Increased Expression of Mineralocorticoid Receptor in Human Ileum after Total Colectomy: Immunohistochemical and Immunoblotting Studies

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Aldosterone is known to regulate transmembrane ion transport and water absorption through its binding to mineralocorticoid receptor (MR) in the mammalian colon. A possible role of aldosterone has been suggested in increased water absorption from remnant ileum in the patients with total colectomy. We, therefore, studied immunolocalization of MR in remnant ileal mucosa in the patients with total colectomy in order to study the role of aldosterone in water and sodium absorption in these patients. Immunohistochemical localization of MR was performed in ileal mucosa of 7 patients with total colectomy and 5 cases of normal ileum obtained from the resection of ascending colon due to carcinoma by using a polyclonal antibody raised against a mineralocorticoid receptor fusion protein as a primary antibody and a biotin-streptavidin system for immunostaining. Immunoblotting was also performed. Positive MR immunoreactivity was observed in both cytoplasm and nucleus of absorptive cells of ileum of total colectomy patients but not in control normal ileum. Immunoblotting revealed the presence of an approximately 100 kDa immunoreactive product corresponding to mineralocorticoid receptor. Aldosterone is considered to act on ileal mucosa following total colectomy and the aldosterone dependent sodium transport and water absorption may be accelerated in ileal mucosa after total colectomy, which is consistent with the postoperative decrease in the stool volume observed in these patients. In conclusion, increased expression of the mineralocorticoid receptor in remnant ileum may play an important role in intestinal adaptation in total colectomized patients.

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Total colectomy followed by various forms of reconstruction, such as ileostomy, ileo-rectal anastomosis and ileo-anal anastomosis are often indicated for surgical therapies in patients with ulcerative colitis and familial polyposis coli (Goligher 1984). Output of fluid volume in total colectomized patients has been reported to be from 1,000 ml to 1,500 ml in an early postoperative period (Jagelman 1988). The volume generally decreases up to 500 ml by the time of discharge, but a subclinical state of mild chronic dehydration and sodium depletion persists in these patients (Gallagher et al. 1962). Gallagher et al. (1962) postulated the possible roles of aldosterone action in decrease of fluid output in the total colectomized patients because of high urinary potassium-sodium ratios observed after the operation. It is well-known that aldosterone stimulates sodium absorption not only in tubular systems of kidney, but also in the large intestine (Levitan and Ingelfinger 1965; Edmonds and Marriott 1969). In the mammalian colons, aldosterone is known to increase sodium absorption, potassium secretion and transmucosal potential difference (Edmonds and Marriott 1967, 1970).

In our previous reports, we examined the immunohistochemical distribution of the mineralocorticoid receptor in human tissues with a polyclonal antibody raised against a mineralocorticoid receptor fusion protein (Fukushima et al. 1991a; Sasano et al. 1992). These results demonstrated that mineralocorticoid receptor was expressed in the kidney or in various exocrine glands such as pancreas, salivary gland, mammary gland and sweat gland (Sasano et al. 1992) and colon (Fukushima et al. 1991a). In the present investigation we examined the immunohistochemical distribution of the mineralocorticoid receptor in remnant ileal mucosa in 7 patients with total colectomy in order to study the role of aldosterone in post-operative water and sodium absorption and possibly in “intestinal adaptation” of these patients.

**MATERIALS AND METHODS**

**Patients**

Clinical data of control subjects and the total colectomized patients are summarized in Table 1. Two to three specimens were obtained at surgery, or sampled at endoscopy with permission of the patients. Endoscopic examination was performed to investigate the grade of inflammation of the ileal pouch when a complication of pouchitis was clinically suspected before and/or after covering-ileostomy closure. Bowel movements of these patients were approximately five times a day after covering-ileostomy closure. Electrolyte balance and renal function were all within normal limits in these patients. Patients were free from serious complications and allowed a normal diet.

**Antibody**

The antibody was kindly donated by Dr. Zygmunt Krozowski, Baker Medical Institute, Prahran, Australia. The production of fusion protein and the subse-
sequent immunization protocol in rabbits were previously described in detail (Fukushima et al. 1991a; Sasano et al. 1992).

**Immunohistochemistry**

The process of tissue fixation and immunostaining were previously described in details by the authors (Fukushima et al. 1991b). Briefly, the specimens were fixed in periodate-lysine-2% paraformaldehyde (MacLean and Nakane 1974) at 4°C for 6 hr, then washed in phosphate-buffered saline (PBS) containing increasing concentrations of sucrose. The specimens were embedded in O.C.T. compound (Miles Pharmaceutical, Naperville, IL, USA), and rapidly frozen in dry ice-ethanol. Frozen sections of 6 microns in thickness were prepared for immunostaining. A Biotin-StreptAvidin Immunostaining system (Biogenex Laboratories Co. Dublin, CA, USA) was used. Briefly, the procedure involves successive incubations with the first antibody at optimal dilutions (×2,000) for 12 hr at 4°C. Endogenous peroxidase in the tissue sections was inactivated by incubation of the sections with 100% methanol containing 0.25% hydrogen peroxide. After washing in PBS, the sections were reacted with goat biotinylated anti-rabbit IgG for 12 hr at 4°C. Sections were then washed with PBS and incubated in StreptAvidin-enzyme conjugates for 45 min at room temperature. The reaction products were visualized by incubation with 0.025% dianinobenzidine (DAB) solution containing 0.01M sodium azide and 0.006% hydrogen peroxide, and counter stained with methyl green. The specificity of histochemical staining was
confirmed by replacing the primary antibodies with pre-immune rabbit serum.

**Immunoblotting (Western blotting)**

Preparation of cytosol and partial purification of receptor: Specimens examined in immunoblotting were three cases of grossly normal portion of kidney resected for renal cell carcinoma, grossly normal appearing mucosa of ascending colon and terminal ileum resected for adenocarcinoma, and ileal mucosa from the patient with familial polyposis coli who was received total colectomy with ileo-rectal anastomosis 15 years ago followed by ileo-anal anastomosis. Mucosa was separated from muscular layer in the gastrointestinal tract at the time of sampling. Specimens were frozen immediately after resection and were subsequently stored at \(-80^\circ\text{C}\). Specimens were homogenized in 3 vol of TM buffer which consisted of 10 mM Tris, 60 mM Na\(_2\)Mo\(_4\), and 1 mM dithiothreitol, pH 7.4, and centrifuged at 100,000 \(\times\) g for 1 hr at 4°C (Krozowski et al. 1989). The cytosol (2.5 ml) was mixed with 0.1 ml of DEAE-Sepharose previously equibrated in TM buffer for 1 hr at 4°C, and centrifuged at 1,000 \(\times\) g for 5 sec. The sediment was washed with TM buffer 3 times, sodium dodecyl sulfate (SDS) added, the mixture boiled for 3 min and the suspension centrifuged at 1,000 \(\times\) g for 5 sec.

**Immunoprobing**

The supernatants were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 10% acrylamide gels. After electrophoresis proteins were blotted onto nitrocellulose membrane. Nitrocellulose membrane was immunoprobed with MINREC4 antiserum (diluted at 1: 200) or pre-immune serum, overnight and washed with Tween-PBS 4 times. Following incubation with biotinylated goat antirabbit IgG and peroxidase conjugated avidin, immunoreactants were visualized with 4-chloro-1-naphtol solution containing 0.006% hydrogen peroxide.

**Results**

Positive staining was observed on absorptive cells in ileal mucosa after total colectomy but not in control ileal mucosa (Fig. 1A, 2B). Ileal mucosa from patients who had total colectomy 7 and 15 years ago demonstrated much more intense MR immunoreactivity than that in those from patients undergoing colectomy two years ago. Ileal mucosa which were clinically considered to be out of contact with the stool, including those obtained from anal side of loop ileostomy or pouch before loop ileostomy closure, were also positive for MR. Immunoreactivity was absent when pre-immune serum was used instead of MINREC4.

Immunoblotting analysis revealed the appearance of a 100 kDa protein in the remnant ileal mucosa after total colectomy (Fig. 2). The bands were also observed in normal kidney and ascending colon, but not in the normal ileum (Fig. 2).
DISCUSSION

The human gastrointestinal tract is highly efficient in absorbing and secreting water and electrolytes required for normal homeostasis. Of all the gastrointestinal tract, water absorption is carried out most efficiently in the colon under physiological conditions (Kalser 1985). Therefore, severe diarrhea and subsequent fluid loss as well as sodium deficit are observed in patients with total colectomy immediately after the resection. On the other hand, it is also well
known that the volume of effluent decreases over a seven to ten day period, with the stool becoming less watery, suggestive of the increased intestinal fluid absorption during this period (Jagelman 1988). However, the mechanism of increased fluid retention in these patients is not completely understood and controversy persists over the development of an enhanced capacity for electrolyte and water absorption in the remnant intestine (Smiddy et al. 1960; Kanaghinis et al. 1963; Edmonds 1971; Prado et al. 1974; Hawker et al. 1980).

In the present study, we examined whether immunoreactivity of mineralocorticoid receptor was observed in remnant ileal mucosa after total colectomy in order to study possible role of aldosterone in increased intestinal fluid absorption. Immunoreactivity of mineralocorticoid receptor was negative in the control ileum, whereas immunohistochemical detection of mineralocorticoid receptor was observed in the ileal absorptive cells after total colectomy. These findings indicate the increased expression of the receptor and subsequent increase in aldosterone-dependent sodium and water absorption in this portion of the gut. Ladas et al. have reported that ileal effluent after total colectomy has a much higher potassium concentration than that of normal subjects (Ladas et al. 1986). In addition, Levitan et al. reported that increased ileal fluid potassium concentrations are observed in response to mineralocorticoid administration (Levitan and Goulston 1967). Increased effluents generally result in a loss of water and salt in patients.
undergoing total colectomy, conditions which stimulate the renin-angiotensin-
aldosterone system and consequently increases the level of aldosterone production
(Gallagher et al. 1962). In our clinical study at Tohoku University Hospital, the
majority of colectomized patients demonstrated high plasma aldosterone concen-
tration as compared with normal subjects (unpublished observations), although
there are some conflicting reports claiming that aldosterone concentrations are
within normal limits in ileostomates (Issacs et al. 1976; Turnberg et al. 1978).
Our present study indicates that circulating aldosterone acts not only on the
kidney but also on remnant ileum, and increased and/or de novo expression of
mineralocorticoid receptor and increased aldosterone output may play an impor-
tant role in intestinal adaptation in these patients.

It is well-known that immunohistochemical methods are associated with
various methodological variations, especially sensitivity of detection. Therefore
we cannot exclude the possibility that the amount of mineralocorticoid receptor in
the control ileum is below the lower limit of immunohistochemical detection.
However, we have demonstrated the presence of an approximately 100 kDa
protein by immunoblotting, which is consistent with the reported molecular
weight of the receptor, in the cytosol of remnant ileum of the patient with total
colectomy but not in control ileum. Therefore, immunoreactivity observed in the
absorptive cells of remnant ileum of the patients with total colectomy indicates
the presence of mineralocorticoid receptor, which has been detected in other
mineralocorticoid target tissues employing MINREC4 (Sasano et al. 1992).

The mechanism of induction or increased expression of mineralocorticoid
receptor in the remnant ileum is still unclear, but it is possible that some unknown
humoral factors may cause an induction and/or increased expression of the
mineralocorticoid receptor in the remnant ileal mucosa. Induction by humoral
factors would be consistent with the detection of receptor in samples of gastro-
intestinal tract which was not in direct contact with luminal contents. Further
investigations are required to clarify possible factors which may modulate
mineralocorticoid receptor expression in remnant ileum of patients undergoing
total colectomy. These factors can contribute to clinical improvement of pos-
toperative management of the patients with total colectomy.

References

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