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A comparison of the effects of hyperosmotic salicylate solutions in closed and perfused rectal in-situ loops of rats

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The effects of presentation of hyperosmotic solutions of sodium salicylate to the closed and perfused rectal loop preparations commonly used in assessing drug absorption have been compared. Epithelial cell loss was quantified in control and treated loops. Tissue damage was significantly greater ($P < 0.01$) in treated perfused loops. Fluid efflux, which was associated with a small but significant change in the haematocrit value, was noted in both systems.

Closed or perfused in-situ intestinal loop preparations are commonly used to assess gastrointestinal absorption in anaesthetized animals. Houston & Wood (1980) have commented that the volume of the solution, the degree of fluid loss, and the elements within the non-drug compartments, are factors that complicate the interpretation of the data. Perfusion of the loop may alter absorption by mixing the bulk phase with the solution which is in intimate contact with the membrane, and which has been depleted of drug. This may favour absorption by maintenance of the concentration gradient. In addition, the positive force developed during perfusion may cause physical changes in the dimensions of the perfused loop and increase cell loss. Winne (1979) demonstrated a change in the rate of solute absorption following a reduction in the dimensions of the unstirred layer, and a number of studies have shown that excessive luminal fluid flux is associated with epithelial damage (Kameda et al 1968; Thomas et al 1984). The presentation of a hyperosmotic solution to the epithelial surface causes an outflow of water into the lumen with hypersecretion of mucus and, in some instances, epithelial cell loss. Under these conditions the thickness and structure of the unstirred layer are also perturbed.

In a static system, movement of water into the lumen re-establishes an equilibrium between the bulk phase tonicity and that of the cells, while in a perfused loop system the osmotic stress remains relatively constant. Consequently, epithelial changes should be greater in the latter system. The two in-situ methods were compared in loops of rat large bowel exposed to hyperosmotic solutions in order to test this hypothesis.

Methods

Male 200 g Wistar rats fasted overnight were anaesthetized with pentobarbitone sodium i.p. (90 mg kg^{-1}). Either a closed or a perfused in-situ loop was prepared using the same 5 cm segment of rectum in each animal. The volume of the loop was approximately 1 ml for both preparations. Krebs buffer (pH 7·9, osmolarity 288 mOsm kg^{-1}) and 2% (w/v) sodium salicylate in Krebs buffer (pH 7·9, osmolarity 519 mOsm kg^{-1}) were used as the control and experimental solutions, respectively; both contained the non-absorbable marker [^{14}C]polyethylene glycol 4000 (3 μCi ml^{-1}). This was included to allow the determination of the cumulative efflux from the time of introduction of the test solution. A Sage syringe pump (model 351) was used in the perfusion system. Two perfusion rates (0·1 ml and 0·2 ml min^{-1}) were used with the salicylate solutions, and the lower rate alone for the Krebs buffer. The [^{14}C]PEG content of samples of luminal contents (closed loop) and luminal outflow (perfused loop) taken at regular intervals was determined by scintillation counting. From these data the net changes in luminal volume were calculated. Tail tip blood samples were taken at the start and end of the experiment and the haematocrit value measured. After 45 min the animals were killed and the rectal loop processed for histology. Sites of epithelial cell loss, and epithelial cell thinning which indicate sites of cell loss (Johnson et al 1978), were counted as sites of damage in 6 randomly selected slides of longitudinal profiles through 6 control and 6 experimental loops. Counts were expressed as damage sites/0·5 mm rectum. Data were subjected to appropriate parametric and nonparametric statistical tests.

Results and discussion

Closed loops exhibited minimal fluid flux in the presence of Krebs buffer, but a 2% solution of sodium salicylate induced a gradual but significant increase in the luminal volume which reached a plateau level of about 60% by 40 min (Fig. 1). In perfused loops, the buffer induced an approximately 30% increase in luminal volume which subsequently fell to less than 5% after 30 min. Luminal volume change reached plateau values almost immediately in the experimental perfused loops (Fig. 2). These levels were maintained for the duration of the experiments and after 30 min were significantly greater than control. At the higher perfusion rate, the percentage volume change in the first 20 min of the experiment was approximately half that seen at the slower rate. Thus, the presentation of the fluid at higher flow rates did not elicit a corresponding increase in water flux. This would appear to indicate that the tissue was responding maximally at the lower flow rate and was therefore unable to increase water

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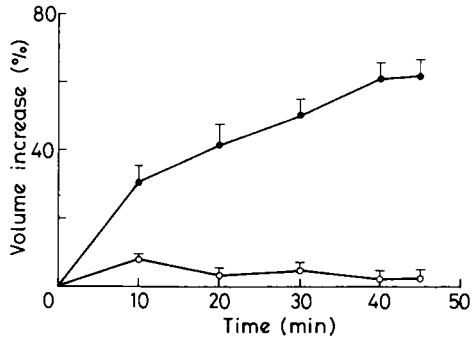

FIG. 1. Fluid efflux expressed as a % volume increase against time in closed loops exposed to Krebs buffer (○), and 2% (w/v) sodium salicylate in Krebs buffer (●). Mean ± s.e.m., n = 6.

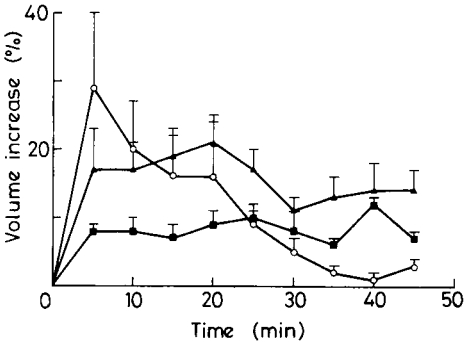

FIG. 2. Fluid efflux expressed as % volume increase against time in perfused rectal loops exposed to Krebs buffer (○), and 2% (w/v) sodium salicylate (▲) in Krebs buffer at a perfusion rate of 0·1 ml min^{-1}; and 2% (w/v) sodium salicylate (■) in Krebs buffer at a perfusion rate of 0·2 ml min^{-1}. Mean ± s.e.m., n = 6.

flux into the lumen in response to the higher osmotic load.

It is not possible to make a direct comparison between fluid flux in the two loop techniques. The one is characterized by a falling osmotic load which eventually will approach tissue tonicity. The other presents a more uniform osmotic load to the system, the gradient of which is determined by the perfusion rate (Winne 1979); and the system responds by reaching a steady state. Winne (1979) has shown that intestinal absorption of a number of compounds increases as the perfusion rate rises in part because of the flattened inflow to outflow gradient.

The luminal fluid efflux was in part reflected in the changed haematocrit values at the end of the experiment and indicated an apparent decrease in extracellular fluid volume (Table 1). This parameter is rarely considered in absorption studies and although the reduction in plasma volume in all cases was small it should certainly be considered when excessive fluxes occur as it is associated with important haemodynamic changes.

Table 1. Haematocrit values from closed and perfused rectal loop animals. Mean ± s.e.m., n = 6. The paired data from each animal was analysed using a one tailed *t*-test.

Solution	Haematocrit time zero	Haematocrit time 45 min
Closed Loop		
Krebs	47·5 ± 0·3	48·2 ± 0·4
2% Sodium salicylate	47·0 ± 0·3	49·2 ± 0·4*
Perfused loop		
Krebs 0·1 ml min^{-1}	48·2 ± 0·3	50·6 ± 0·4
2% Salicylate 0·1 ml min^{-1}	49·6 ± 0·6	51·9 ± 0·6*
2% Salicylate 0·2 ml min^{-1}	49·2 ± 0·4	51·0 ± 0·5*

* $P < 0·01$.

Table 2. Mucosal damage in closed and perfused rectal loops. Mean counts/0·5 mm rectum ± s.d., n = 6. Data from each experimental group was compared with its corresponding control using the Mann-Whitney U-test.

Treatment	Sites of damage (counts/0·5 mm)
Closed loops	
Krebs	3·3 ± 0·6
2% Sodium salicylate	3·5 ± 1·1
Perfused loops	
Krebs 0·1 ml min^{-1}	3·5 ± 0·5
2% Sodium salicylate 0·1 ml min^{-1}	4·8 ± 0·5*
2% Sodium salicylate 0·2 ml min^{-1}	7·4 ± 0·8*

* $P < 0·01$.

The extent of mucosal damage (Table 2) was changed significantly between control and experimental groups in the constant perfusion system. In addition, there was a clear relationship between perfusion rate and damage. O'Driscoll & Corrigan (1983) made a similar observation when they assessed the dry weight of material filtered from perfusates. It seems very likely that damage in the perfusion loops can be related to (a) a constantly higher osmotic load and (b) the physical disturbance by shearing forces of the microenvironment, particularly the unstirred layer, adjacent to the epithelium. Winne (1978) and Thomson & Dietschy (1980) have shown that reductions in the dimension of this layer significantly increased intestinal absorption; however, it is possible that when this is accompanied by epithelial damage then different constraints may determine the barrier to absorption.

Thomson & Dietschy (1980) have commented that it is invalid to compare kinetic constants derived from different in-vitro preparations directly. The data presented here further illustrate differences between the mucosal response in closed and perfused loop systems subjected to hyperosmotic stress. These mucosal responses will undoubtedly influence the process of drug absorption and should be considered when making the choice between the two systems.

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Accumulation of nifedipine after multiple doses

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The single and multiple dose pharmacokinetics of oral nifedipine capsules, 10 mg, have been examined in five patients with peripheral vasospasm. After a single dose, nifedipine was rapidly absorbed in three and slowly absorbed in two patients. Mean bioavailability parameters included a t_{max} of 2·9 h, a C_{max} of 33·3 ng ml^{-1} and a $AUC_{0-8\,h}$ of 113·3 ng h ml^{-1}. After multiple dosing with either 10 or 20 mg every 8 h for 10 days the mean t_{max} at steady state was 2·1 h while the mean dose-corrected (to 10 mg) C_{max} and $AUC_{0-8\,h}$ were 51·9 ng ml^{-1} and 146·9 ng h ml^{-1}, respectively. The mean elimination rate constant was 0·173 h^{-1} after both single and multiple doses. The mean extent of accumulation of nifedipine, defined as the ratio of $AUC_{0-8\,h}$ (steady state)/$AUC_{0-8\,h}$ (single dose), was 1·3; we concluded that nifedipine accumulates in the body when it is administered every 8 h. This should be taken into account when predicting steady state serum concentrations and haemodynamic effects of nifedipine from single dose kinetic data.

Nifedipine, a calcium channel-blocking agent typically administered in multiple doses, has a mean elimination half-life in normal volunteers, following the administration of a single, 10 mg, oral dose, of 3·4 to 8 h (Foster et al 1983; Raemsch & Sommer 1983). From these figures significant accumulation of nifedipine dosed 3 or 4 times a day would be expected. However, there do not seem to be any reports including pharmacokinetic data obtained following its repeated administration that could substantiate its accumulation characteristics. Raemsch & Sommer (1983) found no accumulation or any changes in its pharmacokinetics after administering 10 mg three times daily for one week to normal volunteers.

Recently we were able to study the pharmacokinetics of nifedipine after single oral dose and multiple oral doses to a group of patients who were part of an experimental protocol to evaluate the effectiveness of nifedipine in ameliorating peripheral vasospasm. Because of the scarcity of data relating to the steady state pharmacokinetics of the drug it was thought that the study would provide insight into the accumulation characteristics of nifedipine.

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Materials and methods

Five patients (3 males, 2 females) were involved; they were aged 21–31 years (mean 23·6 yrs) and non-obese with a mean body weight of 56·5 ± 4·9 kg. Renal and hepatic function tests were within normal limits. The patients gave informed consent and they were hospitalized for the duration of the study. Because pain and other symptoms were part of their disorder, various medications, including flurazepam, hydroxyzine, indomethacin and pethidine (meperidine) were permitted during the study. One patient was receiving phenytoin. We confirmed that these medications did not interfere with the assay for nifedipine and that they had not been previously reported in the literature to alter the disposition of nifedipine. But pethidine has been reported to inhibit gastric emptying and to delay the rate, but not the extent, of absorption of paracetamol (Nimmo et al 1975), however, the patients had access to the drug as needed and the daily dose throughout was essentially unchanged.

On the first day a single, 10 mg, oral dose of nifedipine (Procardia, Pfizer Inc., New York) was administered to each patient after at least 2 h without food. Blood (5 ml) was obtained predose and at 0·5, 1·0, 2·0, 3·0, 4·0, 5·0, 6·0, 7·0 and 8·0 h post dose. Blood pressure and pulse were monitored at each sampling time. Serum was collected from the blood samples and frozen at −20 °C for future nifedipine analysis. All blood and serum samples were protected from light.

After the response to the single dose was evaluated, nifedipine, 10 mg every 8 h, was taken by the patients for the next 5 days. At this point, based on the individual responses to the drug, the dose was continued or increased to a maximum of 60 mg per day. Four of the five patients were able to tolerate 20 mg, and the fifth 10 mg every 8 h. These doses were continued for 5 days