamine were entirely distributed in a 5000-ml blood volume, the blood histamine concentration would increase by 4–8 ng/ml. In contrast, patients with a newly diagnosed, malignant tumor had an average blood histamine level approximately 80 ng/ml above normal. It is not clear how the tumor-bearing individual manages such high levels without manifesting symptoms of excess histamine. Similar results occur in tumor-bearing animals who display elevated blood histamine but are less prone to hypovolemic shock than normal animals following an i.v. histamine injection (Lynch and Salomon 1977). Explanations include an alteration in the number and/or affinity of histamine receptors in the presence of a malignant tumor and/or rapid sequestration of histamine such that it becomes relatively inaccessible.

Alterations in histamine receptor sensitivity have been reported. Patients with solid tumors were found to have a decreased sensitivity to intradermal histamine which did not revert to normal until 2 months after successful resection of the tumor (Burtin et al. 1986). Again, correspondence is found in animal studies, where tumor-bearing animals with elevated blood histamine exhibit a decrease in immediate hypersensitivity reactions (Burtin et al. 1982; Scheinmann et al. 1979). Even cultured cancer cells exhibit a homologous loss of H_2 receptor activity after exposure to added histamine (Emami et al. 1985).

Our finding that blood levels of histamine are increased in cancer patients with a newly diagnosed solid tumor is in apparent conflict with the results of Burtin et al. (1983). They reported that blood histamine decreased below normal with the extent of the fall indicative of the extent of the malignancy. Within 14 months following surgical removal of the primary tumor, blood histamine levels returned to the normal range. An explanation might lie in the length of time the primary tumors were resident before blood samples were collected. In the present study we found increases in blood histamine only in patients with a newly diagnosed primary tumor. Individuals with older tumors, with or without current therapy, had blood histamine levels in the normal range. Perhaps, in humans, like hamsters (Moore et al. 1978), histidine decarboxylase activity and subsequent nascent histamine synthesis decline when a tumor progresses beyond a specific stage.

Acknowledgements. Many health-care professionals assisted in aspects of this study. The authors gratefully acknowledge the cooperation of Drs. James Adamson, Daniel Gronk, Stanley Davis, Pierce Sloss, and Eugene Van Wie, and the technical assistance of Sara Christiansen, Betty Peterson, Jenny Sneckenberg, and Fred Ullrich. Particular thanks are due to Ann Atkins and Joan Muvick for their efforts in the collection and preparation of many of the samples.

This study was supported by the N. I. H. (CA 41143).

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Received June 10, 1988/Accepted July 5, 1988