FREE GLUTAMINE CONTENT AND GLUTAMINASE ACTIVITY IN THE GASTRIC MUCOSA OF PATIENTS WITH PRECANCEROUS DISEASES AND CARCINOMA OF THE STOMACH

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The free glutamine content and phosphate-dependent glutaminase activity were determined in biopsy specimens of tissue from gastric polyps, malignant tumors, and ulcers and the surrounding mucosa from patients with precancerous diseases and with carcinoma of the stomach. The glutamine content in the carcinomas was not significantly different from that in normal gastric mucosa. The glutamine level in areas of mucosa remote from the tumor was considerably higher than normally. Gastric polyposis was characterized by absence of glutamine in the mucosa outside the lesion. Marked glutaminase activity was found in the carcinomas, but its level was indistinguishable from normal or from that in patients with precancerous diseases.

KEY WORDS: glutamine; glutaminase; precancerous diseases of the stomach; carcinoma of the stomach.

There is evidence in the literature of the important role of glutamine in neoplastic processes. Glutamine is an essential component of the medium for growth of the cells of certain tumors in tissue culture [7,11,14]. It is also utilized intensively by various tumors of animals and man [2,4]. One of the biochemical reactions of glutamine utilization is its hydrolytic deamination catalyzed by glutaminase. This reaction has been found to be either depressed [1,8] or intensified [10,13] in malignant tumors. Correlation has been established for many tumors between the rate of growth, the activity of phosphate-dependent glutaminase, and morphological structure [9,12]. The study of glutamine metabolism in the process of carcinogenesis and, in particular, the elucidation of the specificity of its metabolism in precancerous diseases [3] and carcinoma of the stomach, is of great interest.

The object of this investigation was to study the free glutamine content and phosphate-dependent glutaminase activity in the gastric mucosa of normal subjects and of patients with precancerous diseases and carcinoma of the stomach.

EXPERIMENTAL METHOD

The free glutamine content [6] and phosphate-dependent glutaminase activity were determined in areas affected by ulcer, by polyposis, and by carcinoma and in areas of gastric mucosa remote from them in patients with chronic atrophic gastritis, atrophic gastritis with metamorphosis of the glands into the intestinal type ("metamorphosis gastritis"), gastric ulcer, polyposis, and carcinoma of the stomach. Persons with a normal gastric mucosa formed the control group. The diagnosis was made on the basis of roentgenological, gastroscopic, and histological investigations of the patients. Tissue was obtained by direct vision intravital gastric biopsy using the "Olympus" fibrogastroscope. A tissue homogenate (the ratio of tissue to water 1:50) was used. Phosphate-dependent glutaminase activity was determined from the quantity of ammonia formed during hydrolysis of glutamine in the presence of phosphate. The composition of the incubation mixture was: 0.2 ml of a 0.05 M neutral solution of L-glutamine, 0.4 ml of homogenate, 0.4 ml of 0.5 M phosphate buffer, pH 8.3. The

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TABLE 1. Free Glutamine Content and Glutaminase Activity in Normal Gastric Mucosa and in Precancerous Diseases and Carcinoma of the Stomach (M± m)

State of subjects from whom material was investigated	No. of subjects investigated	Glutamine, μg N/ mg protein		Glutaminase activity, μg N/ mg protein	
		area of lesion	mucosa outside lesion	area of lesion	mucosa outside lesion
Normal	10	—	10,50±3,23	—	45,99±7,93
Atrophic gastritis	15	—	13,04±4,12	—	52,85±7,84
"Metamorphosis gastritis"	10	—	5,35±1,0	—	0
Gastric ulcer	25	16,82±2,44	19,44±4,75	67,07±15,21	69,09±12,92
Gastric polyposis	10	17,65±6,67	0	32,41±4,75	41,19±7,78
Carcinoma of the stomach	13	13,91±3,17	24,23±5,0	52,43±10,03	62,81±18,15

samples were incubated for 2 h at 37°C. For each sample there were appropriate controls enabling corrections to be made for spontaneous ammonia formation from glutamine (sample without homogenate), for ammonia formation in the homogenate through the action of enzymes on preformed substrates (sample without glutamine), and for the presence of ammonia in the reagents (sample without glutamine or homogenate). The reaction was stopped by the addition of 1 ml of saturated K_2CO_3 solution. The ammonia liberated was trapped in 1 N sulfuric acid solution. The nitrogen content was then determined in all the samples [5]. The glutamine content and glutaminase activity were expressed in μg nitrogen/mg protein. Protein was determined by Lowry's method.

EXPERIMENTAL RESULTS AND DISCUSSION

The mean content of free glutamine in normal human gastric mucosa was 10.5± 3.23 μg/mg (Table 1). In precancerous states of the mucosa the glutamine level was a little different from normal. In polyposis, no free glutamine could be found in an area of the mucosa remote from the lesion. Tissue from ulcerative lesions and polyposis, however, contained large quantities of glutamine. Its content in polyps was significantly higher than in the surrounding gastric mucosa ($P < 0.05$). In the normal mucosa and in chronic atrophic gastritis and gastric ulcer (in mucosa affected by the ulcer and also at a distance from it) glutamine was found in more than half of the subjects examined, but it was found in only two of 10 patients with "metamorphosis gastritis," and it was not found in a single sample of mucosa outside the lesion in patients with polyposis, although it was constantly present in the polyps.

In patients with carcinoma of the stomach the glutamine content in the tumor tissue did not differ significantly from normal. The glutamine level in gastric mucosa remote from the tumor was twice as high as in normal tissue ($P < 0.05$) and in the carcinoma itself ($P < 0.02$). Glutamine was found in the tumor tissue of most patients with carcinoma of the stomach. If it was absent from the tumor tissue, it was likewise not found either in the surrounding gastric mucosa of the same patients.

Determination of phosphate-dependent glutaminase activity in areas of ulceration, polyposis, carcinoma, and normal mucosa in patients with chronic atrophic gastritis, gastric ulcer, and polyposis and carcinoma of the stomach revealed no significant differences (Table 1). Like free glutamine, active glutaminase could not be detected in every case. The mucosa of the patients with "metamorphosis gastritis" in general contained no glutaminase. The absence of changes in total activity of phosphate-dependent glutaminase does not rule out possible abnormalities of its isozyme spectrum.

The substantial increase in the glutamine content in the mucosa surrounding the tumor from patients with carcinoma of the stomach and the absence of glutamine in the surrounding mucosa of patients with polyposis of the stomach point to different relations between the tumor and the surrounding mucosa in benign and malignant processes.

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