Different Responses of Apolipoprotein A-I, A-IV, and B Gene Expression during Intestinal Adaptation to a Massive Small Bowel Resection in Rats

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Gene expression of apolipoproteins (apo) A-I, A-IV, and B, the predominant protein components of chylomicrons, was investigated in the residual ileum after a massive small bowel resection in rats. A Northern blot analysis showed that the apo A-IV mRNA level, but not the apo A-I and B mRNA levels, in the ileum was significantly higher in the resected rats than in the sham-operated rats 24 h and 2 wk post-surgery. RT-PCR coupled with a primer extension assay revealed that the apo B-48 mRNA/apo B-100 mRNA ratio, i.e., apo B mRNA editing, in the ileum was unchanged by the resection. It is thus concluded that, among the major intestinal apolipoproteins, apo A-IV is the only one whose gene expression is influenced by loss of the proximal intestine.

Key words: apolipoprotein; apo B mRNA editing; intestine; intestinal resection; rat

The small intestine is capable of adaptive changes in its mucosal structure and function. For example, massive small bowel resection is an established animal model for intestinal adaptation. In response to a resection, increased crypt cell proliferation in the remnant small intestine results in hyperplasia with tall villi, deep crypts, and enhanced absorption per unit length of the intestine. The phenomena for these adaptive changes have been well documented, and the mechanisms for these responses have been extensively studied. However, the molecular response in enterocytes to intestinal resection is not yet fully understood.

We have previously reported that a massive small bowel resection resulted in a significant increase in the apolipoprotein (apo) A-I (1.2-fold) and apo A-IV (3.2-fold) mRNA levels in the residual ileum of rats 2 wk after the surgical procedure. Moreover, the apo A-IV mRNA level, but not that of apo A-I, in the residual ileum began increasing at 1 h post-resection, achieved a maximum by 12 h and remained stable to 24 h under fasting conditions. These observations suggest that the enterocytes in the residual ileum responded to the massive small bowel resection by increasing the apo A-I and apo A-IV expression in different ways. Apo B is another member of the apolipoprotein multigene family which is abundantly expressed in the enterocytes and is the predominant protein component of chylomicrons (CM). Since it could be expected that the lipid uptake and transport would be increased during intestinal adaptation to a massive small bowel resection, it is of interest to know whether the gene expression of apo B would respond to loss of the small intestine. Apo B circulates in the plasma in two molecular forms, which have been referred to on a centile scale as apo B-100 and apo B-48. Human apo B-100 is synthesized predominantly by hepatocytes, and apo B-48, an amino terminal half part of apo B-100, is synthesized by enterocytes in the small intestine. In contrast, rat hepatocytes synthesize both apo B-100 and B-48. Both peptides are the translational products of a single apo B gene encoding apo B mRNA that undergoes post-transcriptional conversion of cytosine at nucleotide position 6666 to uracil, thereby replacing the glutamine codon (CAA) with an inframe stop codon (UAA). This process is referred to as apo B mRNA editing. In the present study, we investigated the apo A-I, A-IV, and B mRNA levels and apo B mRNA editing during intestinal adaptation to a massive small bowel resection in rats.

Materials and Methods

Male Wistar rats (Japan SLC, Hamamatsu, Japan), which were 5 wk old at the start of the experiment, were housed in individual cages in a temperature-controlled (23 ± 2°C) room with a dark period from 8:00 p.m. to 8:00 a.m. After 5 d of acclimatization, the rats weighing 139 ± 2 g (n = 24) were divided into two groups of 12 for a small bowel resection and 12 for a sham operation, all the rats being fasted for 18 h before surgery. They were anesthetized by an intraperitoneal injection of Nembutal (sodium pentobarbitol, 35 mg/kg of body wt; Abbott Laboratories, North Chicago, IL, U.S.A.). The resected rats underwent 85% jejunoileal resection and subsequent end-to-end anastomosis as previously described. For the sham-operated rats, the abdominal cavity was simply opened and exposed for about 40 min, the same length of time as that required for the resection. None of the rats was allowed any food for 24 h post-operation. Six rats of each group were sacrificed 24 h post-operation under this fasting condition. They were anesthetized by an intraperitoneal injection of Nembutal, and after laparotomy, the 5 cm of ileal segment just proximal to the ileocecal valve was excised. The 5 cm of jejunal segment 5-cm distal to the Treitz ligament was also excised from the sham-operated rats. The luminal contents were washed out with 5 ml of ice-cold saline. The intestinal mucosa was scraped off with a slide glass, snap-frozen in liquid nitrogen, and stored at −80°C for RNA extraction. The other rats were fed on a purified diet containing (as w/w) 25% casein, 65% sucrose, 5% corn oil, 4% mineral mixture, and 1% vitamin mixture for 2 wk. On the last day of the experiment, the animals were sacrificed without fasting, and the intestinal mucosa was obtained and stored as already described.

Total RNA was isolated by the acid guanidium-phenol-chloroform method, using isogen (Nippon Gene, Tokyo) from intestinal mucosa. Samples of total RNA (30 µg/lane) were electrophoresed on denaturing 2.2% formaldehyde, a 1% agarose gel, and transferred to a nylon membrane (Hybond N+, Amersham International, Amersham, U.K.). Blots were hybridized with a 54-base oligonucleotide probe.

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(5′-TCTTGTGCTCAGAAAAGAGCTCAGTGGTGCTCAAAGCGCCTTCCCTCTTAA-3′) constructed to be complementary to 211–264 encoding partial rat apo B cDNA clone pRBS.9 The probe was 3′-tailed labeled with digoxigenin, using a DIG oligonucleotide tailing kit (Boehringer Mannheim, Mannheim, Germany). Prehybridization, hybridization, and washing were carried out with a DIG luminescence detection kit (Boehringer Mannheim) as recommended by the manufacturer. After the detection, each filter was then sequentially rehybridized with the digoxigenin-labeled apo A-I and apo A-IV probes of the 54-base oligonucleotide,9 and the detection was similarly carried out. The relative quantity of mRNA was evaluated by densitometric scanning.

The endogenous apo B mRNA editing ratio was analyzed by RT-PCR coupled with a primer extension/dideoxynucleotide chain termination assay.10 Total RNA samples were treated with DNase RQ1 (Promega, WI, U.S.A.) to remove genomic DNA and subjected to RT-PCR by using Moloney murine leukemia virus reverse transcriptase (GIBCO BRL, MD, U.S.A.) and EX-Taq polymerase (Takara, Otsu, Japan) with a down-stream primer (5′-CAGGATATGATCCTGTCGAAGC-3′) and up-stream primer (5′-ATCTGACTGGGAGACAAAGTAG-3′) as previously detailed.10 This reaction produced an amplicon of 275 bp flanking the edited base. The size of the PCR products evaluated by 2% agarose gel electrophoresis was consistent with the predicted size (data not shown). The RT-PCR products were purified with a QIAquick PCR purification kit (Qiagen, Studio City, CA, U.S.A.) as recommended by the manufacturer, and the products were annealed to 100 pmol of an antisense 5′-digoxigenin end-labeled primer (5′-ATCATATAATCTCTTACTGTA-3′; 8 nucleotides downstream from the edited base) in a buffer (75 mM Tris-HCl at pH 7.5, 25 mM MgCl2, 75 mM NaCl at 70 °C for 15 min after denaturation at 95 °C for 5 min. An enzyme solution (3 μl) containing 2.5 U T7 DNA polymerase (Pharmacia Biotech, Tokyo, Japan), 2 mm dATP, dCTP, TTP, and 5 mm dideoxy GTP was then added to the mixture, which was incubated at 42 °C for 30 min. The extension products were resolved by 7% urea/20% PAGE, this being followed by electrophoretic transfer to a nylon membrane (Hybond N+). Dyes with the extension products was carried out with a DIG luminescence detection kit (Boehringer Mannheim) as recommended by the manufacturer. By this method, the primer annealed to the apo B-100 cDNA was extended to the cytosine at nucleotide 6666 to generate a product of 33 nucleotides, and the primer annealed to apo B-48 cDNA was extended to the next upstream cytosine to generate a product of 38 nucleotides. The ratio of edited to unedited apo B cDNA was evaluated by densitometric scanning.

Each result is expressed as the mean ± SE. A statistical comparison of the means was carried out by Student’s t-test.

Discussion

Apo A-I, apo A-IV, and apo B are expressed mainly in the enterocytes in the small intestine and hepatocytes.11

Table I. Effect of a Massive Small Bowel Resection on the Apolipoprotein B, A-I, and A-IV mRNA Levels in the Ileum of Rats 24 h and 2 wk after Surgery

<table>
<thead>
<tr>
<th></th>
<th>Apo B mRNA</th>
<th>Apo A-I mRNA</th>
<th>Apo A-IV mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>89.3 ± 8.9</td>
<td>87.9 ± 6.7</td>
<td>31.2 ± 2.9</td>
</tr>
<tr>
<td>Resection</td>
<td>90.9 ± 7.8</td>
<td>80.6 ± 7.2</td>
<td>98.1 ± 12.5</td>
</tr>
<tr>
<td>p</td>
<td>&gt; 0.1</td>
<td>&gt; 0.1</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

2 wk after surgery

<table>
<thead>
<tr>
<th></th>
<th>Apo B mRNA</th>
<th>Apo A-I mRNA</th>
<th>Apo A-IV mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>92.3 ± 15.5</td>
<td>91.5 ± 15.9</td>
<td>33.7 ± 3.1</td>
</tr>
<tr>
<td>Resection</td>
<td>129.1 ± 23.8</td>
<td>118.6 ± 19.2</td>
<td>111.2 ± 12.7</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.1</td>
<td>&lt; 9.1</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

* Values for apo B, apo A-I, and apo A-IV mRNA in the ileum are expressed relative to the average values for the jejunal of the sham-operated rats which were normalized to 100. Each value is the mean ± SE (n = 6).

Results

Figure 1 shows representative Northern blots of intestinal RNA from fasted rats 24 h post-operation and from fed rats 2 wk post-operation. Values for apo B, apo A-I, and apo A-IV mRNA in the ileum, which are shown in Table I, are expressed relative to the average values for the jejunal of the sham-operated rats normalized to 100. The ileal apo B and apo A-I mRNA levels were no different between the two groups 24 h after the surgical procedure. Two weeks after the operation, the apo B and apo A-I mRNA levels in the residual ileum of the resected rats tended to be higher than those in the sham-operated rats. In marked contrast, the ileal apo A-IV mRNA levels were significantly higher in the resected rats than in sham-operated rats 24 h and 2 wk post-operation.

Figure 2 shows representative results of the primer-extension assay of PCR-amplified apo B cDNA in the rat intestine 24 h and 2 wk after surgery. The upper, middle, and lower bands show apo B-48-type (edited) cDNA, apo B-100-type (unedited) cDNA, and the free primer, respectively. The percentage of edited apo B transcripts is shown in Table II. There was no significant difference in ileal apo B mRNA editing between the sham-operated and resected rats, all the intestinal regions thus containing about 80% of edited-apo B transcripts.

![Fig. 1. Representative Northern Blots of Small Intestinal RNA from Sham-operated and Resected Rats 24 h (left) and 2 wk (right) after the Operation.](image)

Lane 1, sham-jejunum; lane 2, sham-ileum; lane 3, resected-ileum.

![Fig. 2. Apolipoprotein B mRNA Editing Detected by a Primer Extension/Dideoxynucleotide Chain Termination Analysis in the Small Intestine of Sham-operated and Resected Rats 24 h (left) and 2 wk (right) after the Operation.](image)

TAA and CAA represent edited and unedited apo B mRNA, respectively. Lane 1, sham-jejunum; lane 2, sham-ileum; lane 3, resected-ileum.
Table II. Effect of a Massive Small Bowel Resection on Endogenous Apolipoprotein B mRNA Editing in the Intestine of Rats 24 h and 2 wk after Surgery

<table>
<thead>
<tr>
<th></th>
<th>24 h</th>
<th>2 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-jejunum</td>
<td>82.2 ± 13.1</td>
<td>84.3 ± 14.4</td>
</tr>
<tr>
<td>Sham-ileum</td>
<td>78.4 ± 14.2</td>
<td>79.6 ± 10.5</td>
</tr>
<tr>
<td>Resection-ileum</td>
<td>80.3 ± 12.0</td>
<td>82.9 ± 13.4</td>
</tr>
<tr>
<td><em>p</em></td>
<td>&gt; 0.1</td>
<td>&gt; 0.1</td>
</tr>
</tbody>
</table>

* The percentage of edited apo B transcripts assessed by a primer-extension analysis of PCR-amplified apo B cDNA is expressed as the ratio of apo B-48-type cDNA (TAA) to total apo B cDNA (TAA + CAA). Each value is the mean ± SE (*n* = 6).

These apolipoproteins are the major protein components of triglyceride (TG)-rich lipoproteins, i.e., CM and very-low-density lipoprotein (VLDL), and are thus responsible for the transport of dietary lipids. Under normal conditions, only the proximal small intestine is actively involved in TG absorption, the distal intestine being much less efficient in CM production than the proximal intestine. When the proximal small intestinal absorption fails because of mucosal injury or surgical resection, the distal small intestine is able to assume the function of TG absorption, thereby limiting steatorrhea. In this condition, it is theoretically possible for the lipid uptake, apolipoprotein synthesis, CM assembly and output to be enhanced in the enterocytes of the distal small intestine. In the present study, we investigated the changes in mRNA expression of three apolipoproteins in the residual ileum after a proximal intestinal resection in rats, and the results show that apo A-IV was the only one whose mRNA expression was rapidly up-regulated by loss of the proximal intestine. Two wk after surgery, the ileal apo A-I and B mRNA levels tended to be higher in the resected rats than in the sham-operated rats. Since the adapted small intestine after the resection showed hyperplasia with tall villi, the slight and non-significant decreases in the apo A-I and B mRNA levels appear to have resulted from a possible increase in the number of villus-associated enterocytes expressing the apolipoproteins.

A number of reports have demonstrated that, among the three apolipoproteins (apo A-I, apo A-IV, and apo B), apo A-IV is the only one whose expression, synthesis, and lymphatic secretion were stimulated by the ingestion or intestinal delivery of lipid. In this respect, in the fed state (2 wk post-resection), it is possible that the facilitated uptake of dietary lipid that would normally be absorbed in the proximal intestine was, at least partly, responsible for the specific increase in apo A-IV mRNA expression in the residual ileum after the resection. Additionally, previous work by us and others has suggested that biliary lipid plays an important role in apo A-IV gene expression, synthesis and lymphatic secretion in the small intestine. In the fasted state (24 h post-resection), in which biliary lipids account for the major amount of lipid delivered to the intestine, it is therefore suggested that the increased availability of biliary lipid in the intestinal lumen was responsible for increase in apo A-IV mRNA. Further investigations should be conducted to clarify which component of bile is responsible for up-regulating apo A-IV expression and whether the content of the biliary component would be influenced by the feeding status and/or proximal intestinal resection. In respect of the extrapolation of data to humans, mice could be a superior experimental model to rats, in which biliary secretion is continuously secreted into the intestinal lumen due to the lack of a gallbladder.

It has been shown that the plasma level of peptide YY (PYY) and ileal PYY mRNA were increased after a massive small bowel resection in rats. Thus, PYY has been postulated to be a trophic factor involved in intestinal adaptation after a proximal bowel resection. PYY is synthesized in L-cells located in the distal bowel and is released in response to intestinal nutrients, especially lipid. Kalogeris et al. have demonstrated that a continuous intravenous infusion of PYY significantly increased the intestinal apo A-IV synthesis and secretion in rats. Although it should be elucidated whether apo A-I and apo B expression would respond to PYY, a specific increase in apo A-IV mRNA in the residual ileum may be at least partly mediated by such gastroenteric hormones as PYY.

It is not yet clear what are the physiological implication that only apo A-IV responded to loss of the proximal intestine with a rapid increase in mRNA expression. In the fed state, the ileal enterocytes would adapt to a small bowel resection by increasing the size of CM particles to facilitate the transport of absorbed lipid, since the lymphatic output of apo A-I and apo B is known to be unchanged by intestinal TG intake. In the fasted state, bile is the major source of lipid delivered to the intestine. It has been shown that the addition of phosphatidylcholine to a duodenal infusate in lymph-annulated animals increased the lymphatic output of newly synthesized TG. Since resection of the proximal intestine would increase the luminal availability of bile-derived PC in the residual ileum, the ileal enterocytes may secrete the expanded size of VLDL which is the only lipoprotein produced by the small intestine in fasted animals. Under these conditions, only apo A-IV gene expression seems to be closely related to the lipid content in the TG-rich lipoprotein particles. In addition, we have previously demonstrated an increase in the apo A-IV protein together with its mRNA in the residual ileum. Thus, apo A-IV may play a role in the assembly and output of the expanded TG-rich lipoproteins in the residual ileum. To confirm this, it will be necessary to determine the composition of the TG-rich lipoprotein particles secreted by the residual ileum after a proximal intestinal resection.

A series of observations by Tso and co-workers have suggested that apo A-IV acted centrally to inhibit food intake, gastric secretion, and gastric emptying. In this respect, another possible implication of the specific response of apo A-IV is that the up-regulation of apo A-IV gene expression in the residual ileum may contribute to maintaining a certain plasma level of apo A-IV that is necessary to regulate the feeding behavior and/or gastric functions. We have indeed demonstrated a transient decrease in plasma apo A-IV after a proximal bowel resection which was followed by recovery to the control level. In patients with short-bowel syndrome, delayed gastric emptying would be required for the effective digestion and absorption of nutrients, and the up-regulation of apo A-IV expression in the residual ileum may be involved in this possible adaptation.
In the present study, all the intestinal regions examined, i.e., the jejunum and ileum of sham-operated rats and the residual ileum of resected rats, contained a comparable ratio of edited apo B transcripts, being consistent with the results of the previous report [28] and demonstrating that intestinal apo B mRNA is extensively edited in all regions of the small intestine with no horizontal or vertical gradient detectable. Intestinal apo B mRNA editing is responsible for the biosynthesis of apo B-48, the protein component of chylomicrons. The present data demonstrate that apo B mRNA editing is not associated with the possible compensation of CM assembly and output during small intestinal adaptation.

In conclusion, the present results demonstrate that ileal apo A-I and B mRNA expression and apo B mRNA editing were constitutive, but that apo A-IV was highly sensitive to loss of the small intestine. Further investigations will be necessary to elucidate the physiological implications of the specific upregulation of apo A-IV gene expression in the residual ileum.

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References