DUODENAL MUCOSAL PROTECTION BY BICARBONATE SECRETION AND ITS MECHANISMS

Proximal portion of duodenum is exposed to intermittent pulses of gastric H+ discharged by the stomach. This review summarizes the mechanisms of duodenal mucosal integrity, mainly the role of mucus-alkaline secretion and the mucous barrier protecting surface epithelium against gastric H+. The mucous barrier protects the leaky duodenal epithelium against each pulse of gastric H+, which penetrates this barrier and diffuses into duodenocytes, but fails to damage them due to: a) an enhanced expression of cyclooxygenase-1 (COX-1), with release of protective prostaglandins (PG) and of nitric oxide (NO) synthase (NOS) with, however, production of NO, stimulating duodenal HCO₃⁻ secretion and b) the release of several neurotransmitters also stimulating HCO₃⁻ secretion such as vasoactive intestinal peptide (VIP), pituitary adenylate-cyclase activating polypeptide (PACAP), acetylcholine, melatonin, leptin and ghrelin released by enteric nerves and mucosal cells. At the apical duodenocyte membrane at least two HCO₃⁻/Cl⁻ anion exchangers operate in response to luminal H⁺ to provide adequate extrusion of HCO₃⁻ into duodenal lumen. In the basolateral portion of duodenocyte membrane, both non-electrogenic (NBC) and electrogenic (NBCₑ) Na⁺-HCO₃⁻ cotransporters are activated by the exposure to duodenal acidification, causing inward movement of HCO₃⁻ from extracellular fluid to duodenocytes. There are also at least three Na⁺/H⁺ (NHE1-3) amiloride-sensitive exchangers, eliminating H⁺ which diffused into these cells. The Helicobacter pylori (Hp) infection and gastric metaplasia in the duodenum with bacterium inoculating metaplastic mucosa and inhibiting HCO₃⁻ secretion by its endogenous inhibitor, asymmetric dimethyl arginine (ADMA), may result in duodenal ulcerogenesis.

Key words: Duodenal mucosal barrier, duodenal HCO₃⁻, prostaglandins, nitric oxide, CCK, vagal nerves, sodium-bicarbonate cotransport, H⁺/Na⁺ exchanger, HCO₃⁻/Cl⁻ exchanger
INTRODUCTION

The duodenal cluster unit consists of a group of organs, including duodenum itself, stomach, pancreas, liver and biliary tree. These organs originate embryologically from the closely related structures, whose functions are regulated, at least in part, by the duodenum. The lining of the duodenum is equipped with a variety of receptors sensitive to chemical (pH, osmolarity, and nutrients) and physical factors (pressure and contractility of duodenum) that activate neuro-hormonal mechanisms maintaining the integrity of the duodenal mucosa and also involved in the control of gastric, pancreatic and hepato-biliary functions.

This article is designed to overview the mucoso-protective and anti-ulcer mechanisms of the duodenum, which is intermittently exposed to various irritants emptied by the stomach, especially the aggressive factors such as gastric acid ($H^+$) and pepsin as well as other irritants present in the ingested food, contaminated by bacteria and their toxins or originating from the stomach or biliary tree.

Since its identification in the stomach by Prout in 1823 of $HCl$ (1), its secretory mechanisms have been the subject of extensive investigations during last century leading to discovery of such stimulants as vagal nerves by Pavlov in 1886 (2), gastrin by Edkins in 1906 (3), and histamine by Popielski in 1916 (4). With the synthesis of antagonists of $H_2$-receptors discovered by Black (5) and proton pump inhibitors by Sachs et al. (6), that were found to inhibit all forms of gastric secretion including that induced by meal, a major break-through occurred in gastric physiology (Fig. 1).

Gastric acid and pepsin as aggressive factors

Gastric acid (and pepsin), is considered as major aggressive factor against the gastro-duodenal mucosa as outlined in 1910 by German surgeon, K. Schwarz (7), who formulated famous dictum "No acid - no ulcer", implying that the presence of acid is the "condition sine qua non" of ulcer formation (see Fig. 1). It is of interest that in the same publication, Schwarz considered "the mucosal resistance" as important factor contributing to the possible ulcerogenesis. Schwarz's dictums related to pathogenesis of peptic ulcer formation was not challenged until Allen and Garner (8) and then Flemstrom and Garner (9) provided direct evidence for the ability of gastro-duodenal mucosa to respond to the action of gastric acid with an immediate and abundant secretion of $HCO_3^-$. Apparently, the secretion of $HCO_3^-$ was soon found to be an active, metabolism-dependent transport occurring along the entire gastrointestinal tract.

Duodenal alkaline secretion

As peptic ulcers in humans develop both in the stomach and duodenum, especially in its upper portion, "duodenal cup", we used dogs with canulated pouches prepared from the oxyntic and antral portions of the stomach and with
loops fashioned from the proximal and distal duodenum, a direct evidence was obtained evidence that, indeed, upper GI mucosa possesses the ability to secrete HCO₃⁻, the most effective in this respect being proximal part of the duodenum, though some less impressive alkaline secretion was also noticed in distal duodenum and in the gastric antrum and corpus (10). Another study on the in situ perfused proximal duodenum including duodenal bulb kept between two balloons in conscious dogs, showed higher HCO₃⁻ secretion than that released by the isolated (and externally denervated) proximal duodenal loop of similar length, suggesting an important role of extrinsic autonomic innervation in the maintenance of this secretion (11). Interestingly, such denervation of the proximal duodenum resulted also in the elimination of acid-induced duodeno-gastric inhibitory reflexes controlling gastric H⁺ secretion (12). Actually, the balance between protective and aggressive factors acting on the mucosa plays a decisive role in the pathogenesis of mucosal lesion or integrity (Fig. 2).

As expected from the concept of Boldyreff's (13), who first recognized at the end of 19th century the cyclic periodicity of motor and secretory gastrointestinal functions, it was found that also the duodenal alkaline secretion shows periodicity...
in phase with migrating motor complex (MMC), reaching peaks at phases II and III and nadir at phase I of this MMC (10). Feeding interrupts this periodicity and induces more uniform alkaline secretion. The rise in HCO$_3^-$ at the phase II and III has been attributed to the alkaline stimulation by increments in plasma motilin and ghrelin and activation of cholinergic neurons of enteric nervous system (ENS) as this alkaline secretion can be suppressed by atropine (11). Since at phase II/III, there is also an increase in gastric acid secretion and enhanced gastric emptying with progressive motor activity, it is possible that gastric H$^+$ discharged into the duodenum acts on luminal duodenal HCO$_3^-$ to release CO$_2$, raising its partial pressure, $pCO_2$, stimulating additionally HCO$_3^-$ secretion (11).

Vagal excitation, such as achieved with sham-feeding in dogs, was found to result in a dramatic stimulation of proximal duodenal alkaline secretion that could be partly attenuated using atropine (11), suggesting that vagal-cholinergic innervation plays an important role in the regulation of sham-feeding-induced duodenal HCO$_3^-$ secretion. Unlike vagally-induced duodenal HCO$_3^-$ secretion, that was relatively little affected by suppression of prostaglandin (PG) biosynthesis with indomethacin (11), basal and HCl- or arachidonic acid-induced duodenal alkaline secretion was found to be PG-dependent. These acidic stimulants applied on the
Duodenal mucosa caused concentration-dependent increase in the \( \text{HCO}_3^- \) as well as in mucus glycoprotein secretion (14). The increase in duodenal \( \text{HCO}_3^- \), brought about by arachidonic acid were prevented by the pre-treatment with indomethacin similarly as those induced by HCl. As mucus layer covering the epithelial surface of the duodenum is the first line of mucosal defense against chemical, predominantly acidic irritant, as well as the mechanical, bacterial or enzymatic insults, the components responsible for these protective functions appear to be highly glycosylated mucus glycoproteins. Following arachidonate application, mucus glycoproteins were significantly enriched in phospholipids and this certainly enhanced the protective qualities of the mucus gel.

Using similar to our technique of duodenal perfusion technique in humans, Isenberg and his colleagues (15) found that in normal subjects, the proximal duodenal \( \text{HCO}_3^- \) secretion reached higher values than that in distal duodenum and that it was greatly enhanced by mucosal acidification or administration of exogenous PGE\(_2\). In contrast, duodenal ulcer (DU) patients show reduced \( \text{HCO}_3^- \) response to luminal acid, despite of higher endogenous PGE\(_2\) release by proximal duodenum due to overexpression of COX-2 and this deficiency of alkaline secretion has been attributed to mucosal infection with Hp, inflammation and scarring (16). The eradication of Hp was found to increase duodenal alkaline secretion and this could be attributed to the recovery of duodenal mucosa from the inflammation induced by Hp infecting the gastric metaplastic areas in the duodenum (16). These and other studies seem to confirm that both in humans and animals, PGE\(_2\) is a potent stimulant of duodenal \( \text{HCO}_3^- \) secretion and appears to be responsible, at least in part, for basal and acid-induced alkaline secretion (12, 13, 16, 17). The mucus-alkaline secretion occurs in the mucosa of the entire gastrointestinal tract, the most extensive being that of duodenum, where mucous cells exhibit high activity of carbonic anhydrase and active exchanger \( \text{HCO}_3^- /\text{Cl}^- \) extruding \( \text{HCO}_3^- \) into the duodenal lumen (Fig. 3).

Neuro-hormonal mechanisms of alkaline secretion

In addition to PG, numerous other substances both naturally occurring or used therapeutically, have been reported to contribute to the stimulation of duodenal alkaline secretion. We reported that sucralfate and colloidal bismuth citrate (DeNol), administered in humans in peptic ulcer therapy, increased significantly duodenal \( \text{HCO}_3^- \) secretion and this has been attributed to PG-dependent stimulation, but as bismuth is known to suppress \( H. \text{pylori} \) (Hp) activity, this effect could be also explained by the eradication or, at least, suppression of this \( \text{bacterium} \) infection in these subjects though testing for Hp was not available at this time (20).

Since administration of L-nitro-arginine derivatives, such as L-NNA, was reported to inhibit gastric and duodenal alkaline secretion and these effects were reversed by the addition of L-arginine to L-NNA, endogenous nitric oxide (NO)
has been also implicated in the regulation of duodenal HCO\textsubscript{3}\textsuperscript{-} secretion (21). This is in keeping with the observation that exogenous donors of NO, such as glycerine trinitrate, or stimulants of sensory nerves releasing CGRP such as capsaicin (at lower doses) were also effective stimulants of gastro-duodenal alkaline secretion (21). This has been confirmed recently by Takeuchi et al. (22), who documented that NO, like PG, is involved in acid-induced duodenal HCO\textsubscript{3}\textsuperscript{-} secretion and could be attributed to the upregulation of constitutive NO synthase (cNOS) (Fig. 4).

Yao et al. (23) identified in in vitro preparation of rabbit duodenal mucosa the HCO\textsubscript{3}\textsuperscript{-} secretory pathways related to vasoactive intestinal peptide (VIP) and cyclic AMP. Glad et al. (24) confirmed in anesthetized pigs that VIP and its chemical analog, pituitary adenylate cyclase-activating polypeptide (PACAP), stimulate duodenal as well as hepatobiliary HCO\textsubscript{3}\textsuperscript{-} secretion. Sjoblom and Flemstrom (25) reported recently that melatonin, a neurotransmitter released by neurons of the enteric nervous system of the gut, is also an effective stimulant of duodenal alkaline secretion in anesthetized rats and suggested that this indole is involved in H\textsuperscript{+}-induced alkaline secretion acting via MT\textsubscript{2} receptors. Furthermore, sensory nerves in the duodenal...
mucosa, activated by acid or small doses of topical capsaicin, have been proposed to be involved in the stimulation of alkaline secretion ether by axon-reflex with the release of sensory neuropeptides such CGRP that in turn activates the release and action of NO (21). The overall mechanisms operating in the duodenum in response to topical application of H⁺ involve COX-PG, NOS-NO and sensory nerves-CGRP-NO systems. Furthermore, novel hormones such as PYY, ghrelin, melatonin VIP, orexins or CCK have been proposed to contribute to duodenal HCO₃⁻ secretin (Fig. 4).

Quantitatively, the amounts of HCO₃⁻ secreted by canine proximal duodenum are only a small portion of the amounts of maximally stimulated gastric H⁺ secreted in the stomach (11). Jarbur et al. (26) who quatitated gastric acid and duodenal alkaline secretion in fasting humans reported that the amounts of HCO₃⁻ produced in the duodenum under basal conditions were similar to duodenal loads of gastric H⁺ and this production may be mediated, at least in part, by increased pCO₂ generated in the duodenal lumen from the HCO₃⁻ hydrolysis by gastric H⁺ during phase III of MMC (see Fig. 4).

Mucus-alkaline secretion and formation of protective mucosal barrier in the stomach differ from those in the duodenum. In the stomach, gastric mucosa is covered by tight epithelial cells with continuous surface mucus layer to which HCO₃⁻ is secreted by goblet cells, creating the pH gradient across mucus layer that neutralizes any H⁺ diffusing from the gastric lumen towards the surface epithelial cells and thus preventing their acidification and damage (Fig. 5). The total amount of HCO₃⁻ secreted by the gastric mucosa is relatively small when

Fig. 4. Involvement of various factors activated by duodenal acid in the mechanisms of HCO₃⁻ secretion in duodenal mucosa.
compared to maximal gastric H⁺ secretion, but due to the fact that HCO₃⁻ is secreted into the thin layer of adherent mucus gel, this HCO₃⁻ secretion is highly effective in neutralizing the penetrating luminal H⁺. By contrast the duodenum is covered, however, by a leaky epithelium and has thicker mucus gel with more abundant secretion of HCO₃⁻, which is thought to be the primary defence mechanism against gastric H⁺ discharged to the duodenum. Although, the duodenum is supplied with large amounts of HCO₃⁻ originating from the pancreas and biliary tree and secreted in response to duodenal acidification due to release of secretin, the major "chemical battlefield" with gastric H⁺ entering the duodenum is the duodenal bulb. The bulb is located just distal to gastric antrum and proximal to the pancreatично-biliary ducts and uniquely exposed to a highly variable pH environment due to peristaltically conveyed pulses of concentrated gastric H⁺ discharged by the stomach to duodenum. Since the duodenum does not have the inherent acid protective structural properties of the stomach with intercellular tight junctions, it evolved very efficient means for defence against gastric H⁺. The proximal duodenum could be compared to the titration chamber with gastric H⁺ neutralized mostly by the HCO₃⁻ originating from the bulbar
mucosa and secreted due to local action on duodenocytes of numerous mediators including already mentioned PG, NO, Ach, melatonin, VIP, PACAP and probably also motilin-like peptide, ghrelin PYY leptin, as well as CCK, all secreted by intestinal mucosal cells and released into the duodenal lumen (see Fig. 4).

Duodenocyte membrane transport systems

It should be emphasized that HCO₃⁻ does not increase immediately upon the start of acid perfusion of duodenal bulb but rather somewhat later after acid challenge when H⁺ ions already diffused into duodenocytes and reduce their intracellular pH (pHᵢ). To understand the sequence of events following duodenal acid challenge, it is necessary to identify the apical and basolateral transport mechanisms in duodenocytes. It has been proposed (26, 27), the apical duodenocyte membrane is equipped with active exchangers HCO₃⁻/Cl⁻, extruding HCO₃⁻ to duodenal lumen in exchange for Cl⁻ (AE - anion exchanger) and several types of Na⁺/H⁺ exchangers (NHE1-3) eliminating the H⁺ from the duodenocytes back to duodenal lumen (Fig. 6). The AE has been related to cystic fibrosis transmembrane conductance regulator (CFTR) protein and found to be expressed predominantly in a pical membrane of duodenocytes in duodenal crypts (28, 29). At the baso-lateral duodenocyte membrane both electroneutral Na⁺-HCO₃⁻ cotransport (NBC₁) and electrogenic cotransport NBC (NBCₙ₁) as well as Na⁺/H⁺ exchanger (NHE) are

![Fig. 6. Duodenocytes are equipped with numerous transporters at the apical and basolateral membrane.](image-url)
present (30). Kaunitz and Akiba (31) summarizing the protective duodenal response to gastric acid challenge in humans pointed out that luminal \( H^+ \) diffusing into the duodenocytes, lowers pH, and initially decreases the buffer power of these cells but subsequently decrease \( HCO_3^- \) secretion due to the decrease of CFTR conductance. Lowering pH, immediately increases, however, the activity of basolateral NBC\(_1\) and promotes an inward movement of \( HCO_3^- \) from extracellular fluid to increase the cellular content of \( HCO_3^- \) and its secretion to duodenal lumen through CFTR-related \( HCO_3^-/Cl^- \) exchanger. Furthermore, acidic intracellular pH increases the activity of basolateral NHE and enhances an extrusion of \( H^+ \) into the submucosal space with increase of cellular alkali load. Luminal \( H^+ \) in the duodenum also increases the mucus secretion from goblet cells, resulting in the increase of the thickness of mucus gel. This increase in mucus secretion is accompanied by an enhancement of mucosal blood flow mediated by the \( H^+ \) stimulation of the sensory nerves releasing CGRP and NO causing vasodilation in the mucosa. \( H^+ \) in duodenum may also release ghrelin and leptin into duodenal lumen from the surface epithelial cells and these peptides, similarly to CCK already reported to stimulate duodenal \( HCO_3^- \) should also be considered as candidate stimulants of duodenal alkaline secretion of physiological importance.

Fig. 7. Hipotetical events leading to duodenal ulcerogenesis related to \( H. pylori \) infection, increased acid load, duodenitis and ADMA production.
Duodenal ulcer patients may not only exhibit excessive gastric H\(^+\) secretion and accelerated gastric emptying of H\(^+\), thus enhancing duodenal acidification and mucosa damage, but also produce a potent endogenous asymmetric dimethylated arginine derivative (ADMA) that reduces duodenal HCO\(_3^-\) response to gastric H\(^+\) and thus favours the damage of duodenal mucosa and ulcer formation in these patients (32) (Fig. 7).

**CONCLUDING REMARKS**

This overview provides an insight into how duodenal mucosa defends itself against the damage by pulses of concentrated gastric H\(^+\) entering the duodenum. Unlike in the stomach, where mucosal barrier with tight epithelial cells and covering mucus layer actually prevent luminal H\(^+\) from entering and damaging the surface epithelium, in the *duodenum* the epithelial cells are leaky permitting luminal H\(^+\) to diffuse into the duodenocytes and acidify their interior with subsequent activation of the baso-lateral Na\(^+-\)HCO\(_3^-\) cotransporter, leading to alkali load in these cells and excessive HCO\(_3^-\) secretion due to activation of apical membrane HCO\(_3^-\)/Cl\(^-\) exchangers. Acidic pH of duodenocytes also activates basolateral Na\(^+\)/H\(^+\) exchangers causing extrusion of H\(^+\) from duodenocytes and acidification of submucosal tissue with stimulation of capsaicin receptors on afferent nerves leading to increased mucosal blood flow and increased thickness of mucus-gel layer enhancing duodenal protective activity. In certain conditions such as Hp infection, the inflammatory changes in the mucosa result in decrease in mucus-alkaline secretion, partly due to ADMA effect and mucosal resistance to acid injury leading to the formation of peptic ulcerations. Eradication of Hp restores the ability of duodenal mucosa to produce HCO\(_3^-\) due to reduction in ADMA formation and the restoration of the integrity of duodenal mucosa.

**REFERENCES**


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