Repair of the surface epithelium after saponin-induced colonic mucosal injury in the rat

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Abstract The superficial colonic epithelia of rats were exposed to 1.0% saponin solution for 3 min and fixed at various periods thereafter. The repair or restitution process was observed by light as well as by transmission and scanning electron microscopy. The exposure of the luminal surface to saponin resulted in uniform and extensive damage to the superficial epithelial cells without affecting the cells in the crypts. At 3 min after saponin treatment, the damaged epithelial cells exfoliated from the mucosa and the basal lamina was exposed. Within 15 min, most of the exposed basal lamina was covered by squamous to low-cuboidal epithelial cells, probably migrating from the crypts. These epithelial cells extended large lamellipodia over the denuded basal lamina. After 15 min the damaged surface was completely covered with epithelial cells, which became columnar at 1 h. Tight junction protein ZO-1 became positive along the restituted epithelium. Proliferating-cell nuclear antigen (PCNA) staining showed that proliferation of epithelial cells occurred after the restitution. These results suggest that saponin treatment serves as a good model system to study colonic restitution, which is carried out by rapid migration from the remaining crypt cells, followed by cellular proliferation. Rapid formation of tight junctions spanning the damaged regions allows rapid restoration of the barrier function of the colonic epithelium.

Key words Restitution · Ultrastructure · Cell migration · Colon · Saponin

Introduction

An uninterrupted sheet of epithelial cells connected by tight junctions serves as a structural basis of the mucosal barrier in the gastrointestinal tract. Superficial cell damage that breaks the mucosal barrier but is not accompanied by macroscopic hemorrhagic lesions probably occurs relatively frequently during the normal course of food and drug intake. Such mild forms of intestinal epithelial injury also commonly occur in many diseases. The superficial defects in the gastrointestinal tract are restored by the process called restitution. Restitution is defined as a process by which epithelial integrity and continuity are rapidly reestablished following injury, before cell proliferation or an extensive inflammatory response occurs. It occurs rapidly after the injury by the migration of the remaining viable cells. Most of the studies on restitution have focused on the repair processes in the gastric mucosa after various types of surface injury that did not cause deep erosions.

In the colon, studies have attempted to create a model for experimental colitis by using bile acids, carrageenan, alcohol, or acetic acid. Nonetheless, little is known about the etiology of colitis and even less about the cellular mechanisms of colonic epithelial repair. Most studies of colonic wound repair have focused on acute injury that destroys epithelial cells down to the deep crypts. These investigations provided knowledge concerning the long-term repair process of the colonic mucosa, but might not reflect usual pathophysiological conditions. Ultrastructural observations of experimental superficial wound healing in the rectum and colon have shown restitution by cell migration and suggested its important role in maintenance of the colonic mucosal barrier.

The purpose of the current study was to develop a model system for superficial mucosal damage in the colonic mucosa in vivo and to observe the restitution process. We have developed a highly reproducible in vivo model of intestinal epithelial injury induced by saponin, which enabled us to monitor and evaluate, by thin-section electron microscopy and scanning electron microscopy, the sequence of morpho-
logical events of repair within the first minute to 7 days after superficial damage. In addition, formation of tight junctions and cellular proliferation were monitored by immunohistochemical staining for zonula occludens-1 (ZO-1) and proliferating-cell nuclear antigen (PCNA), respectively.

**Materials and methods**

**Tissue preparation**

Male Wistar rats, weighing 200–250 g, were fasted overnight in wire-bottomed cages with water ad libitum. Under sodium pentobarbital anesthesia, 1 ml of 1.0% saponin in physiological saline solution was injected into the colonic lumen via the anus by use of a 7-cm-long metal syringe. The distal colon was distended with the saponin solution so that the mucosal surface would be uniformly exposed to the saponin solution. After 3 min, the saponin was quickly rinsed out with 3 ml of physiological saline.

After various intervals ranging from 1 min to 7 days, the colon was exposed under pentobarbital anesthesia. Each rat was killed by cervical dislocation after the introduction of fixative into the colon, which was fixed in situ for 10 min. Control rat colons received physiological saline for 3 min instead of saponin. Three rats were used for each group. Five nearly equally sized pieces of each colon were cut open from the antimesenteric side. The fixed specimens were pinned to wax plates with the mucosal side up and processed for light and electron microscopic observation.

**Light and transmission electron microscopy**

Tissues for light microscopy as well as for transmission electron microscopy were fixed in 2.5% glutaraldehyde/2.0% formaldehyde in 0.1 M sodium cacodylate buffer in situ and for an additional 12–24 h at room temperature. The tissues were postfixed in 1% OsO₄ treated with 0.5% uranyl acetate, dehydrated with a graded series of ethanol, and embedded in Epon. Semithin sections for light microscopy were cut, stained with lead citrate and uranyl acetate, and examined with a H-800 electron microscope (Hitachi, Tokyo, Japan).

**Scanning electron microscopy**

Tissues for scanning electron microscopy were obtained from areas adjacent to those used for plastic embedding. After the initial fixation, specimens were postfixed for 2 h in 1% OsO₄ dehydrated in ethanol, critical-point dried with liquid CO₂, and coated with gold. They were then examined with a S-800 scanning electron microscope (Hitachi).

**Immunohistochemistry**

For PCNA staining, specimens were fixed in 10% formalin and embedded in paraffin. Sections (4 µm thick) were cut and mounted on glass slides coated with poly t-lysine. They were then dewaxed and immunostained by the peroxidase-antiperoxidase (PAP) method with primary incubation with anti-PCNA antibody (1:50; DAKO, Glostrup, Denmark) overnight. Diaminobenzidine was employed as a chromogen. Specimens were counterstained with hematoxylin. For ZO-1 staining, cryostat sections (5 µm thick) of freshly frozen specimens were cut and fixed in 3% formaldehyde in phosphate-buffered saline for 1 min. Immunofluorescence staining was carried out as previously described using rabbit anti-ZO-1 antibody (1:200; Zymed, South San Francisco, CA, USA) and Cy3-labeled donkey anti-rabbit IgG (1:1000; Jackson Immunoresearch, West Grove, PA, USA), with nuclear counterstaining with DAPI (2 µg/ml, 4',6-diamidino-2-phenylindole dihydrochloride; Boehringer-Mannheim, Mannheim, Germany). Fluorescence as well as the corresponding Nomarski-differential interference-contrast images was recorded with an Olympus AX-70 fluorescence microscope equipped with a PXL-1400 cooled-CCD (charge-coupled device) camera (Photometrics, Tucson, AZ, USA) and analyzed with IPLab Spectrum software (Signal Analytics, Vienna, VA, USA).

**Results**

Light microscopic observation of mucosal destruction by saponin and restitution

Exposure to 1.0% saponin solution for 3 min specifically and uniformly destroyed the superficial colonic epithelium of rats without causing grossly detectable hemorrhage or hyperemia. The superficial epithelium was widely and extensively damaged (Fig. 1b). The saponin damage was uniform and confined to the superficial mucosa. No detectable hyperemia or hemorrhage was observed. Necrotic superficial epithelial cells exfoliated, and the lamina propria underneath was exposed 1 min after saponin exposure. Cells in the crypts remained undamaged. As early as 5 min after the saponin treatment, flattening of the epithelial cells located next to the denuded regions was observed (Fig. 1c), suggesting the migration of the remaining viable epithelial cells to recover the exposed basal lamina. At 15 min after saponin treatment, the lesion was mostly covered with epithelial cells (Fig. 1d), although some of the surface epithelial cells were squamous to cuboidal in shape. The intercellular space between them was markedly wide. By 60 min, most of the superficial mucosa was restituted with columnar epithelial cells (Fig. 1e). By 180 min, the surface epithelial cells appeared columnar (Fig. 1f), some of them containing apical mucous granules typical of normal colonic mucosa (data not shown). Exposure of the mucosa to physiological saline did not alter the morphology of the colon, and the surface had normal-appearing columnar mucous cells (Fig. 1a).