Clinical Science and Molecular Medicine (1978), 55, 293-300

Analytical subcellular fractionation of jejunal biopsy specimens: enzyme activities, organelle pathology and response to corticosteroids in patients with non-responsive coeliac disease

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(Received 24 October 1977; accepted 8 May 1978)

Summary

1. Jejunal biopsies from five patients with non-responsive coeliac disease have been subjected to analytical subcellular fractionation and enzymic microassay in order to compare the organelle pathology of this group with untreated but gluten-sensitive patients.

2. Compared with the gluten-sensitive group these non-responsive patients showed marked reduction of the endoplasmic reticulum enzymes, normal activities of lysosomal enzymes and slightly less severely reduced brush border activities.

3. It is suggested that the present biochemical studies in combination with previous clinical reports and measurements of DNA and protein synthesis by cultured mucosal biopsies delineate non-responsive coeliac disease as a distinct entity.

4. The patients were treated with oral prednisolone (20 mg daily) for between 5 and 9 weeks and the properties of the jejunal biopsies restudied.

5. Although morphologically there was only a partial restoration of the villus architecture the enzymic alterations and organelle abnormalities returned essentially to normal values.

Key words: brush border, corticosteroids, cytosol, disaccharidase, jejunal biopsy, microsomes, non-responsive coeliac disease, plasma membrane, subcellular fractionation.

Introduction

Coeliac disease is an important cause of the malabsorption syndrome and is associated with gross morphological abnormalities of the small intestinal mucosa. Although there has been some discussion on the exact definition of the disease, it is clear that most patients show a considerable improvement in the villus architecture and enterocyte structure and function after exclusion of gluten from the diet. There is, however, a small proportion of adult patients, variously estimated at between 10 and 20%, who do not show a villus regeneration after gluten withdrawal. It is a matter for conjecture whether these patients should be classified as having coeliac disease or should be designated as showing another form of the coeliac syndrome. Certainly some of these patients, who we have referred to as having non-responsive coeliac disease (Jones & Peters, 1977), have earlier in the course of their disease shown a morphological and clinical response to gluten withdrawal. At present it is possible to categorize a patient as having non-responsive coeliac disease only after several months of close observation on a strict gluten-free diet. If distinct biochemical differences can be detected in these patients it is likely that it will be possible to predict which patients will fail to respond to a gluten-free diet. In the present study, using analytical subcellular fractionation techniques combined with enzymic analysis, differences between these two groups of patients with different types of coeliac disease have been explored.
**TABLE 1. Clinical details of patients with non-responsive coeliac disease**

Abbreviations: GFD, gluten-free diet; SVA, subtotal villus atrophy; PVA, partial villus atrophy; NH, normal histology.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Clinical details</th>
<th>Steroid therapy</th>
<th>Morphological response</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.D.</td>
<td>72</td>
<td>F</td>
<td>Previous malabsorption with SVA, responding clinically and histologically to GFD. Relapse after gluten ingestion with persistent SVA despite GFD. Minimal malabsorption</td>
<td>Prednisolone: 20 mg daily, 5 weeks</td>
<td>SVA → PVA</td>
</tr>
<tr>
<td>K.G.</td>
<td>56</td>
<td>F</td>
<td>Malabsorption with SVA for 10 years. Initial clinical response to GFD but persisting SVA in spite of strict GFD and ACTH (40 units daily) for 1 year. Severe malabsorption</td>
<td>Prednisolone: 20 mg daily, 5 weeks</td>
<td>SVA → PVA</td>
</tr>
<tr>
<td>M.C.</td>
<td>31</td>
<td>F</td>
<td>Coeliac disease aged 1½ years. Initial response to GFD with relapse after gluten ingestion. Persistent SVA despite 4 years GFD. Minimal symptoms</td>
<td>Prednisolone: 20 mg daily, 6 weeks</td>
<td>SVA → NH</td>
</tr>
<tr>
<td>F.C.</td>
<td>57</td>
<td>M</td>
<td>Coeliac disease aged 46 with symptoms from childhood. Initial clinical and histological response to GFD. Relapse after gluten ingestion with persistent SVA despite 3 years GFD. Moderate malabsorption</td>
<td>Prednisolone: 20 mg daily, 9 weeks</td>
<td>SVA → PVA</td>
</tr>
<tr>
<td>W.W.</td>
<td>58</td>
<td>M</td>
<td>Coeliac disease diagnosed aged 40 years with symptoms from adolescence. No response to GFD with SVA, cryoglobulinemia and focal glomerulitis. Moderate malabsorption</td>
<td>Prednisolone: 20 mg daily, 7 weeks</td>
<td>SVA → PVA</td>
</tr>
</tbody>
</table>
The early recognition of non-responsive coeliac disease patients is probably important as these patients may progressively deteriorate, with a fatal outcome (Booth, 1970). These patients appear to be improved by corticosteroid treatment but there have been few studies on the biochemical changes which occur in the small intestine after this treatment.

**Methods**

**Patients**

Jejunal biopsies were obtained with the Crosby capsule from patients undergoing routine diagnostic biopsies or biopsies taken during the assessment of their response to treatment. They were examined by dissecting and conventional light microscopy.

Five patients with non-responsive coeliac disease were studied on at least two occasions. They had received a strict gluten-free diet for at least 3 years before study. Clinical details are given in Table 1. Four of the patients had previously clearly responded to a gluten-free diet but had relapsed after gluten ingestion with subsequent failure to respond to a strict gluten-free diet. All patients showed subtotal villus atrophy. While still taking a strict gluten-free diet the patients were treated with oral prednisolone (20 mg daily) and restudied after 5–9 weeks. Four of the patients showed morphological improvement in the appearance of their biopsies. The studies reported in this paper have been approved by the local ethical committee.

**Biochemical studies**

Portions of the biopsies were homogenized in sucrose solution (0.3 mol/l) containing disodium EDTA (1 mmol/l), pH 7.2, and ethanol (20 mmol/l) and subjected to analytical subcellular fractionation by sucrose-density-gradient centrifugation, and subsequent enzymic analysis of the gradient fractions was performed as described previously (Peters, 1976). The enzyme activities and distribution data are compared with similar data obtained from control subjects (Peters, 1976) and patients with gluten-sensitive coeliac disease (Peters, Jones & Wells, 1978).

**Results**

**Biochemical studies**

Table 2 shows the specific activities of the organelle marker enzymes in biopsy homogenates from control subjects, patients with coeliac disease and patients with non-responsive coeliac disease who have received corticosteroid therapy. Comparison of the responsive and non-responsive groups shows that the first six enzymes listed are located to the brush border, although at least some of the enzymes have a secondary extra brush-border component. Apart from alkaline phosphatase, these marker enzymes are higher in the non-responsive than in the gluten-sensitive groups. There is no difference in the catalase activity between the two groups but three of the four acid hydrolases are significantly lower in the non-responsive group.

### Table 2: Enzymic activities of biopsy homogenates

Specific activity is expressed as the mean value ± SE (munit/mg of protein) and the number of specimens assayed is shown in parenthesis. Control values are from Peters (1976). Total protein is expressed as mg ± SEM.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>EC no.</th>
<th>Specific activity</th>
<th>Control</th>
<th>Untreated responsive coeliac disease</th>
<th>Non-responsive coeliac disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Untreated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>3.1.3.1</td>
<td>64.7±6.3 (15)</td>
<td>16.9±9.5*(11)</td>
<td>12.6±3.4†(4)</td>
<td>58.1±3.0†(4)</td>
</tr>
<tr>
<td>Leucyl-2-naphthylamidase</td>
<td>3.4.11.1</td>
<td>4.25±0.78 (9)</td>
<td>2.81±0.37 (5)</td>
<td>3.11±0.08 (4)</td>
<td>6.60±0.9 (3)</td>
</tr>
<tr>
<td>γ-Glutamyl transferase</td>
<td>2.3.2.2</td>
<td>12.1±1.4 (11)</td>
<td>5.45±0.87 (7)</td>
<td>8.09±1.0 (5)</td>
<td>15.3±1.4 (3)</td>
</tr>
<tr>
<td>Total α-glucosidase (pH 6.0)</td>
<td>3.2.1.20</td>
<td>9.54±1.0 (13)</td>
<td>3.10±0.6 (11)</td>
<td>3.11±0.6 (9)</td>
<td>9.65±1.50 (4)</td>
</tr>
</tbody>
</table>

(Continued)
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>EC no.</th>
<th>Specific activity</th>
<th>Control</th>
<th>Untreated responsive coeliac disease</th>
<th>Non-responsive coeliac disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Untreated</td>
</tr>
<tr>
<td>Zn2+-resistant α-glucosidase</td>
<td>3.2.1.20</td>
<td>8.49 ± 2.03  (8)</td>
<td>1.03 ± 0.32 (5)</td>
<td>2.76 ± 0.70 (4)</td>
<td>11.9 ± 0.21 (3)</td>
</tr>
<tr>
<td>(pH 6-0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tris-sensitive α-glucosidase</td>
<td>3.2.1.20</td>
<td>8.52 ± 1.32  (5)</td>
<td>—</td>
<td>2.11 ± 0.4 (3)</td>
<td>10.9 ± 1.68 (3)</td>
</tr>
<tr>
<td>(pH 6-0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catalase</td>
<td>1.11.1.6</td>
<td>79.9 ± 5.5  (15)</td>
<td>99.1 ± 12.1 (7)</td>
<td>90.5 ± 12.5 (8)</td>
<td>113 ± 12.9 (3)</td>
</tr>
<tr>
<td>N-Acetyl-β-glucosaminidase</td>
<td>3.2.1.30</td>
<td>6.08 ± 0.47  (14)</td>
<td>7.51 ± 0.15 (10)</td>
<td>4.72 ± 0.40 (8)</td>
<td>8.89 ± 0.79 (4)</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>3.1.3.2</td>
<td>39.1 ± 3.8  (7)</td>
<td>41.2 ± 2.5 (7)</td>
<td>40.0 ± 2.8 (4)</td>
<td>37.3 ± 3.9 (3)</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>3.2.1.31</td>
<td>3.05 ± 0.20  (7)</td>
<td>4.40 ± 0.25 (5)</td>
<td>0.625 ± 0.07 (3)</td>
<td>—</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>3.2.1.23</td>
<td>1.79 ± 0.93  (4)</td>
<td>1.65 ± 0.47 (6)</td>
<td>0.570 ± 0.101 (3)</td>
<td>1.56 ± 0.3 (3)</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>1.11.1.37</td>
<td>3720 ± 310  (15)</td>
<td>2150 ± 220 (8)</td>
<td>2250 ± 220 (8)</td>
<td>3470 ± 110 (3)</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>1.11.1.37</td>
<td>387 ± 40    (5)</td>
<td>530 ± 24  (4)</td>
<td>745 ± 90 (5)</td>
<td>254 ± 40 (3)</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>3.2.1.20</td>
<td>1.13 ± 0.10  (14)</td>
<td>0.957 ± 0.131 (8)</td>
<td>0.554 ± 0.105 (4)</td>
<td>1.91 ± 0.21 (3)</td>
</tr>
<tr>
<td>(pH 8-0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn2+-sensitive α-glucosidase</td>
<td>3.2.1.20</td>
<td>1.96 ± 0.43  (10)</td>
<td>1.73 ± 0.24 (4)</td>
<td>0.610 ± 0.22 (4)</td>
<td>2.46 ± 0.06 (3)</td>
</tr>
<tr>
<td>(pH 6-0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tris-resistant α-glucosidase</td>
<td>3.2.1.20</td>
<td>1.30 ± 0.38  (7)</td>
<td>—</td>
<td>0.601 ± 0.070 (3)</td>
<td>2.79 ± 0.22 (3)</td>
</tr>
<tr>
<td>5'-Nucleotidase</td>
<td>3.1.3.5</td>
<td>41.1 ± 4.1  (8)</td>
<td>38.1 ± 2.5 (4)</td>
<td>38.0 ± 2.5 (3)</td>
<td>38.1 ± 5.0 (3)</td>
</tr>
<tr>
<td>Total protein (mg)</td>
<td>—</td>
<td>1.25 ± 0.14  (25)</td>
<td>0.465 ± 0.070 (14)</td>
<td>0.651 ± 0.12 (9)</td>
<td>0.691 ± 0.14 (4)</td>
</tr>
</tbody>
</table>

* Student's t-test was used to compare the untreated gluten-sensitive group with non-responsive group.
† Non-responsive group and prednisolone-treated non-responsive group were compared with the control group.

Malate dehydrogenase activity is significantly lower in both untreated gluten-sensitive and non-responsive groups compared with controls, but between the two groups there is no difference. Lactate dehydrogenase activity is elevated in the untreated responsive group compared with controls and the activity in the non-responsive group is elevated even more.

The two endoplasmic reticulum markers Zn2+-sensitive α-glucosidase and α-glucosidase (pH 8-0) are significantly reduced in the non-responsive compared with the gluten-sensitive group.
Subcellular fractionation in non-responsive coeliac patients

From comparison of the effect of prednisolone on the non-responsive group it is clear that in parallel with the morphological and clinical response there are significant changes in the activities of certain enzymes in the biopsy homogenates. All six brush-border enzymes, catalase, the peroxisomal marker, two of the three acid hydrolases and malate dehydrogenase, all show significant increases.

Lactate dehydrogenase, however, shows a decrease in activity and this is significantly lower than the control values. The three endoplasmic reticulum enzymes, $\alpha$-glucosidase (pH 8·0), Zn$^{2+}$-sensitive and Tris-resistant $\alpha$-glucosidases, are all increased significantly above control values.

Subcellular fractionation

Fig. 1 and Fig. 2 compare the activity and distribution in the sucrose gradients of the various organelle marker enzymes in the non-responsive group with the control subjects.

Alkaline phosphatase, the brush-border marker enzyme, is reduced to one-fifth of control activity with a replacement of the distinct brush-border component at a density of 1·21 and a small broad peak with a modal density of 1·14.

Malate dehydrogenase is significantly reduced but this decrease in activity is solely confined to the particulate mitochondrial component. N-Acetyl-$\beta$-glucosaminidase is decreased and there is an increase in the relative proportions of soluble to particulate activity.

$\alpha$-Glucosidase, assayed at pH 8·0, shows a 50% decrease in activity compared with control values and there is a striking decrease in the lower density components. Lactate dehydrogenase, a cytosol enzyme, shows a marked increase in activity but the gradient distribution remains unaltered.

Fig. 2 shows the distribution of additional enzymes. Zn$^{2+}$-resistant $\alpha$-glucosidase, $\alpha$-glucosidase (pH 6·0) and $\gamma$-glutamyl transpeptidase are all significantly reduced with a loss of the brush-border components.

Alkaline phosphatase shows a distinct brush-border peak but the activities in the denser regions of the gradients do not quite reach control values. $\alpha$-Glucosidase (pH 8·0) activity is significantly elevated and the increase is uniform throughout the
Alkaline phosphatase
Malate dehydrogenase
10-
5-
15
1-
1.05 1.15 1.25 1.35 1.45 1.55
15
10
5
0
Relative Frequency
Density
Alkaline phosphatase (×0.90)
Malate dehydrogenase (×0.93)

Catalase
N-Acetyl-β-glucosaminidase
(×1.41)
(×1.46)
glucosidase (pH 8.0)
Lactate dehydrogenase (×0.75)
α-Glucosidase
(×1.69)

Fig. 3. Isopycnic centrifugation of postnuclear supernatant from jejunal biopsies from patients with non-responsive coeliac disease treated with prednisolone. For details see the legend to Fig. 1. Percentage recoveries: alkaline phosphatase, 105 ± 8 (4); malate dehydrogenase, 83 ± 5 (3); catalase, 99 ± 13 (3); N-acetyl-β-glucosaminidase, 74 ± 6 (4); α-glucosidase, pH 8.0, 109 ± 5 (3); lactate dehydrogenase, 110 ± 6 (3).

gradient whereas lactate dehydrogenase shows a disproportionate decrease in the activity found within the sucrose gradient.

Zn2+-resistant α-glucosidase and γ-glutamyl transpeptidase activities (Fig. 4) are significantly elevated. Zn2+-sensitive α-glucosidase, the endo-

plasmic reticulum marker, shows elevated activities, particularly of the soluble component.

Discussion

The data in this paper demonstrate important enzymic and organelle differences between jejunal biopsy specimens from patients with untreated but gluten-sensitive coeliac disease and patients with non-responsive coeliac disease, both groups showing subtotal villus atrophy. The results will be discussed under subheadings related to the individual organelles comparing the results with those previously reported on control tissue (Peters, 1976) and gluten-sensitive coeliac disease (Peters et al., 1978).

Basal–lateral (plasma) membrane. No significant difference has been demonstrated between the activity of the basal–lateral membrane marker 5'-nucleotidase or the distribution of the membrane fragments in the sucrose gradient in the various groups of patients studied. As discussed previously (Peters et al., 1978), this result would tend to suggest that the enterocyte damage in coeliac disease is not mediated via this membrane.

Endoplasmic reticulum. The activities of the marker enzymes for this organelle are reduced in the non-responsive group compared with the untreated gluten-sensitive group and the controls. This result is in agreement with measurements of protein synthetic rates in cultured biopsy specimens (Jones, L'Hirondel & Peters, 1976), when it was found that the rate of incorporation of [14C]leucine in protein is considerably lower in the non-responsive than in the gluten-sensitive group.

The subcellular fractionation experiments show an increase in the ratio of rough to smooth endoplasmic reticulum, suggesting an increase in the proportion of membrane protein synthesis (Dallner, Siekevitz & Palade, 1966). The response to prednisolone shows a marked increase in the activities of the endoplasmic reticulum marker enzymes and normalization of the ratio of rough to smooth membranes. The effect of prednisolone on the normal human gut has not been investigated but animal studies (Batt & Peters, 1976) have demonstrated increased activities of brush-border enzymes and of RNA, suggesting an effect on protein synthesis. Recent studies (Scott, Batt & Peters, 1977) have confirmed this hypothesis. The increase in the activities of brush-border enzymes and the striking effect on the endoplasmic reticulum markers suggests that prednisolone exerts a similar action on the intestine of patients with coeliac disease.
**Mitochondria.** The activities of mitochondrial enzymes are similarly depressed in both untreated responsive and non-responsive coeliac disease, although in the non-responsive group the ratio of extramitochondrial to intramitochondrial enzyme is greater. It is likely that the decrease in mitochondrial enzyme activity is secondary to the mucosal damage with the increased extra- to intra-mitochondrial activities reflecting damage to the organelle itself. The raised activities of lactate dehydrogenase, particularly in the non-responsive group, probably reflect a compensatory response to mitochondrial damage.

In the experimental animal corticosteroids increase the activity of mitochondrial enzymes, possibly as an adaptive response to the increased absorptive-digestive capacity of the brush border (Batt & Peters, 1976), and it is likely that in the coeliac disease mucosa a similar effect occurs.

**Peroxisomes.** The activities of catalase are unaffected by the coeliac disease process although the fractionation procedure indicates an increased proportion of cytosolic activity, probably due to increased peroxisomal fragility. The increased activities of catalase in the steroid-treated patients is of interest, particularly as both peroxisomal and cytosolic components are increased. Animal studies, however, demonstrated no effect of prednisolone on catalase (Batt & Peters, 1976).

**Lysosomes.** In the biopsies from patients with untreated but responsive coeliac disease increased activities of acid hydrolases and enhanced lysosomal fragility were demonstrated (Peters, Heath, Wansbrough-Jones & Doe, 1975; Peters et al., 1978). The non-responsive patients have total activities of acid hydrolases lower than normal although there is also evidence of enhanced lysosomal fragility. The reason for the difference in lysosomal activity in the two groups of coeliac patients is unclear. It may be related to the load of gluten, as the non-responsive patients will have received a strict gluten-free diet for many months. Morphological studies have demonstrated prominent lysosomes in untreated gluten-responsive coeliac disease (Curran & Creamer, 1963; Riecken, Stewart, Booth & Pearse, 1966; Rubin, Ross, Sleisenger & Weser, 1966) and it is possible that the gluten or its partial degradation products accumulating within lysosomes are responsible for these morphological changes.

**Brush borders.** It is clear that this organelle is the most severely affected by the coeliac disease process. Enzyme activities may be reduced to as little as one-fifth of control values and the fractionation data indicate an almost complete loss of this organelle. From comparison of the brush border in both subtypes of coeliac disease it appears that the decrease of enzyme activity is more marked for the gluten-sensitive than the non-responsive form.

The enzyme activities and density-gradient properties of the brush border show a remarkable response to corticosteroid therapy. The enzyme activities return to normal and, for certain enzymes, are increased to supranormal values. The subcellular fractionation studies also indicate a restoration of the brush-border pattern to normal although the biopsies show a partial villus atrophy and this morphological picture in gluten-sensitive coeliac disease is only associated with a partial restoration of brush-border enzymes and fractionation properties (Peters et al., 1978).

Corticosteroids have been shown, by using morphological techniques, to produce a remission in spite of continued gluten ingestion in patients with coeliac disease (Wall, Douglas, Booth & Pearse, 1970) and there are isolated reports of their use in non-responsive coeliac disease (Lepore, 1958; Pink & Creamer, 1967; Booth, 1970; Hillman, 1972; Hamilton, Chambers & Wynn-Williams, 1976). The mechanism of this beneficial effect of corticosteroids in non-responsive coeliac disease is unclear. A possible role as an immunosuppressant has been emphasized (Booth, 1970; Katz, Falchuk, Strober & Swachman, 1976) and the reports of the successful use of azathioprine in non-responsive coeliac disease supports this claim (Hillman, 1972; Hamilton et al., 1976). A further suggestion is that corticosteroids protect the enterocytes from lysosome-mediated autolysis (Wall et al., 1970). A characteristic feature of the gluten-sensitive coeliac disease lesion is the enhanced crypt cell division (Booth, 1970; Wright, Watson, Morley, Appleton, Marks & Douglas, 1973) and it is possible that corticosteroids will normalize this effect.

The present studies, in combination with previous biochemical studies showing a distinct DNA (Jones & Peters, 1977) and protein synthesis rate (Jones et al., 1976), clearly delineate the non-responsive form of coeliac disease from the gluten-sensitive variant. The biochemical studies indicate reduced endoplasmic reticulum and lysosomal enzymes in the non-responsive group but the brush border is less severely affected. This distinction of non-responsive coeliac disease was hitherto possible on clinical grounds only after prolonged strict gluten withdrawal. It is clear from the present study that this group of patients mostly respond fairly
rapidly to a modest dose of corticosteroids but probably more important is the suggestion that these patients are at particular risk from complications. One of the patients (K.G.) in the present study has subsequently died of intestinal ulceration with malignancy. In the follow-up of the study of non-responsive and responsive coeliac disease reported by Barry & Read (1973) complicating malignancies have developed more frequently in the non-responsive than in the gluten-sensitive group.

Acknowledgments

We are grateful to Dr C. C. Booth for his advice and interest. The expert technical assistance of Mr Peter White and the secretarial assistance of Ms Jean de Luca are gratefully acknowledged. This work is supported by the Medical Research Council and The Wellcome Trust.

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