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Effect of Fluoride on the Intestinal Epithelial Cell Brush Border Membrane

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Fluoride is being ingested from many sources such as drinking water, food, dental health products, pesticide, insecticide, etc. Fluoride consumed by man and animals is chiefly absorbed in the intestine (Stookey et al. 1964). Chronic fluoride exposure causes mottled teeth and osteosclerosis. Over-fluoridation (126 mM) of drinking water have been reported to cause nausea, vomiting and diarrhoea (Waldbott 1981). Furthermore, the effect of acute and low concentrations of fluoride on gastric secretion, ion transport and other disorders have also been studied (Waldbott 1977; Reed and Smy 1980; Suketa 1980; Assem and Wan 1982; Shayiq et al. 1984). Fluoride also causes alterations in the permeability of membranes and membrane bound enzymes (Kaul 1974; Martin et al. 1980).

The intestinal cell lining plays an important role in digestion and absorption. It automatically becomes the most exposed site of contact to fluoride following ingestion. Our earlier study have shown significant alterations in the formation of lipid peroxides in rat intestine following oral administration of fluoride (Shayiq et al. 1986). The present study was undertaken to investigate the damage of rat intestinal epithelium *in situ* caused by relatively high and low fluoride concentrations.

MATERIALS AND METHODS

Male albino rats (150-170 g) were procured from animal breeding facility of Industrial Toxicology Research Centre, Lucknow and fasted overnight with water *ad libitum*. Laparotomy on each rat was performed under light ether anaesthesia. The intestine was thoroughly washed with normal saline through two cuts, one slightly distal to the duodeno-jejunal junction and the other at the distal end of the ileum. Four loops each 10 cm length were prepared from the upper end of intestine using sterile threads with a space of 1.0 cm in between the two loops. Loops were filled with 2.0 ml sodium fluoride solutions of 24, 48 and 96 mM, respectively, through the proximal opening with

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the help of a syringe fitted with a blunt needle. The proximal opening was then immediately ligated. Fourth loop received distilled water and served as control. The intestine was left in situ and the abdomen kept closed. Proper breathing and anaesthesia of the animal was kept under watch throughout the experiment. After 30 min loops were removed, gently blotted and luminal contents drained into graduated tubes. Loops were washed with demineralized water. The luminal fluid was made up to desired volume and used for various estimations. The intestine was immediately placed in cold saline and everted using a glass rod. The epithelial layer was scrapped off with the help of a glass slide and brush border vesicles prepared according to the procedure of Forstner et al. (1968).

Alkaline phosphatase and ATPase activities in luminal fluid and brush border preparations were determined according to Weiser (1973) and Hidalgo et al. (1983), respectively. Enzyme activities are expressed in terms of specific activity (μmoles of reaction product liberated/min/mg protein). Protein was determined according to the method of Lowry et al. (1951) using bovine serum albumin as standard. Extraction of lipids was carried out according to Folch et al. (1957). Total phospholipids and sterols were estimated using the procedures described by Wagner et al. (1962) and Zlatkis et al. (1953), respectively. SDS-PAGE was carried out according to Laemmli (1970) using 4% acrylamide concentration in the collecting gel and 8% in the separating gel.

RESULTS AND DISCUSSION

Table 1 shows the effect of fluoride on luminal fluid and brush border enzymes. A concentration-dependent increase of alkaline phosphatase in the luminal fluid indicates the release of enzyme from the brush border following fluoride treatment. Concomitantly, a concentration-dependent decline in the phosphatase activity was evident in the brush border preparations. Activation as well as inhibition of phosphatases by fluoride in chicks have been reported by Griffith et al. (1963). Similarly, ATPase activities of brush border preparations also declined significantly. These results indicate that higher fluoride concentrations cause substantial damage to the intestinal brush border membrane. Activities of Ca^{2+}, Mg^{2+} and Ca^{2+} + Mg^{2+}-ATPase also declined (32-51%; $p < 0.05$) in the luminal fluid following fluoride treatment. However, there were no significant differences in ATPase activities with increasing concentrations of fluoride. It has been shown that fluoride depletes ATP content of cells (Burn 1962). Our earlier study on isolated intestinal epithelial cells also indicated that fluoride inhibits glycolysis and stops ion transport as a consequence of ATP disappearance (Shayiq and Kidwai 1986). Interference of fluoride with the mobilization of Ca^{2+} and Mg^{2+} and enzymes dependent on these metals has also been reported (Riekstniece et al. 1965). Like EDTA, fluoride is also an efficient chelator of Ca^{2+} and, therefore, the role of Ca^{2+} in maintaining the integrity of the intestinal membrane could be affected by fluoride. Further, the inhibitory effect of fluoride on intestinal Na^{+}/K^{+} ATPase has been related with the altered mobilization of ions (Opit et al. 1966).

Table 1. **Effect of fluoride on intestinal brush border enzymes**

Treatment (mM)	Alkaline phosphatase	Ca^{2+}-ATPase	Mg^{2+}-ATPase	Ca^{2+}+ Mg^{2+} -ATPase
Luminal fluid				
0	0.144 ± 0.007	0.119 ± 0.010	0.098 ± 0.009	0.095 ± 0.008
24	0.297 ± 0.021*	0.058 ± 0.007*	0.064 ± 0.004*	0.054 ± 0.004*
48	0.366 ± 0.042*	0.058 ± 0.005*	0.064 ± 0.006*	0.055 ± 0.006*
96	0.466 ± 0.050*	0.061 ± 0.003*	0.067 ± 0.006*	0.050 ± 0.005*
Brush border				
0	2.215 ± 0.186	1.790 ± 0.142	1.996 ± 0.203	3.800 ± 0.380
24	1.710 ± 0.164*	1.119 ± 0.105*	1.193 ± 0.109*	1.062 ± 0.091*
48	0.976 ± 0.087*	1.072 ± 0.101*	1.050 ± 0.110*	0.743 ± 0.069*
96	0.680 ± 0.072*	0.880 ± 0.068*	1.008 ± 0.093*	0.670 ± 0.072*

Values are mean specific activities ± S.D. from 4-6 experiments. *$P < 0.05$

Table 2 shows the effect of fluoride on total phospholipids and sterols. Total protein content of luminal fluid increased significantly with the increasing concentrations of fluoride. Simultaneously, it declined in the brush border preparations indicating the release of some proteins either from the membrane (superficially bound) or some intracellular proteins. Significantly higher sterol contents in the luminal fluid following fluoride treatment further indicates the release of sterols from the brush border membrane. A concentration dependent decline in the sterol content of brush border membrane additionally supported the theory of membrane damage. Phospholipid contents could not be detected in luminal fluid up to 48 mM fluoride concentration. However, at higher concentration some phospholipids appeared to be dissociated from the membrane and were released into the luminal fluid. As such no significant change in the phospholipid content of brush border membrane was evident following fluoride treatment.

Table 2. **Effect of fluoride on lipid components of intestinal brush border**

Treatment (mM)	Cholesterol	Phospholipid
Luminal fluid*		
0	3.92 ± 0.31	ND
24	4.52 ± 0.54	ND
48	6.36 ± 0.51^{+}	ND
96	8.40 ± 0.64^{+}	3.60 ± 0.41
Brush border**		
0	85.14 ± 6.85	138.6 ± 11.3
24	71.22 ± 6.92	140.1 ± 12.4
48	56.88 ± 4.26^{+}	142.7 ± 13.8
96	48.74 ± 3.79^{+}	128.8 ± 11.9

Values are mean ± SD from 4-6 animals. * μg/ml; ** μg/g wet tissue; $^{+}P < 0.05$; ND = not detectable.

To elaborate the release of proteins and polypeptides from brush border membrane following fluoride treatment, an analysis of the luminal fluid was performed by electrophoresis in SDS-containing polyacrylamide gels (Fig. 1). It revealed a complex protein composition ranging from apparent molecular weight of 15,000 to 400,000 daltons. As compared to the control, additional minor protein/polypeptide bands were visible following the treatment of higher fluoride concentrations. Furthermore, a concentration-dependent increase in the intensities of various major protein bands was also evident when equal amounts of proteins were used for electrophoresis. These findings also demonstrate the release of either membrane bound or intracellular proteins

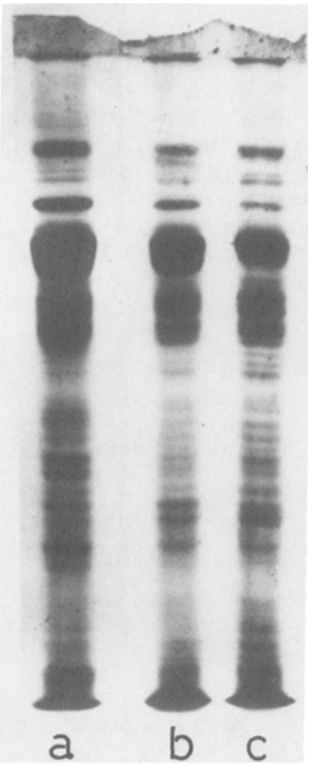

Figure 1. SDS-gel electrophoresis pattern of luminal fluid following NaF treatment (a) 96 mMNaF (b) 24 mMNaF (c) Control

with the increasing fluoride concentrations suggesting at least partial damage to the microvillus membrane.

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