ONCOLOGY

In Vitro Effects of Folic Acid on γ-Glutamyltransferase and Glutathione Reductase Activities in Malignant Lung and Thymus Tumors

A. A. Karelin, R. N. Korotkina, G. N. Matskevich, V. V. Borisov, A. A. Vishnevskii, L. V. Polikarpova, and A. G. Kunitsyn

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In vitro effects of folic acid (10^{-5}, 10^{-4}, and 10^{-3} M) on activities of γ-glutamyltransferase and glutathione reductase, the enzymes involved in glutathione metabolism, were studied in tissue samples obtained after surgical treatment of the lungs and thymus. Folic acid did not change γ-glutamyltransferase activity in lung cancer tissue, but in thymoma tissue this substance in a concentration of 10^{-3} M inhibited it by 16%. Folic acid had no effects on glutathione reductase activity in benign tumors and normal lung and thymus tissues, but increased this activity in thymoma and lung cancer tissues. Activation of glutathione reductase was probably related to binding of folic acid in the allosteric center of the enzyme, which probably induced conformational changes in the catalytic center, acceleration of electron transport from $NADPH_2$ to oxidized glutathione via flavin adenine nucleotide, and intense production of reduced glutathione.

Key Words: *malignant tumors; lungs; thymus; folic acid; γ-glutamyltransferase; glutathione reductase*

Glutathione metabolism in non-small-cell lung cancer cells contributes to the resistance of these tumors to radio- and chemotherapy [5]. In this respect, studies of enzymes involved in glutathione metabolism during malignant transformation of lung cells are of considerable importance.

Our previous experiments showed that γ-glutamyltransferase (γ-GT, EC 2.3.2.4) activity in tumor tissues from patients with lung cancers and thymomas 70-fold surpasses that in healthy individuals [3]. Activities of other enzymes involved in glutathione metabolism, glutathione reductase (GR, EC 1.6.4.2), glutathione S-transferase (EC 2.5.1.18), and glutathione peroxidase (GSH-Px, EC 1.11.1.9) also increase in malignant tumors of the lungs and thymus.

It was reported that intracellular accumulation of glutathione in human malignant tumors is probably associated with activation of γ-GT [6,8]. Therefore, the use of folic (pteroylglutamic) acid (FA) or its antagonists (aminopterin and amethopterin) as γ-GT inhibitors is a promising approach to the therapy of patients with lung and thymus cancers.

FA and its analogues were shown to inhibit activity of soluble γ-GT from rat liver and grafted hepatoma G-27 [1,2]. However, *in vitro* effects of FA on γ-GT and GR activities in malignant tumors of human lungs and thymus are still unknown.

Since GR is an oligomer consisting of 2 subunits with a molecular weight of 50 kDa [12], we assumed that FA acts as an allosteric regulator of enzyme activity.

A. V. Vishnevskii Institute of Surgery, Russian Academy of Medical Sciences, Moscow

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Here we studied *in vitro* effects of FA on γ-GT and GR activities in neoplastic tissues of the lungs and thymus (thymoma).

MATERIALS AND METHODS

We examined tissues obtained during surgical treatment of malignant tumors of the lungs and thymus: thymoma (n=7), malignant tumors of the lungs (central and peripheral cancers, n=15), and benign tumors of the lungs (tuberculoma and hamartoma, n=8). We also studied samples from the lung and thymus parenchyma containing no atypical cells (normal tissues). The samples were frozen and stored at -20°C. Immediately before examination, these samples were scissored on ice and homogenized in a Potter-Eveliem homogenizer. γ-GT activity was measured as described elsewhere [11]. The amount of γ-GT catalyzing the formation of 1 nmol p-nitroaniline from L-γ-glutamyl-p-nitroaniline per mg protein over 1 h was taken as one unit of enzyme activity. GR activity was measured as described elsewhere [7] and expressed in nmol degraded $NADPH_2$ per mg protein over 1 min. The concentration of total protein in homogenates was estimated by the method of Lowry [10]. FA was used in concentrations of 10^{-5}, 10^{-4}, and 10^{-3} M. The samples incubated without FA served as the control. The results were analyzed by Student's t test.

RESULTS

Since the γ-glutamyl residue in FA should have high affinity for the γ-GT active center (due to structural similarity), competitive inhibition of γ-GT with FA can be expected. However, it was reported that FA inhibits γ-GT in a noncompetitive manner [2].

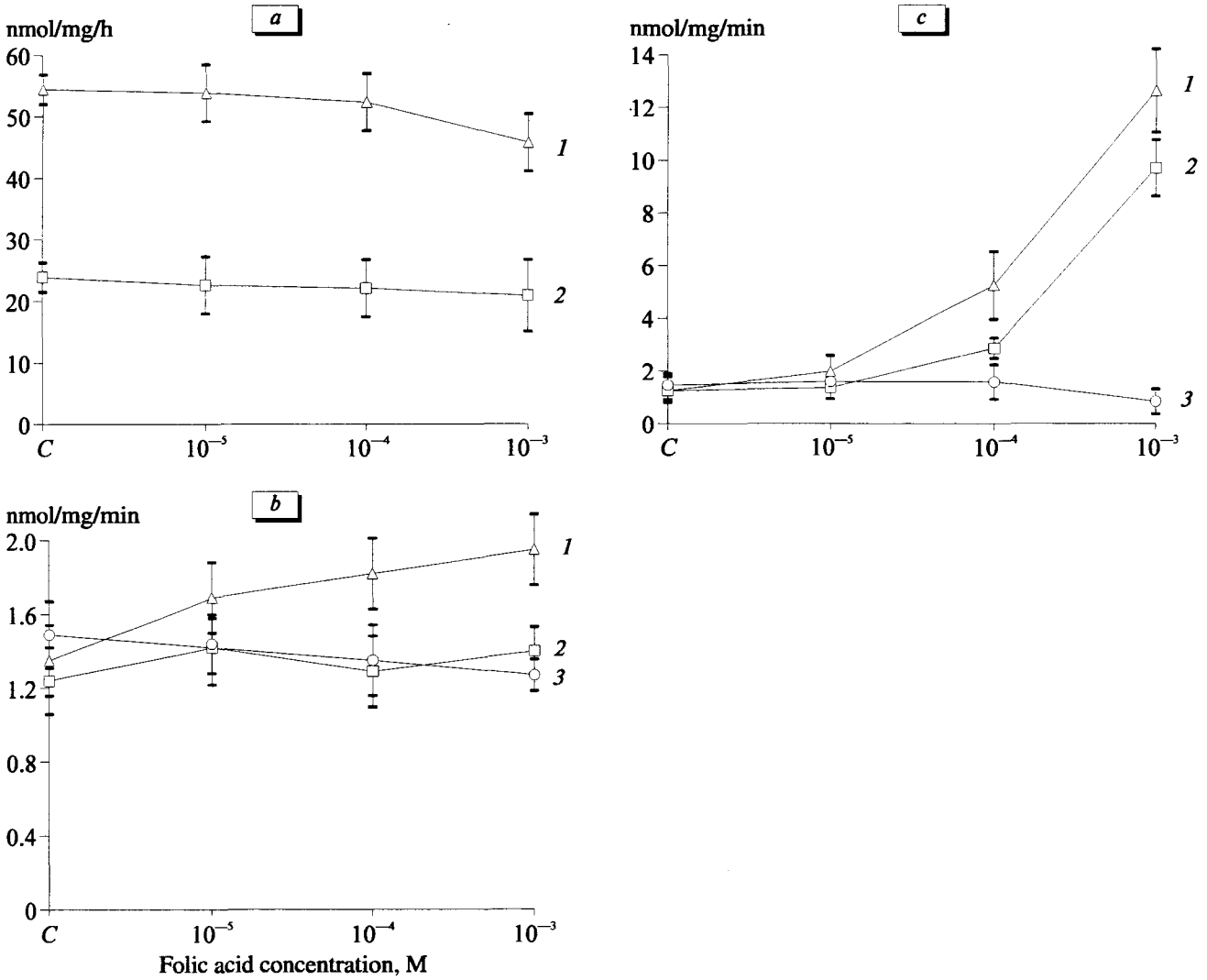

Fig. 1. Effects of folic acid on activities of γ-glutamyltransferase (*a*) and glutathione reductase (*b*, *c*) in benign and malignant tumors of the thymus and lungs. *a*: Malignant thymoma (*1*) and lung cancer (*2*); *b*: lung cancer (*1*), benign tumor of the lungs (hamartoma, *2*), and normal lung parenchyma (*3*); *c*: malignant thymoma in the absence (*1*) and presence of 8 M urea (*2*), normal thymus tissue (*3*). *C*: control.

FA in concentrations of 10^{-5} and 10^{-4} M had no effect on γ-GT activity in lung cancer and thymoma tissues (Fig. 1, *a*), but in a dose of 10^{-3} M this substance inhibited enzyme activity in thymoma tissue by 16%. In lung cancer tissues, 10^{-3} M FA only slightly decreased γ-GT activity.

In lung cancer tissue, FA in concentrations of 10^{-5}, 10^{-4}, and 10^{-3} M increased GR activity by 25, 34.6, and 44.2%, respectively (Fig. 1, *b*).

FA had no effect on GR activity in hamartoma tissue.

FA did not activate GR in normal lung parenchyma tissue containing no atypical cells.

FA in concentrations of 10^{-5}, 10^{-4}, and 10^{-3} M increased GR activity in thymoma tissue by 58.8, 320.9, and 1000%, respectively (Fig. 1, *c*).

We also studied the effect of treatment with a denaturing agent (8 M urea) on FA-induced activation of GR in thymoma tissue homogenates. In the presence of 8 M urea, the activating effect of FA persisted, but was less pronounced. FA in concentrations of 10^{-4} and 10^{-3} M increased GR activity by 114 and 800%, respectively, while in a concentration of 10^{-5} M this substance practically did not change enzyme activity.

It should be emphasized that FA did not activate GR in normal thymus tissues, while in a concentration of 10^{-3} M, this substance even decreased enzyme activity.

Thus, human thymoma tissues hold much promise for studying the effects of FA on GR activity.

FA (as a metabolic regulator) probably binds to the allosteric center of GR, which leads to conformational changes in its catalytic center, acceleration of electron transport from $NADPH_2$ to oxidized glutathione (via flavin adenine nucleotide), and intense production of reduced glutathione. This mechanism of GR activation is confirmed by the following facts: first, FA is not structurally similar to GR substrates, $HADPH_2$ and oxidized glutathione, and second, treatment of the enzyme with 8 M urea attenuates its activation with FA (Fig. 1, *c*).

FA-induced activation of GR from lung and thymus cancer cells probably has much biological importance. Rapidly growing cancer cells require DNA synthesis and folate coenzymes [13]. Ribonucleotide reductase (EC 1.17.4.1) is a key enzyme catalyzing the synthesis of deoxyribonucleotides (as DNA precursors), in particular $NADPH_2$-dependent reduction of ribonucleotide diphosphates to deoxyribonucleotide diphosphates [15]. Electrons are transferred from $NADPH_2$ to the active center of ribonucleotide reductase via reducing sulfhydryl compounds (thioredoxin and glutaredoxin) [9]. Glutathione generated by GR appeared to be the source of reducing equivalents for ribonucleotide reductase [14]. GR transfers the reduction potential from glutathione to ribonucleotide reductase [14]. These data explain the interrelation between FA-induced activation of GR in lung and thymus cancer cells and DNA synthesis necessary for rapidly growing neoplastic cells.

FA-induced activation of GR in malignant tumors of human lungs and thymus is of particular interest. This phenomenon is important for studying the mechanisms of regulation of GR and other disulfide reductases. Measurements of FA-stimulated GR activity in biopsy samples can be used in clinical practice for the diagnostics of small lung neoplasms (1.5-3 cm in diameter), including hamartoma, tuberculoma, and cancer.

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