Mechanistic Studies on Effervescent-Induced Permeability Enhancement

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Purpose. To determine the mechanism(s) by which effervescence induces penetration enhancement of a broad range of compounds ranging in size, structure, and other physiocochemical properties across rat and rabbit small intestinal epithelium.

Methods. Effervescent induced penetration enhancement was investigated *in vitro* by utilization of a modified Ussing chamber diffusion cell apparatus and *in vivo* by single-pass intestinal perfusion.

Results. Carbon dioxide (CO_2) bubbling directly onto rabbit ileum epithelium induced an increase in drug permeability. Mechanistic studies indicated that effects due to CO_2 bubble evolution, such as increased drug dissolution rates, mucus thinning/stripping, and pH buffer effects did not contribute to increases in drug flux. Cellular enzyme (5'-ND and LDH) and total protein release assays did not indicate cell membrane perturbation and/or damage. CO_2 bubbling induced a reduction in transepithelial electrical resistance (TEER) indicating epithelial disruption due to a structural change of the paracellular pathway. This was further substantiated by a MW dependence on paracellular marker flux. In addition, tissue recovery was relatively rapid, ≈20 min.

Conclusions. CO_2 bubbling directly onto the intestinal epithelium induced enhanced drug permeability due to an alteration of the paracellular pathway. This, in addition to fluid flow and membrane hydrophobicity concepts, may account for observed increases in drug flux.

KEY WORDS: effervescence; carbon dioxide; penetration enhancer; transport; oral drug delivery; small intestine.

INTRODUCTION

Effervescent preparations have been utilized in oral drug delivery for more than 200 years. In the eighteenth century, saline cathartics were the first effervescent formulations to be developed (1). Since that time, numerous preparations utilizing effervescent technology have been produced including; stomach distress medications (e.g. Alka-Seltzer®), vitamin supplements (e.g. calcium), and analgesics.

Widespread acceptance of effervescent delivery systems is based primarily on two characteristic properties: (1) a palatable saline taste masking the unpleasant taste commonly associated with therapeutic agents; and (2) a buffer effect. While these two properties are important, effervescence has also been shown to strip the mucus layer and influence gastrointestinal (GI) physiological processes such as altering stomach motility patterns and increasing mucosal and gastric secretions (2).

Effervescent dosage forms have been demonstrated to promote rapid and/or enhanced oral absorption for a number of drug compounds (e.g. caffeine, disulfiram) in comparison to other formulations (3–5). Although previous studies have shown differences in bioavailability and other pharmacokinetic parameters, most have neglected to determine the exact reasons and postulate that the differences are due to increased drug dissolution rates or a buffering/solubility mechanism. This paper will focus on the permeability enhancement effects of effervescence on a variety of drugs across intestinal epithelium with subsequent examination of enhancement mechanism(s).

MATERIAL AND METHODS

Materials

[^{3}H]Polyethylene glycol 900 (PEG 900; 2.23 mCi/g), [^{3}H]mannitol (22.5 mCi/mmol), [^{14}C]caffeine (53.3 mCi/mmol), [^{14}C]benzoic acid (22.0 mCi/mmol), [^{3}H]polyethylene glycol 4000 (PEG 4000; 2.0 mCi/g), and [^{3}H]tetracycline (1.0 mCi/mmol) were purchased from NEN Research Products (Wilmington, DE). [^{14}C]Diazepam was purchased from Amersham Life Sciences, Inc. (Arlington Heights, IL). Tetracycline, benzoic acid, mannitol, and sodium pentobarbital were obtained from Sigma Chemical Co. (St. Louis, MO). Caffeine, PEG 900, and PEG 4000 were purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). Ketamine hydrochloride was procured from Fort Dodge Laboratories (Fort Dodge, IA), and xylazine was purchased from Phoenix Pharmaceuticals, Inc. (St. Louis, MO). All other reagents were analytical grade and were used as received.

In Vitro Transport Studies

New Zealand male albino rabbits (Bakkom's Rabbitry; Viroqua, WI) weighing between 5.0–5.5 lbs were maintained on an unrestricted diet with standard caging facilities and a 12 hour light/dark cycle. The research conducted in all animal studies adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised, 1985). The rabbits were sacrificed by 10 ml i.v. injection of 5% sodium pentobarbital solution into a marginal ear vein. The abdominal wall was exposed and a surgical incision down the midline was performed. An 8 to 10 cm ileum segment was excised and placed in ice cold saline. The epithelium was exposed via longitudinal incision along the mesenterium, washed with ice cold saline, and mounted onto a modified Ussing diffusion apparatus. Sorenson's buffer, 7.0 ml (pH 6.8; 300 mOsm) was added to the receiver chamber. 0.15% mannitol, benzoic acid, caffeine, tetracycline, PEG 900, and PEG 4000 buffer solutions (35 ml) were spiked with [^{3}H]mannitol (7.0 μCi), [^{14}C]benzoic acid (7.5 μCi), [^{14}C]caffeine (3.5 μCi), [^{3}H]tetracycline (11.5 μCi), [^{3}H]PEG 900 (12.0 μCi), or [^{3}H]PEG 4000 (10.0 μCi), respectively. [^{14}C]Diazepam (3.5 μCi) was placed in 35 ml pure buffer. For each respective mucosal to serosal transport study, radioactive drug solution (7.0 ml) was inserted into the donor chamber. Solutions were prewarmed and maintained at 37°C by a circulating water bath. In stirrer experiments, electronically driven stirrers (Jim's Manufacturing, Inc.; Iowa City, IA) were placed in both chambers ≈1.0 cm from the tissue surface to ensure adequate mixing. Due to unstirred water layer effects on drug permeability, the rate at which the solution was stirred was chosen as the point in which an increase in the stir rate did not induce a subsequent rise in drug permeability. In CO_2 or nitrogen studies, the donor

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chamber stirrer was removed and replaced with a 1.5 mm diameter tube allowing direct gas bubbling (rates ≈1.0, 10.0, and 100.0 ml/min) onto the epithelial surface without inducing a temperature change within the chamber. The tissue was oxygenated by the inclusion of <5% oxygen. The solutions were also monitored for pH changes. One ml samples were removed from the receiver chamber at designated time points and replaced with an equal amount of buffer. Permeability coefficients (P) were determined by equation (1):

$$P = \frac{V}{A \times C_0}\left(\frac{dM}{dt}\right) \quad (1)$$

where V = volume (7.0 ml), A = tissue surface area (0.785 cm^2), C_0 = initial donor concentration (100%), and dM/dt = steady state slope of % drug transported vs. time profile.

Tissue Recovery Studies

Male Sprague-Dawley rats (Harlan Sprague-Dawley, Madison, WI) weighing 400–450g were fasted for 20 to 24 hours with an unrestricted water supply prior to each study. The rats were anesthetized with 0.4 ml intramuscular (IM) injection of ketamine (100 mg/ml) and xylazine (10 mg/ml) mixture with additional 0.1 ml IM injections every 30 to 45 minutes as necessary to maintain anesthesia. Body temperature was maintained at 37°C by a heating pad. All solutions were prewarmed to 37°C.

A 10 cm ileum segment was cannulated ending 15 cm proximal to the ileo-cecal valve and performed so as to not inhibit blood flow. The segment was reinserted into the abdominal cavity and the abdominal wall sutured to prevent heat loss. At experimental conclusion, the rat was sacrificed via heart injection of 3.0 ml sodium pentobarbital.

The cannulated segment was cleansed by perfusion of Sorenson's phosphate buffer (pH 6.8; 300 mOsm) for 30 min at a rate of ≈0.5 ml/min by a cassette perfusion pump (Monostat, Inc., New York, NY). The drug solution following the cleansing buffer contained 0.15% benzoic acid in isotonic Sorenson's buffer with the addition of [^{14}C] benzoic acid (5.0μCi) and [^{3}H] PEG 4000 (1.0μCi). The non-absorbable marker, ^{3}H-PEG 4000, was used as an indicator of water flux. The radiolabeled solution was perfused (≈0.5 ml/min) through the intestinal segment and the perfusate collected over 15 min intervals. Samples were assayed for disintegrations per minute (dpm) utilizing a dual labeled quench correcting program on a TriCarb 2000CA scintillation counter (Packard Instrument Co., Inc., Downers Grove, IL). In CO_2 experiments, CO_2 was bubbled (rate ≈ 5.0 ml/min) into the tygon tubing containing the radiolabeled solution just prior to its entry into the proximal portion of the cannulated segment.

The fraction of benzoic acid (BA) absorbed, F_a, from the perfusate was calculated by equation (2):

$$F_a = \left[\left(\frac{[^3H]PEG_{in}}{[^3H]PEG_{out}}\right) \times \left(\frac{[^{14}C]BA_{out}}{[^{14}C]BA_{in}}\right)\right] \quad (2)$$

where in = inlet concentration and out = outlet concentration. In tissue recovery studies, CO_2 was bubbled into the duodenum for 20 minutes and then discontinued.

Lactate Dehydrogenase

In vivo single pass perfusion samples were assayed for LDH activity utilizing an LDH enzyme kit (#228-10, Sigma Chemical Co.), which allows for spectrophotometric determination (λ = 340 nm, Hitachi U3000 Spectrophotometer) of NADH formation due to the interconversion of lactate to pyruvate in the presence of LDH. Perfusate (50 μl) was combined with 1.0 ml reagent solution and mixed by inversion within a cuvette. Solution absorbance was monitored over a 90 sec interval. The absorbance change is proportional to LDH activity within the sample. The assay was run at 30°C.

5′-Nucleotidase

5′-ND is a cell membrane-bound enzyme located within a variety of tissues including intestinal and nasal epithelium. *In vivo* single-pass perfusate samples were individually assayed for 5′-ND activity following the procedures outlined in the 5′-ND enzyme kit (265-UV; Sigma Chemical Co.), which provides a method for spectrophotometric determination (λ = 340 nm) of 5′-ND activity. The assay was run at 30°C.

Total Protein Release

Total protein release from intestinal epithelium exposed to *in vivo* single-pass perfusion techniques was determined by a modification of the Ohnishi and Barr method, which is sensitive to protein concentrations between 15 and 100 mg/dl (6). This method (micro-protein kit, #690; Sigma Chemical Co.) provides a means for obtaining protein concentrations via absorbance spectroscopy (λ = 730 nm).

Electrophysiology

A dual pair electrode system was used within an in-vitro diffusion cell apparatus. The inner electrode pair were placed ≈ 1.0 cm from either side of the rat duodenum tissue surface bathed in 7.0 ml Sorenson's buffer (pH 6.5, 300 mOsm). Current was applied via a dc power supply (Hewlett Packard, Palo Alto, CA) across the membrane in a stepwise fashion from 0 to 15 μA in 5.0 μA intervals. Resistance measurements were obtained from the resultant transmembrane potential values, subtracting background resistance of the bathing medium.

RESULTS

Effervescence and Drug Transport

Permeability studies were conducted on a wide spectrum of drug compounds varying in size, structure, and other properties. As listed in Table 1, permeability coefficients for mannitol, PEG 900, tetracycline, caffeine, benzoic acid, and diazepam increased over that of stir experiments when CO_2 was bubbled (100 ml/min) onto the intestinal epithelium. The exception was PEG 4000, a compound which permeates the epithelium primarily via the paracellular pathway at low rates. When analyzing the extent of change between stirrer and CO_2 permeability coefficients, mannitol (log $K_{o/w} = -3.10$) and tetracycline (log $K_{o/w} = -1.47$) obtained a greater-fold increase (5.26 and 6.52, respectively) than the more hydrophobic drugs; caffeine (log $K_{o/w} = 0.00$), benzoic acid (log $K_{o/w} = 1.87$), and diazepam

Table 1. Permeability Coefficient for Stir, Carbon Dioxide, and Nitrogen Experiments

Compound	Permeability Coefficient (x 10^{-5}cm/sec) Stirrers[a] (S.D.)	Carbon Dioxide[a] (S.D.)	Nitrogen[b,c] (S.D.)
Tetracycline	0.18 (0.013)	1.20 (0.32)[c],*	1.23 (0.38)**
Caffeine	1.97 (0.57)	6.74 (0.81)[c],*	5.69 (0.72)**
Benzoic Acid	2.01 (1.04)	6.50 (1.35)[c],*	5.58 (1.55)**
Mannitol	0.23 (0.02)	1.21 (0.27)[c],*	NA[g]
PEG 4000	0.115 (0.011)	0.151 (0.037)[c]	NA
PEG 900	0.213 (0.015)	0.747 (0.13)[c],* 0.646 (0.53)[d],* 4.30 (0.94)[e,f]	NA
Diazepam	3.12 (0.85)	6.13 (0.64)[d],*** 6.04 (1.4)[d],*** 2.16 (0.64)[e]	NA

* Significant difference from stirrer experiments (2-sample t-test, $p < 0.01$).
** Significantly different from stirrer experiments (2-sample t-test, $p < 0.02$).
*** Significant difference from stirrer and 1.0 ml/min CO_2 (2-sample t-test, $p < 0.02$).
[a] Average of 3 to 8 experimental repetitions.
[b] Average of 3 to 4 experimental repetitions.
[c] Bubbling rate = 100 ml/min; equivalent to the rate generated by 3/4 Alka-Seltzer® tablet.
[d] Bubbling rate = 10 ml/min.
[e] bubbling rate = 1.0 ml/min.
[f] significant difference from stirrer ($p < 0.08$) and higher CO_2 bubbling rates ($p < 0.02$).
[g] NA = values not available, S.D. = standard deviation ($\times 10^{-5}$ cm/sec).

(log $K_{o/w}$ = 2.82), which increased 3.62, 3.23, and 1.96-fold, respectively. Increased benzoic acid absorption was also seen with in vivo single pass duodenum (data not shown) and ileum (Fig. 5) perfusion studies confirming results obtained *in vitro*.

Nitrogen and Drug Transport

The influence of nitrogen bubbling onto rabbit intestinal tissue is shown in Table 1. Nitrogen induced an equivalent enhancement effect on tetracycline, caffeine, and benzoic acid permeability coefficients in comparison to CO_2 studies.

CO_2 Bubbling Rate and Drug Transport

PEG 900 permeability was not affected by reducing the bubbling rate to 10 ml/min. Comparison of PEG 900 permeability coefficient obtained with 100 ml/min and 10 ml/min rates were found to be insignificantly different ($p > 0.05$). However, a decrease in permeability was observed on further reduction to 1.0 ml/min with the permeability coefficient value falling between that obtained for stirrer and higher bubbling rates (10 and 100 ml/min).

Diazepam permeability also was not reduced with a decrease in CO_2 bubbling rate to 10 ml/min. When examining diazepam permeability coefficient values between the 100 and 10 ml/min bubbling rates, they were not found to be significantly different ($p > 0.05$). With a 100-fold rate reduction, diazepam permeability returned to levels equivalent to stirrer experiments.

Paracellular Marker Transport

Results of mannitol, PEG 900, and PEG 4000 permeability coefficients are listed in Table 1. In both CO_2 and stir experiments, rabbit ileum permeability coefficients followed the sequence: mannitol > PEG 900 > PEG 4000. As the MW of the compound increased, there was a reduction in the extent of permeability enhancement induced by CO_2 bubbling.

Membrane Perturbation/Damage

Results of the LDH, 5′-ND, and total protein release experiments are shown in Figures 1–3. The positive control, 1.25% sodium deoxycholate (NaDOC), induced higher levels of LDH, 5′-ND, and total protein release into the perfusate over that of CO_2 and buffer experiments. When comparing CO_2 to buffer, there was statistically no significant difference ($p > 0.05$) at any of the 5′-ND or total protein release time points. The same is true for the LDH assay except at the 5 min time point ($p < 0.02$).

Transepithelial Electrical Resistance

TEER experimental results are shown in Figure 4. Normal duodenum tissue resistance values are considered to fall within the "leaky" range of 75 to 100 $\Omega \times cm^2$ (7). In the presence of CO_2 bubbling, resistance values fell to levels significantly ($p < 0.04$) below control experiments indicating a structural alteration of the epithelial membrane.

Tissue Recovery

Results of *in vivo* rat ileum tissue recovery experiments are presented in Figure 5. The fraction of benzoic acid disappearing

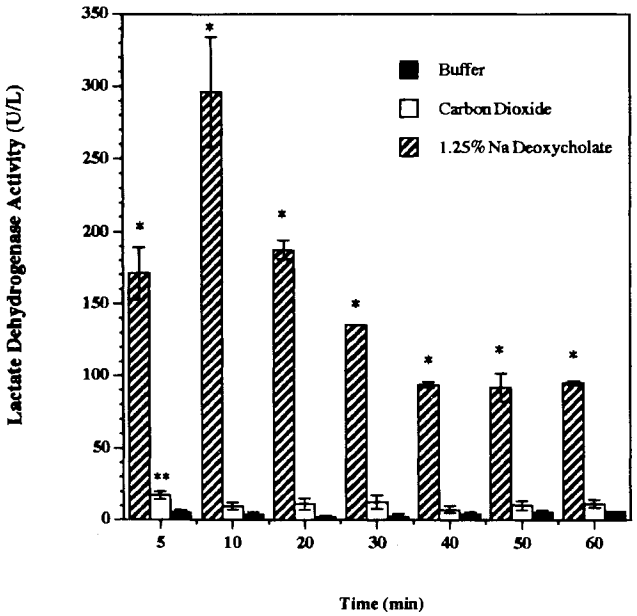

Fig. 1. Lactate dehydrogenase enzyme activity after *in vivo* single-pass perfusion of rat ileum tissue with buffer, carbon dioxide, or 1.25% sodium deoxycholate (NaDOC). Error bars represent ± SEM of 3 to 4 experimental repetitions. *Significant difference (t-test, $p < 0.02$) between NaDOC and carbon dioxide or buffer experiments. **Significant difference (t-test, $p < 0.04$) from 5 min stir value.

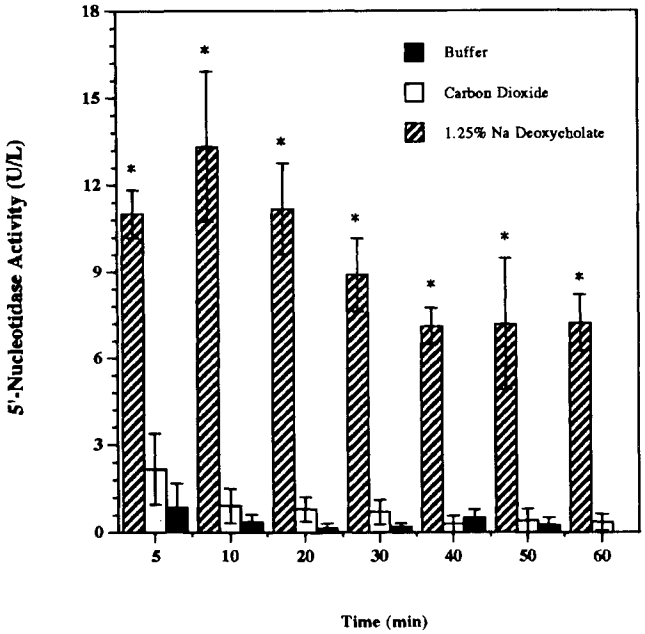

Fig. 2. 5'-Nucleotidase enzyme activity after *in vivo* single-pass perfusion of rat ileum tissue with buffer, carbon dioxide, or 1.25% sodium deoxycholate (NaDOC). Error bars represent ±SEM of 3 to 4 experimental repetitions. *Significant difference (t-test, $p < 0.01$) between NaDOC and carbon dioxide or buffer experiments.

from the perfusate increased when CO_2 was bubbled into the segment. After discontinuation of the bubbling, a relatively rapid linear decline to non-CO_2 experimental levels was observed.

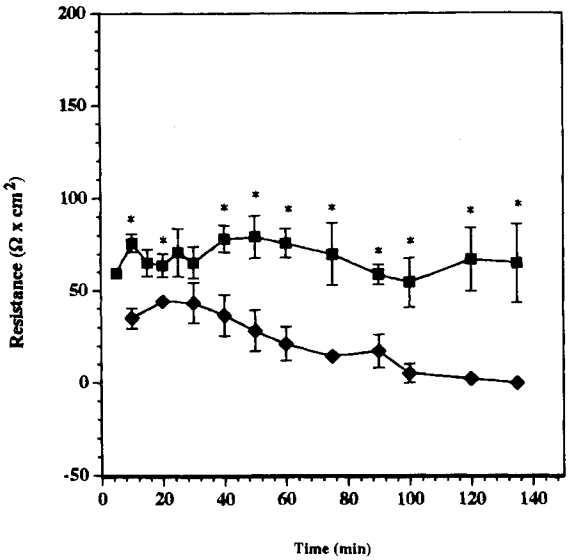

Fig. 4. Rat duodenum tissue resistance profile associated with (■) stirrers and (◆) carbon dioxide bubbling (rate ≈ 100 ml/min). Error bars are ±S.D. of 4 experiments. *Indicates significant difference (t-test, $p < 0.04$).

DISCUSSION

Permeability coefficients obtained with stir experiments were comparable to previously published results (8). As expected, the permeability coefficients correlated to the drug's partition coefficient with hydrophilic drug (mannitol, PEG's, tetracycline) flux less than their hydrophobic counterparts (benzoic acid, diazepam). Tetracycline, caffeine, mannitol, benzoic

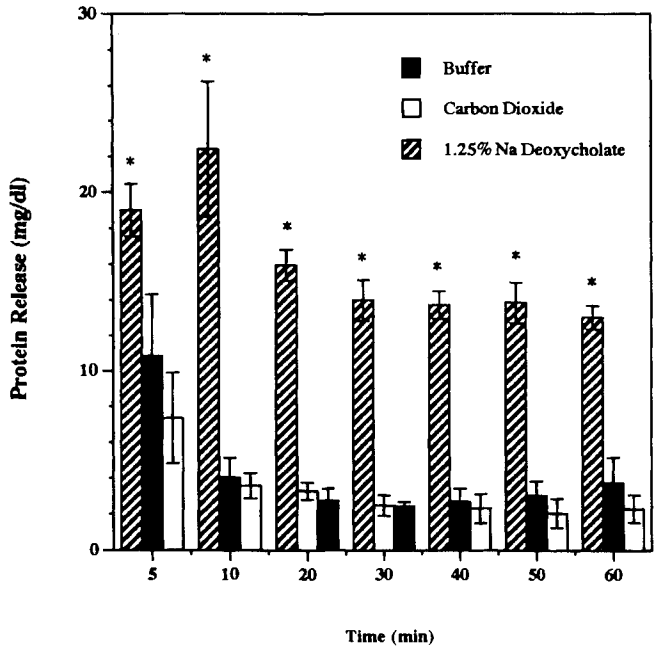

Fig. 3. Protein release after *in vivo* single-pass perfusion of rat ileum tissue with buffer, carbon dioxide, or 1.25% sodium deoxycholate (NaDOC). Error bars represent ±SEM of 3 to 4 experimental repetitions. *Significant difference (t-test, $p < 0.02$) between NaDOC and carbon dioxide or buffer experiments.

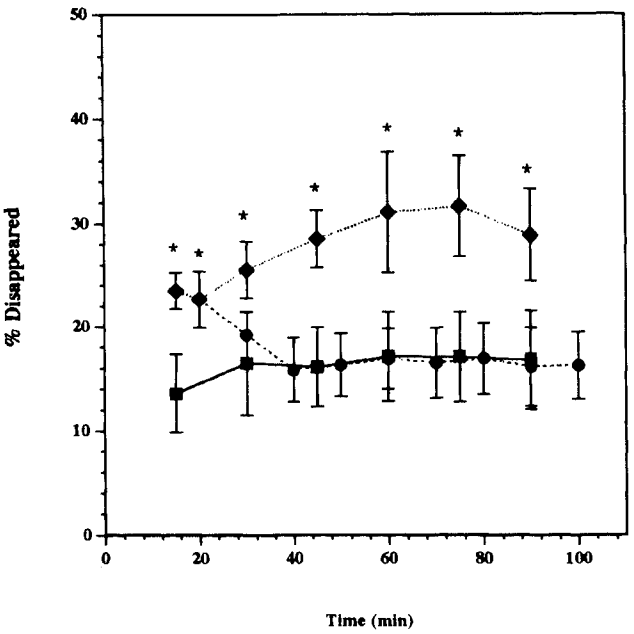

Fig. 5. Tissue recovery as determined by percent benzoic acid disappearing from perfusate solution. *In vivo* single-pass perfusion of rat ileum undergoing (■) carbon dioxide (rate ≈ 5 ml/min), (◆) control, and (●) recovery studies. Error bars represent S.D. of 4 experiments. *Significant difference (t-test, $p < 0.04$).

acid, PEG 900, and diazepam permeability rates were enhanced across the rabbit ileum in the presence of carbonation. In addition, stirrer and CO_2 bubbling experiments did not induce tissue toxicity as indicated by MTT cell viability measurements (2). These results may be explained by potential effects induced by CO_2 as a chemical entity or bubble evolution at the tissue surface including: (1) an alteration in pH tissue gradient; (2) a buffer effect; (3) solvent drag due to increased fluid flow or pressure gradients; (4) thinning or stripping of the mucus layer; (5) disruption of the epithelial barrier, and/or (6) increased membrane hydrophobicity. Since mucus stripping would lead only to faster steady state diffusion conditions and not alter drug permeability rates, this mechanism was disregarded as a potential explanation.

The bubbling rate for initial Ussing chamber experiments was 100 ml/min, chosen to mimic effervescent production similar to a single Alka-Seltzer® tablet. When the CO_2 bubbling rate was reduced 100-fold, a decrease in permeability for both PEG 900 and diazepam was encountered. However, the reduction was greater for diazepam, a hydrophobic compound, than for PEG 900, a polar compound. The combined results of the bubbling rate and permeability experiments indicated that the hydrophilic compounds were affected to a greater extent. This may indicate a structural change of the paracellular pathway (i.e. tight junction), creation of new or widening pre-existing aqueous pores within the cell membrane, and/or induction of greater solvent flux across the membrane. The enhancement associated with hydrophobic drugs could in part be due to increased flux through the paracellular route, but this effect would be limited. A better explanation would involve the concept of membrane hydrophobicity, in which CO_2 molecules partition within the membrane producing an increased hydrophobic environment leading to enhanced nonpolar drug flux.

The results also indicate that nitrogen induced equivalent enhancement on tetracycline, caffeine and benzoic acid. CO_2 and nitrogen are absorbed within and across cell membranes, but nitrogen is incapable of altering intracellular or extracellular pH. Since nitrogen and CO_2 induced similar permeability enhancement, alteration in pH was discarded as potential mechanistic explanation.

A variety of methods have been employed for determining the extent of epithelial cell damage or perturbation upon exposure to chemicals. Tissue microscopy has been widely used for visual examination but is costly, time consuming, and incorporates researcher subjectivity. Within the past decade, measurement of cellular enzyme or protein release has become increasingly popular.

LDH, 5′-ND, and protein release assays provide an alternative method to quantitatively measure subtle changes occurring within the intestinal epithelium. Lactate dehydrogenase (LDH) is a cytosolic enzyme whose leakage is indicative of the extent of cell membrane porosity and/or lysis providing a measure for cell damage (9,10). Previous studies have indicated that early enzyme release begins when membrane damage is reversible (11,12). Results indicated significant LDH leakage into the perfusate after epithelium exposure to the bile salt, sodium deoxycholate (NaDOC). Bile salts partly promote penetration enhancement by damaging cell membranes through lipid and protein extraction (13,14). LDH release is due to extensive membrane stretching and/or pore creation allowing enzyme movement across the bilayer (15).

5′-ND is a cell membrane bound enzyme whose release into the intestinal perfusate indicates the level of membrane perturbation. Gradual removal of 5′-ND or other membrane proteins disrupt the orientation of the lipid bilayer and increases membrane fluidity. These effects have previously been shown to be associated with enhanced drug flux (16).

The protein release assay is not only indicative of membrane perturbation but also membrane damage. Therefore, the protein released is an additive value corresponding to membrane and cytosolic proteins.

The combination of the three assays provide substantial evidence as to the lack of membrane damage and/or perturbation due to CO_2 bubbling. Therefore, mechanical disruption of the transcellular pathway does not play significantly in the permeation enhancement mechanism.

The three hydrophilic markers utilized for comparison are known to traverse the intestinal epithelium by the paracellular space (17,18). When examining carbon dioxide's influence on paracellular marker permeability, a correlation between probe molecular weight and permeability coefficient is evident. Rabbit ileum permeability followed the sequence: mannitol > PEG 900 > PEG 4000. This is indicative of CO_2 imparting an effect on the paracellular pathway in that there is a reduction in probe permeability with increased molecular weight. This type of effect has been previously shown in experiments by Lane, et. al., (19) in which rat intestinal permeability for various paracellular probes ranging from mannitol (MW 182.17) to inulin (MW 5000) were examined in the presence of penetration enhancers known to elicit tight junction opening. Additionally, Nakanishi et al. (20) showed that the paracellular enhancing effects of EDTA on rectal permeability decreased as the MW increased. At a certain MW, a cutoff in the permeability enhancement should occur due to the fact that the tight junction would still obtain its barrier characteristics. This is observed, in which the cutoff point occurs with PEG 4000.

CO_2 also induced a decrease in transepithelial electrical resistance (TEER). This is indicative that the epithelium is undergoing a morphological change where the integrity of the tight junction and/or cell membrane is disrupted (e.g. pores, protein leaching). According to previously mentioned results, there is no indication of cell membrane damage or perturbation due to contact between the tissue surface and CO_2 bubbles. Therefore, the only plausible explanation is a structural integrity effect on the tight junction. This result corresponds to previous experimental findings, where a decrease in intestinal TEER values correspond to changes in tight junctional integrity and enhanced permeability to hydrophilic drugs (21,22). These findings also support the increased permeability effect of CO_2 in which the hydrophilic drugs, mannitol and tetracycline, have greater enhancement in comparison to the hydrophobic drug, diazepam.

An important issue in delivering therapeutic agents in combination with a penetration enhancer is safety. Most penetration enhancers influence drug permeability by altering tissue morphology leading to breakdown in tissue integrity. The issues of most importance concern tissue irritation, membrane damage, and the rate of damage and recovery. Tissue recovery rate imparted by an absorption enhancer is related to the extent of toxicity (23). A favorable enhancer is one that induces minimal

toxicity with relatively fast tissue recovery rates (1–2 hours). This limits the opportunity for absorption of harmful environmental substances. Therefore, it is essential to study tissue recovery rates in addition to penetration enhancement effects.

The disruptive effect of CO_2 seem to be transient with epithelial barrier properties reestablished within a short period of time, 20 minutes. Therefore, carbonated delivery systems can be safely used, with rapid tissue recovery after effervescence discontinuation.

In conclusion, permeability enhancement induced by CO_2 bubbling was associated with an alteration of the paracellular pathway. This, in addition to fluid flow and membrane hydrophobicity concepts, may account for observed increases in drug flux.

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