Inhibition of Gastric Mucosal Carbonic Anhydrase by Taurocholic Acid and Other Ulcerogenic Agents

Eero Kivilaakso, MD, Helsinki, Finland

Gastric mucosa contains unusually high concentrations of carbonic anhydrase [1]. In addition to parietal cells, where it seems to contribute to the formation and secretion of hydrogen ions, this enzyme is equally abundant in the epithelial surface cells [2]. There is increasing evidence to suggest that it has a protective function at this location. Inhibitors of carbonic anhydrase have been shown to enhance the susceptibility of canine gastric mucosa to ulceration when exposed to luminal acid [3], a finding recently confirmed by studies with rabbit antral mucosa [4] and rat gastric glandular mucosa [5]. Similarly, inhibition of carbonic anhydrase provokes ulceration in isolated sacs of amphibian gastric mucosa [6,7]. It seems that carbonic anhydrase is needed for the utilization of ambient HCO_3^- by the mucosa because it protects against luminal acid [5,7]. If carbonic anhydrase does have a protective function in the gastric mucosa, it is possible that inhibition of mucosal carbonic anhydrase contributes to mucosal injury in some ulcerogenic situations. The present study investigates this possibility by assessing the influence of some well-established ulcerogenic agents on the activity of gastric carbonic anhydrase.

Material and Methods

Studies in vitro: After overnight fast, Spraque-Dawley rats (250 to 300 g) were anesthetized with intraperitoneal urethane (1 g/kg body weight). Whole-body perfusion with 0°C saline was performed through a polyethylene catheter inserted into the abdominal aorta until the venous effluent was completely clear of blood. The stomach was removed and opened along the greater curvature and the mucosa was cleansed thoroughly of its contents with 0°C saline. The stomach was stretched on a flat surface and the superficial layer of the glandular portion of the gastric mucosa was scraped off with the edge of a glass slide. The mucosal scraping was added to 3 ml of H_2O at 4°C and homogenized. The homogenate was centrifuged at 10,000 *g* for 15 minutes at 4°C. Erythrocyte contamination of the homogenate was assessed by measuring sample hemoglobin [8], but no measurable amounts of hemoglobin were detected.

Carbonic anhydrase activity of the supernatant was determined by the micromethod of Maren [9], which involves measuring the time taken for H_2CO_3, produced by hydrating carbon dioxide at 0°C, to decrease the pH of Na_2CO_3-$NaHCO_3$ buffer to 7. Enzyme activity was expressed as enzyme units (EU) according to the following equation: EU = uncatalyzed time − catalyzed time/catalyzed time.

In experiments involving inhibitory agents, the agent was added to the reaction vessel containing the mucosal enzyme 2 minutes before addition of the buffer. The uncatalyzed reaction time was determined with a solution containing the test agent but not the enzyme. For each agent, the concentration producing a 50 percent inhibition in enzyme activity (I_{50}) was determined from a plot of the percentual enzyme inhibition against the log of the inhibitor concentration [10]. At each step, the reaction time was repeatedly determined at least five times and the average reaction time was used for further calculations. The variation between each repeat was always less than ±10 percent.

Studies in vivo: After overnight fast the rats were anesthetized with urethane (1 g/kg body weight). The esophagogastric junction was ligated preserving the vagi, and the stomach was emptied and meticulously cleansed with warm saline through a catheter inserted through the pylorus. The stomach was filled with 3 ml of test solution containing the agent under study in 150 mM sodium chloride or 100 mM hydrochloric acid plus 50 mM sodium chloride at 37°C. After 30 minutes the animal was perfused with 0°C saline and the gastric mucosa was processed for determination of carbonic anhydrase activity as described above. Before centrifugation, samples for estimation of total protein [11] and DNA [12] were taken from the homogenate. Activity of the mucosal carbonic anhydrase was expressed as enzyme units (EU) per milligrams of protein.

In experiments with intravenous administration of the test agent, the stomach was cleansed and filled with 150 mM sodium chloride only. Acetazolamide (100 mg/kg body

From the Second Department of Surgery, Helsinki University Central Hospital, Helsinki, Finland. Supported by grants from the Sigrid Jusélius Foundation and the Paulo Foundation, Helsinki, Finland.

Requests for reprints should be addressed to Eero Kivilaakso, MD, Second Department of Surgery, Meilahti Hospital, 00290 Helsinki 29, Finland.

TABLE I Effect of Various Ulcerogenic Agents on the In Vitro Activity of Carbonic Anhydrase in the Gastric Mucosa of the Rat*

Test Agent	Reaction Time Determinations (n)	I_{50}
Taurocholic acid	6	8.94 ± 0.49 mM
Acetylsalicylic acid	6	10.40 ± 1.68 mM
Ethanol	6	14.50 ± 1.09 % vol/vol
Acetazolamide	6	0.63 ± 0.06 μM
Lysolecithin	6	No effect
Urea	6	No effect

* For each test agent, the concentration causing 50 percent inhibition of enzyme activity is given.

TABLE III DNA Contents of Gastric Mucosal Homogenate Obtained From Rat Stomachs Exposed to Luminal Sodium Chloride (NaCl) or Taurocholic Acid (TCA) with Hydrochloric Acid (HCl)

Group	Reaction Time Determinations (n)	DNA Contents (μg/mg protein)
Control (NaCl)	6	38.5 ± 1.8
TCA, 20 mM + HCl, 100 mM	6	42.5 ± 0.77

weight) was given as an intravenous bolus 15 minutes before the start of carbonic anhydrase determination. Intravenous acetylsalicylic acid (30 mg/kg body weight) was freshly prepared by dissolving 0.3 g of $NaHCO_3$ and 0.3 g acetylsalicylic acid in 10 ml of water [*13*] and was given as a slow intravenous infusion 15 minutes before the start of carbonic anhydrase determination. All chemicals were obtained from Sigma Chemical, Co., St. Louis, MO. The acetazolamide (Diamox®) was from Lederle Laboratories, Pearl River, NY.

Statistical analysis of the data was performed using Student's t test for unpaired variates.

Results

Effects in vitro: The effects of the various ulcerogenic test agents on the activity of carbonic anhydrase derived from gastric mucosa are given in Table I. For each agent, the mean concentration causing a 50 percent inhibition in the enzyme activity (I_{50}) is given. Taurocholic acid, acetylsalicylic acid, and ethanol all had a clear inhibitory action on the activity of carbonic anhydrase. Furthermore, their I_{50} values were within a concentration range that might be expected to be present within the stomach. Similarly, acetazolamide, a specific inhibitor of carbonic anhydrase, displayed a strong inhibitory action on the enzyme. In contrast, the two other agents tested, lysolecithin (in a concentration up to 10 mg/ml) and urea (in a concentration up to 1 M), had no measurable influence on the activity of gastric carbonic anhydrase.

Effects in vivo: Specific activities of carbonic anhydrase in gastric mucosa exposed to the various test agents in vivo are given in Table II. Exposure of the gastric mucosa to taurocholic acid significantly inhibited the activity of mucosal carbonic anhydrase. The degree of inhibition was greater in the presence of acid (−47.2 percent) than in the absence of it (−21.6 percent). Exposure of the gastric mucosa to the other agents that were tested did not cause any significant inhibition of mucosal carbonic anhydrase activity. Intraluminal ethanol, both in the presence and absence of acid, caused some decrease in the activity of gastric carbonic anhydrase, but intraluminal acetylsalicylic acid had no effect whatsoever. However, administration of acetylsalicylic acid intravenously did cause a slight but significant decrease ($p < 0.05$) in the activity of carbonic anhydrase in the gastric mucosa. Similarly, systemic intravenous administration of acetazolamide (100 mg/kg body

TABLE II Effect of Various Ulcerogenic Agents, Administered Intragastrically or Intravenously (IV), on the Carbonic Anhydrase Activity in the Gastric Mucosa of the Rat

Test Agent	Reaction Time Determinations (n)	Specific Enzyme Activity (EU/mg protein)	Percentual Effect (%)	p Value
Control (NaCl)	12	19.9 ± 1.2	...	...
TCA, 20 mM	6	15.6 ± 1.2	−21.6	<0.05
TCA, 20 mM + HCl, 100 mM	6	10.5 ± 1.6	−47.2	<0.01
ASA, 20 mM	6	20.0 ± 0.89	+ 0.5	NS
ASA, 20 mM + HCl, 10 mM	6	22.4 ± 1.9	+12.6	NS
Ethanol, 20% vol/vol	6	16.7 ± 1.3	−16.1	NS
Ethanol, 20% vol/vol + HCl, 100 mM	6	17.1 ± 2.1	−14.1	NS
ASA, 30 mg/kg (IV)	6	16.2 ± 0.49	−18.6	~0.05
Acetazolamide, 100 mg/kg (IV)	6	0.73 ± 0.13	−96.3	<0.01

EU = enzyme units; NaCl = sodium chloride; HCl = hydrochloric acid; TCA = taurocholic acid; ASA = acetylsalicylic acid; NS = not significant.

weight) caused a profound inhibition ($p < 0.01$) in the activity of gastric carbonic anhydrase.

DNA contents of the mucosal homogenate were determined in the control group and in the group with mucosal exposure to taurocholate and hydrochloric acid (where the decrease of carbonic anhydrase activity was the greatest). The results are given in Table III. No difference in DNA content between the two groups was observed, suggesting that the decrease in carbonic anhydrase activity was not due to exfoliation of cellular elements from the mucosa during exposure to taurocholic acid and hydrochloric acid.

Comments

Several agents, such as sulfonamides and halides, are known to inhibit erythrocytic carbonic anhydrase [*1*], but information on agents capable of inhibiting gastric carbonic anhydrase is much scantier. Newsome and Leitch [*14*] showed that ethanol, in a relatively low concentration (I_{50} 16.2 percent vol/vol), had an inhibitory action on carbonic anhydrase derived from gastric mucosa. The present study confirms this finding and indicates that, in addition, other ulcerogenic agents, such as taurocholic acid and acetylsalicylic acid, are also capable of directly inhibiting carbonic anhydrase derived from gastric mucosa. Furthermore, this inhibitory action seems to occur in relatively "physiologic" concentrations, since, for example, a mean concentration of 5.3 mM of bile salts has been measured in gastric contents of patients with gastric ulcer [*15*], and ingestion of two aspirin tablets (1,000 mg) with a glass of water into an empty stomach would produce a peak concentration of 10 to 20 mM of acetylsalicyclic acid in the gastric contents. Thus, the I_{50} concentrations measured in the present study for these agents (and ethanol) are not far out of the range of concentrations that would be expected to be encountered within the stomach. The two other agents tested, urea and lysolecithin, did not have any influence on the activity of gastric carbonic anhydrase, even though very high concentrations were used. This suggests that the "uremic gastritis" that sometimes occurs in association with renal failure is probably not caused by inhibition of gastric carbonic anhydrase due to elevated blood urea, and that the ulcerogenic action of lysolecithin [*16*] is also mediated by some other mechanism.

Of the three ulcerogenic agents that inhibit gastric carbonic anhydrase in vitro, only taurocholic acid had a clear inhibitory influence on the enzyme under in vivo conditions, even though relatively high concentrations were used. One explanation for this may be that the inhibitory action of these agents is highly reversible and is therefore readily abolished during the assay procedure when the inhibitor is no longer present. Another possibility is that the agents do not get access to the action site of the enzyme when instilled into a healthy stomach with intact epithelial lining. However, in an ulcerogenic situation, the permeability of the gastric mucosa is usually increased [*17,18*], allowing presumably better entry of luminal agents into the mucosa. It is also possible that the carbonic anhydrase directly exposed to the luminal agents at the apical membrane of surface epithelial cells is quantitatively negligible, although its functional role in the protection of the mucosa against luminal acid may be vital. Therefore, even complete inhibition of this species of carbonic anhydrase may not materially decrease the total activity of gastric carbonic anhydrase.

In contrast to acetylsalicylic acid administered intragastrically, acetylsalicylic acid administered intravenously did cause a slight but significant inhibition of carbonic anhydrase activity in the gastric mucosa. Previous studies have shown that acetylsalicylic acid can also induce lesions in the gastric mucosa when it is administered by extragastric routes [*13,19*], but the mechanism of this ulcerogenic action has still remained largely obscure. The present studies suggest that one factor that possibly contributes to ulceration under this condition might be inhibition of gastric carbonic anhydrase. There is also some evidence that acetylsalicylic acid and inhibitors of carbonic anhydrase can mutually potentiate each other's toxicity [*20*], a phenomenon that is possibly also mediated by synergic inhibition of carbonic anhydrase.

The ulcerogenic action of bile acids, acetylsalicylic acid, and ethanol is usually attributed to their ability to break the gastric "mucosal barrier" with resultant increased diffusion of luminal hydrogen ions into the mucosal tissue. The present studies suggest that, in addition, inhibition of gastric carbonic anhydrase may contribute to the ulcerogenic potency of these agents. Recent studies have indicated that inhibition of carbonic anhydrase with acetazolamide decreases the ability of the gastric mucosa to withstand tissue acidity, since ulceration occurs after administration of this drug at tissue pH which is normally tolerated without damage [*4,5*]. It seems that, in addition to increasing hydrogen ion back diffusion and consequent mucosal acidification, taurocholic acid also impairs mucosal tolerance to acid in a similar manner [*4*]. In light of the present findings it is possible that this enhanced mucosal sensitivity to tissue acidity might be mediated by direct inhibition of gastric carbonic anhydrase by taurocholic acid.

Summary

Carbonic anhydrase, an enzyme catalyzing hydration of CO_2 and vice versa, is exceptionally abundant in the gastric mucosa, including the surface epithelial cells where it seems to have a protective function. The present study evaluates various ulcerogenic agents in terms of their ability to influence the activity of carbonic anhydrase in the gastric

mucosa. Taurocholic acid, acetylsalicylic acid, and ethanol all significantly inhibited carbonic anhydrase derived from gastric mucosa of the rat in in vitro conditions, and this inhibition occurred in concentrations that are likely to be present within the stomach. In contrast, lysolecithin and urea had no effect on carbonic anhydrase activity. In in vivo situations, intragastric taurocholic acid (20 mM) likewise significantly inhibited the activity of carbonic anhydrase in the gastric mucosa. The inhibition was stronger in the presence of luminal acid (hydrochloric acid, 100 mM) than in the absence of it. In contrast, intragastric acetylsalicylic acid (20 mM) or ethanol (20 percent vol/vol) had no effect. Yet, intravenous acetylsalicylic acid (20 mg/kg body weight) did have a slight but significant inhibitory action. The results indicate that taurocholic acid is able to inhibit gastric mucosal carbonic anhydrase, a feature which may contribute to its ulcerogenic action. Even though acetylsalicylic acid and ethanol likewise inhibit gastric carbonic anhydrase in in vitro conditions, their action in in vivo situations remains questionable.

Acknowledgment: The technical assistance of Merja Kirsi is gratefully acknowledged.

References

1. Maren TH. Carbonic anhydrase: chemistry, physiology and inhibition. Physiol Rev 1967;47:495–781.
2. O'Brien P, Rosen S, Trencis-Buck L, Silen W. Distribution of carbonic anhydrase within the gastric mucosa. Gastroenterology 1977;72:870–4.
3. Werther LJ, Hollander F, Altamirano M. Effect of acetazolamide on gastric mucosa in canine vivo-vitro preparations. Am J Physiol 1965;209:127–33.
4. Kivilaakso E, Barzilai A, Schiessel R, Fromm D, Silen W. Experimental ulceration of rabbit antral mucosa. Gastroenterology 1981;80:77–83.
5. Kivilaakso E. High plasma HCO_3^- protects gastric mucosa against acute ulceration in the rat. Gastroenterology 1981;81:921–7.
6. Davies RE, Edelman J. The function of carbonic anhydrase in the stomach. Biochem 1951;50:190–4.
7. Kivilaakso E, Barzilai A, Schiessel R, Crass R, Silen W. Ulceration of isolated amphibian gastric mucosa. Gastroenterology 1979;77:31–7.
8. Zade-Oppen AMM. A method for the determination of hemoglobin in dilute solutions. Acta Soc Med Uppsal 1960;65: 249–57.
9. Maren TH. A simplified micromethod for the determination of carbonic anhydrase and its inhibitors. J Pharmacol Exp Ther 1960;130:26–9.
10. Carg LC. Inhibition of carbonic anhydrase of rat liver by halides. J Pharmacol Exp Ther 1977;200:434–8.
11. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. J Biol Chem 1951;193:265–75.
12. Burton K. A study of the conditions and mechanism of the diphenylamine reaction for the calorimetric estimation of deoxyribonucleic acid. Biochem J 1956;62:315–23.
13. Bugat R, Thompson MR, Aures D, Grossman MI. Gastric mucosal lesions produced by intravenous infusion of aspirin in cats. Gastroenterology 1976;71:754–9.
14. Newsome LR, Leitch GJ. Direct inhibition of gastrointestinal carbonic anhydrase by ethanol. Digestion 1978;17:370–3.
15. Rhodes J, Barnardo DE, Phillips SF, Rovelstad RA, Hoffmann AF. Increased reflux of bile into the stomach in patients with gastric ulcer. Gastroenterology 1969;57:241–52.
16. Kivilaakso E, Ehnholm C, Kalima TV, Lempinen M. Duodenogastric reflux of lysolecithin in the pathogenesis of experimental porcine stress ulceration. Surgery 1976;79:65–9.
17. Gordon MJ, Skillman JJ, Zervas NT, Silen W. Divergent nature of gastric mucosal permeability and gastric acid secretion in sick patients with general surgical and neurosurgical disease. Ann Surg 1973;178:285–94.
18. Kivilaakso E, Silen W. Pathogenesis of experimental gastric-mucosal injury. N Engl J Med 1979;301:364–9.
19. Brodie DA, Chase BJ. Role of gastric acid in aspirin-induced gastric irritation of the rat. Gastroenterology 1967;53: 604–10.
20. Anderson CJ, Kaufman PL, Sturm RJ. Toxicity of combined therapy with carbonic anhydrase inhibitor and aspirin. Am J Ophthalmol 1978;86:516–9.