HISTOPATHOLOGICAL EFFECTS OF SODIUM FLUORIDE ON THE DUODENUM OF RABBITS

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SUMMARY: To gain a better understanding of how fluoride causes gastrointestinal distress, we conducted an experimental study on rabbits to evaluate damage to the gastroduodenal tissue by sodium fluoride. Young albino rabbits weighing 400-600 g were injected daily with 5, 10, 20, and 50 mg NaF/kg bw for fifteen weeks and then sacrificed. Histopathological examination of the duodenum revealed erosion and necrosis of surface mucosa, hemorrhages, necrosis of Brunner's gland, clumped submucosa, and hypertrophy of muscles in muscularis mucosae in increasing severity according to the dose of NaF. Most noteworthy was the loss of mucosal layer in direct proportional to the amount of fluoride administered.

Keywords: Albino rabbits, Brunner's gland, Duodenum histopathology, Gastroduodenal hemorrhages, Intestinal mucosa, Sodium fluoride.

INTRODUCTION

Fluoride is known to enter the circulation by absorption from gastric and duodenal mucosa. The duodenum, proximal jejunum, distal ileum, and colon absorb fluoride proportionally to its luminal concentration via passive diffusion.

Acute and chronic studies in animals and human have shown that sodium fluoride causes gastrointestinal damage. In inhabitants of endemic fluorosis areas, gastric symptoms including loss of appetite, nausea, anorexia, abdominal pain, flatulence, constipation, and intermittent diarrhea are often present. In cases of osteofluorosis, intestinal disorders have also been reported. The objective of this paper was to assess fluoride-induced damage to the duodenum in experimental rabbits.

MATERIALS AND METHODS

Sixty young albino rabbits, weighing 400-600 g, from Kaila Scientific Corporation, Agra, India, were allowed to acclimatize for one week and were then divided into five groups and caged separately. Groups II, III, IV, and V were subcutaneously administered by hypodermic syringe sodium fluoride (Loba Chemie, Bombay, 99% purity, GR Grade) dissolved in double distilled water in doses of 5, 10, 20, and 50 mg/kg bw/day, respectively, for fifteen weeks. Group I (control) received only the vehicle. All the animals had ad libitum access to tap water and were maintained on a standard pellet diet obtained from Hindustan Lever Laboratory Animal Feed, India.

After 15 weeks the control and test animals were sacrificed under ether anaesthesia. The duodenum was carefully dissected out, blotted free of

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blood, washed in normal saline, and fixed in Bouin’s fluid and Carnoy’s fixative for histopathological examination. The tissue was dehydrated in tertiary butyl alcohol, cleared in amyl acetate, and embedded in paraffin. Sections were cut at 7 µm and stained with iron haematoxylin and eosin. Gastro-duodenal damage was evaluated by microscopic examination.

RESULTS

Group I (Control): The duodenal, mucosa and submucosa had normal appearance (Figures 1 and 2).

Group II (5 mg NaF/kg bw/day): The rabbits of this group exhibited necrosis of the duodenal mucosal layer and clumped submucosa (Figure 3) as compared to the controls (Figures 1 and 2). The duodenal muscles showed atrophy (Figure 4).

Group III (10 mg NaF/kg bw/day): The histioarchitecture of the duodenum showed corrosion of surface mucosa. The submucosa appeared clumped (Figure 5). There were diffuse punctate hemorrhages in the mucosa (Figure 6), and the muscles exhibited hypertrophy.

Group IV (20 mg NaF/kg bw/day): The duodenum of animals of this group showed hemorrhages and necrosis of cells of folds. There was loss of surface mucosa, vacuolization, pyknotic nuclei, and necrosis of Brunner’s glands. Clumped submucosa and hypertrophy of muscles in the muscularis mucosae (Figures 7 and 8) were noted as compared to the control.

Group V (50 mg NaF/kg bw/day): Histopathological examination of the duodenum showed hyperatrophiied muscles in the muscularis mucosae (Figure 9). There was loss of surface mucosa and necrosis of Brunner’s glands. The lumen of glands widened due to disintegration of cells (Figure 10), and there was marked necrosis of surface mucosa, disintegration of columnar epithelium of folds, and clumping of submucosa. (Figures 11 and 12).
Figure 1. Structure of normal duodenum in a rabbit of control group x 100.

Figure 2. Gastroduodenal mucosa in a rabbit of control group (magnified view) x 400.
Figure 3. Necrosis of mucosal layer, clumped submucosa, and atrophy of muscles in duodenum of a rabbit treated with 5 mg NaF/kg bw/day x 400.

Figure 4. Nuclear pyknosis and cytoplasmic changes in mucosa of duodenum in a rabbit treated with 5 mg NaF/kg bw/day x 400.
Figure 5. Corrosion of surface mucosa, clumped submucosa (SM), hyperatrophied muscles (MS) and necrosis of Brunner's gland (BG) in a rabbit treated with 10 mg NaF/kg bw/day x 100.

Figure 6. Duodenal mucosa showing necrosis in a rabbit treated with 10 mg NaF/kg bw/day x 100.
Figure 7. Duodenum of a rabbit treated with 20 mg NaF/kg bw/day showing obliteration of mucosal folds, necrosis, disintegration and loss of mucosa, clumped sub-mucosa (SM) and atrophic muscles x 100.

Figure 8. Vacuolization and pyknotic nuclei in Brunner’s gland of a rabbit treated with 20 mg NaF/kg bw/day x 400.
Figure 9. Hypertrophied muscles in the duodenum of a rabbit treated with 50 mg NaF/kg bw/day x 400.

Figure 10. Brunner's gland (BG) showing necrosis in a rabbit treated with 50 mg NaF/kg bw/day x 400.
Figure 11. Acute necrosis of mucosal folds along with loss of surface mucosa, broken villi, pyknosis, and clumped submucosa in the duodenum of a rabbit treated with 50 mg NaF/kg bw/day x 100.

Figure 12. Acute necrosis and disintegration of cells in gastroduodenal mucosa of a rabbit treated with 50 mg NaF/kg bw/day x 400.
DISCUSSION

In this study, fluoride-induced histopathological changes in the duodenum included surface abrasion of mucosa, clumped submucosa, diffuse punctate hemorrhages, necrosis of crypt cells and Brunner’s glands, and hypertrophy of musculature. These damaging effects of fluoride on gastroduodenal mucosa in rabbits are in accord with those observed by Susheela and Das, who recorded abrasion and cell degeneration in the duodenal mucosa of rabbits subjected to 10 mg NaF/kg bw/day for 24 months. Later, they found severely damaged gastroduodenal mucosa in human subjects caused by the toxic action of fluoride resulting in dyspeptic symptoms.

In rats exposed to 100-ppm fluoride in their drinking water, cytoplasmic degranulation and vacuolization in crypt cells, hydrophic degeneration in lamina propria and muscular tissue, increase in the number of goblet cells, broken tips of villi, and nuclear pyknosis in the small intestine have been reported. Earlier studies on acute and chronic fluoride intoxication in experimental animals and in humans showed that NaF caused extensive gastroduodenal damage in the form of erosion, erythema, petechiae, denudation of the mucosa, and degeneration of the epithelial cells. The structural damage was attributed to rapid penetration of hydrofluoric acid across the gastrointestinal cell membrane, an interpretation that accounts for the occurrence of the duodenal ulceration found in fluoridated rabbits during the present investigation.

Gastritis and spastic bowels, hemorrhagic vomiting, severe epigastric pain, abdominal pain, diarrhea and abdominal distension during fluoride intoxication have been reported. These gastric disturbances were attributed to the action of fluoride on gastric mucosa. Czerwinski and Lankosz observed gastric duodenal ulcers in 12% of aluminum factory workers. Klemmer and Hadler also found gastric ulcers in patients with skeletal fluorosis due to habitual inhalation of an organofluoride anesthetic.

Pribilla accounted for four cases of acute intoxication with silicofluoride. According to him the gastric mucosae of the duodenum were moderately loose and showed white and red corroded areas. The entire small intestine exhibited petechial hemorrhages and congestive changes. More or less similar pathological changes were noticed in the fluoride-treated rabbits during the present study. Numerous small erosions extending about one third of the total depth of gastroduodenal mucosa occurred. These lesions were confluent and amounted to a loss of almost the entire gastroduodenal mucosa.
REFERENCES