Effects of sodium fluoride on water and acid secretion, soluble mucus and adherent mucus of the rat stomach

Kamel Gharzouli DEA PhD, Smain Amira Mphil, Seddik Khennouf Mphil, Akila Gharzouli DEA PhD

In addition to its widely accepted cariostatic effect and its possible preventive or therapeutic roles in osteoporosis (1,2), fluoride is harmful when taken acutely in large doses or chronically in moderate doses. Among the soft tissues, the stomach is exposed to the highest concentrations of fluoride when dental products, in concentrations of 12 to 640 mmol/L, are inadvertently swallowed, or during osteoporosis treatment (50 mg fluoride/day) (2).

Mucosal injury, ranging from erythema to mucosal erosions, of the upper gastrointestinal tract was observed in patients undergoing fluoride therapy (otosclerosis) or presenting with skeletal fluorosis as a result of high levels of fluoride in drinking water (greater than 1 mg/L) (3). Localized hemorrhagic areas of the gastric mucosa are visible after ingestion of a single dose of sodium fluoride 20 mg (53 mmol/L) by healthy volunteers (4). Rat gastric mucosa injury, induced by a single dose of sodium fluoride 10 or 50 mmol/L in hydrochloric acid 100 mmol/L, is observed microscopically within 10 mins and continues to develop for the next hour (5).

Besides its damaging effects on gastric mucosa, fluoride was shown to inhibit basal and histamine-stimulated acid secretion, but increase water secretion in the cat and rat stomach (6-8). By using the chambered dog gastric wall as a model, it has been shown that sodium fluoride induces a net...
secretion of water, sodium, potassium and mucus accompanied by a marked loss of acid from the luminal side (9). In addition, perfusion of rat stomach with sodium fluoride (pH 3.2) led to a net decrease of transmural electrical potential difference (10).

Fluoride is rapidly absorbed by the stomach as hydrofluoric acid (11). The fractional absorption is inversely related to the luminal pH and, therefore, to the concentration of hydrofluoric acid (12). The injurious effects of fluoride may result from acid accumulation in the interstitial fluid, and inhibition of a variety of enzymes (13) and membrane transport systems by ionic fluoride (14).

The present study examined the combined effects of sodium fluoride and luminal pH on water and acid output, and soluble and adherent mucus. To evaluate both kinds of mucus, fucose and galactose were used to represent the soluble fraction and mucus-bound Alcian blue to represent adherent mucus.

**ANIMALS AND METHODS**

Female Wistar rats, weighing between 200 and 250 g, were deprived of food for 48 h. They were allowed free access to water until 1 h before the experiment. During the fasting period, the animals were kept individually in cages with wide-mesh wire bottoms to prevent coprophagy. The animals were then anesthetized by an intraperitoneal injection of urethane (1.2 g/kg). The stomach was ligated at the level of the cardia and filled, through the pylorus, with a precisely measured volume (1 mL) of the test solution and immediately closed with a pyloric ligation, with care being taken to avoid main vessel supplies. The abdomen was sutured, and the animal was kept under a heating lamp. One hour later, the stomach was rapidly removed and freed from its mesenteric attachments. The stomach content was drained and completely recovered by washing with 10 mL isotonic saline. The recovered solution was weighed and centrifuged at 1300 g for 10 mins at room temperature to remove contaminating debris. Samples with more than 0.5 mL of sediment were discarded. Supernatants were used for acid and glycoprotein determinations. The stomach was then blotted dry, weighed and submitted to adherent mucus determination.

**Experimental design:** To test the effect of fluoride and acidity, animals were randomly divided into six groups (n=8 to 11). Rat stomachs were filled with one of the following test solutions: isotonic saline (sodium chloride 150 mmol/L); sodium fluoride 5 mmol/L; sodium fluoride 20 mmol/L; hydrochloric acid 50 mmol/L; sodium chloride. All of these test solutions were made isotonic by the addition of the appropriate amount of sodium chloride. The isotonic saline and sodium fluoride 50 mmol/L solutions served as control conditions.

**Adherent gastric mucus measurement:** The amount of mucus adhering to the gastric wall was measured using Alcian blue dye according to the method described by Corne et al (15). This cationic dye binds to glycoproteins and soluble mucopolysaccharides to insoluble complexes without penetrating mucosal cells. The stomach was opened along the greater curvature, and the glandular portion was excised, immersed for 2 h in 0.1% Alcian blue and dissolved in sucrose 0.16 mol/L buffered with sodium acetate 0.05 mol/L (pH adjusted to 5.8 with hydrochloric acid). The unbound dye was removed by two subsequent washings of 15 and 45 mins in sucrose 0.25 mol/L. The mucus-bound dye was eluted by tissue immersion for 2 h in magnesium chloride 0.5 mol/L. The resulting solution was shaken briefly with an equal volume of diethyl ether to dissolve particles that may interfere with the spectrophotometric determination. The optical density of the aqueous phase was then measured at 605 nm and converted to micrograms of Alcian blue per gram of wet glandular tissue by comparison with a standard curve obtained from dilution of 0.1% Alcian blue.

**Glycoprotein determination:** Glycoprotein concentration of the gastric content was estimated by galactose and fucose determination. Glycoprotein separation from the luminal washings was carried out by ethanol precipitation (16). Absolute ethanol (15 mL) was added to 5 mL of the supernatant obtained from the luminal content. The resulting suspension was kept overnight at 4°C for complete precipitation, after which the precipitate was collected by centrifugation (8700 g for 30 mins at 4°C). The pellet was washed with ethanol (5 mL) and submitted to a second centrifugation. The final pellet was then dissolved in 10 mmol/L sodium hydroxide (10 mL). Galactose and fucose concentrations were determined according to the phenol-sulphuric acid method (17) and to the technique described by Dische and Shettles (18), respectively. Galactose and fucose contents were expressed in µg/g wet tissue.

**Fluid and acid determination:** Fluid output (mL/g of wet tissue) was determined by subtracting the recovered volume from that of the filling solution. Acid was determined by titration of 0.5 mL of the sample with sodium hydroxide 10 mmol/L to pH 7 (Metrohm Titrator, Herisau, Switzerland). Acid output (µEq/g wet tissue) was calculated as the difference between the total recovered amount and that of the filling solution; negative values indicate a loss from the lumen.

**Statistical analysis:** Data are expressed as means ± SEM. The statistical significance of differences was determined by one-way ANOVA followed by Dunnett’s multiple test. The level of significance was fixed at 5%. Correlations were determined by the least squares method.

**RESULTS**

**Fluid output:** Filling the stomach with hydrochloric acid 50 mmol/L had no effect on fluid output compared with isotonic saline (P>0.05). The pooled fluid output was 0.184±0.047 mL/g (n=18). However, the luminal fluid was increased by 280% and 340% compared with controls when the stomach was exposed to sodium fluoride 5 and 20 mmol/L, respectively (P<0.05) (Figure 1). Sodium fluoride, at both concentrations, in acidified medium (50 mmol/L hydrochloric acid) also resulted in a significant increase of 380% and 256%, respectively (P<0.05) (Figure 1).
Acid output: Acid output was dependent on both medium pH and sodium fluoride concentration in the lumen (Figure 2). At low concentration of sodium fluoride (5 mmol/L), acid output did not change significantly with respect to controls, whereas sodium fluoride 20 mmol/L induced a net reduction of titratable acidity from the lumen (P<0.05). The combination of sodium fluoride, at both concentrations, with hydrochloric acid caused a marked reduction of titratable acidity in the gastric lumen (P<0.05). The observed increase of acid loss ranged from 65% to 82%.

Sugar output: Under control conditions, the total recovered amounts of galactose and fucose were 124±22 µg/g (n=8) and 32.8±1.9 µg/g (n=8), respectively. Stomach exposure to hydrochloric acid 50 mmol/L had no significant effect on galactose and fucose contents. However, sodium fluoride (5 or 20 mmol/L) in the presence or absence of hydrochloric acid induced a net increase of galactose and fucose in the luminal fluid (Figures 3,4). Galactose output variation induced by sodium fluoride in acidic medium increased from 60% to 119.7% when the concentration was brought from 5 to 20 mmol/L. In contrast, fucose output increase was less pronounced (from 40.2% to 69.8%). Similar variations were also observed in acid-free medium and indicate that the effect of sodium fluoride was pH-independent. A high correlation was observed between galactose and fucose contents whether the luminal fluid was rendered acidic or not (P<0.001; Table 1). The ratio of fucose to galactose was not changed by sodium fluoride and/or hydrochloric acid (Table 1). The pooled mean ratio was 0.291±0.029 (n=50).

Alcian blue recovery: Stomach treatment with hydrochloric acid 50 mmol/L did not alter the amount of mucus-bound Alcian blue (Figure 5). The pooled value was 156.0±9.5 µg/g (n=18). Both concentrations of sodium fluoride reduced the recovered dye. This effect was independent on both luminal pH and sodium fluoride concentration (Figure 5). Despite that sodium fluoride induced a decrease of bound-dye parallel to an increase of galactose and fucose in the lumen, there...
was no significant correlation between these two parameters (Table 1).

DISCUSSION

The results indicate that sodium fluoride 5 and 20 mmol/L in combination with hydrochloric acid 50 mmol/L induced a net increase of fluid output accompanied by a reduction of titratable acidity present in the lumen of the rat stomach. It has been shown that an intermediate concentration of sodium fluoride (10 mmol/L) was able to reduce basal and histamine-stimulated acid secretion and to induce a loss of acid after filling the stomach with hydrochloric acid 100 mmol/L (6). In normal saline, sodium fluoride 5 mmol/L did not affect acid output. Thus, it appears that the effect of sodium fluoride depends on luminal pH. In the ex vivo dog stomach, the threshold concentration of fluoride able to induce a net loss of acid is close to 1 mmol/L but in the presence of hydrochloric acid is 149 mmol/L (19). Concentrations of sodium fluoride above 5 mmol/L in hydrochloric acid 50 mmol/L (the present study) and 10 mmol/L in hydrochloric acid 140 mmol/L (19) had no further effect on acid output. The role of luminal acidity is to reduce the threshold concentration of fluoride by the formation of a greater amount of hydrofluoric acid molecules. An increase of water output has already been reported in rat (6) and dog (19). This was accompanied by an increase of sodium and potassium (8, 19).

Numerous data have accumulated to show that the rate of fluoride absorption by the stomach is rapid and inversely related to the pH of the luminal content (6, 12). Fluoride is

<table>
<thead>
<tr>
<th>Sodium fluoride 5 to 20 mmol/L without hydrochloric acid (n=18)</th>
<th>Gal versus Fuc</th>
<th>Alcian blue versus Gal</th>
<th>Alcian blue versus Fuc</th>
<th>Fluid versus Gal</th>
<th>Fluid versus Fuc</th>
<th>Fuc/Gal</th>
</tr>
</thead>
<tbody>
<tr>
<td>r=0.944</td>
<td>r=0.069</td>
<td>r=0.047</td>
<td>r=0.633</td>
<td>r=0.610</td>
<td>0.29±0.02</td>
<td></td>
</tr>
<tr>
<td>P&lt;0.001</td>
<td>P&gt;0.05</td>
<td>P&gt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium fluoride 5 to 20 mmol/L with hydrochloric acid 50 mmol/L (n=19)</td>
<td>r=0.869</td>
<td>r=0.126</td>
<td>r=0.91</td>
<td>r=0.490</td>
<td>r=0.260</td>
<td>0.28±0.02</td>
</tr>
<tr>
<td>r=0.869</td>
<td>r=0.126</td>
<td>r=0.91</td>
<td>r=0.490</td>
<td>r=0.260</td>
<td>0.28±0.02</td>
<td></td>
</tr>
<tr>
<td>P&lt;0.001</td>
<td>P&gt;0.05</td>
<td>P&gt;0.05</td>
<td>P&lt;0.05</td>
<td>P&gt;0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fuc Fucose; Gal Galactose

Table 1: Correlation between the different components in the gastric secretion and Alcian blue recovery after filling a rat stomach with acid-free or acidic sodium fluoride solutions.
thought to be passively absorbed by the epithelia in the hydrofluoric acid form (11). Fluoride with a pK 3.4 forms hydrofluoric acid easily when present in acidic medium. Therefore, when the stomach is exposed to fluoride, the concentration of hydrofluoric acid increases and establishes a chemical gradient. Hydrofluoric acid molecules are highly diffusible (20) and enter the mucosa rapidly, where they dissociate into ionic fluoride and hydrogen. The increase of water, sodium and potassium secretion may result from the damaging effect of accumulated fluoride and acid into the mucosa. The effects of fluoride on the stomach resemble those of acetylsalicylic acid (pK 3.6), which causes gross mucosal damage and increases mucus leakage (21) as a result of cytotoxic concentrations of drug anions in the mucosa (22). Gross mucosal damage induced by fluoride has been reported with elevated concentrations of fluoride and acidity. They range from intense redness of the corpus mucosa to localized hemorrhagic areas (4,9,23). Inspection of stomachs after treatment with sodium fluoride with or without hydrochloric acid did not reveal any consistent damage pattern, apart from redness of the corpus, which is frequently observed with sodium fluoride 20 mmol/L plus hydrochloric acid 50 mmol/L. The decrease in stomach blood flow induced by sodium fluoride (8,23) accounts for the redness of the stomach observed after exposure to sodium fluoride. It has been shown that the treatment of cultured porcine endothelial cells with sodium fluoride results in increased formation of endothelium-derived relaxing factor (24). The vascular stasis resulting from endothelial smooth muscle relaxation may delay the disposal of excessive intramucosal acid from the mucosa and lead to gastric damage.

Estimation of luminal mucus in gastric washings has been used extensively as an index of secretion. For example, pentagastrin, histamine and prostaglandins have been reported to increase luminal glycoproteins (25-29). However, various phenomena may induce variation in soluble mucus – a shift to increase luminal glycoproteins (25-29). However, various phenomena may induce variation in soluble mucus – a shift to increased mucus secretion and mucosal ultrafiltration (21) as a result of cytotoxic concentrations of drug anions in the mucosa (22). Gross mucosal damage induced by fluoride has been reported with elevated concentrations of fluoride and acidity. They range from intense redness of the corpus mucosa to localized hemorrhagic areas (4,9,23). Inspection of stomachs after treatment with sodium fluoride with or without hydrochloric acid did not reveal any consistent damage pattern, apart from redness of the corpus, which is frequently observed with sodium fluoride 20 mmol/L plus hydrochloric acid 50 mmol/L. The decrease in stomach blood flow induced by sodium fluoride (8,23) accounts for the redness of the stomach observed after exposure to sodium fluoride. It has been shown that the treatment of cultured porcine endothelial cells with sodium fluoride results in increased formation of endothelium-derived relaxing factor (24). The vascular stasis resulting from endothelial smooth muscle relaxation may delay the disposal of excessive intramucosal acid from the mucosa and lead to gastric damage.

Conclusions
Fluoride acts as a barrier-breaking agent, inducing ultrafiltration of fluid from the interstitium into the gastric lumen accompanied by an increased acid back-diffusion, an outpouring of glycoproteins and a reduction of adherent mucus. The rapid penetration of fluoride in the mucosa as hydrofluoric acid molecules may play an amplifying role where once fluoride has induced local vascular stasis, intramucosal acidity increases and ultimately leads to a pronounced mucosal damage. Results of the present study suggest that patients under prolonged fluoride therapy may suffer from gastric damage if luminal acidity is not lowered with inhibitors or neutralized with antacids.

Acknowledgements: The present study was supported by grants (F1901/04/95) from the Ministère de l’Enseignement Supérieur et de la Recherche Scientifique and the Agence Nationale pour le Développement de la Recherche en Santé, Algeria. The International Foundation for Science (Stockholm, Sweden) is also acknowledged for its support to Kamel Gharzouli (F/2564-1).

References

G. gharzoul.vp
Mon Jun 14 16:37:38 2000