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Effect of 16,16-dimethyl prostaglandin E_2 on the gastric mucosal barrier

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SUMMARY 16,16-dimethyl prostaglandin E_2 (16DM) can protect the gastric mucosa from injury and yet apparently damages the gastric mucosal barrier. The effect on the gastric mucosal barrier of 16DM (26·2 μmol/l), a dose 50 times the ED50 for inhibition of acid secretion, was investigated in Heidenhain pouches in four dogs by measuring plasma shedding from the pouches after the topical application of histamine (2·7 mmol/l) and ionic fluxes. The results were compared with those using 30% ethanol, a known barrier breaker. The topical application of histamine after three hours' perfusion with 30% ethanol led to plasma shedding at a rate of 7·5 ($\pm$2·6) ml/h, which was significantly greater than the rate of 1·2 ($\pm$1·4) ml/h after three hours' perfusion with 16DM and of 1·5 ($\pm$1·7) ml/h in the control group. Ethanol also caused an increase in the flux of H^+, Na^+, and Cl^-, indicating an increase in mucosal permeability, whereas 16DM increased the flux of Na^+ and Cl^- but not of H^+. It is concluded that 16DM does not damage the gastric mucosal barrier but stimulates the secretion of fluid containing Na^+ and Cl^-.

16,16-dimethyl prostaglandin E_2 (16DM) is a synthetic methyl analogue of prostaglandin E_2 (PGE_2) and a highly potent inhibitor of gastric acid secretion (Robert and Magerlein, 1973). Unlike PGE_2, 16DM is stable in an acid medium and therefore is effective topically as well as parenterally (Robert *et al.*, 1976), and is 50 times more potent than PGE_2 (Robert *et al.*, 1976). In addition, 16DM can protect the gastric mucosa from injury by a wide variety of ulcerogenic stimuli in a dose 100 times less than the threshold dose for acid inhibition (Robert *et al.*, 1977), indicating that this protective action is not the result of acid inhibition.

The combined actions of acid inhibition and mucosal protection should make 16DM a valuable agent in the prevention and treatment of peptic lesions, and another methyl analogue of PGE_2, 15-methyl prostaglandin E_2, has been studied clinically with encouraging results (Fung *et al.*, 1974a, b). However, ionic flux studies of 16DM in canine Heidenhain pouches have indicated that it 'breaks' the gastric mucosal barrier (O'Brien and Carter, 1975; Bolton and Cohen, 1976) an action which would damage rather than protect the gastric mucosa, though measurement of potential difference across isolated gastric mucosa suggests that 16DM 'tightens' the barrier (Bowen *et al.*, 1975). This study was undertaken to resolve this controversy.

Histamine applied to the intact gastric mucosa is not absorbed and has no effect (Code, 1956) but, if the mucosal barrier is broken, topically applied histamine will pass into the interstitial space and cause an increase in capillary permeability and plasma shedding (Davenport, 1966). Topical histamine was applied to canine Heidenhain pouches after pretreatment with 16DM or 30% ethanol, a known barrier breaker (Davenport, 1967), and the effect on plasma shedding compared. In addition, confirmatory ion flux studies were performed.

Methods

The study was performed in four female mongrel dogs weighing approximately 20 kg, prepared by antrectomy, gastroduodenal anastomosis, and fashioning of a Heidenhain pouch. The pouches opened to the surface through a wide bore metal cannula. The pouch in this model is vagally denervated and should not secrete acid unless subjected to an exogenous stimulus. Before the dogs were included in this experiment they were tested to confirm that they fulfilled this criterion. One month

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was allowed to elapse between surgery and the start of the experiment. The experiments were carried out in a random sequence. At least 48 hours were allowed between successive tests in any dog and each experiment was performed twice in each dog. After an 18 hour fast the dogs were placed on a Pavlov table and the pouches perfused by gravity infusion from a reservoir at the height of the pouch, to avoid distension. The perfusate entered the bottom of the pouch and was withdrawn from the top, thereby allowing maximum contact between the perfusate and mucosa. A tap on the withdrawal line was used for repeated sampling and the perfusate was returned to the reservoir by a roller pump. The volume of the pouch represented approximately one quarter of the perfusate volume.

Each experiment was divided into two perfusion periods, the first of three hours and the second of one hour. In the control experiments the pouches were perfused for the first period with 90 ml of a solution containing 120 mmol H^+/l, 5 mmol Li^+/l, and 5 g polyethylene glycol (PEG)/l as a volume marker, and made isosmotic at 300 mmol/l with sodium chloride. Samples were taken at 30 minute intervals and analysed for H^+, Li^+, Na^+, Cl^-, and PEG. The dogs received 100 mg Evan's Blue at the start of each experiment and a blood sample was taken at 90 minutes. After three hours the perfusate was changed and replaced for the second period by 90 ml of an acid free solution containing 5 mmol Li^+/l, 5 g PEG/l and made isosmotic with sodium chloride, to which was added histamine di-HCl 2·75 mmol/l (0·5 mg/ml). This solution was perfused for one hour, samples were taken at 10 minute intervals and analysed for Li^+, Na^+, Cl^-, and PEG and a blood sample was taken at 30 minutes (Table 1). H^+ was measured by titration against 0·1 N NaOH using an automatic titrimeter (Radiometer Copenhagen). Li^+ and Na^+ were measured by flame photometry (Corning) and Cl^- on a chloride meter (Corning). PEG was measured by the turbidimetric method of Malawer and Powell (1967). The samples were centrifuged before analysis to remove any cell debris and mucus which might interfere with turbidimetry.

Table 1 *Protocol of experiments*

	First period	*Second period*
Duration	3 hours	1 hour
Control pouch perfusate	Acid/saline	Acid-free + histamine
Experimental perfusates	Control + 16DM Control + ethanol	Control Control

Evan's blue, 100 mg, was given intravenously at the start of the first period. Blood samples were taken halfway through both periods. The perfusate was completely changed between periods.

The net ion flux (NIF) for each ion was calculated for each perfusion period by measuring the NIF during the six sampling periods in each perfusion period, and taking the mean of the last five values as the NIF for the whole perfusion period (Chung *et al.*, 1973). The volume changes during each perfusion period were calculated from the PEG concentrations. In subsequent experiments either ethanol 30% v/v or 16DM 26·2 μmol/l (10 μg/ml) was added to the first period perfusate, the second period perfusate being unchanged. The dose of 16DM used is approximately 50 times greater than the ED50 for the inhibition of stimulated acid secretion in a canine Heidenhain pouch (Robert *et al.*, 1976). This dose was chosen to be in the same range as the 15 μg/ml used by O'Brien and Carter (1975) which appeared to cause barrier damage and was equal to the largest of three doses studied in our original permeability studies (Bolton and Cohen, 1976).

An aliquot of the perfusate taken at the end of each perfusion period and of the plasma taken halfway through each period were centrifuged until optically clear and the Evan's blue concentration measured spectrophotometrically at 570 nm. From this data the volume of plasma in each perfusate was calculated (Davenport and Kauffman, 1975).

Results

The results are set out in Tables 2 and 3 as the means and one standard deviation. As the same animals were used throughout, the data were paired and the significance assessed using Wilcoxon's rank sum test. In the first period of perfusion the topical application of 30% ethanol caused a significant increase in the lumen to plasma flux (back diffusion) of H^+ and in the plasma to lumen flux of Na^+ and Cl^- (Table 2). This increase in ionic flux was associated with a highly significant increase in the volume of fluid produced by the pouch and its plasma content. The topical application of 16DM 26·2 μmol/l (10 μg/ml) caused a highly significant increase in the plasma to lumen flux of Na^+ and Cl^-, but was without effect on lumen to plasma flux of H^+. 16DM also caused a highly significant increase in the volume of fluid produced and in its plasma content (Table 2).

In the second period, after withdrawal of the ethanol and the addition of topical histamine in an acid free solution, there remained a significant increase in the plasma to lumen flux of Na^+ and Cl^-. After withdrawal of 16DM and the addition of topical histamine there was no significant change in the fluxes of Na^+ or Cl^- (Table 3). In this period the plasma to lumen flux of Na^+ and Cl^- after ethanol was significantly greater than after 16DM.

After ethanol histamine caused a highly significant

Table 2 *Ionic fluxes and volumes of fluid and plasma produced under basal conditions (control) and in response to topical application of ethanol or 16DM during first perfusion period*

	No. of tests	First period flux (μmol/30 min)			First period volume (ml/3 h)	
		Lumen to plasma	Plasma to lumen		Total	Plasma
Experiment		H^+	Na^+	Cl^-		
Control	8	187·4 ±95·9	386·6 ±95·9	193·1 ±224·6	24·0 ±6·4	0·9 ±0·8
Ethanol (30% v/v)	7	623·7† ±256·3	1216·5† ±299·0	540·3* ±178·9	49·2† ±12·3	3·7† ±1·6
16DM (26·2 μmol/l)	8	166·7 ±173·1	912·7† ±438·0	763·2† ±470·0	47·1† ±16·7	3·7† ±2·8

Results expressed as mean ± SD. †$P \leqslant 0{\cdot}01$. *$P \leqslant 0{\cdot}05$ when compared with the control values.

Table 3 *Ionic fluxes and volumes of fluid and plasma produced in second period in response to topical histamine in control group and after pretreatment with ethanol or 16DM*

	No. of tests	Second period flux (μmol/10 min)		Second period volume (ml/1 h)	
		Plasma to lumen		Total	Plasma
Experiment		Na^+	Cl^-		
Control	8	167·7 ±179·6	127·2 ±205·6	15·9 ±6·8	1·5 ±1·7
After ethanol	7	616·3* ±269·6	605·9* ±262·2	39·3† ±13·5	7·5† ±2·6
After 16DM	8	293·0 ±342·0	250·5 ±304·9	20·7 ±14·1	1·2 ±1·4

Results expressed as mean ± SD. †$P \leqslant 0{\cdot}01$. *$P \leqslant 0{\cdot}05$ when compared with control values.

increase in the volume of fluid produced but after 16DM the histamine had no effect (Table 3). The histamine also caused a highly significant increase in plasma shedding after ethanol but had no effect on plasma shedding after 16DM (Table 3). The volumes of fluid produced and plasma shed in response to histamine after ethanol were not only significantly greater than the controls but also significantly greater than after pretreatment with 16DM.

Lithium was added to the perfusate with the intention of using the ion as an indicator of permeability by measuring the lumen to plasma flux (Chung *et al.*, 1973). As the results were variable and doubt has been cast on the validity of the use of this ion as an indicator of H^+ diffusion (Saik and Brown, 1978), the lithium data have not been included.

Discussion

The results following the application of ethanol 30% v/v demonstrate gastric mucosal barrier damage. There was a significant increase in the back diffusion of H^+ and of influx of Na^+ and Cl^-. In addition, there was an increase in the fluid produced, probably secondary to the local release of histamine caused by the back diffusion of H^+ (Davenport, 1966), and in plasma shedding. The effect of 16DM in the first period was similar to that of ethanol but there were important differences. There was a significant increase in the plasma to lumen flux of Na^+ and Cl^- but not in the lumen to plasma flux of H^+. Damage to the gastric mucosal barrier is associated with an increase in the permeability to all the ions present. The effect of 16DM in apparently increasing the permeability to only two of the three ions measured is therefore unlikely to represent gastric mucosal barrier damage unless 16DM caused a selective alteration in permeability. More probably the effect is due to secretion of fluid containing Na^+ and Cl^- and this is supported by the increase in fluid produced by the pouches and plasma shedding caused by 16DM. When applied topically to isolated *in vivo* strips of gastric fundus 16DM causes an increase in mucosal blood flow under basal conditions (Cheung and Lowry, 1976) and therefore in these basal pouches 16DM probably produced such an increase. This could cause transudation and plasma loss from the capillaries and account for the volume changes recorded here. In addition, it is possible that 16DM has a direct stimulatory effect on non-parietal cell secretion and we have recently demonstrated, using an acid free perfusate, that the fluid produced by gastric pouches in response to 16DM contains not only Na^+ and Cl^- but also HCO_3^- (Bolton and Cohen, 1977, 1978).

Important differences in the response of the

pouches to topical histamine after pretreatment with ethanol or 16DM were demonstrated in the second period. After pretreatment with ethanol, histamine caused a significant increase in the plasma to lumen flux of Na^+ and Cl^-, and in the volume of fluid produced and plasma shed. The amount of fluid produced and plasma shed per unit time in the second period was much greater than in the first period. If this effect was due solely to endogenous histamine, it should have been in the same range as in the first period; the large increase in the second period suggests that exogenous histamine was absorbed across the broken gastric mucosal barrier. The plasma to lumen flux of Na^+ and Cl^- in this period was probably due to the Na^+ and Cl^- content of the fluid transudate produced in response to the absorbed histamine. After pretreatment with 16DM, topical histamine had no effect on the plasma to lumen flux of Na^+ and Cl^-, nor did it increase the volume of fluid produced or its plasma content.

It has recently been suggested that changes in the ionic permeability of the gastric mucosal barrier are mediated by histamine (Rees *et al.*, 1977). Consequently, the use of histamine in these experiments could be criticised. Two studies have shown that exogenous histamine may increase ionic permeability, but in one (Moody and Davis, 1970) the histamine was given intravenously and in the other (Fiocca *et al.*, 1974) it was applied to the serosal surface of isolated gastric fundus. In the second period the controls showed a slight increase in the output of fluid and plasma and in the plasma to lumen flux of Na^+ and Cl^- per unit time when compared with the first period. However, as the perfusate in the second period was identical in all three groups, a comparison between the groups in this period is valid. The pronounced increase in plasma to lumen flux of Na^+ and Cl^- and in the secretion of fluid and plasma occurring only after pretreatment with ethanol, but not after 16DM, indicates that histamine cannot have been significantly absorbed across the gastric mucosa after its exposure to 16DM and therefore 16DM cannot have caused gastric mucosal barrier damage.

The interpretation of flux data from canine Heidenhain pouches is based on the assumption that the pouches are non-secreting and that the alteration in the Na^+ and H^+ content of the perfusate represents the passive diffusion of these ions across the gastric mucosa. This work suggests that the increase in the plasma to lumen flux of Na^+ produced by 16DM is not the result of passive diffusion, but of active secretion and, if this is true, Na^+ flux clearly cannot be used to measure permeability in the presence of this agent. Previous reports of the barrier damaging effect of 16DM (O'Brien and Carter, 1975; Bolton and Cohen, 1976) have relied heavily on the Na^+ flux data, the changes in H^+ flux being much smaller.

It is concluded that 16DM does not break the gastric mucosal barrier, but stimulates a non-parietal cell secretion. As 16DM has a dual acid inhibitory and cellular protective action and as it does not damage the gastric mucosal barrier it is possible it will yet prove to be a valuable prophylactic and therapeutic agent in the management of peptic lesions.

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