Enterocyte function in progressive systemic sclerosis as estimated by the deconjugation of pteroyltriglutamate to folic acid

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SUMMARY As a measure of enterocyte function, the deconjugation of pteroyl-l-glutamyl-y-L-glutamic acid to folic acid and subsequent active absorption was measured in 19 patients with progressive systemic sclerosis and compared with 14 controls. The absorption step of folic acid was identical in the two groups, while deconjugation of pteroyl-l-glutamyl-y-L-glutamyl-y-L-glutamic acid was significantly decreased in the patients with progressive systemic sclerosis. This observation suggests a primary epithelial defect of the small intestine in patients with progressive systemic sclerosis.

Progressive systemic sclerosis (PSS) is a systemic disease characterised by excessive deposits of collagen in skin and various internal organs. The small intestine is involved in about 40% of all progressive systemic sclerosis cases. The cell kinetics and the ultrastructure of the enterocyte has been shown to be changed in progressive systemic sclerosis patients, and a primary epithelial defect of the small intestine has previously been suggested.

Folates are absorbed in the upper jejunum by a rather complicated two stage process. Practically all folates in food are present as glutamic acid conjugates and their absorption requires hydrolysis to pteroylmonoglutamate forms by a specific γ-carboxypeptidase at the mucosal brush-border, and subsequent transport across the enterocyte by an energy-dependent process.

The aim of the present study was, as a measure of enterocyte function, to estimate the deconjugation of a synthetic pteroyltriglutamate (TGA) (Fig. 1) to folic acid (PGA) and the subsequent active absorption in progressive systemic sclerosis patients in reference to a group of controls.

Methods

Patients Nineteen patients (mean age 57 years, range 28–78, 17 women, two men), with progressive systemic sclerosis consented, after being properly informed, to participate in the study. All progressive systemic sclerosis patients were being treated with collagen synthesis inhibitors (D-penicillamine and glutamine).

Controls The control group (n=14, mean age 37 years, range 16–62, 12 women, two men) is taken from an earlier investigation on the impact of antiepileptic treatment

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Fig. 1 Structure of the synthetic pteroyltriglutamate (TGA).
on folate absorption, and from five healthy volunteers. The patients were all otherwise healthy outpatients with first time seizures and, at the time of the investigation, without any previous or present medication.

**CHEMICALS**

Pteroylglutamic acid (PGA, folic acid) (Sigma Chemical Co, St. Louis, USA) Pteroyl-L-glutamyl-γ-L-glutamic acid (Niels Clausen-Kaas, Chemical Research Laboratory, Farum, Denmark). Both substances were ground with lactose powder and dispensed into gelatine capsules, each containing 5 μmol.

**INVESTIGATIONS**

During admission each patient was subjected to two experiments. The folate absorption of a 5 μmol oral dose of pteroylglutamate was measured and, after one week, the absorption of a 5 μmol oral dose of folic acid was measured. During each experiment, blood was drawn before and 30, 60, 80, 100, 120, 150, 180, 240, 300, 330, and 360 minutes after pteroylglutamate or folic acid administration. During the experiment the patients received a light lunch without vegetables, and were allowed to drink mineral water, tea, and coffee ad libitum.

The treatment of the progressive systemic sclerosis patients with collagen synthesis inhibitor was discontinued before each absorption test. The last dose was administered at noon the day before the test, and first reintroduced after termination of the experimental procedure.

**DETERMINATION OF FOLATE**

Plasma samples were stored at −20°C. Each patient’s specimens, from both experiments, were assayed in one batch, using a modified lactoglobulin binding method, sensitivity 2.5 nmol/l, equally sensitive for folic acid and 5-methyl-tetrahydrofolic acid.

**KINETIC MODEL**

Pteroylglutamate is a synthetic substrate for γ-carboxy-peptidase. At the mucosal brush border it is degraded to folic acid and eventually absorbed as such. In other studies multiple small intestinal biopsies were obtained from all progressive systemic sclerosis patients included in the present study. Surface architecture, reflecting absorptive area, was in all of the patients found normal when surveyed in the dissecting microscope. The distribution of folate is with physiological doses rather complex and characterised by a sum of saturable processes in the liver, tissues and kidneys. By raising the dose to 5 μmol (for comparison: the minimal dietary requirement is only 0.1 μmol daily) these processes are saturated and rendered negligible, and first order plasma kinetics apply. In a first order system the following applies:

\[
\text{AUC} = \frac{\text{dose absorbed}}{C_l}
\]

Consequently, the ratio of AUC\(_{\text{TGA}}\)/AUC\(_{\text{PGA}}\) of every patient or control equals the fraction of TGA dose absorbed in relation to PGA dose absorbed in this particular patient or control:

\[
\frac{\text{AUC}_{\text{TGA}}}{\text{AUC}_{\text{PGA}}} = \frac{\text{TGA dose absorbed}}{\text{PGA dose absorbed}}
\]

which simplifies to:

\[
\frac{\text{TGA dose absorbed}}{\text{PGA dose absorbed}}
\]

The ratio of AUC\(_{\text{TGA}}\)/AUC\(_{\text{PGA}}\) can therefore be used as an estimate of the intestinal brush-border γ-carboxy-peptidase activity.

**STATISTICAL ANALYSIS**

The dispersion of distributions was compared by the F-test. The means were thereafter compared by Student’s t test (Table).

<table>
<thead>
<tr>
<th>Type of quantity*</th>
<th>PSS patients (n=19) (mean±SD)</th>
<th>Controls (n=14) (mean±SD)</th>
<th>Level of significance (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC(_{\text{PGA}}) min - nmol/l</td>
<td>33034±12117</td>
<td>34469±7614</td>
<td>NS</td>
</tr>
<tr>
<td>AUC(_{\text{TGA}}) min - nmol/l</td>
<td>20117±7957</td>
<td>27517±5425</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>AUC(<em>{\text{TGA}})/AUC(</em>{\text{PGA}}) ratio</td>
<td>0.631±0.157</td>
<td>0.810±0.086</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

*For abbreviations see footnote.

**Results**

The results from the absorption studies are listed in the Table and displayed graphically in Figures 2 and 3. The means of areas under folate plasma concentration curves after folic acid dosing (Fig. 2) are virtually identical in the two groups, contrary to the area under curve after pteroylglutamate dosing, which is significantly lower in the progressive systemic sclerosis group (20117±7957 min - nmol/l) than in the control group (27517±5425 min - nmol/l) (p<0.01). As an individual measure of the intestinal brush-
Enterocyte function in progressive systemic sclerosis

Fig. 2  Mean of plasma folate (over fasting level) after administration of 5 μmol PGA (full lines) and of TGA (dotted lines) in 19 PSS patients (○○○) and in 14 controls (●●●).

border γ-carboxy-peptidase the ratio of AUC\textsubscript{TGA}/AUC\textsubscript{PGA} for each patient and control is shown in Figure 3. The ratio is found significantly decreased in the progressive systemic sclerosis patient group (p<0.001).

The control group comprises younger individuals than the progressive systemic sclerosis patient group. Age and AUC\textsubscript{TGA}/AUC\textsubscript{PGA} ratio, however, were found to be totally uncorrelated by linear regression (r=0.29), rendering the groups comparable.

Discussion

Folatepolyglutamates are deconjugated by a specific γ-carboxy-peptidase at the intestinal mucosal brush-border. The present study uses the ratio absorbed pteroyltriglutamate absorbed folic acid as a test of enterocyte function in progressive systemic sclerosis patients in reference to a group of controls. As every participant acts as his own control in the estimation of this ratio, the ratio reflects the degree of TGA deconjugation to folic acid in every particular participant.

As seen from Fig. 2 the active absorption of folic acid is nearly identical in the two groups, whereas the pteroyltriglutamate deconjugation is significantly lower in the progressive systemic sclerosis patient group (Fig. 3). As the mucosal absorptive area, judged by multiple small intestinal biopsies, was found normal in all patients, a decreased γ-carboxy-peptidase activity of the mucosal brush border in the progressive systemic sclerosis group is the most likely reason for this.

Impaired intestinal motility in progressive systemic sclerosis may give rise to bacterial overgrowth which again may lead to high folate concentrations as a consequence of folate elaboration by the intestinal bacteria. Three of our progressive systemic sclerosis patients had bacterial overgrowth, but did not differ significantly from the rest of the group (Fig. 3).

The progressive systemic sclerosis patient group comprises more older individuals than the group of controls. The folate absorption kinetics, however, are found totally uncorrelated to age, which leaves the higher age mean in the progressive systemic sclerosis group without significance.

All progressive systemic sclerosis patients were in long term medication with collagen inhibitors. This medication was discontinued in time to allow a total clearing of the last dosage before the absorption test. This excludes a direct interference of D-penicillamine or glutamine on the absorption; yet secondary effects of the longterm collagen inhibitive treatment cannot be entirely discounted.

The decreased intestinal absorptive capacity for pteroyltriglutamate in progressive systemic sclerosis patients does not necessarily imply folate malabsorption during the much lower, physiological folatepolyglutamate concentrations, but it does lend further evidence to the theory of a primary epithelial defect in progressive systemic sclerosis, which has been previously suggested.

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References


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