Ultrastructural Alterations and Quantitative Changes in the Mineral Content of Colonic Mucosa in a Diabetic Rat Model

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Abstract: Background: Scarce information is available regarding the effects of diabetes on the colonic mucosa. Objective: The present study was undertaken to investigate the effect of diabetes on the ultrastructure and trace elements of the rat colonic mucosa. Methods: Twenty adult male Wister albino rats were randomly divided into two groups; control and diabetic, 10 animals each. The diabetic group received intravenous injection of a single dose of streptozotocin (STZ) (30 mg/ml). Rats were sacrificed at the end of 8 weeks with an overdose of ether anesthesia. The proximal colon was immediately removed and processed for scanning electron microscope (SEM) and energy dispersive X-ray analysis (EDAX) using Quanta 250 FEG-EDAX for element detection. Results: revealed that colonic crypts of the diabetic animals showed enlarged, irregular crypt openings, with excessive exfoliation at the periphery. Numerous lymphocytes and activated monocytes were observed on the crypt surfaces and in the lamina propria. Accumulation of collagen fibres in the sub-epithelial layer and degranulation of apical mucin secreting goblet cells could be seen. Enterocytes showed significant decrease in P and Ca and significant increase in Na, K and Si (P < 0.05). The Goblet cells showed decrease in P, Ca and Cl while there was significant increase in Na and K (P < 0.05). Conclusions: It could be concluded that using the scanning electron microscope (SEM) with energy dispersive X-ray analysis (EDAX) opens new perspective to study the pathophysiological effect of diabetes on colonic mucosa.

Key words: Diabetes · Mucosa · Mineral content and rat model

INTRODUCTION

Up to 60% of the patients with diabetes mellitus develop gastrointestinal (GI) symptoms, such as constipation and diarrhea [1, 2]. Diarrhea was noted also in rats with streptoztocin-induced chronic diabetes [3]. The occurrence of colonic disturbance in diabetes was accompanied with thickened subepithelial collagen layer [4], impaired mucosal absorption of fluid and electrolytes [3] and autonomic neuropathy of enterocytes [5].

The present study was undertaken to investigate the effect of diabetes on rat colonic mucosa using scanning electron microscope (SEM) with energy dispersive X-ray analysis (EDAX). The latter is a powerful analytical tool that allows elemental identification and quantitative compositional information and characterization of biological specimens [6]. This will allow synchronous examination of the ultrastructure and trace elements changes of the cells of the colonic mucosa. The present study was undertaken to investigate the effect of diabetes on the ultrastructure and trace elements of the rat colonic mucosa.

MATERIALS AND METHODS

Induction of Diabetes: A total of 20 male Wistar rats, of 200-250g body weight, were randomly divided into control and diabetic groups, 10 animals each. The control group was injected with the vehicle (phosphate-buffered saline, PBS). The diabetic group was made diabetic by intravenous injection of a single dose of streptozotocin (STZ). The injection was freshly prepared immediately before use by dissolving STZ (30 mg/ml) in (PBS) and injected into the penile vein of the recipient rats, under light ether anesthesia, at a dose of 40 mg/kg body weight. Rats were maintained with free access to standard diet.
Statistical Analysis: Results were presented as means ± SEM. The differences between the two groups were tested by the Student's t test. The differences were considered significant at P < 0.05.

RESULTS

SEM of Normal Colonic Rat Mucosa: The mucosal surface of the normal proximal colon is composed of orderly and closely packed rounded or polygonal cryptal units with centrally located oval or rounded openings. Some of these cryptal mouths were seen thrusting out mucus. Each cryptal unit is being delineated by a furrow or cleft (intercryptal cleft) (Fig. 1). Each unit consists of concentrically arranged colonic absorptive epithelial cells and mucous–secreting goblet cells (Fig. 2). Goblet cells were scattered throughout the surface colonic epithelium. Many of these goblet cells were seen embracing the mouth of colonic crypts. Moreover, their luminal surfaces are characterized by short, sparse microvilli and apical mucigen granules (Fig. 2). In most instant a thick layer of mucus coat was seen covering the cryptal colonic surface (Fig. 3). The colonic surface epithelial cells were columnar with a thick carpet of uniform surface microvilli). The colonic crypt showed minimal collagen fibers and minimal supepithelial collagen deposit (Fig. 4).

SEM of Diabetic Colonic Rat Mucosa: The shape of crypt openings in diabetic mucosal surface were distorted, enlarged and they were irregularly spaced (Fig. 5,6). Some cryptal mouths were seen extruding mucin (Fig. 6). Excessive exfoliation was observed at the periphery of colonic crypts (Fig. 7). Marked inflammatory changes were also observed on the cryptal colonic surfaces and in the lamina propria. Most of cellular infiltrates were numerous lymphocytes activated monocytes and activated leucocytes (Fig. 8). Prominent excessive collagen fibres accumulation could be seen in the sub-epithelial layer of colonic mucosa (Fig. 9). Moderate collagen fibres accumulation was seen around the colonic crypts and in-between the crypts (Fig. 10). Widespread degranulation of apical mucin-secreting goblet cells (goblet-cell depletion) was observed (Fig. 11).

Mineral Analysis of Enterocytes: The enterocytes of the diabetic mucosal surface showed significant decrease in P and Ca while there was significant increase in Na, K and Si (P < 0.05). The changes in the other minerals; C, N, O & Au (gold coating) were not significant (Table 1, Figs. 12 vs. 13).
Table 1: Control vs. diabetic (multi-point analyses) of mineral weight % ratio rat enterocytes of colonic mucosa. Values are presented as mean ± SEM

<table>
<thead>
<tr>
<th>Element</th>
<th>Control</th>
<th>Diabetic</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon (C)</td>
<td>47.3983±3.89</td>
<td>57.1383±3.1379</td>
<td>0.06426</td>
</tr>
<tr>
<td>Nitrogen (N)</td>
<td>9.15± 0.92824</td>
<td>7.99273±1.76593</td>
<td>0.28417</td>
</tr>
<tr>
<td>Oxygen (O)</td>
<td>13.7067±3.02037</td>
<td>13.196±2.49112</td>
<td>0.44848</td>
</tr>
<tr>
<td>Sodium (Na)*</td>
<td>1.3533±0.1646</td>
<td>2.099±0.14874</td>
<td>0.00182</td>
</tr>
<tr>
<td>Phosphorus (P)*</td>
<td>7.085±1.23525</td>
<td>3.283±1.06857</td>
<td>0.01711</td>
</tr>
<tr>
<td>Sulphur (S)</td>
<td>4.2128±2.63834</td>
<td>7.585±1.53825</td>
<td>0.14554</td>
</tr>
<tr>
<td>Potassium (K)*</td>
<td>1.3867±0.40676</td>
<td>3.771±0.71153</td>
<td>0.00587</td>
</tr>
<tr>
<td>Calcium (Ca)*</td>
<td>1.076±0.21753</td>
<td>0.605±0.13368</td>
<td>0.0408</td>
</tr>
<tr>
<td>Gold (Au)</td>
<td>46.75±1.25</td>
<td>40.5±5.5</td>
<td>0.31025</td>
</tr>
<tr>
<td>Silica (Si)*</td>
<td>0.286±0.03945</td>
<td>0.858±0.14806</td>
<td>0.00572</td>
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</tbody>
</table>

* Significant differences at P < 0.05

Table 2: Control vs. diabetic (multi-point analyses) of mineral weight % ratio rat goblet cells of colonic mucosa. Values are presented as mean ± SEM

<table>
<thead>
<tr>
<th>Element</th>
<th>Control</th>
<th>Diabetic</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon (C)</td>
<td>27.825±6.69925</td>
<td>41.33±5.15665</td>
<td>0.07062</td>
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<tr>
<td>Nitrogen (N)</td>
<td>7.69±0.74951</td>
<td>8.393±0.66953</td>
<td>0.24999</td>
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<tr>
<td>Oxygen (O)</td>
<td>7.4533±1.75326</td>
<td>7.633±1.58205</td>
<td>0.47037</td>
</tr>
<tr>
<td>Sodium (Na)*</td>
<td>1.147±0.27491</td>
<td>2.037±0.21482</td>
<td>0.01271</td>
</tr>
<tr>
<td>Phosphorus (P)*</td>
<td>4.806±1.87396</td>
<td>4.406±1.41931</td>
<td>0.03155</td>
</tr>
<tr>
<td>Sulphur (S)</td>
<td>4.675±0.125</td>
<td>5.162±0.43367</td>
<td>0.32157</td>
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<tr>
<td>Potassium (K)*</td>
<td>1.172±0.27102</td>
<td>2.492±0.25291</td>
<td>0.0037</td>
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<tr>
<td>Calcium (Ca)*</td>
<td>0.726±0.05575</td>
<td>0.54±0.16074</td>
<td>0.00586</td>
</tr>
<tr>
<td>Gold (Au)</td>
<td>58.5±5.37742</td>
<td>47.25±3.17214</td>
<td>0.12163</td>
</tr>
<tr>
<td>Chlorine (Cl)*</td>
<td>0.487±0.08892</td>
<td>0.102±0.03425</td>
<td>0.0034</td>
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* Significant differences at P < 0.05

Fig. 1: Scanning electron micrograph of normal rat proximal colonic mucosa, showing some cryptal mouths thrusting out mucous

**Mineral Analysis of Goblet Cells:** The Goblet cells of the diabetic mucosal surface showed significant decrease in P, Ca and Cl while there was significant increase in Na and K (P < 0.05). The changes in the other minerals; C, N, O, S & Au (gold coating) were not significant (Table 2, Figs. 14 vs. 15).
Fig. 2: Scanning electron micrograph of normal rat proximal colonic mucosa, showing apical mucin granules of the goblet cells

Fig. 3: Scanning electron micrograph of normal rat proximal colonic mucosa, showing the highly trabeculated and layered mucous coat of the colonic mucosa. Note the passage of mucin out of the goblet cells over the surrounding epithelial cells. Note well demarcated epithelial cell borders

Fig. 4: Scanning electron micrograph of normal rat proximal colonic mucosa, showing freeze-fractured ethanol-dehydrated tissue. The colonic crypt showed minimal subepithelial collagen deposit
Fig. 5: Scanning electron micrograph of the diabetic rat proximal colonic mucosa, showing that the shape of crypt opening of the diabetic mucosal surface are distorted, collapsed and irregularly spaced.

Fig. 6: Scanning electron micrograph of the diabetic rat proximal colonic mucosa, showing obliteration of crypts opening together with loss of inter-cryptal clefts. Note remnants of mucosal coat.

Fig. 7: Scanning electron micrograph of the diabetic rat proximal colonic mucosa, showing diffuse surface edema of the cryptal cell lining and numerous exfoliations.
Fig. 8: Scanning electron micrograph of the diabetic rat proximal colonic mucosa, showing marked inflammatory infiltrates in the lamina propria

Fig. 9: Scanning electron micrograph of rat diabetics’ colonic proximal mucosa, showing prominent excessive collagen fibres accumulation in the sub-epithelial layer of colonic mucosa

Fig. 10: Scanning electron micrograph of rat diabetics’ colonic proximal mucosa, showing excessive collagen fibres accumulation around the colonic crypts and in-between the crypts
Fig. 11: Scanning electron micrograph of the diabetic rat proximal colonic mucosa, showing widespread de granulation of the apical mucin secreting goblet cells (goblet cell depletion)

Fig. 12: Graph representing the auto peak ID of mineral contents of control rat enterocytes of colonic mucosa
Fig. 13: Graph representing the auto peak ID of mineral contents of diabetic rat enterocytes of colonic mucosa

Fig. 14: Graph representing the auto peak ID of mineral contents of control rat Goblet cells of colonic mucosa
**DISCUSSION**

The present study includes scanning electron microscope (SEM) with energy dispersive X-ray analysis (EDAX) that allows the analysis of elements in the biological specimens to investigate the effect of diabetes on rat colonic mucosa. The SEM study showed that the shape of crypt openings in diabetic mucosal surface was distorted, enlarged and irregularly spaced. Few cryptal mouths were seen extruding mucin. Similar observations were seen in the mouse model of dextran sulfate sodium (DSS)-induced colitis [7]. Ultrastructural and histochemical changes were also seen in the colonic mucosa of the rats suffering from type I diabetes [8]. The authors concluded that absorptive cells have notable importance for proper function of the colon, absorbing water and nutrients. In type I diabetes, hyperglycemia leads to remarkable alterations in cell structure.

The mechanisms underlying increased prevalence of the GI symptoms in Diabetes mellitus are poorly defined and controversial [9]. Reports that diarrhea in streptozotocin-induced diabetic rats is caused by an impaired adrenergic regulation of intestinal fluid and electrolyte transport [3, 10]. Other authors [5] reported the occurrence of autonomic neuropathy, colonic dilatation and loss of fecal consistency in streptozotocin-diabetics rat. Moreover, the disturbance in ion transport may cause diarrhea, which can cause life threatening dehydration. This is supported by the findings of the present study that goblets and enterocytes showed significant decrease in P and Ca while there was significant increase in Na, K and Si (P < 0.05).

The GI symptoms in patients with diabetes could be caused by disturbed GI motility, as well as abnormal secretion and absorption [1]. Mucins act as the core constituent of the GI mucosal layer and are secreted mainly by goblet cells that are dispersed throughout the epithelial layer [11, 12]. The present study showed widespread degranulation of apical mucin-secreting goblet cells (goblet-cell depletion). Excessive exfoliation was observed at the periphery of the colonic crypts. Our finding of goblet-cell depletion and disappearance of the thick mucus coat with exfoliation of the surface epithelium supported the findings of the other authors.
Marked inflammatory changes were also observed on the cryptal colonic surfaces and in the lamina propria. Most of the cellular infiltrates were numerous lymphocytes and activated monocytes. Prominent excessive accumulation of collagen fibres could be seen in the sub-epithelial layer of colonic mucosa. Uncontrolled streptozotocin-induced diabetes leads to accumulation of collagen [13]. This is supported by the finding of other authors [4,14,15] who reported a marked thickening of the sub-epithelial collagen layer (SCL) in diabetic patients. In agreement of previous studies [4,13-15], the present work confirmed a prominent excessive collagen fibres accumulation which was seen in the sub-epithelial layer of colonic mucosa, mainly around colonic crypts, in-between the crypts and the bases of the crypts.

Calcium is one of the most important minerals in vertebrate physiology. The ionic form of calcium (Ca2+) is also an intracellular messenger that mediates aspects of muscle contraction, nerve transmission, enzyme and hormone secretion and many other biological processes, such as cell cycle regulation and programmed cell death [16]. The reported decrease in Ca2+ in goblet cells and enterocytes emphasis the significant role played by Ca2+ in the GI physiological function and in the occurrence of digestive disease [17]. Also studies suggested that dairy calcium and vitamin D inhibit the development of colorectal cancer (CRC) [19]. Epidemiological findings and the results from Ca2+ supplementation trials in patients as well as in studies in experimental animals showed effective chemoprevention of cancer colon with Ca2+, alone or in conjunction with vitamin D [20]. Also Calcium deficiency may cause abnormal epithelial proliferation and increases colon cancer risk [21]. Other authors [22] suggested that calcium signaling had a vital role in the proliferation and migration of SW620 colon cancer cells.

CONCLUSIONS

It could be concluded that using the scanning electron microscope (SEM) with Energy dispersive X-ray analysis (EDAX) opens new perspective to study the effect of diabetes on the individual cells of the colonic mucosa. Colonic mucosa, mucus secretion of the goblet cells, as well as the submucosa, is extremely important for the function and transit of substances in this organ. However, the damage arising from STZ-induced diabetes, in the form of alteration of the mineral contents seen in the enterocytes and goblet cells of the diabetic colonic mucosa may be one of the earliest mechanisms of aberrations of bowel mucosa cells seen in diabetic patients. Restoration of colonic mineral contents and the colon osmolality may ameliorate the effect on diabetic colitis.

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Abbreviations:

STZ Streptozotocin
SEM Scanning electron microscope
EDAX Energy dispersive X-ray analysis
PBS Phosphate-buffered saline
GI Gastrointestinal
CRC Colorectal cancer
SCL Sub-epithelial collagen layer
DSS Dextran sulfate sodium

REFERENCES


