CASE REPORTS

Intestinal fatty acid binding protein is available for diagnosis of intestinal ischaemia: immunochemical analysis of two patients with ischaemic intestinal diseases

T Kanda, H Fujii, M Fujita, Y Sakai, T Ono, K Hatakeyama

Abstract
Mesenteric infarction and other acute ischaemic intestinal diseases are still a challenging diagnostic problem. Based on animal experiments, intestinal fatty acid binding protein (I-FABP), which is uniquely localised to the bowel, has recently been proposed as a new serum marker for intestinal ischaemia. This paper reports on two cases with acute intestinal ischaemic diseases, and the measurement of serum I-FABP by western blot analysis. The concentrations of ordinary serum markers were normal and the bowel necrosis was not diagnosed until surgical exploration. Immunochemical analysis showed that the I-FABP concentrations in the patients’ serum samples were high at the time of admission, and that I-FABP was undetectable in the samples obtained after bowel resection and in healthy control subjects. This paper suggests that I-FABP is released into the circulation in the acute phase of intestinal ischaemia and that I-FABP can be used in establishing the diagnosis of ischaemic intestinal diseases.

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Keywords: intestinal ischaemia, fatty acid binding protein.

Acute intestinal ischaemic diseases representing superior mesenteric artery occlusion are important diseases in abdominal emergencies, in many cases requiring surgical intervention.1, 2 These diseases are difficult to diagnose precisely, mostly because no clinical signs or laboratory tests are of specific diagnostic value, resulting in high morbidity and mortality. Although a reliable marker for intestinal ischaemia has been required, there is no biochemical marker available to date. Recently, a series of studies on rodent experimental models strongly suggested that intestinal fatty acid binding protein (I-FABP), which exhibits specific localisation to the epithelium of the small bowel,3 is a promising serum marker for intestinal ischaemic injury.4–6 In the clinical setting, however, it remains uncertain whether I-FABP is released into the circulation at the time of intestinal ischaemia, or whether the released I-FABP in the serum is detectable or not. To consider these questions, we have analysed the serum samples of two patients with ischaemic diseases of the small intestine using a specific antiserum against human I-FABP. We present the case histories and the promising data obtained with this useful tool for the diagnosis of acute intestinal ischaemia.

Methods

CLINICAL SAMPLES
Pre and postoperative blood samples were obtained from two patients with acute intestinal ischaemic disorders, confirmed by laparotomy, and analysed retrospectively. Peripheral venous blood was taken, and the serum was frozen at −20°C until analysis. Control serum samples were collected from healthy volunteers.

HUMAN I-FABP STANDARDS
A recombinant human I-FABP was used as the antigen for immunisation and as the standard for western blot analysis. The expression strategy is described below. The human I-FABP cDNA was amplified by means of the polymerase chain reaction (PCR)7 using a human ileal cDNA library as a template. The sense primer carried the first seven amino acids of human I-FABP with an NdeI site at the start codon, whereas the antisense primer carried the last seven amino acids of it with a BamHI site.8 The PCR product was subcloned into the unique NdeI and BamHI sites in the vector, pET3a. The protein expressed was purified to homogeneity by gel filtration, and confirmed by partial protein sequencing and amino acid analysis.

I-FABP DETERMINATION
We have prepared an antiserum against human I-FABP because the antiserum against rat I-FABP was much less sensitive to human I-FABP.4 Specific antiserum was raised in

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As electrocardiography showed ST-T wave abnormalities, she was admitted to hospital with the tentative diagnosis of ischaemic heart disease. Next morning, however, her vital signs rapidly worsened with peritoneal signs, and surgical exploration was performed.

Laparotomy showed that transmural necrosis was complete in the jejunum, ileum, and ascending colon. Pulsation of the superior mesenteric artery had ceased at its origin. An operative diagnosis of intestinal infarction resulting from superior mesenteric artery occlusion was made. The patient was not considered to be suitable for extensive bowel resection and died the next day.

Case 2
A 57 year old man presented at hospital in the evening, complaining of abdominal pain. He had received subtotal gastrectomy with gastrojejunostomy for gastric cancer two months before. He was prescribed analgesics and went home, but the pain increased gradually, followed by vomiting. He was admitted to the hospital the next morning with the diagnosis of bowel obstruction resulting from postoperative adhesion. The Table gives the results of the initial laboratory investigations. Blood gas analysis showed metabolic acidosis: pH, 7.39; plasma bicarbonate concentration, 14.2 mmol/l; base excess, −8.0 mmol/l.

The patient presented with severe dehydration and parenteral fluid therapy was started. His complaint worsened, however, and so he had an emergency operation at midnight. Laparotomy showed volvulus of the small bowel, resulting from rotation about the mesentery adhered to the jejunal effenter loop. About 150 cm of the small intestine was necrotised transmurally, and thus excised. Postoperatively, the patient developed multiple organ failure, which eventually led to death on the 36th postoperative day.

Results
The method of immunochemical analysis used was specific for human I-FABP and sensitive to concentrations more than 0.4 µg/ml (Figs 1 and 2).

On analysis of the serum samples from eight healthy volunteers (aged 25-59 years; five men), I-FABP was proved to be undetectable (Fig 1; lanes 5 and 6).

Western blot analysis exhibited I-FABP in two samples of the patient with superior mesenteric artery obstruction (Fig 1; lanes 7 and 8). The serum I-FABP showed a high value (about 0.6 µg/ml), at the time of admission or three hours after the onset (Fig 1; lane 7). It was also positive on the next day, just before operation, with a comparatively low value below 0.4 µg/ml (Fig 1; lane 8).

Serum obtained from the patient with bowel volvulus (case 2) was analysed throughout the pre and postoperative periods. The concentrations of I-FABP in the serum samples were estimated to be between 0.4 and 0.8 µg/ml at the time of admission and just before

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### Data for haematological and biochemical examinations on admission

<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>8.6*</td>
<td>9.4*</td>
</tr>
<tr>
<td>RBC</td>
<td>4.4</td>
<td>2.6*</td>
</tr>
<tr>
<td>Hb</td>
<td>14-9</td>
<td>7.4*</td>
</tr>
<tr>
<td>PCV</td>
<td>43-0</td>
<td>22.6*</td>
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<tr>
<td>Platelets</td>
<td>160</td>
<td>228</td>
</tr>
<tr>
<td>AST (7-38)</td>
<td>24</td>
<td>17</td>
</tr>
<tr>
<td>ALT (4-35)</td>
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<td>13</td>
</tr>
<tr>
<td>LDH (150-450)</td>
<td>567*</td>
<td>356</td>
</tr>
<tr>
<td>ALP (77-244)</td>
<td>154</td>
<td>109</td>
</tr>
<tr>
<td>CPK (49-228)</td>
<td>133</td>
<td>ND</td>
</tr>
<tr>
<td>BUN (7-20)</td>
<td>13-0</td>
<td>28.0*</td>
</tr>
</tbody>
</table>

WBC = total leucocyte count (×10³ cells/mm³); RBC = total erythrocyte count (×10⁹ cells/mm³); Hb = blood haemoglobin concentration (g/dl); PCV = packed cell volume (% of packed red cells); platelets = total thrombocyte count (×10⁹ cells/mm³); AST = serum aspartate aminotransferase activity (IU/l); ALT = serum alanine aminotransferase activity (IU/l); LDH = serum lactate dehydrogenase activity (IU/l); ALP = serum alkaline phosphatase activity (IU/l); CPK = serum creatine phosphokinase activity (IU/l); BUN = blood urea nitrogen (mg/dl). *Abnormal values. ND = not determined.

Normal values in the hospital are expressed in parentheses.

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Figure 1: Detection of intestinal fatty acid binding protein (I-FABP) in serum by immunochemical analysis. The patient (case 1) and normal control (12-5 µl each) serum samples were subjected to 10% polyacrylamide gel electrophoresis,10 and then probed with an antiseraum specific for human I-FABP. Prestained molecular weight markers (Amersham) and recombinant human I-FABP standards were run and transferred simultaneously. Lanes 1-4 are human I-FABP standards, 5 ng (0.4 µg/ml) I-FABP serum equivalent), 10 ng (0.8 µg/ml), 20 ng (1.6 µg/ml), and 40 ng (3.2 µg/ml), respectively. Lanes 5 and 6 show control serum samples obtained from a 39 year old healthy man and a 27 year old healthy woman, respectively. Lanes 7 is the serum obtained from the patient with superior mesenteric artery occlusion (case 1) at the time of her admittance to the emergency unit, and lane 8, her serum just before operation. I-FABP can be seen only in the serum from the patient (arrow). Molecular sizes are shown in kilodaltons.
Figure 2: Detection of I-FABP in serum by immunochemical analysis. Lanes 1–4 are human I-FABP standards, 5 ng (0.4 μg/ml I-FABP serum equivalent), 10 ng (0.8 μg/ml), 20 ng (1.6 μg/ml), 40 ng (3.2 μg/ml), respectively. Lanes 5–11 show the serum of the patient with coeleusis of the small intestine (case 2); lane 5, serum at the time of admission; lane 6, just before operation; lane 7, on the third postoperative day (POD3); lane 8, POD11; lane 9, POD14; lane 10, POD18; and lane 11, POD24. I-FABP can be seen only in the preoperative serum (arrow). Molecular sizes are shown in kilodaltons.

Discussion
Acute intestinal ischaemia is a serious clinical disorder,\(^1\) with mesenteric infarction having the extremely high mortality of about seventy per cent.\(^1\) Diagnosis and early surgical intervention are essential if clinical outcome of this disease is to be improved. Unfortunately, it is not easy to make a precise diagnosis of intestinal ischaemia, especially in the initial phase, mainly because no clinical signs or laboratory tests are of specific diagnostic value. Although selective arteriography is regarded as a method of accurate diagnosis, with an additional benefit of vasodilator infusion,\(^1\) it is invasive, cannot be applied for some disorders (for example, non-occlusive infarction or venous ischaemia), and most importantly, is not available at every institute where a patient is seen.

This situation led us to attempt to establish a biochemical marker for intestinal ischaemia. Several serum enzymes have been evaluated in the clinical setting, such as lactate dehydrogenase, creatine phosphokinase, aspartate aminotransferase, alkaline phosphatase, dibamine oxidase, and hexosaminidase.\(^1\) None have proved efficacious clinically, either because of low specificity and sensitivity, or failure to identify intestinal ischaemia before the start of gangrene. We are interested in I-FABP because of its unique characteristics, including its low molecular mass of 15 kilodaltons, high content in enterocytes (2–3% of cytosolic proteins), and specific localisation to the small intestine.\(^2\) Moreover, I-FABP is expressed preferentially in the mucosal villus,\(^5\) which is the first region affected by intestinal ischaemic injury. These features imply that I-FABP could be a potential serum marker for early intestinal ischaemia. In fact, earlier studies\(^4\) with rodent intestinal ischaemic models showed that I-FABP escaped from the ischaemic bowel into the circulation, and that the value of serum I-FABP increased dramatically even in the reversible phase of ischaemia. Little is known, however, about the distribution of I-FABP in the human intestine. There has been no reports that I-FABP is released into the circulation in humans. It remains uncertain whether or not serum I-FABP is detectable in the clinical setting of intestinal ischaemia. We have attempted to measure the I-FABP concentrations in the serum samples from two patients with ischaemic bowel diseases caused by different aetiologies (one with superior mesenteric artery occlusion and the other with intestinal volvulus). A specific antiserum against human I-FABP was required for the immunochemical analysis because of the lower immunoreactivity of the anti-rat I-FABP antibody.

I-FABP was not detected in serum from all eight normal volunteers on western blot analysis with the specific antiserum against human I-FABP, while it was detected in the serum of both patients. In case 2, the positive signal of I-FABP on immunoblotting disappeared after resecting of the necrotised intestine. These findings strongly suggested that necrosis of the small bowel gives rise to the release of I-FABP into the circulation. The concentrations of serum I-FABP before the operation were estimated to be 0.2–0.6 μg/ml in case 1 and to be around 0.5 μg/ml in case 2. These results are compatible with the previous data obtained in animal experiments (0.4–0.5 μg/ml in superior mesenteric artery occlusion\(^4\) and 0.25 μg/ml in segmental ischaemia of the jejunum\(^6\)).

In case 1, it is noteworthy that I-FABP appeared in the serum and was present at a high concentration at the time of admission, when she had only severe epigastric pain of three hour duration – that is, no objective abnormality pointing to an abdominal emergency. On the other hand, aspartate aminotransferase, alkaline phosphatase, and creatine phosphokinase showed normal values, as well as aspartate aminotransferase, alkaline phosphatase, and lactate dehydrogenase, in case 2, showing that I-FABP is superior to other bowel ischaemic markers so far proposed.

We used an immunocytochemical technique for detection of serum I-FABP and found that seven hours were needed to complete the analysis. This is too long when dealing with the ischaemic bowel. Both patients presented here, however, had surgical exploration more than 24 hours after their admission, and unfortunately, they both died. Delayed excision of the necrotised bowel can cause sepsis, and occasionally leads to multiple organ failure. It has been reported that the longer the preoperative time is, the worse the postoperative outcome with regard to mortality.\(^24\) Therefore, to improve the survival rate, this method of immunochemical analysis might be of practical value.

To bridge the gap between experimental studies and clinical application, we have analysed patients with acute bowel ischaemia. These two cases are insufficient to prove the general validity of I-FABP as a bowel ischaemic marker, and some questions remain to be answered. What extent of bowel insult is necessary for detection? Does the escape of I-FABP from the injured bowel occur in other intestinal disorders, such as acute bacterial
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enterocolitis and prolonged simple obstruction of the intestine? Future studies are needed on a large scale in a variety of bowel disorders to consider these questions. The case reports presented here, however, provide two direct pieces of evidence that (a) I-FABP is released into the circulation in acute intestinal ischaemic disease, and (b) the serum I-FABP released can be detected on immunochemical analysis. For the purpose of practical use, the development of an enzyme linked immunosorbent assay for serum I-FABP in humans is now in progress.

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