Changes in potential difference across the human buccal mucosa with buffered or unbuffered aspirin and salicylate

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SUMMARY The potential difference (PD) across the gastric mucosa is an index of mucosal integrity, and is lowered by topical application of irritants such as aspirin. There are basic similarities in the PD across the buccal and gastric mucosae, and we have therefore investigated the actions of various salicylates in buffered or un-buffered solution on buccal PD in human subjects. Aspirin (at pH 2) and soluble aspirin (pH 4.4) applied topically reduced buccal PD, but this fall was abolished by buffering to pH 7. Sodium salicylate likewise reduced buccal PD at pH 4 and pH 6, but not when buffered to pH 7. Two other soluble aspirin mixtures also reduced buccal PD, indicating insufficient buffering capacity to prevent topical irritancy. Ingestion of aspirin (600 mg), avoiding topical contact with the buccal mucosa, did not alter buccal PD. Paracetamol applied topically likewise failed to reduce buccal PD. Measurement of buccal PD may be useful in the preliminary assessment of the gastrointestinal irritation provoked by anti-inflammatory and other compounds.

The transmural electropotential difference (PD) across the gastric mucosa has been used as an index of the integrity of the gastric mucosa in animals¹⁻² and man³⁻⁵. A reduction in gastric PD has been observed after the topical application of irritants such as aspirin, bile salts, and ethanol in animals¹⁻² and in man⁴⁻⁷. These effects occur concurrently with the other characteristics of gastric 'barrier' damage such as the back-diffusion of acid from the gastric lumen into the mucosa and the increase in luminal concentration of sodium ions⁸⁻¹⁰. Such changes in gastric PD also correlate with histologically demonstrable damage to the human mucosa after aspirin ingestion.⁷

Because of the basic similarities in the magnitude, polarity, and the responses of the PD across the buccal mucosa and the gastric mucosa in man¹¹ it may be possible to predict easily the ability of aspirin-like drugs and other potentially irritant compounds to damage the gastric mucosa after topical application, by measuring changes in buccal

mucosal PD. In the present study, we have therefore investigated the actions of various formulations of acetylsalicylic acid and sodium salicylate, and of paracetamol on buccal PD in man.

A preliminary report on this work was presented to the Clinical Section of the British Pharmacological Society.¹²

Methods

POTENTIAL DIFFERENCE MEASUREMENTS

Experiments were performed on a total of 50 healthy volunteers between the ages of 19 and 30 years to whom the protocol was explained and who gave their written informed consent. Subjects were required to refrain from taking any aspirin or aspirin-like drugs within one week of the study and any subject with a history of aspirin-sensitivity was excluded.

Buccal mucosal potential difference was measured essentially as described by Houston¹¹ using a probe electrode constructed from a Perspex (lucite) tube which contained a silver-silver chloride junction contact with a short column of saline agar (NaCl 150 mmol/l with 2% w/v agar). The skin electrode

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was also constructed of Perspex and contained a silver-silver junction in contact with a layer of saline-agar (Fig. 1). This electrode was strapped over a bleb raised on the volar surface of the forearm by the intradermal injection of sterile saline solution (150 mmol/l, a procedure which eliminates the skin potential.¹³ Since changes in pressure on the mouth electrode have been shown to alter buccal PD measurements,¹¹ a restraining device was used upon which the head could rest. Buccal PD was recorded on a millivoltmeter (Orion, model 701) and displayed on a chart recorder (Fig. 1). The individual PD measurements, taken over a three minute period, varied by less than 2 mV from the average value for that period.

The experimental protocol is shown in Fig. 1. The subject was required to rinse the mouth with deionised water (20 ml), and, after expelling the contents, the buccal PD was measured at 30 second intervals over a three minute control period. The drug under study, dissolved or suspended in deionised water (20 ml) immediately before use, was then swilled around the buccal cavity for three minutes, without the subject swallowing the liquid. The contents were expelled and the mouth rinsed with distilled water (100 ml in consecutive 20 ml aliquots) and the test buccal PD measured over a three minute period as before. The pH values of the test and reference solution, measured on a Radiometer P4M62 pH meter, remained steady over a 15 minute experiment period, and were not altered by a three minute contact with the buccal mucosa (three experiments).

DRUGS

Acetylsalicylic acid powder (Wellcome Research Laboratories), sodium salicylate (British Drug House), soluble aspirin tablets BP (300 mg; Boots Co. Ltd), aspirin tablets BP (300 mg; Boots Co. Ltd), paracetamol tablets (Boots Co. Ltd), Codis tablets (soluble aspirin 600 mg, codeine 12 mg; Reckitt and Colman), and Paxidine (soluble aspirin 500 mg, codeine 8 mg; Boots Co. Ltd).

STATISTICAL EVALUATION

From the values of buccal PD taken during the three minute measurement period, the average control and test values were calculated for that subject. The data from all subjects in each group is expressed in terms of buccal PD (mV) or change in buccal PD (Δ mV) and shown as mean $\pm$ SE mean of (n) values. The statistical significance of the data was evaluated using Student's *t* test for paired or unpaired data where appropriate. $P < 0.05$ was taken as significant.

Results

EFFECTS OF SOLUBLE ASPIRIN

Soluble aspirin tablets (300–1200 mg) added to deionised water (20 ml) produced a solution of pH 4.4–4.5. In a study using 11 subjects, soluble aspirin (300 mg) did not significantly alter PD, whereas soluble aspirin (600 and 1200 mg) signifi-

Fig. 1 Measurement of potential difference (PD) across the human buccal mucosa.

cantly ($P < 0.01$) reduced the PD (Table 1). The change in PD was not significantly different ($P > 0.05$) between the 600 mg or 1200 mg dose of soluble aspirin.

In a series of experiments with another five subjects it was observed that, when the soluble aspirin (600 mg) solution was buffered to pH 7.2 by the addition of sodium bicarbonate, the fall in PD was abolished (Table 2).

The changes in PD with soluble aspirin (600 mg) in the two series of experiments shown in Tables 1 and 2 (30 ± 4 and 19 ± 3 mV) were not significantly

Table 1. *Effect of soluble aspirin (BP), sodium salicylate, and buffered salicylate on PD across buccal mucosa in man.*

Drug	Dose (mg)	pH	ΔPD (mv)	n	
Soluble aspirin	300	4.5	4 ± 2	5	NS
	600	4.5	19 ± 3	11	†
	1200	4.5	15 ± 2	5	†
Sodium salicylate	1200	6.3	12 ± 3	8	*
Sodium salicylate + NaHCO ₃	1200	7.3	3 ± 1	5	NS

Data shown as change in PD (ΔPD) are the mean $\pm$ SE mean from (n) subjects. The pH refers to the pH of the solution when the dose specified was suspended in 20 ml distilled water. The level of statistical significance of the change in PD from control values is shown by * $P < 0.05$; † $P < 0.01$.

Table 2. *Effect of soluble aspirin, buffered soluble aspirin, and two soluble aspirin mixtures on buccal mucosal PD.*

Drug	Dose (mg)	pH	ΔPD (mv)	(n)	
Soluble aspirin	600	4.5	30 ± 4	5	†
Soluble aspirin + NaHCO ₃	600	7.2	2 ± 3	3	NS
Soluble aspirin + codeine (Codis)	12	4.3	21 ± 2	5	†
Soluble aspirin + codeine (Paxidine)	500	8	20 ± 1	5	†

Results, shown as PD change, (ΔPD) are the mean $\pm$ SE mean from (n) subjects. The level of statistical significance of the change from control values is shown by † $P < 0.01$.

Table 3. *Buccal mucosal potential difference before and after treatment with sodium bicarbonate-citric acid and mixtures at various pH values.*

PD (mv)			
pH	Control	Test	
6.4	-39 ± 6	-38 ± 5	NS
4.6	-39 ± 6	-36 ± 4	NS
2.6	-39 ± 6	-37 ± 6	NS

Results are shown as PD mean $\pm$ SE mean of values from seven subjects. In each case, there is no significant difference between control and test value.

different from each other. However, to reduce any variation in the response of PD, comparisons between different drugs or treatments are best made using a crossover design within a given group of subjects.

EFFECTS OF ASPIRIN

In a study using seven subjects, aspirin powder (1200 mg) suspended in deionised water (20 ml) giving a pH of 2.4, produced a marked fall in PD (Fig. 2). A comparable fall was observed with soluble aspirin (1200 mg, pH 4.5) as found in the previous studies. However, when the aspirin powder (1200 mg) was dissolved in sodium bicarbonate solution to give a final solution of pH 7.2, no significant fall in buccal PD was observed (Fig. 2).

In control experiments on these seven subjects, solutions adjusted to 2.6, 4.6, and 6.4 with citric acid and sodium bicarbonate had no significant effect on control buccal PD (Table 3).

EFFECTS OF SODIUM SALICYLATE

Sodium salicylate (1200 mg) dissolved in deionised water, and adjusted to pH 4.4 with citric acid produced a significant fall in buccal PD in seven subjects (Fig. 2). In another series of eight subjects, the fall in PD produced by sodium salicylate (1200 mg) at pH 6.3 was abolished when the solution was adjusted to 7.3 with sodium bicarbonate (Table 1).

Fig. 2 *Effect of aspirin, soluble aspirin, buffered aspirin, and sodium salicylate (1.2 g) on buccal mucosal potential difference (PD) in seven subjects. Results are shown as mean $\pm$ SE mean where the level of the statistical significance is shown by ** $p < 0.01$; *** $p < 0.001$.*

EFFECTS OF SOLUBLE ASPIRIN-CODEINE MIXTURES

In another five subjects, the actions of two proprietary mixtures of soluble aspirin and codeine were investigated. As shown in Table 2, these mixtures gave solutions of pH 4.3–4.4 and produced a significant fall in buccal PD.

EFFECTS OF SYSTEMICALLY-ADMINISTERED ASPIRIN

To determine whether the effect of aspirin was solely a topical action on the buccal mucosa, three subjects rapidly swallowed two intact aspirin tablets (600 mg) with a draught of water (50 ml) avoiding any prolonged contact with the buccal cavity, after control values of buccal PD had been measured. Thirty minutes after aspirin ingestion, a time close to the peak plasma levels of aspirin,¹⁴ buccal PD was again determined. There was no significant change in the post-aspirin value (-42 ± 5 mV) as compared with the pretreatment control value (-38 ± 4 mV; $P > 0.05$).

EFFECT OF PARACETAMOL

In another three subjects, a suspension of paracetamol (500 mg) in deionised water (20 ml; pH 4.7) had no significant effect ($P > 0.05$) on buccal PD (PD was -39 ± 4 mV before and -36 ± 3 mV after paracetamol).

Discussion

The present study confirms the findings of Houston¹¹ that, like other areas of the gastrointestinal tract,¹⁵ the human buccal mucosa generates a significant bioelectric potential difference (PD). The magnitude and polarity of this PD (-35 to -55 mV) is comparable with that reported for the human gastric mucosa by Andersson and Grossman,³ and others. The ionic basics of the gastric mucosal PD is not clearly defined but involves the flux of ions including H^+ , Na^+ , and Cl^- , and, because of the different environment of stomach and buccal cavity, it is unlikely that the ionic mechanisms underlying buccal PD are comparable. Nevertheless, acetylsalicylic acid in acidic solution can reduce buccal PD, as found with human gastric PD.^{4–6} Light microscopy studies have shown areas of cellular disruption and microscopic erosions which accompany such changes in gastric mucosal PD⁷ and likewise the change in buccal PD may reflect topical irritation, as other studies have shown that aspirin can cause cellular damage when applied to the buccal mucosa.¹⁶ The

fall in buccal PD appears to depend on the presence of free H^+ , as the fall in PD induced by aspirin was abolished by buffering the solution to pH 7 with sodium bicarbonate; this was also found with the gastric mucosa by both Murray *et al.*,⁵ and Bowen *et al.*⁷ In control experiments, acidic solutions alone did not alter buccal PD. In the current study, the abolition of the PD response to aspirin in neutral solution did not appear to depend solely on the reduction of the lipid soluble non-dissociated species. A marked fall in PD was observed at both pH 2.5 and 4.5 and, as aspirin has a dissociation constant (pK_a) of 3.5, this represents a degree of ionisation of 10% and 90% respectively.

Sodium salicylate also caused a significant fall in buccal PD at pH 4.4 and pH 6.3, but not when buffered to pH 7.2. Salicylate likewise reduces PD across the canine gastric mucosa,¹⁷ which again may reflect topical irritation in the presence of luminal H^+ ions.

The fact that PD across the gastric or buccal mucosa did not change with highly buffered aspirin could suggest that formulations of aspirin or similar drugs providing a neutral pH would have less acute topical gastric irritancy. In the current study, soluble aspirin BP and two proprietary aspirin-containing mixtures giving solutions of pH 4 provoked falls in PD indicating that the buffering capacity of the mixtures was insufficient. Reformulation of soluble aspirin BP, as well as certain proprietary soluble aspirin mixtures, may be indicated. Furthermore, the buffering capacity of new formulations must take into account the low pH of the gastric contents and must be sufficient to raise the gastric pH to neutrality. Thus, it has been shown that oral administration of a highly buffered aspirin mixture does not reduce human gastric PD.⁵ Although acute administration of a relatively low dose of buffered aspirin mixture has been reported to cause negligible damage to the gastric mucosa, as assessed by histological examination,⁷ a recent study indicated that in higher doses (3.9 g per day) buffered aspirin offered little or no protection to the gastric and duodenal mucosa¹⁸. Enteric coated tablets did appear, however, to cause less irritation.^{18, 19} In the present study, paracetamol also failed to reduce buccal PD, perhaps reflecting its less irritant action on the buccal mucosa, as suggested for the gastric mucosa.

On chronic administration, topical irritation of the gastric mucosa by non steroid anti-inflammatory agents is likely to be only one of the factors provoking gastrointestinal damage. Thus sodium salicylate, which, like aspirin, can reduce gastric PD and initiate back-diffusion of hydrogen ions across the mucosa,¹⁷ causes less gastrointestinal

bleeding than aspirin (as determined by faecal excretion of Cr⁵¹ labelled erythrocytes) when administered to man.^{20 21} Interestingly, sodium salicylate, unlike aspirin, does not inhibit prostaglandin cyclo-oxygenase (the enzyme responsible for the biosynthesis of prostaglandins) in the gastrointestinal tract, when administered in anti-inflammatory doses in the rat.^{22 23} This suggests that the fall in gastric or buccal PD after topical application of sodium salicylate is not a consequence of inhibition of prostaglandin biosynthesis. Such a suggestion is supported in the current work where systemic pre-treatment with aspirin (avoiding local contact with the buccal mucosa) failed to change buccal PD at a time of peak aspirin plasma levels, although it is not known how the buccal tissue levels of aspirin so obtained compare with those after local administration. Likewise, Ivey and colleagues²⁴ have shown that intravenously-administered aspirin, in doses which should be sufficient to inhibit endogenous prostaglandin synthesis, failed to alter gastric PD in man, as also shown in animal studies by Bugat and co-workers.²⁵ These findings could also suggest that endogenous prostaglandin formation is not a major factor in maintaining the PD across the gastric or buccal mucosa.

From our previous studies, it was suggested that topical irritation, as reflected by changes in PD and acid back diffusion, is insufficient to initiate marked gastric bleeding, but, when combined with the inhibition of gastric mucosal prostaglandin formation, it leads to extensive gastric damage.²⁶ Thus, reduction of topical irritancy alone is unlikely to provide non-steroid anti-inflammatory agents devoid of gastrointestinal toxicity, and animal studies have clearly demonstrated that aspirin and similar compounds can cause gastric damage when administered by the intravenous route.^{25 26} The rational development of non-steroid anti-inflammatory drugs for clinical use with less gastrointestinal toxicity should therefore encompass elimination of both the inhibitory effects on gastrointestinal prostaglandin production and also the topical irritant actions. Our recent studies have suggested that anti-inflammatory compounds which selectively inhibit prostaglandin production at inflammatory sites yet do not inhibit prostaglandin production in the gastrointestinal tract can be achieved.²² Such compounds can then be formulated to reduce or prevent any topical irritancy, if present.

Although the ionic basis for the buccal PD is as yet unknown, the present findings suggest that studies on human buccal PD may be predicative of local irritant effects on the gastric mucosa and may provide a rapid, preliminary assessment of the

activity of newer anti-inflammatory compounds before detailed studies on the human gastric mucosa.

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