Relationships between excretion of steroid hormones and tryptophan metabolites in patients with breast cancer

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The growth and maintenance of the mammary gland requires both steroid and polypeptide hormones. Malignant growth in this tissue may also be hormonally dependent. As a consequence, the ablation of hormone-producing organs, such as the pituitary and adrenal glands, may benefit about half the patients with advanced breast cancer after mastectomy. Not all patients respond to endocrine ablation, however, and methods are required for selecting the patients who are most likely to benefit. Since hormonal imbalance is an etiological feature of breast cancer, the selection of patients for adrenalectomy could be based on either measurement of hormone levels themselves or other biochemical parameters that reflect this change in hormonal status. Of the methods based on levels of endogenous hormones, the discriminant method (1) is the most widely used. The results obtained in a large prospective trial in Guernsey (Channel Islands) (2) indicate the discriminant is of prognostic value even before the disease is manifest. In the discriminant method, the hormonal imbalance is reflected in changes in urinary levels of 17-hydroxycorticosteroids and etiocholanolone. An alternate method uses the ratio of urinary 11-deoxy-17-oxosteroids (3). As sensitive metabolic changes may be apparent before significant alterations in hormonal levels can be measured, an attempt has been made to correlate the discriminant with possible biochemical indicators of hormonal imbalance. The choice of tryptophan metabolites for this purpose was prompted by the work of Rose (4, 5), who attributed the elevation of the excretion of tryptophan metabolites in breast cancer patients to a stimulation of hepatic catabolism by estrogens. In the work to be described, the discriminant, urinary steroid ratio, and the excretory pattern of tryptophan metabolites were measured concurrently in a large group of breast cancer patients and in normal women.

Methods

A simplified method for the separation and quantitative estimation of xanthurenic acid (XA), kynurenine (LK), 3-hydroxy-L-kynurenine (3HK), and 3-hydroxyanthranilic acid (3HA) was developed (Bell, E. M., W. I. P. Mainwaring and R. D. Bulbrook, manuscript in preparation). The method involves three stages: 1) thin-layer chromatography on cellulose (6); 2) elution from the plate; and 3) quantitative measurement of fluorescence. In the latter stage, 3HA and XA were measured directly, whereas 3HK and LK were measured after hydrolysis to 3HA and anthranilic acid, respectively, using kynureninase from Neurospora crassa. The initial stages of the isolation of the enzyme were conducted as described by Jakoby and Bonner (7) but the final purification stage involved stepwise elution from diethylaminoethyl (DEAE)-cellulose. At this purity, the enzyme is infinitely stable at -15 C. The recovery of metabolites in individual experiments was calculated from the recoveries of known amounts of authentic standards from the urine sample under investigation. The specificity of method was checked at higher levels of metabolites by spectrophotometric comparison of the eluted metabolites with standard compounds. The fluorescence spectra did not require correction except for that of XA in which background interference (probably the 8-methyl ether of XA) necessitated the use of a correction procedure (8). Chromatographic separations were performed in the dark.

Individuals under test were fasted overnight and given 5.0 g L-tryptophan (4) orally in milk or orange juice at 7:30 AM. Consistency in the time of administration of tryptophan was prompted by the work of Rapoport et al. (9). Urine was collected for 8 hr thereafter, pooled, and frozen. Samples may be stored at -15 C for up to 3 months without deleterious effects. After gradual thawing, 5 to 25-μl aliquots of urine were analyzed in quadruplicate.

1 From the Imperial Cancer Research Fund, London, and the Breast Cancer Unit of Guy's Hospital, London.
together with duplicate samples supplemented with known amounts of authentic standards. Aliquots were also taken for the determination of the 11-deoxy-17-oxosteroid:17-hydroxycorticosteroid ratio.

Results

The individual metabolites XA, 3HK, 3HA, and LK were calculated as milligrams excreted per 8 hr after tryptophan loading and the summated values were termed total metabolites. Both the individual and total values of the metabolites were plotted against the urinary steroid ratio and the regression, correlation, and probability were calculated for each experimental group.

The values of individual metabolites were not particularly meaningful, whereas those of the total tryptophan metabolites were significant when plotted against the urinary steroid ratio. As shown in Fig. 1, there was a firm correlation in these parameters in normal women, with \( P < 0.02 \). Somewhat higher rates of excretion of tryptophan metabolites were found at the lower steroid ratios. The correlation between the steroid ratio and the tryptophan metabolites only applied in women who were not using estrogen-based contraceptive preparations, for such users excreted abnormally high amounts of tryptophan metabolites. In addition, the lowered urinary steroid ratio mimicked that found in some breast cancer patients.

When the total tryptophan metabolites were calculated in breast cancer patients (Fig. 2), there was more scatter in the results and many patients excreted vastly increased amounts of tryptophan metabolites compared with the normal controls. The slope of the regression line was much steeper and the correlation slightly less definite \( (P < 0.05) \). Of outstanding interest was the finding that patients with low urinary steroid ratios tended to have the abnormally high excretory levels of tryptophan metabolites. These high levels of tryptophan metabolites were not present in postmenopausal cancer patients (Fig. 3), but most patients in this experimental group had low urinary steroid ratios.

The age of the women undergoing the tests had a significant bearing on the levels of tryptophan metabolites excreted after tryptophan loading. In normal women (Fig. 4), the highest excretion rates were found in those at the menopause, between the ages of 42 to 47. Confirmation of this important trend demands the future investigation of many more women of postmenopausal age. The relation-
ship between the menopause and a high rate of excretion of tryptophan metabolites was particularly striking in women with breast cancer (Fig. 5). The women with the abnormally high rates of excretion of total tryptophan metabolites were generally in the 40- to 50-year age group.

Discussion

All the available evidence suggests that the excretory products of tryptophan reflect changes in the catabolic processes in the liver (10) even in cancer patients. Rose (4, 5) for example, showed the abnormal level of tryptophan excretion in cancer patients before and after mastectomy so that the results reflect a general underlying hormonal disturbance rather than the presence of the tumor alone. Many hormones including cortisol (11) are known to influence the activity of hepatic enzyme tryptophan pyrrolase, and in the cancer patients with a low urinary steroid ratio there is almost certainly a higher level of total cortisol relative to the metabolites of the androgens, such as etiocholanolone. Furthermore, because there is decreased binding of cortisol by transcortin in breast cancer patients (12), a higher proportion of the more active, unbound cortisol will result. These changes in the level of steroid hormones could act synergistically in potentiating a higher tryptophan pyrrolase activity, the rate-limiting step in the catabolism of tryptophan (13). The higher level of cortisol will selectively stimulate the synthesis of apoenzyme protein, whereas the reduced level of androgens will result in a decrease in general anabolic processes, with the direction of tryptophan into the catabolic pathway rather than into the general amino acid pool for protein synthesis. The sparing of tryptophan could also lead to direct inductive effects, whereby the saturation of the available tryptophan pyrrolase with heme cofactor is ensured (15). This overall scheme provides a fairly satisfactory explanation of high excretion rates of tryptophan in the group of patients with a low urinary steroid ratio.

Thus far, the metabolic patterns in the cancer group have been discussed solely with reference to the contribution made by androgens and corticosteroids to the general hormonal imbalance. Rose (4, 5) suggests that variations in the levels of estrogens may also be pertinent. The present findings are entirely compatible with the data of Rose (4, 5), in that a lower excretion rate of tryptophan metabolites was found in the older, postmenopausal group, many of whom were in the lower urinary steroid ratio group.

Overall, there was a pronounced difference between the normal and cancer groups, which suggests that a complex hormonal imbalance is perhaps a predilection to the malignant process. Changes in androgens, corticosteroids, and probably estrogens are responsible for this imbalance and this may be satisfactorily demonstrated either by measurements of urinary steroids or by the excretion of tryptophan metabolites.
References


Discussion

D. P. Rose: The possibility of a relationship between the altered hormonal environment of breast cancer and an abnormal urinary excretion of tryptophan metabolites was suggested by the finding of disturbed metabolism of this amino acid in subjects receiving estrogens (1).

The results obtained from my initial study (2) are given in the figure. This shows the total amount of a 5-g oral dose of L-tryptophan excreted as 3-hydroxykynurenine, xanthurenic acid, and 3-hydroxyanthranilic acid by a group of normal women, 20 patients who had been treated for breast cancer by mastectomy alone, and 24 patients treated by prophylactic oophorectomy and mastectomy. None of the cancer patients had evidence of tumor recurrence at the time of the investigation, and in all cases it was at least 3 months since the completion of their course of postoperative radiotherapy.

High excretions of metabolites occurred in 13 of the patients treated by mastectomy alone, and I suggested that this may have been a reflection of an increased secretion of estrogens, or a defective production of androgens. Of the 24 patients treated by oophorectomy, 12 excreted less tryptophan metabolites than did the controls, perhaps because reduction in estrogen activity had impaired the capacity for conversion of tryptophan to nicotinic acid ribonucleotide. The elevated levels of metabolites...
in the urine of eight of the oophorectomized patients may have been due to an increase in estrogen production by the adrenal glands, and it could be that these are the patients for whom a combination of bilateral oophorectomy and adrenalectomy is the most suitable endocrine ablative procedure.

The elevated urinary excretion of tryptophan metabolites in breast cancer is certainly not due to surgical intervention or a metabolic response to radiotherapy for it occurs also in untreated cases of breast cancer (3).

Studies of tryptophan metabolism in carcinoma of the breast, either alone or in conjunction with urinary steroid assays, may provide a guide to the most appropriate form of endocrine therapy for a particular patient. In addition, the report by Bulbrook and Hayward (4) of an apparent association between abnormal urinary hormone excretion and the subsequent development of breast cancer suggests that the determination of tryptophan metabolites in urine could be used for the detection of "high risk" women in the population with respect to these tumors. This has been made a practical proposition with the introduction of automated methods of assay by Dr. Brown and his co-workers.

J. E. Leklem: Did you notice any 8-methyl ether of xanthurenic acid co-chromatographing with xanthurenic acid?
W. I. P. Mainwaring: Yes, there is a possibility that the contaminant mentioned is this compound. However, using the Allen correction that I suggested, we managed to get satisfactory analyses without having to take this contaminant into further consideration.

J. E. Leklem: Did the extent of disease in the breast cancer group correlate with the level of metabolites?
W. I. P. Mainwaring: We haven't looked into it. This work is in a relatively preliminary stage at the moment but this type of question will be looked into in the near future.

References