Mucosal and Serum Alkaline Phosphatase Activities in Milk Allergy

N Iyngkaran, FRCP*
M Yadav, PhD**
C G Boey, BS***

* Departments of Paediatrics and ** Pathology,
University Hospital, 59100 Kuala Lumpur
** Department of Genetics & Cellular Biology,
University of Malaya, 59100 Kuala Lumpur

Summary

The effect of cow's milk protein (CMP) challenge on the levels of alkaline phosphatase (ALP) in the upper jejunal mucosa and the serum were studied in 25 infants clinically suspected to have cow's milk allergy. Following CMP provocation 3 groups were identified. Group 1 consisted of 10 infants who had clinical and histological reaction to CMP challenge. All 10 infants had significant depletion in the levels of tissue and serum ALP. Group 2 consisted of 5 infants who had histological reaction but no clinical reaction. Tissue ALP was depressed in 3 but not in 2 following CMP challenge. Serum ALP were essentially unaltered in all 5. Group 3 consisted of 10 infants who clinically and histologically tolerated CMP challenge. Tissue and serum ALP were not depressed in any. Estimation of sucrase levels in the mucosa and xylose absorption before and after CMP challenge were also performed for comparison with changes of tissue and serum ALP levels. The clinical significance of the changes in serum ALP level is discussed.

Key Words: Alkaline phosphatase, Milk allergy, Diarrhoea, Xylose, Mucosal sucrase

Introduction

Measurement of alkaline phosphatase (ALP) in serum has been extensively used in clinical medicine and the activity reflects the function of the enzyme produced by the liver, bone and intestine. While serum ALP levels have been used as diagnostic indicators in bone and liver disease, its value as a marker in intestinal pathology is less well appreciated. A number of gastrointestinal diseases are associated with an increase in the levels of serum ALP. Cow's milk protein (CMP) provocation in infants allergic to cow's milk cause severe damage to the jejunal mucosa and mucosal repair begins rapidly on removal of the provocation diet. We have earlier demonstrated that the ALP levels in the jejunal biopsy were depleted significantly following milk provocation in allergic infants and were unchanged in normal controls. Moreover, we have noted similar decreases in the enzyme in rectal mucosa following oral milk challenge. However, there is little information on the level of serum ALP in milk allergy. The present study was conducted to evaluate the effect of oral milk provocation-diet on the mucosal and serum ALP activity in infants with cow's milk protein sensitive enteropathy (CMPSE).

Patients and Methods

Patients

Twenty-five infants with diarrhoea clinically suspected to be due to cow's milk allergy were studied. The mean age of admission was 67 days (range 3 to 390 days) and at onset of diarrhoea was 23 days (range 2
to 88 days). The mean duration of diarrhoea was 22 days (range 1 to 60 days). Investigations on admission included examination of the stools for virus and bacterial pathogens and parasites. Stools were positive for *Salmonella* sp. in 2 infants, *Shigella* sp. in 1, *Escherichia coli* in 2 and rotavirus in 1.

After appropriate correction of fluid and electrolyte imbalance, the infants were started on a lactose and cow's milk protein-free formula such as Pregestimil, Nutramigen or Prosobee.

**Milk challenge studies and biopsies**

The infants were discharged when their stools were normal and they were feeding well, and gaining weight. They were readmitted 6 to 8 weeks later for CMP challenge studies which were carried out as described previously. The infants were challenged with a low lactose cows’ milk protein formula namely, Lactolac (Cooperative Condens Fabriek, Holland).

A proximal jejunal biopsy was performed immediately before and 20-24 hours after milk provocation. The biopsy specimen was divided for histopathological microscopical examination and for enzyme assay.

**Assay for enzymes and oligosaccharidases**

Serum ALP levels were estimated in pre- and post-challenge blood specimens collected before and 24 hours after CMP challenge. One hour blood xylose test before and after milk provocation, alkaline phosphatase levels and sucrase activities in the small bowel mucosa were estimated as described by us previously.

**Results**

**Clinical, histological and enzymatic characteristics of the Infants**

At readmission all 25 infants were clinically well and showed satisfactory weight gain.

The histological examination of the jejunal mucosa of the infants on a protein exclusion diet for 6-8 weeks showed a normal healthy mucosa with long and slender villi which were generally about three times the length of the crypts of Lieberkuhn. Following challenge with CMP the patients developed a wide range of histological appearance of the mucosa and clinical response to the provocation diet. Based on the clinical response and the histological appearance of the mucosa, the patients could be divided into 3 groups. In Group I and II patients, there was severe villous shortening and in some cases the villi were totally effaced. The histological score was highly significant in Group I and II patients of the pre- and post-challenge mucosa. In Group III patients there was no difference between the pre- and post-score of the mucosal histology. The characteristics of the patients in the 3 groups and the enzyme changes in the mucosa and blood xylose levels in the blood are summarised below.

**Group 1** consisted of 10 infants who had clinical and histological reaction to CMP challenge. In all 10 infants there was significant depression in the post challenge levels of blood xylose, mucosal sucrase and mucosal ALP and serum ALP compared to their respective pre-challenge levels.

**Group 2** consisted of 5 infants who had histological reaction but not clinical symptoms. Four of the 5 infants had significant depression of mucosal sucrase activity. Tissue ALP levels and blood xylose levels were depressed in 3 and not depressed in 2. The serum ALP levels were essentially unaltered in all 5.

**Group 3** consisted of 10 infants who clinically and histologically tolerated CMP. The sucrase, blood xylose, mucosal and serum ALP levels after CMP change did not show any significant alteration over their respective pre-challenge value.

The histological, enzymological and blood xylose results are summarised in Table I.

**Discussion**

The major finding in this study was that in infants with symptomatic cow's milk protein sensitive enteropathy (CMPSE), depletion in ALP levels in the mucosa following CMP challenge was associated with depletion in serum ALP levels. The changes in tissue and serum ALP levels closely paralleled the depression in sucrase activity and blood xylose levels in these infants and reflected the mucosal injury sustained from the CMP challenge.
As the intestine is a source of serum ALP it was inferred that depletion of mucosal ALP in infants with CMPSE would be reflected in corresponding changes in serum ALP activity. This inference was proven correct in infants with symptomatic CMPSE whose changes in serum ALP closely paralleled changes in mucosal ALP. However, the degree of depression of serum ALP was much lower, 19% (range 9-39%) compared to that of mucosal ALP (70%). The serum and mucosal ALP levels were not significantly altered in asymptomatic CMPSE unlike sucrase activity and blood xylose levels.

Previously, Wolf suggested that ischaemic and erosive disorders of the stomach, and small and large intestines were associated with elevated levels of serum ALP. This was based on the assumption that in those pathologies the damaged enterocyte discharged its content of ALP into the mucosa and submucosa from where it reached the general circulation via the villous capillaries or more circuitously through the lacteals. However, our present findings do not concur with the inference of Wolf.

In the intestinal mucosa besides the mature enterocyte, the white blood cell is another source of ALP. Inflammatory cell migration into the mucosa of the small and large bowel enteropathies. It follows that the level of

| Table 1 |
| Changes in histological score, mucosal sucrase, mucosal alkaline phosphatase, serum phosphatase and blood xylose levels in 25 infants before and after cow’s milk protein (CMP) challenge |

<table>
<thead>
<tr>
<th>Patients</th>
<th>Histology</th>
<th>Sucrase</th>
<th>Blood xylose</th>
<th>Mucosal alkaline phosphate</th>
<th>Serum alkaline phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre Post</td>
<td>% Change</td>
<td>Pre Post</td>
<td>% Change</td>
<td>Pre Post</td>
</tr>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>2 11.5</td>
<td>83</td>
<td>5</td>
<td>2.06</td>
<td>60</td>
</tr>
<tr>
<td>SD</td>
<td>±1.8</td>
<td>±2.1</td>
<td>±17.2</td>
<td>±1.9</td>
<td>±1.3</td>
</tr>
<tr>
<td>Hest</td>
<td>p&gt;0.01</td>
<td>p&gt;0.01</td>
<td>p&gt;0.01</td>
<td>p&gt;0.01</td>
<td>p&gt;0.01</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1.3</td>
<td>8.9</td>
<td>85.2</td>
<td>5.9</td>
<td>2.8</td>
</tr>
<tr>
<td>SD</td>
<td>±1.5</td>
<td>±1.7</td>
<td>±14.5</td>
<td>±2.1</td>
<td>±1.1</td>
</tr>
<tr>
<td>Hest</td>
<td>p&gt;0.01</td>
<td>p&gt;0.05</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Group 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>2.1</td>
<td>2.7</td>
<td>–</td>
<td>5.8</td>
<td>6.1</td>
</tr>
<tr>
<td>SD</td>
<td>±1.7</td>
<td>±2.1</td>
<td>±2.5</td>
<td>±2.5</td>
<td>±6.2</td>
</tr>
<tr>
<td>Hest</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Group 1: Clinical and histological reaction to CMP challenge.
Group 2: Histological reaction without clinical symptoms to CMP challenge.
Group 3: No histological or clinical reaction to CMP challenge.

Hest: Student Hest; statistical significance taken as p<0.05
NS: Pre- and post-challenge values are statistically not significant (p>0.05)
a: Histological appearance scored as described in reference 6
b: Micromoles per minute per gram tissue nitrogen
c: Milligrams per deciliter
d: Micromoles p-nitrophenyl phosphatase hydrolysed per minute per gram tissue protein
e: International units
mucosal ALP in these situations will be the net effect of two opposing factors, (i) positive contribution of ALP by the migrating inflammatory cells and the new epithelial cells originating from multiplication of undifferentiated cells in the villous crypts, and (ii) loss of ALP from the destruction of mature enterocytes.

In CMP induced villous atrophy, enterocyte destruction and increased infiltration of the mucosa with inflammatory cells are constant features but the severity and extent of each can be variable along the gut. Thus the ALP loss as a result of enterocyte damage can be compensated by the ALP contributed by the migrating leukocytes and new epithelial cells produced in the crypts. However, in our studies depletion of mucosal ALP is a constant finding in symptomatic CMPSE. It may be inferred that in symptomatic CMPSE villous damage is so severe and extensive that the loss of ALP from enterocyte damage far exceeds that contributed by migrating leukocytes and new epithelial cells. We have shown that mucosal damage in symptomatic CMPSE can involve both the small and large bowel. It would appear therefore that the severity and extent of mucosal damage and the degree of inflammatory cell migration will determine the amount of mucosal ALP released which in turn will determine the level of serum ALP.

References