

# Lafutidine, a Protective H<sub>2</sub> Receptor Antagonist, Enhances Mucosal Defense in Rat Esophagus

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## Abstract

**Background** Luminal acid or CO<sub>2</sub> induces a hyperemic response in the esophagus, via activation of acid sensors on capsaicin-sensitive afferent nerves (CSAN). Since disruption of the hyperemic response to luminal CO<sub>2</sub> acidifies the interstitium of the esophageal mucosa, the hyperemic response may maintain interstitial pH (pH<sub>int</sub>). We hypothesized that acid-related hyperemia maintains pH<sub>int</sub>, preventing acid-induced injury in the esophageal mucosa.

**Methods** We examined the effects of capsaicin (Cap) or lafutidine (Laf), a mucosal protective H<sub>2</sub> antagonist, on the regulation of pH<sub>int</sub> and blood flow in rat esophagus using ratiometric microimaging and laser-Doppler measurements of the lower esophageal mucosa of living rats. The esophagus was topically superfused with pH 7.0 buffer, or a pH 1.0 or pH 1.0 + pepsin (1 mg/ml) solution with or without Laf.

**Results** Cap (30 or 100 μM) or Laf (0.1 or 1 mM) dose-dependently increased blood flow, accompanied by increased pH<sub>int</sub>. The pH 1.0 solution increased blood flow without pH<sub>int</sub> change, whereas Laf (1 mM) increased blood flow and pH<sub>int</sub> during acid exposure. The effects of Laf were abolished by ablation of CSAN. Perfusion of the

acidified pepsin solution gradually decreased pH<sub>int</sub>, inhibited by Laf perfusion.

**Conclusions** Activation of CSAN by Laf with or without acid, accompanied by hyperemia, increased pH<sub>int</sub>, preventing acidified pepsin-induced interstitial acidification. Stimulation of the capsaicin pathway with compounds such as Laf enhances mucosal protection from acid-related injury in the upper gastrointestinal tract.

**Keywords** Esophageal blood flow · Interstitial pH · Capsaicin-sensitive afferent nerves · Pepsin · Interstitial acidification

## Introduction

The upper gastrointestinal mucosa responds to luminal acid by increasing HCO<sub>3</sub><sup>-</sup> and mucus secretion, increasing mucosal blood flow, and by increasing epithelial cellular buffering in order to protect the mucosa from acid-induced injury. These mucosal defense mechanisms appear to be coordinated by the activation of submucosal acid sensors [1, 2]. The duodenal mucosa is protected from injury due to luminal acid by the ‘capsaicin pathway.’ In the duodenum, the large luminal acid load originating from the stomach is converted to the H<sup>+</sup> equivalent CO<sub>2</sub> at the cell surface prior to absorption by the duodenal mucosa. CO<sub>2</sub> at the enterocyte surface is absorbed into the enterocyte, facilitated by ecto-carbonic anhydrases (CAs), followed by reconversion to H<sup>+</sup> by cellular CA and transport across the enterocyte basolateral membrane into the subepithelial interstitium [1, 3]. Subepithelial H<sup>+</sup> interacts with submucosal acid sensors such as transient receptor potential vanilloid 1 (TRPV1) expressed on submucosal capsaicin-sensitive afferent nerves (CSAN), releasing the vasoactive compounds

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such as calcitonin gene-related peptide (CGRP) and nitric oxide (NO), increasing mucosal blood flow [3, 4]. In contrast to the “leaky” duodenal mucosa, the esophageal mucosa, with tight intercellular junctions, responds to luminal acid with hyperemia and increased pre-epithelial layer gel thickness, but with no change of interstitial pH ( $\text{pH}_{\text{int}}$ ) [5]. Nevertheless,  $\text{CO}_2$ , rather than  $\text{H}^+$ , traverses the epithelial layers, interacting with epithelial and neuronal CAs and acid sensors, inducing the hyperemic response via the activation of CSAN [6]. The esophageal mucosa thus shares parts of the capsaicin pathway with the duodenum as a mucosal protective mechanism from injury due to refluxed gastric acid.

The hyperemic response to luminal acid is a well-studied defense mechanism in the esophagus [7–9]. Hyperemia is believed to enhance the supply of  $\text{HCO}_3^-$  to the mucosa and the removal of  $\text{H}^+$  and  $\text{CO}_2$  from the mucosa during acid exposure.  $\text{HCO}_3^-$  delivery is an important esophageal mucosal protective factor [10]. Luminal perfusion of a high  $\text{CO}_2$  solution or of a pH 1.0 acid solution increases esophageal blood flow without affecting  $\text{pH}_{\text{int}}$  [6]. Disruption of  $\text{CO}_2$ -induced hyperemia by CA inhibition or acid sensor inhibition irreversibly lowers  $\text{pH}_{\text{int}}$ , implicated in mucosal injury and the generation of noxious sensations [6]. These results suggest that the hyperemic response to luminal acid or high  $\text{CO}_2$  maintains  $\text{pH}_{\text{int}}$  in the esophageal mucosa during acid exposure. We thus hypothesized that induction of hyperemia in the esophagus may increase  $\text{pH}_{\text{int}}$ , augmenting mucosal resistance to acid-induced injury. Since the hyperemic response to luminal acid is mediated via the capsaicin pathway, stimulation of acid sensors expressed on CSAN and may also protect the mucosa from injury.

Lafutidine (Laf), a histamine  $\text{H}_2$  receptor antagonist, has a mucosal protective effect in addition to its antisecretory properties via indirect stimulation or sensitization of CSAN [11–15]. Laf is protective against experimental gastric lesions even in the presence of supplementary potent antisecretory therapy, suggesting that it has a direct gastro-protective effect in addition to its antisecretory properties [15]. Laf reduces esophageal injury in the gastric acid reflux model, apart from its antisecretory effect [13]. This protective effect is abolished by selectively ablating CSAN, antagonism of CGRP and inhibition of NO synthase [16, 17], suggesting that the capsaicin pathway is integral to Laf’s protective properties. We further hypothesized that Laf enhances mucosal defenses by activating or sensitizing mucosal acid-sensing mechanisms in rat esophagus.

We thus examined the effect of luminal superfusion of capsaicin or Laf on  $\text{pH}_{\text{int}}$  and blood flow in rat esophagus. Furthermore, we examined the effect of Laf on acidified pepsin-induced changes in  $\text{pH}_{\text{int}}$  and blood flow, a surrogate for esophageal injury. Our results showed that Laf-induced

hyperemia increased  $\text{pH}_{\text{int}}$  and prevented interstitial acidification due to acidified pepsin via the activation of CSAN in rat esophagus.

## Methods

### Chemicals and Animals

Laf was provided by Taiho Pharmaceutical Inc (Tokyo, Japan). Laf, ( $\pm$ )-2-(furfurylsulfinyl)-*N*-[4-[4-(piperidino-methyl)-2-pyridyl]oxy-(*Z*)-2-butenyl]acetamide (431.54 g/mol), is relatively lipophilic ( $\log p = 0.39$ ). 5',6'-carboxyfluorescein (CF) was obtained from Molecular Probes (Eugene, OR, USA). Capsaicin (Cap), porcine pepsin A, HEPES, and other chemicals were obtained from Sigma Chemical (St. Louis, MO, USA). Krebs solution contained (in mM) 136 NaCl, 2.6 KCl, 1.8  $\text{CaCl}_2$ , and 10 HEPES at pH 7.0. The pH 1.0 saline solution was made from 1 N HCl with adding NaCl to adjust isotonicity. Each solution was prewarmed to 37°C using a water bath, and temperature was maintained with a heating pad during the experiment. For stock solutions, capsaicin was dissolved in 10% Tween<sup>®</sup>-80, 10% EtOH, and 80% saline. The solution was diluted with Krebs buffer in order to ensure that final concentrations of Tween<sup>®</sup>-80, and EtOH were less than 0.1%. For vehicle perfusion, Krebs solution with 0.1% solvents was used. Laf was initially dissolved in 1 N HCl and pH was adjusted to pH 7 with the addition of 1 N NaOH. Stock solution of Laf (50 mM) was diluted with pH 7.0 Krebs buffer before use.

All studies were performed with approval of the Veterans Affairs Institutional Animal Care and Use Committee (VA IACUC). Male Sprague–Dawley rats weighing 200–250 g (Harlan, San Diego, CA, USA) were fasted overnight, but had free access to water.

### Measurement of Blood Flow and Interstitial pH ( $\text{pH}_{\text{int}}$ )

Esophageal blood flow and  $\text{pH}_{\text{int}}$  were simultaneously measured in the lower esophagus as previously described [5, 6]. In brief, under isoflurane anesthesia (1.5–2.0%) using a rodent anesthesia inhalation system (Summit Medical Systems, Bend, OR, USA), rats were placed supine on a heating block system warmed with recirculating water (Summit Medical) to maintain body temperature at 36–37°C, as monitored by a rectal thermistor. Prewarmed saline was infused via the right femoral vein at 1.08 ml/h using a Harvard infusion pump (Harvard Apparatus, Holliston MA, USA); blood pressure was monitored via a catheter placed in the left femoral artery using a pressure transducer (Kent Scientific, Torrington, CT, USA). The lower esophageal mucosa was exposed and a concave stainless-steel disk (16 mm in diameter and 1–2 mm deep) with a 3-mm central

aperture was fixed watertight on the mucosal surface with a silicone plastic adherent (Silly Putty, Binney & Smith, Easton, PA, USA). The serosal surface of the esophagus was supported with a right-angle laser-Doppler flow probe (R-type, Transonic, Ithaca, NY, USA) just below the chambered mucosa. A thin plastic coverslip was fixed to the disk with the silicone adherent to permit closed superfusion with solutions at a rate of 0.25 ml/min by means of a Harvard infusion pump. Two PE-50 polyethylene perfusion lines were inserted into the chamber to enable rapid changes of the perfusate.

Esophageal blood flow was measured as the voltage output of the laser-Doppler instrument (model BLF21, Transonic) and expressed relative to the stable level (the basal level) ~30 min after the perfusion was started. Blood flow was continuously recorded with a strip chart recorder and read every 5 min.

To measure  $\text{pH}_{\text{int}}$ , the pH-sensitive, fluorescent indicator CF (5 mg/kg) in saline was intravenously injected 5 min before the start of the experiment. Fluorescence of the microscopically observed chambered area of esophageal mucosa at 515-nm emission was recorded. Readings were taken approximately 10 s before and after each measured time point. The paired readings at 495- and 450-nm excitation needed to calculate a fluorescence ratio were thus taken at a maximum of 20 s apart. The paired images were captured every 5 min and analyzed by selecting three areas of esophageal submucosa between microvessels, which were followed throughout the experiment. In vitro calibration was accomplished using an aqueous solution containing 0.2  $\mu\text{M}$  CF. In vitro calibration in solution and in excised esophageal tissue revealed that the measurable pH range using CF is between pH 6.0 and 7.5, as previously reported [5]. We have confirmed that despite a gradual decrease in the fluorescent intensity over time, the fluorescence ratio was stable during the 60-min experimental period [5, 6]. We have also shown that there is a good correlation between the measured  $\text{pH}_{\text{int}}$  and arterial blood pH [6], since in vivo calibration is technically difficult.

#### Experimental Protocol

The exposed esophageal mucosa was superfused with solutions via a mucosally placed perfusion chamber. Blood flow was stabilized during continuous perfusion of pH 7.0 Krebs buffer, after which the time was set as  $t = 0$  min. At  $t = -5$  min, CF was injected IV. The esophageal mucosa was then superfused with pH 7.0 Krebs buffer from  $t = 0$  until  $t = 10$  min (basal period); the perfusate was then changed to pH 7.0 Krebs containing vehicle, Cap (30 or 100  $\mu\text{M}$ ), or Laf (0.1 or 1 mM) from  $t = 10$  until  $t = 20$  min (challenge period). The perfusate was changed to the pH 7.0 solution from  $t = 20$  until  $t = 35$  min (recovery period). For acid-perfusion experiments, the

mucosa was superfused with a pH 1.0 acid solution with or without pepsin (1 mg/ml) or Laf (1 mM) from  $t = 10$  until  $t = 45$  min (challenge period).

Ablation of CSAN was accomplished with high-dose capsaicin pre-treatment (125 mg/kg sc) as described previously [18]. Capsaicin-treated or vehicle (10% Tween<sup>®</sup>-80, 10% EtOH, and 80% saline)-treated rats were studied 10–14 days after the injections. Completeness of de-afferentation was assessed by the 0.1%  $\text{NH}_4\text{OH}$  eye drop test. The esophageal mucosa of capsaicin-treated or vehicle-treated rat was superfused with an acid solution and Laf (1 mM) as described above.

#### Statistics

All data from six rats in each group were expressed as means  $\pm$  SEM. Comparisons between groups were made by one-way ANOVA followed by Fisher's least significant difference test.  $p$  value less than 0.05 were taken as significant.

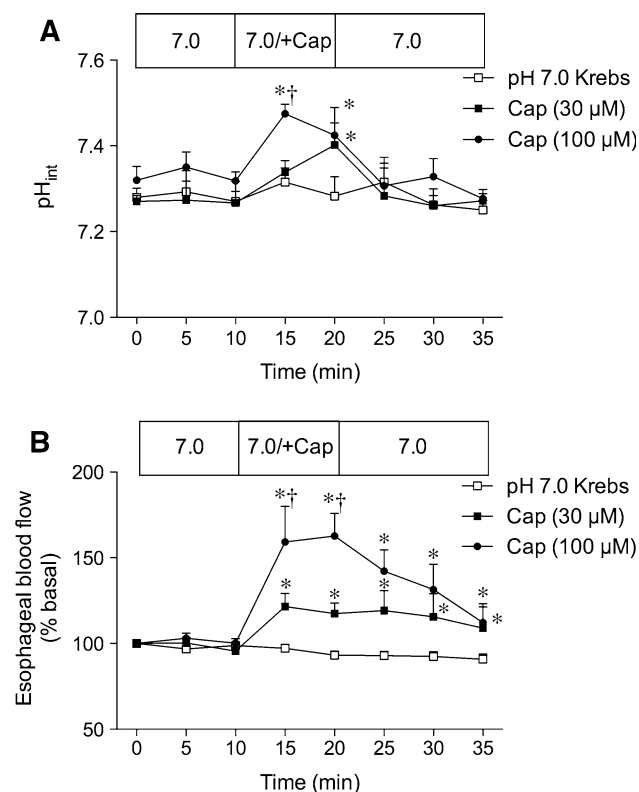
## Results

#### Effect of Luminal Perfusion of Cap or Laf on Esophageal $\text{pH}_{\text{int}}$ and Blood Flow

We first examined the effects of the TRPV1 agonist Cap on  $\text{pH}_{\text{int}}$  and mucosal blood flow. Cap (30 or 100  $\mu\text{M}$ ) increased esophageal blood flow (Fig. 1b), consistent with prior studies [7], accompanied by the interstitial alkalinization (Fig. 1a). This result correlates the induction of hyperemia with increased  $\text{pH}_{\text{int}}$ . Similarly, Laf (0.1 or 1 mM) increased blood flow, accompanied by a  $\text{pH}_{\text{int}}$  increase (Fig. 2a, b). The effects of Laf were abolished by prior de-afferentation as a result of high-dose capsaicin pretreatment (Cap-t) (Fig. 3a, b), suggesting that Laf-induced hyperemia is mediated by the activation of CSAN.

#### Effect of Laf on Acid-Induced Changes in Esophageal $\text{pH}_{\text{int}}$ and Blood Flow

Since Laf increased  $\text{pH}_{\text{int}}$  and blood flow via the activation of CSAN, we hypothesized that Laf enhances mucosal protection during acid exposure in the esophagus. We perfused Laf during the basal and also during the challenge periods in order to maximize its effects on mucosal defenses. A pH 1.0 solution had no effect on  $\text{pH}_{\text{int}}$ , but increased blood flow as previously reported [5, 6] (Fig. 4a, b). Laf (1 mM) pretreatment increased  $\text{pH}_{\text{int}}$  during coperfusion with a neutral solution. Subsequent coperfusion of Laf with a pH 1.0 solution increased  $\text{pH}_{\text{int}}$  more than the  $\text{pH}_{\text{int}}$  in the pH 1.0 group during the acid challenge period (Fig. 4a). In

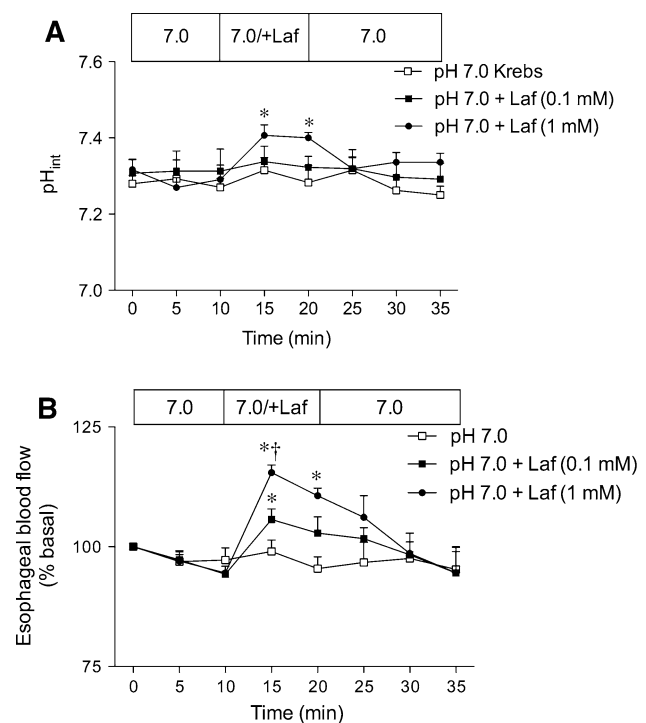


**Fig. 1** Effect of luminal capsaicin (Cap) on interstitial pH ( $pH_{int}$ ) and blood flow in rat esophagus.  $pH_{int}$  (a) and blood flow (b) were monitored during the luminal perfusion of Cap (30 and 100  $\mu$ M) in rat esophagus under in vivo microscopy. Cap increased the blood flow, accompanied by  $pH_{int}$  increase. Data are expressed as mean  $\pm$  SEM ( $n = 6$ ). \*  $p < 0.05$  versus pH 7.0 Krebs group, †  $p < 0.05$  versus Cap (30  $\mu$ M) group

the Laf group, increased  $pH_{int}$  was accompanied by increased blood flow during the basal period, followed by an enhanced hyperemic response to acid perfusion compared to the pH 1.0 group (Fig. 4b). Cap-t abolished the Laf-induced  $pH_{int}$  increase during the basal and acid challenge periods, and further acidified  $pH_{int}$  during the acid challenge period (Fig. 4a). This result is consistent with a previous report showing that Cap-t irreversibly lowers  $pH_{int}$  during luminal perfusion of a high  $CO_2$  solution, consistent with mucosal injury [6]. Furthermore, Cap-t abolished the hyperemic response to acid + Laf (Fig. 4b). Cap-t also abolished the acid-induced hyperemia associated with interstitial acidification (data not shown). These results suggest that luminal acid stimulates CSAN, and that Laf enhances the hyperemic response to acid via CSAN activation or modulation, with a resultant increase of  $pH_{int}$ .

#### Effect of Laf on Acidified Pepsin-Induced Changes in Esophageal $pH_{int}$ and Blood Flow

We further hypothesized that Laf protects the mucosa from the interstitial acidification accompanying epithelial injury

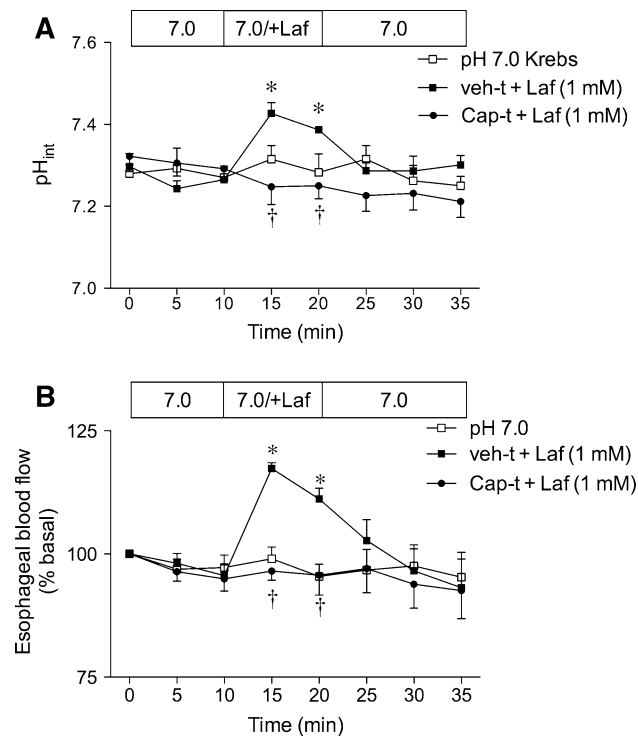


**Fig. 2** Effect of luminal lafutidine (Laf) on  $pH_{int}$  and blood flow. Perfusion of Laf increased esophageal blood flow (b), accompanied by  $pH_{int}$  increase (a). Data are expressed as mean  $\pm$  SEM ( $n = 6$ ). \*  $p < 0.05$  versus pH 7.0 Krebs group, †  $p < 0.05$  versus pH 7.0 + Laf (0.1 mM) group

or increased permeation of acid into the mucosa. We thus examined the effect of acidified pepsin on  $pH_{int}$  and blood flow, since acidified pepsin reduces electrical resistance of rabbit esophagus in vitro and may thus also injure the rat esophagus in vivo [19]. Pepsin (1 mg/ml) was added to the pH 1.0 perfusate in order to compromise the mucosal barrier. Acidified pepsin gradually, but markedly, decreased  $pH_{int}$  (Fig. 5a), consistent with increased  $H^+$  diffusion into the mucosa. This  $pH_{int}$  change was reversed by Laf (1 mM) pre- and co-perfusion, consistent with mucosal protection (Fig. 5a). Perfusion of the acidified pepsin solution transiently increased blood flow as did the acid alone, followed by a second hyperemic response observed at  $t = 40$ – $45$  min (Fig. 5b), associated with a markedly decreased  $pH_{int}$  (Fig. 5a). Laf increased blood flow before and after acidified pepsin superfusion, but prevented the secondary hyperemia observed in acid + pepsin group. These results suggest that Laf treatment prevents the acidified pepsin-induced mucosal injury in the esophagus by protective hyperemia and  $pH_{int}$  increase.

#### Discussion

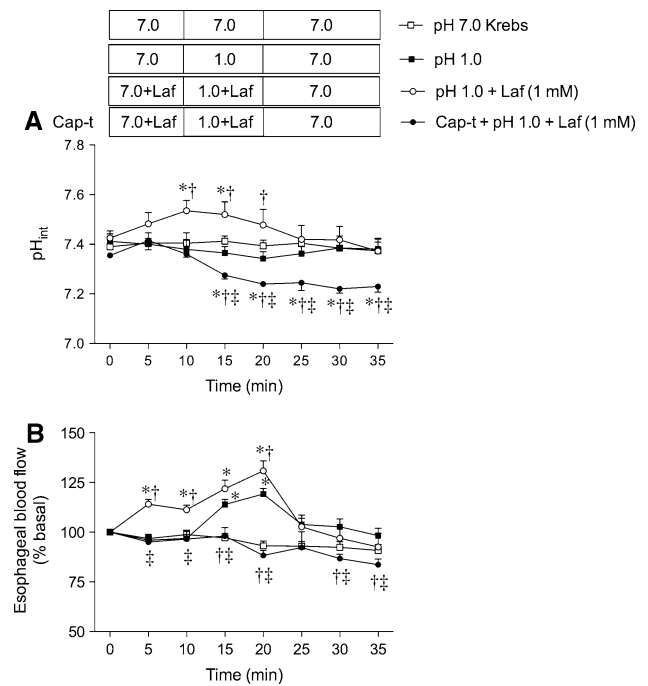
We demonstrated that the induction of hyperemia with luminal Cap or Laf increased esophageal blood flow,



**Fig. 3** Effect of capsaicin pretreatment (Cap-t) on Laf-induced changes in  $pH_{int}$  and blood flow in rat esophagus. Animals were pretreated with vehicle (veh-t) or high-dose capsaicin 10–14 days before the experiments. Cap-t abolished the Laf-induced  $pH_{int}$  increase (a) and hyperemia (b). Data are expressed as mean  $\pm$  SEM ( $n = 6$ ). \*  $p < 0.05$  versus pH 7.0 Krebs group,  $^\dagger p < 0.05$  versus veh-t + Laf group

accompanied by interstitial alkalinization in rat esophagus. Laf enhanced acid-induced hyperemia with increased  $pH_{int}$  via the activation of CSAN, and prevented acidified pepsin-induced interstitial acidification. This is the first study to demonstrate that hyperemia protects the esophageal mucosa due to increased  $pH_{int}$  and that Laf locally enhances esophageal mucosal defense mechanisms, apart from its acid antisecretory effect as an  $H_2$  receptor antagonist.

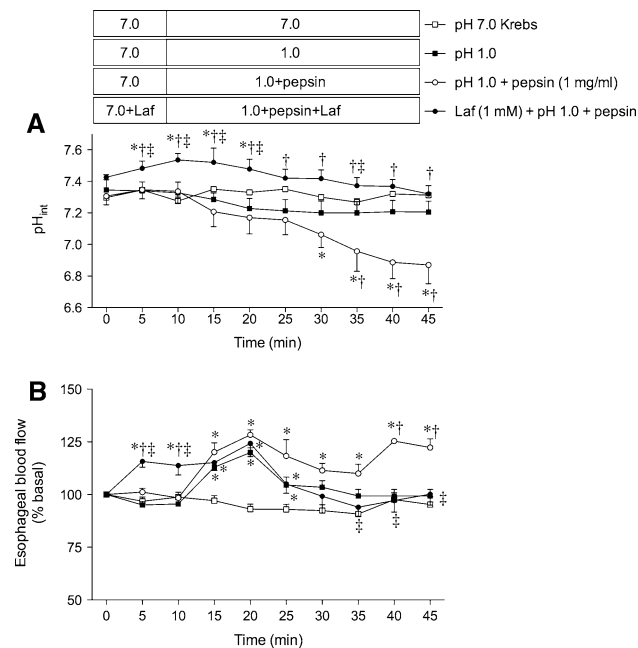
The esophageal mucosa responds to luminal acid by augmenting its intrinsic defense mechanisms such as by increasing mucosal blood flow [5, 7]. Inhibition of the hyperemic response during exposure to high luminal  $pCO_2$  lowers  $pH_{int}$  [6], suggesting that reflex hyperemia maintains  $pH_{int}$  homeostasis. Impairment of reflex hyperemia lowers  $pH_{int}$ , presumably by impairing the ability of the enhanced mucosal blood flow to remove acid and  $CO_2$ , and to deliver  $HCO_3^-$ . In multiple tissues, interstitial acidification is sensed by neuronal chemosensors, producing noxious sensations [20–22]. Thus, a clinical equivalent to the tissue acidification observed in the acid + pepsin group would likely be perceived as heartburn or a similar painful sensation. Furthermore, prolonged or marked acidification



**Fig. 4** Effect of Laf with acid on  $pH_{int}$  and blood flow in rat esophagus. A pH 1.0 solution was perfused with or without Laf (1 mM). Laf was perfused during the basal and challenge periods (0–20 min) in the pH 1.0 + Laf and in the Cap-t (capsaicin pretreated) + pH 1.0 + Laf groups. The boxes above the graphs represent the perfusate combination for the corresponding groups. Pretreatment with Laf increased  $pH_{int}$  (a) and blood flow (b), and enhanced acid-induced hyperemia. Cap-t abolished the effects of Laf perfusion and further decreased  $pH_{int}$ . Data are expressed as mean  $\pm$  SEM ( $n = 6$ ). \*  $p < 0.05$  versus pH 7.0 Krebs group,  $^\dagger p < 0.05$  versus pH 1.0 group,  $^\ddagger p < 0.05$  versus pH 1.0 + Laf group

may eventually injure the affected tissue. Induction of hyperemia via the activation of CSAN increased  $pH_{int}$ , preventing acidified pepsin-induced interstitial acidification, supporting our hypothesis that the hyperemic response maintains  $pH_{int}$ , presumably preventing acid-related injury of the esophageal mucosa.

Activation of the capsaicin pathway can be accomplished by luminal acid perfusion or by TRPV1 agonists such as capsaicin. Capsaicin, which is the pungent component of hot peppers, is perceived as a burning or hot sensation, which limits its use as a therapeutic agent. The precise mechanism by which Laf exerts its effects is unknown; nevertheless, denervation of CSAN abrogates the protective effects of Laf, suggesting involvement of the capsaicin pathway. In vitro studies have failed to document a direct interaction between Laf and TRPV1 [12, 14], whereas Laf potentiates Cap-induced CGRP release from the gastric mucosa [14], suggesting that Laf sensitizes TRPV1 or interacts at alternate components of the capsaicin pathway. Our results demonstrated that luminal Laf perfusion increased blood flow in the absence of perfused luminal acid and that Laf enhanced acid-induced



**Fig. 5** Effect of Laf with acid-pepsin superfusion on  $\text{pH}_{\text{int}}$  and blood flow in rat esophagus. Pepsin (1 mg/ml) with pH 1.0 acid was perfused with or without Laf (1 mM). Laf was perfused during the basal and challenge periods (0–45 min) in the Laf + pH 1.0 + pepsin group. The boxes above the graphs represent the perfusate combination for the corresponding groups. The acid-pepsin solution gradually decreased  $\text{pH}_{\text{int}}$  (a) accompanied by biphasic hyperemia (b). Laf pre-perfusion increased  $\text{pH}_{\text{int}}$  and blood flow, and prevented acid-pepsin-induced interstitial acidification (a). Data are expressed as mean  $\pm$  SEM ( $n = 6$ ). \*  $p < 0.05$  versus pH 7.0 Krebs group, †  $p < 0.05$  versus pH 1.0 group, ‡  $p < 0.05$  versus pH 1.0 + pepsin group

hyperemia, both mediated by CSAN, supporting our hypothesis that Laf sensitizes TRPV1 or submucosal afferent nerves. Since Laf ingestion, perhaps due to its indirect interaction with TRPV1, does not provoke a burning sensation, it might prove useful in the therapy of heartburn, acid-induced esophageal injury, and related disorders.

Another finding is that the acute, local application of Laf enhanced mucosal defenses in the esophagus, regardless of its anti-acid secretory effect, since the perfused segment was isolated from gastric acid. Luminal Laf increases mucosal blood flow, mediated by CSAN, in rat stomach [11], similar to our results. Intragastric application of Laf prevents acid reflux-induced esophageal injury via CSAN in pylorus/forestomach-ligated rats [13], also consistent with our results.

We observed a biphasic hyperemic response during acidified pepsin superfusion. The initial hyperemia is consistent with a direct acid effect, whereas the secondary hyperemia might be due to mucosal injury resulting from the profound interstitial acidification resulting from the increased acid permeability induced by acidified pepsin.

Sustained or repeated superfusion of the rabbit esophageal mucosa over a period of days was necessary to injure the esophagus in one study [23]. In another study, a 90-min esophageal perfusion with a pH 1.4 solution + 0.5% pepsin produced only edema or hyperemia in 8/9 rats [24]. Thus, macroscopic or measurable histologic injury in our acute topical superfusion model may be difficult to produce. Nevertheless, in the rat duodenum and many other organs, irreversible cellular acidification or tissue acidosis usually precedes or is the cause of tissue injury [25].

Laf was topically perfused over the esophageal mucosa in all of the experiments in which it was used, consistent with a direct effect on the mucosa, in contradistinction to the pharmacology of clinically used oral  $\text{H}_2$  receptor antagonists, which must be absorbed from the intestine and transported by the circulation to the gastric mucosa. Since Laf is soluble at strongly acidic pH, Laf in gastric juice may reflux into the esophagus and then protect the esophageal mucosa in humans with acid reflux. Since Laf is not absorbed from the stomach, the gastric juice concentration resulting from an orally administered clinical dose of Laf (10 mg) may reach  $\sim 2$  mM when gastric juice volume is 10 ml, above the 0.1–1.0 mM we used in rats.

In conclusion, the induction of hyperemia via CSAN increased  $\text{pH}_{\text{int}}$ , preventing acid-induced interstitial acidification in rat esophagus. Laf activated CSAN and enhanced mucosal defense mechanisms, protecting mucosa from acid-pepsin injury. Our results support the therapeutic strategy in which compounds stimulating the capsaicin pathway such as Laf enhances mucosal protection from acid-related injury in the upper gastrointestinal tract and thus may uniquely protect from esophageal injury.

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## References

1. Akiba Y, Kaunitz JD. Luminal chemosensing and upper gastrointestinal mucosal defenses. *Am J Clin Nutr.* 2009;90:826S–831S.
2. Kaunitz JD, Akiba Y. Acid-sensing protective mechanisms of duodenum. *J Physiol Pharmacol.* 2003;54:19–26.
3. Mizumori M, Meyerowitz J, Takeuchi T, et al. Epithelial carbonic anhydrases facilitate  $\text{PCO}_2$  and pH regulation in rat duodenal mucosa. *J Physiol.* 2006;573:827–842.
4. Akiba Y, Ghayouri S, Takeuchi T, et al. Carbonic anhydrases and mucosal vanilloid receptors help mediate the hyperemic response to luminal  $\text{CO}_2$  in rat duodenum. *Gastroenterology.* 2006;131:142–152.

5. Tanaka S, Chu S, Hirokawa M, Montrose MH, Kaunitz JD. Direct measurement of acid permeation into rat oesophagus. *Gut*. 2003;52:775–783.
6. Akiba Y, Mizumori M, Kuo M, et al. CO<sub>2</sub> chemosensing in rat oesophagus. *Gut*. 2008;57:1654–1664.
7. Bass BL, Trad KS, Harmon JW, Hakki FZ. Capsaicin-sensitive nerves mediate esophageal mucosal protection. *Surgery*. 1991;110:419–425.
8. McKie LD, Dunkin BJ, Pennanen MF, Dunlap KW, Harmon JW, Bass BL. Esophageal mucosal blood flow: a central role for calcitonin gene-related peptide. *Surgery*. 1994;116:409–417.
9. Tobey NA. Systemic factors in esophageal mucosal protection. *Digestion*. 1995;56(Suppl 1):38–44.
10. Tobey NA, Powell DW, Schreiner VJ, Orlando RC. Serosal bicarbonate protects against acid injury to rabbit esophagus. *Gastroenterology*. 1989;96:1466–1477.
11. Onodera S, Shibata M, Tanaka M, et al. Gastroprotective mechanism of lafutidine, a novel anti-ulcer drug with histamine H<sub>2</sub>-receptor antagonistic activity. *Arzneimittelforschung*. 1999;49:519–526.
12. Mimaki H, Kagawa S, Aoi M, et al. Effect of lafutidine, a histamine H<sub>2</sub>-receptor antagonist, on gastric mucosal blood flow and duodenal HCO<sub>3</sub><sup>-</sup> secretion in rats: Relation to capsaicin-sensitive afferent neurons. *Dig Dis Sci*. 2002;47:2696–2703.
13. Nagahama K, Yamato M, Kato S, Takeuchi K. Protective effect of lafutidine, a novel H<sub>2</sub>-receptor antagonist, on reflux esophagitis in rats through capsaicin-sensitive afferent neurons. *J Pharmacol Sci*. 2003;93:55–61.
14. Nishihara K, Nozawa Y, Nakano M, Ajioka H, Matsuura N. Sensitizing effects of lafutidine on CGRP-containing afferent nerves in the rat stomach. *Br J Pharmacol*. 2002;135:1487–1494.
15. Onodera S, Shibata M, Tanaka M, Inaba N, Yamaura T, Ohnishi H. Gastroprotective activity of FRG-8813, a novel histamine H<sub>2</sub>-receptor antagonist, in rats. *Jpn J Pharmacol*. 1995;68:161–173.
16. Umeda M, Fujita A, Nishiwaki H, Takeuchi K. Effect of lafutidine, a novel histamine H<sub>2</sub>-receptor antagonist, on monochloramine-induced gastric lesions in rats: role of capsaicin-sensitive sensory neurons. *J Gastroenterol Hepatol*. 1999;14:859–865.
17. Ichikawa T, Ota H, Sugiyama A, et al. Effects of a novel histamine H<sub>2</sub>-receptor antagonist, lafutidine, on the mucus barrier of human gastric mucosa. *J Gastroenterol Hepatol*. 2007;22:1800–1805.
18. Akiba Y, Guth PH, Engel E, Nastaskin I, Kaunitz JD. Acid-sensing pathways of rat duodenum. *Am J Physiol Gastrointest Liver Physiol*. 1999;277:G268–G274.
19. Tobey NA, Hosseini SS, Caymaz-Bor C, Wyatt HR, Orlando GS, Orlando RC. The role of pepsin in acid injury to esophageal epithelium. *Am J Gastroenterol*. 2001;96:3062–3070.
20. Steen KH, Reeh PW, Kreysel HW. Dose-dependent competitive block by topical acetylsalicylic and salicylic acid of low pH-induced cutaneous pain. *Pain*. 1996;64:71–82.
21. Hamamoto DT, Ortiz-Gonzalez XR, Honda JM, Kajander KC. Intraplantar injection of hyaluronic acid at low pH into the rat hindpaw produces tissue acidosis and enhances withdrawal responses to mechanical stimuli. *Pain*. 1998;74:225–234.
22. Pan HL, Longhurst JC, Eisenach JC, Chen SR. Role of protons in activation of cardiac sympathetic C-fibre afferents during ischaemia in cats. *J Physiol*. 1999;518:857–866.
23. Lanas A, Royo Y, Ortego J, Molina M, Sainz R. Experimental esophagitis induced by acid and pepsin in rabbits mimicking human reflux esophagitis. *Gastroenterology*. 1999;116:97–107.
24. Arai T, Necheles H. Effect of alternating acid and alkaline digestive fluids on the gastrointestinal tract of the rat. *Proc Soc Exp Biol Med*. 1965;119:169–174.
25. Mizumori M, Ham M, Guth PH, Engel E, Kaunitz JD, Akiba Y. Intestinal alkaline phosphatase regulates protective surface microclimate pH in rat duodenum. *J Physiol*. 2009;587:3651–3663.