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Increased folate catabolism in mice with ascitic tumours

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Summary

1. Folate deficiency is reported in association with certain malignant tumours, and it has been suggested that this arises from increased folate turnover and catabolism in such circumstances.

2. Using an experimental animal model to determine the rate of catabolism of [^{3}H]pteroylglutamate (folic acid) by the quantitative estimation of the two urinary catabolites *p*-[^{3}H]aminobenzoylglutamate and [^{3}H]acetamidobenzoylglutamate, we have measured the rate of folate catabolism in mice with ascitic tumours.

3. There was a significant increase in the rate of catabolism in the mice with tumours compared with controls over a 10 day period. This was associated with the accumulation of ascitic fluid and an increase in the number of tumour cells in the treated animals.

4. The increase in catabolism appeared to be due to increased cell turnover of the tumour rather than an increase in cell mass, as the increase in mass of the tumour was negligible.

Key words: ascitic tumour, folate, pteroylglutamate, tumours.

Introduction

Folate deficiency is known to develop in patients with certain tumours [1, 2] and is presumed to be due to the increased demand for folate coenzymes in rapidly dividing cells. Further support for this hypothesis was obtained when an abnormal pteridine, 6-hydroxymethylpterin, which is a product of folate catabolism, was demonstrated both in tumour cell cultures and in the urine of cancer patients [3].

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The aim of this study was to investigate the possibility that the folate deficiency associated with malignant tumours is due to increased folate catabolism. The rate of catabolism of [^{3}H]pteroylglutamate ([^{3}H]PteGlu) in mice with ascitic tumour cells was measured by estimating quantitatively the two main urinary catabolites, *p*-aminobenzoylglutamate (PABGlu) and acetamidobenzoylglutamate (APABGlu) [4, 5].

Methods

Radiochemicals

The following radiochemicals (with their corresponding specific radioactivities) were supplied by Amersham International, Amersham, Bucks., U.K.: [^{3}H]pteroylglutamate ([3′,5′,7,9(n)-^{3}H]PteGlu; 93 mCi/mg) >95% pure; [^{3}H]hexadecane (2.17 mCi/mg); ^{51}Cr-labelled EDTA (700 μCi/mg).

Animals and procedure

BDF (C67BL/6 x DBA/2) mice of the male sex were randomly assigned to groups of five per metabolic cage. These mice were specially inbred and are particularly suitable for evaluation of the P388 lymphatic leukaemia [6, 7]. All mice were fed on a standard diet containing 0.5 mg of folic acid/kg body wt. [8], and their daily weight and dietary intake and fluid balance were measured. All mice received a single intraperitoneal injection of 4 μCi of [^{3}H]PteGlu and 5 nCi of ^{51}Cr-labelled EDTA, and the pooled urine from each cage was collected daily for 10 days.

Tumour mice. Four groups of five mice each received a single intraperitoneal injection of 100 μl of Locke-Ringer solution containing 10^6 P388 lymphoid leukaemia cells. This tumour line was induced in 1955 in a DBA/2 mouse [6]. It is maintained in DBA/2 mice [7]. At 10 days the

mice were killed by cervical dislocation; the amount of ascitic fluid was measured and the tumour cells were separated, weighed and counted with a haemocytometer [6].

Control mice. Two groups of five mice each received a single intraperitoneal injection of 100 µl of Locke–Ringer solution alone.

Estimation of [^{3}H]PABGlu and [^{3}H]APABGlu

Estimation of these catabolites was performed by alkaline hydrolysis of all *p*-aminobenzoic acid ([^{3}H]PAB)-containing catabolites to [^{3}H]PAB which was extracted by a previously described procedure and the radioactivity estimated [5]. The completeness of the injection and collection techniques was monitored by use of ^{51}Cr-labelled EDTA [5].

Statistics

The difference between the mean daily excretion and cumulative excretion of [^{3}H]PAB catabolites in the control and the tumour groups was determined [9].

Results

Estimation of tumour growth

In the first 3 days each group of mice lost weight in the metabolic cages: mean weight loss per cage of five mice was 3 g in the controls and 9.8 g in the treated mice. Subsequently over the next 7 days all mice gained weight: mean weight gain per cage of five mice was 1.2 g per cage in the controls and 17.2 g per cage in the treated mice. The increase in weight in the treated mice was partly due to an increase in the weight of the tumour cells (5.6 g per cage of five mice), in turn mainly due to the accumulation of ascitic fluid (11.6 g per cage of five mice). The tumour cells accounted for a 5% increase in body weight.

The number of tumour cells also increased from the inoculum of 10^6 P388 lymphoid leukaemia cells per mouse to 330×10^6 per mouse or 1.6×10^9 per cage of five mice. There was no accumulation of ascitic fluid in the control group.

Estimation of total radioactivity

Total excreted radioactivity was highest in the first 3 days in all groups, stabilizing after this time to a slow decline [5]. There was a significant increase in total excretion of radioactivity in the treated group compared with controls from day 4 onwards. Mean (± SEM) daily excretion was $(4.9 \pm 0.5) \times 10^4$ c.p.m. (controls) and $(11.4 \pm 2.8) \times 10^4$ c.p.m. (treated) ($P < 0.001$). Mean (± SEM) cumulative excretion from day 4 to day 10 was $(3.6 \pm 0.5) \times 10^5$ c.p.m. (controls) and $(5.5 \pm 0.5) \times 10^5$ c.p.m. (treated) ($P < 0.001$).

Estimation of [^{3}H]PAB catabolites

Estimation of [^{3}H]PAB catabolites was highest in the first 3 days, stabilizing after this time (Fig. 1). There was a definite increase in these catabolites from day 4 onward in the treated group. Mean (± SEM) daily excretion in the treated group was $(2.6 \pm 0.25) \times 10^4$ c.p.m. and in the control group $(1.7 \pm 0.2) \times 10^4$ c.p.m. ($P < 0.01$). Mean (± SEM)

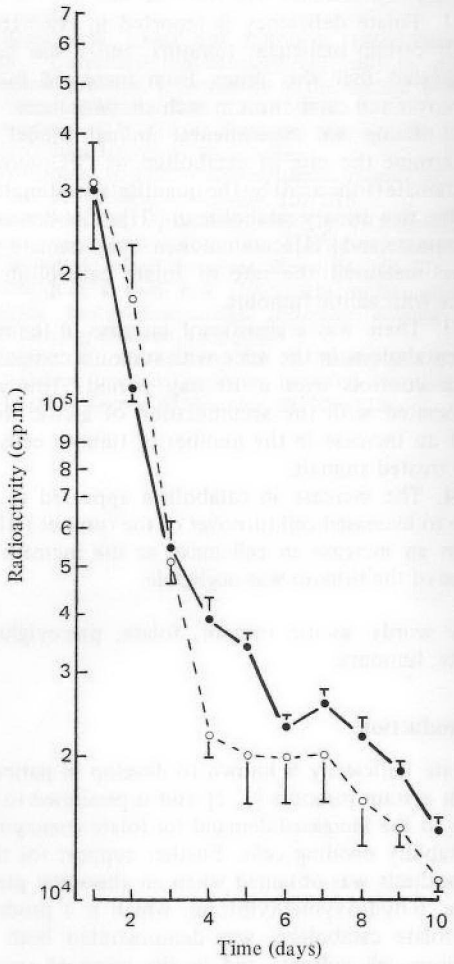

FIG. 1. Rate of folate catabolism in mice with ascitic tumours compared with controls. ●, Mean ± SEM of four groups of five mice with ascitic tumours; ○, mean ± SEM of two groups of five control mice.

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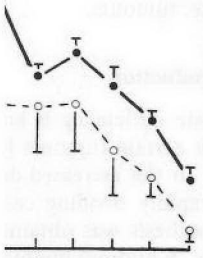

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cumulative excretion from day 4 to day 10 in the treated group was $(1.80 \pm 0.2) \times 10^5$ c.p.m. and in the control group was $(1.20 \pm 0.2) \times 10^5$ c.p.m. ($P < 0.001$).

Discussion

This study demonstrates that there is a 50% increase in the rate of folate catabolism in mice with ascitic tumours. The increased rate of catabolism coincided with the accumulation of ascitic fluid in the treated animals and a substantial increase in both the number and the weight of the malignant cells, implying an active tumour. This suggests that the increased rate of catabolism was due either to an increased demand for folate coenzymes in the malignant cells or to increased folate turnover in the rapidly dividing cells. As there was a 5% increase in the weight of the tumour and a 50% increase in catabolism, it is likely that the increased catabolism was associated with increased cell turnover rather than an increase in weight of the tumour.

These results conflict with a recent study which reported that folate metabolism is decreased in tumour-bearing rats [10]. However, in the latter study the urinary metabolites were measured at 24 and 48 h after administration of radioactive PteGlu. It has been consistently shown that there is a complex pattern of excreted intact folates in the first 2 days after a radioactive dose and that the radioactive catabolic products are not found in the urine until after day 3 [4]. Furthermore, as in that study [10] the two main catabolites, PABGlu and APABGlu, were not estimated after day 3 it is unlikely that folate catabolism was being measured. Measurement of total excretion during this initial equilibration period completely masks any difference that exists in the excretion of folate catabolites [4]. In addition, the study made no attempt to distinguish between excretion of intact folate and of catabolites.

Why the presence of tumour cells, either in man [3] or in mice, should lead to increased folate breakdown remains unclear. It seems reasonable, however, that rapid cell division, dependent as it is on folate participation, might lead to increased turnover of the vitamins.

Acknowledgments

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