Restoration of rat colonic epithelium after in situ intestinal installation of the absorption promoter, sodium caprate

Xuexuan Wang, Sam Maher, David J. Brayden*

UCD School of Agriculture, Food Science and Veterinary Medicine and UCD Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland

*Author to whom all correspondence should be addressed: David J Brayden
Room 214, Veterinary Sciences Centre
UCD, Belfield, Dublin 4, Ireland
Tel: +353 1 7166013
Fax: +353 1 7166219
Email: david.brayden@ucd.ie

Journal: Therapeutic Delivery
Category: Preliminary Communication
Abstract

**Background:** Sodium caprate (C\textsubscript{10}) is an oral absorption promoter that is currently in clinical trials as solid dosage forms for poorly permeable small molecules and peptides. Clinical data with zoledronic acid tablets suggest that significant delivery along with acceptable safety can be achieved from a once-a-week dosing regime. C\textsubscript{10} has surfactant-like properties at the high doses used *in vivo* and therefore we examined its effects on rat intestinal epithelium following instillation.

**Results:** Addition of 100mM concentrations of C\textsubscript{10} with the paracellular flux marker, FITC-dextran 4kDa (FD4), permitted bioavailability of 33% to be achieved. When C\textsubscript{10} was added 10, 30 and 60 min in advance of FD4, enhancement was still present, but was progressively reduced. Histology revealed that the permeability increase was likely related in part to superficial epithelial damage caused in the first few minutes of exposure, which was rapidly repaired within 30-60 min.

**Conclusions:** Design of optimized dosage forms containing C\textsubscript{10} should co-release the payload and promoter close to the epithelium in high concentrations. While C\textsubscript{10} induces some epithelial damage, the remarkable capacity for epithelial repair may render this effect insignificant *in vivo*.

**Keywords:** Sodium caprate, medium chain fatty acids, oral macromolecule delivery, absorption promotion, mucosal injury, epithelial repair
Introduction

One of the major challenges in drug delivery is the need for systems that improve oral bioavailability of poorly permeable molecules. Absorption promoters that increase epithelial permeability have been studied for nearly half a century, but to-date none have been approved. One of the most advanced candidates in clinical trials is sodium caprate (C\textsubscript{10}), the sodium salt of the aliphatic saturated medium chain fatty acid, capric acid (reviewed in [1]). C\textsubscript{10} has been successfully formulated in antibiotic rectal suppositories, which were marketed in Scandanavia and Asia [2, 3]. In preclinical studies, C\textsubscript{10} improved oral macromolecule permeation across Caco-2 monolayers [4], isolated rat and human intestinal mucosa [5], as well as in rat intestinal instillations and perfusions [6]. Recently, the effect of the promoter on oral bioavailability has been successfully demonstrated in man for enteric coated solid-dosage forms with a range of actives including antisense oligonucleotides [7], bisphosphonates [8], low molecular weight heparin [9] and the GnRH antagonist, acyline [10].

The study of co-presentation of promoter and cargo is important in designing solid-dosage formulations in order to optimize delivery of pay-load across the intestinal epithelium. Study of contemporaneous and/or delayed release of C\textsubscript{10} with its associated bioactive cargo may lead to improved designs. Furthermore, while C\textsubscript{10} does not appear to lead to significant or irreversible mucosal toxicity even at the high concentrations required to deliver drugs \textit{in vivo}, compromising the gastrointestinal barrier is often cited as a limitation to the potential use of intestinal promoters, at least in a repeat-dosing format [11, 12]. There is a perception (real or otherwise) that increases in epithelial
permeability, by either opening tight junctions through the paracellular pathway or, more significantly, by transcellular perturbation, may cause unintentional absorption of toxic dietary xenobiotics, microbial antigens or micro-organisms. It is notable that the size of the majority of these substances are far in excess of the molecular weight and diameter of the drugs whose permeation have been enhanced by C\textsubscript{10} to date, the exception being the enabling of virus transported across airway epithelia [13]. In vivo studies suggest that high mM concentrations of the agent required to enable delivery in animal models and human studies pertain more to surfactant-like transcellular mechanisms compared to paracellular effects triggered in vitro [1]. This is corroborated by studies in rodents where the promoter causes superficial mucosal injury [6]. Taking these data into consideration, there is considerable interest in assessing the rate of recovery of the epithelial barrier following enhancement by C\textsubscript{10} in relevant in vivo intestinal models.

The purpose of this study was therefore to examine the duration of C\textsubscript{10} promoting action on intact rat intestinal mucosa and the time taken for the epithelium to restore normal barrier function. We examined the kinetics of C\textsubscript{10}’s promoting action by measuring absorption of a marker molecule, FITC-dextran 4kDa (FD4), in an in situ colonic instillation study in anaesthetized rats. Gross histology was used to give visual evidence of changes to morphology over time. This study confirms that the enhancement window with C\textsubscript{10} is narrow, and that the compromised intestinal epithelial barrier of rats is rapidly restored to its native state within an hour.
Experimental

Animals

All animal experimental protocols were approved by the University College Dublin (UCD) Animal Research Ethics Committee and were approved under animal license from the Irish Department of Health and Children (license reference B100/3709). Male Wistar rats (200-250g) were purchased from the Charles River Laboratories Inc (Margate, UK). Animals were housed under controlled environmental conditions regarding humidity and temperature with a 12:12 hour light/dark cycle. Rats received tap water and standard laboratory chow *ad lib* unless otherwise stated.

Rat colonic instillations

*In situ* intestinal absorption studies were carried out as previously described with minor modifications [14]. Rats were fasted for 12 hours and anaesthesia was delivered by intraperitoneal injection of ketamine (75mg/kg) and xylazine (15mg/kg). Anaesthesia was maintained with isoflurane (1-2%) vaporized in oxygen and administered at a rate of 1.5 L/min. Following midline laparotomy, C\textsubscript{10} (100mM 0.2ml/100g body weight) was instilled into the colonic lumen with a 30G microfine needle for 0, 10 or 30 min before instillation of FD4 (5 mM) into the same segment. Blood samples (~0.2 ml) were withdrawn into heparinized vials by cardiac puncture, and centrifuged at 5000 x g for 15 min at 4°C. Fluorescence intensity of FD4 (\(\lambda_{\text{ex}}/\lambda_{\text{em}} 480/520\text{nm}\)) was measured in plasma samples diluted in borate buffer (0.1 M, pH 8.5) [15] in a spectrofluorimeter (MD Spectramax, Gemini). The concentration of FD4 was calculated from an external standard curve.
The peak concentration \( C_{\text{max}} \) and the time taken to reach \( C_{\text{max}} \) \( (T_{\text{max}}) \) were calculated from the plasma concentration profiles and the area under the plasma-concentration curve (\( \text{AUC}_{0-2\text{h}} \)) was calculated using WinNonLin 5.2® (Pharsight Corporation, USA). The absolute bioavailability (\( F \)) of FD4 over the two hour intestinal instillation \( (F_{0-2\text{h}}) \) was calculated as follows: 

\[
F_{0-2\text{h}} \ (\%) = \left( \frac{\text{AUC}_{0-2\text{h}}}{\text{AUC}_{\text{i.v.} \infty}} \right) \times 100
\]

where \( \text{AUC}_{0-2\text{h}} \) was area under the plasma concentration curve over the period \( (0-2 \ \text{h}) \) and \( \text{AUC}_{\text{i.v.} \infty} \) the area under the plasma concentration versus time \( (0-\infty) \) after i.v. administration of sterile filtered FD4 (40mg/kg) in saline [15].

**Histology of intestinal mucosa**

Intestinal sections were immediately removed from euthanized rats and were opened longitudinally along the mesenteric border, pinned onto a sheet of parafilm to secure orientation, fixed in formalin \( (10\% \ \text{v/v}) \), and embedded in paraffin wax. Tissue sections were cut at 5\( \mu \text{m} \) on a microtome, mounted on adhesive-coated slides, stained with haematoxylin and eosin and examined under light microscopy.

**Data analysis**

Unless otherwise stated, all experiments were carried out on three independent occasions and data was expressed as the mean ± standard error of the mean. Statistical significance was measured by two-tailed Student’s t-tests using GraphPad Prism 5® software and was designated at the level of \( P<0.05 \).
**Results and Discussion**

The capacity of C_{10} to promote the *in situ* absorption of poorly permeable solutes is widely reported across the GI tract of rats (reviewed in [1]). Basal F of FD4 (5mM) after colonic instillation for 120 min was 1%, with a C_{max} of 1.6 ± 0.8µg/ml (Fig. 1). Instillation of 100mM C_{10} with FD4 increased F by 33-fold to 33% and increased the plasma C_{max} by 44-fold to 71.8 ± 8.3µg/ml. In the majority of *in situ* drug delivery models, the promoter and cargo are contemporaneously delivered to the epithelium as aqueous mixtures. Pre-treatment of the intestinal epithelium with C_{10} for 10, 30 or 60 min followed by instillation of FD4 significantly and progressively reduced the enhancement effect of C_{10} as measured by F and enhancement ratios (Table 1). C_{max} averages for the 10, 30 or 60 minute pretreatments of C_{10} were 5-, 8.9- and 8.4-fold lower than when FD4 and C_{10} were co-administered, respectively. Sequential instillations therefore had less of an increase on the C_{max} and F of FD4 than was seen with co-administration, (Table 1). There was an indication of greater enhancement at shorter intervals between addition of the promoter and the payload (Table 1). Staggered administration of FD4 10-60 min after C_{10} instillation still led to an increase in F (4 to 8.7-fold), despite the degree of enhancement dissipating at longer time gaps. These data indicate that the greatest promoting action of C_{10} in an anaesthetized rat instillation model is best achieved when the promoter is co-presented to the intestinal epithelium with FD4 and not when it is presented as a pretreatment. C_{10} was not flushed out of the intestinal lumen before addition of FD4 in this study, but results were not significantly different to when we attempted to flush the lumen (data not shown). This may be because C_{10} is rapidly absorbed with a T_{max} of 10 min [16-18].
Spreading of the promoter/cargo mix in the intestinal lumen may have an impact on enhancement potential [19], as could unpredictable dilution effects in intestinal fluid volumes. This is particularly true in the small intestine of humans where, in addition to rapid absorption of the promoter, the fluid volumes (fasted 105ml, fed 45ml) have a considerable diluting effect compared with those of the large intestine (fasted 13ml, fed 11ml) and rectum (3ml) [20]. In addition, a rapid transit time could also prevent the optimal promoter/drug concentration being presented to the small intestinal epithelium [20-22]. Matching the promoter dissolution to that of the drug for co-release is therefore essential, otherwise they may never reach the intestinal mucosa together. The data in Fig. 1 and Table 1 suggest that this disparity between the rates of release from a solid dosage form could ultimately lead to the promoter quickly reaching the intestinal epithelium, reversibly increasing permeability, but ahead of arrival of payload, hence reducing overall efficacy. This suggests that formulations with C\textsubscript{10} should be designed to achieve the highest concentration of co-released promoter and cargo from onset of release.

The time for the intestinal epithelium to recover from increases in permeability and mucosal injury is under scrutiny since prolonged enhancement could potentially allow xenobiotics, toxins and pathogen entry across the gut wall. In Caco-2 monolayers incubated with C\textsubscript{10}, there was a time- and concentration relationship in recovery of transepithelial electrical resistance (TEER), a surrogate marker of epithelial permeability [23, 24]. Monolayers treated with C\textsubscript{10} (10mM) for 60 min recovered TEER values to 36% of maximum after 5 days, while at lower concentrations recovery was considerably shorter [24]. Similarly, recovery time was considerably shorter, and the extent of
recovery was greater when monolayers were incubated with C\textsubscript{10} for 10-20 min compared to 60 min [24]. In a separate study, the apparent permeability coefficient of FD4 across Caco-2 monolayers was over 7-fold greater at 120 min compared to 20 min in the presence of C\textsubscript{10} [4], which further indicates the impact of the incubation time on the magnitude of the increase in transport. In a rat \textit{in situ} jejunal loop study, promotion of phenol red absorption by C\textsubscript{10} was diminished after just 30 min, perhaps due to rapid absorption of the promoter itself [17]. This effect has also been described in colonic [17] and rectal [18] loops in rats. When cefazolin was co-perfused in jejunum with C\textsubscript{10} (50-100mM) the plasma concentration of the antibiotic significantly increased [25]. Upon cessation of the C\textsubscript{10} perfusion, the plasma levels of cefazolin decreased within 15-30 min. This was not the case with sodium dodecyl sulphate (SDS) however, as the absorption of cefazolin continued to increase following removal of the promoter and did not significantly drop over the following two hours. In a study of epithelial recovery after treatment with, SDS it was shown that the epithelium can take up to 4 hours to recover from an increase in transport [26]. Indeed, recovery of epithelial permeability from a transport induced state has not been demonstrated with all promoting agents to the same extent; for example, in a rectal perfusion with EDTA and PEG, permeability to sulfanilic acid had recovered to only 50\% and 66\% of control after 120 min [27]. Even promoters that are in the same structural class as C\textsubscript{10} do not behave in a consistent manner: both pre- and post incubation of a medium chain mono- di- and tri- glyceride mix of caprylate in the intestines of anesthetized rats for 10 min had no significant effect on cefmetazole absorption compared to co-presentation [28]. In a canine study, enteric-coated formulations containing C\textsubscript{10} led to an increase in the absorption of an oligonucleotide
(ISIS 104838, antisense to TNF-α) [29]. The rapid absorption kinetics of C₁₀ stimulated
design of a pulsatile formulation, characterized by an immediate release of high
concentrations of the promoter with the oligonucleotide cargo, followed by pulsed
replenishment of C₁₀ to sustain the enhancement window. In human patients, the
bioavailability of these formulations ranged from 7-12% [30].

The most conclusive data demonstrating the reversibility of C₁₀ action on permeability is
from a lactulose: mannitol urinary excretion ratio (LMER) study in man [9]. Intra-jejunal
administration of C₁₀ (500 mg) to human subjects increased the ratio up to 20 min after
C₁₀ administration, but not at 40–60 min. In the presence of gastrointestinal permeability
enhancement technology (GIPET®, Merrion Pharmaceuticals Ltd, Dublin, Ireland), a
number of poorly permeable drugs including zoledronic acid (Orazol®, Merrion
Pharmaceuticals, Ireland) have had their oral bioavailability increased in man, and this
was associated with an increase in the LMER by 50% [9]. In comparison with LMER
values seen with aspirin (146-760% [31]) and Crohn’s disease (520% [32]), the increase
in epithelial permeability by C₁₀ is smaller, reversible and disappears when it is absorbed
or removed.

There is a relationship between restoration of normal epithelial permeability and recovery
from epithelial injury [33]. Indeed, a permeability recovery model has been used to
quantify the damage caused by a surfactant in combination with morphology assessment
[26]. In addition to the data on reversibility of the permeability increase induced by C₁₀,
we therefore also assessed the effect of the promoter on gross histology of the epithelium.
Instillation of saline into the colonic lumen had no effect on mucosal morphology and the epithelial surface of control mucosae was unperturbed with no noteworthy damage to enterocytes or goblet cells; there was no sign of damage to the sub-mucosa (Fig. 2A). When the colonic mucosa was instilled with C\textsubscript{10} (100mM) for 10 min, there was some damage to the mucosal surface, with continuous necrosis, moderate cellular infiltration in the lamina propria and sub mucosa (Fig. 2B), confirming our previous dataset at 5 and 10 min [6]. At the longer time points of 30 and 60 min post-administration of C\textsubscript{10}, damage to the intestinal mucosa was rapidly repaired (Fig 2C, D), and this was consistent with the FD4 permeability data (Table 1). These data support similar conclusions made using the rat perfusion model for transport of phenol red in the presence of a detergent [26].

The safety of C\textsubscript{10} has been reviewed with the majority of studies examining toxicity at the experiment end point; a point at which there could be considerable epithelial repair [1]. Such studies may be less informative because of the remarkable capacity of the intestinal epithelium to recovery from injury ([34]), and even the most extreme cases of cellular damage and perturbation can be reversible [19]. The current study shows that C\textsubscript{10} can cause significant injury to the mucosal surface, but that that it is rapidly repaired. In a related study, the effect of C\textsubscript{10} on rat mucosal epithelium was also assessed by scanning- and transmission electron microscopy after 15 min jejunal instillations (unpublished data, personnel communication, Lloyd Tillman, ISIS Pharmaceuticals Ltd, Carlsbad, USA). C\textsubscript{10} did not significantly affect mucosal morphology, even at a considerably higher dose than administered in the current study. There is often disparity between promoter studies, which is confounded by the concentration of the promoter, and the experimental methods.
of the selected gut permeability model. For example, absorption of a 6kDa peptide with a proprietary enhancer platform was significantly greater when the intestinal segment was ligated [19]. Nevertheless, the data from the current study and from previous reports suggest that C10 can cause mucosal perturbation, but that the barrier is rapidly repaired. This is not unique to C10; the intestinal mucosae can recovery from injury caused by other transcellular promoters such as, taurodeoxycholic acid (TDC), SDS and nonylphenoxypolyoxyethylene [11, 26, 33]. However, recovery is often slower with surfactants that have a low critical micellar concentration (CMC), and furthermore, the safety profile of such agents when absorbed remains unclear. C10 itself, while rapidly absorbed, is a dietary fatty acid that is recognized as safe for addition to foods for human consumption, indicating systemic toxicity is unlikely to be an important consideration [1]. The cytotoxicity of C10 reported in cell cultures has led to criticism of the clinical potential of the promoter. However, data from static in vitro models do not effectively translate to in vivo models because of the presence of relevant repair mechanisms in vivo and the propensity for rapid dilution of C10 in the gut. This information has aided in the selection of suitable candidate drugs for enhancement with C10. Clinical trials with oral weekly doses of zoleadric acid were to some extent designed so that the epithelium has considerable time to repair from any injury caused by the promoter between doses [1]. It will be interesting to see safety data from long-term oral daily dosing studies of C10 with other pay-loads. Finally, it is important to put the injury caused by C10 in context with the damage caused by other dietary agents. Constituents of a healthy diet including; food substances, drugs, drug formulations and even bile salts lead to mucosal injury, but cycles of damage and repair are normal physiological processes. Despite such a comprehensive
number of studies involving $C_{10}$, what is unknown is the exact nature of its interaction with intestinal mucosae. Despite *in vitro* data indicating a paracellular mode of action, it is more likely that the promoter acts through transcellular perturbation *in vivo*. When it is in contact with mucosal phospholipid bilayers, the promoter behaves similar to other surface active agents, particularly when present at concentrations in the same order as its CMC. Enhancement may in fact also be due to a non-covalent interaction of either monomeric, micellar or vesicular $C_{10}$ with the candidate drug thereby altering its physicochemical properties to improve drug absorption across the epithelium. Concentrations of $C_{10}$ above its CMC form supra-molecular vesicles which could easily traffic drug across the epithelium. Predicting how $C_{10}$ behaves when in contact with the intestinal wall is difficult considering the variability in composition of lumen fluid particularly pH, lumen salt concentration, and the presence of other surfactants. Progress has been made with the development of fasted-state-simulating intestinal fluid (FaSSIF) and fed state-simulating intestinal fluid (FeSSIF), so it is reasonable to suggest that these should be the starting point for studies to understand the interaction of the promoter with the intestinal epithelium, while in parallel, attempting to understand the mechanism of mucosal injury and restitution.

**Conclusions**

The promoting action $C_{10}$ on permeability is concentration-dependent and is rapidly reversed in rat colonic instillations. The promoter and cargo should ideally be delivered to the epithelium together, and formulations should be designed to sustain the promoting window. Transiently increasing transmucosal permeability with $C_{10}$ is accompanied by
superficial mucosal injury. The intestinal epithelium has the capacity to rapidly recover from any mucosal damage sustained by treatment with C\textsubscript{10}. This provides further evidence that the enhancement action and damage caused by mild non-ionic surfactants like C\textsubscript{10} are closely related \textit{in vivo}, but whether this is a significant safety issue is debatable.

**Executive summary**

- C\textsubscript{10} improves the absorption of FD4, a poorly absorbed molecule
- The promoting action of C\textsubscript{10} is rapidly reversed following rat colonic instillation
- Co-presentation of C\textsubscript{10} with the selected cargo to the intestinal epithelium is essential for optimal promotion
- Delivery platforms that synchronize release of high concentrations of C\textsubscript{10} with cargo and maintain release of high promoter concentrations for a period could improve effectiveness
- C\textsubscript{10} causes mucosal injury in rat colonic instillation that is rapidly reversed
- Increased intestinal permeability and the mucosal injury associated with surfactants are closely related events
- The high capacity of the intestinal epithelium for repair suggests that damage caused by a promoter should be assessed over the entire time-course of its promotion
- Further studies are required in order to ascertain the \textit{in vivo} mechanism of promotion and epithelial repair
Future perspective

C₁₀ is currently one of the most advanced oral enhancers in clinical development for selected cargoes. It has generally recognized as safe (GRAS) status in dietary supplements and there is extensive knowledge from its widespread use as an excipient, which may suggest that it may have less safety hurdles than new chemical entity-type promoters. As a surfactant at high doses used in man, it seems to temporarily damage the epithelium to an extent, an effect unrelated to the tight junction opening action seen at low concentrations \textit{in vitro}. Future work will be focused on how the agent induces damage and how the restitution process takes place and is so effective. Even though C₁₀ is in clinical trials, cargoes will have to be carefully selected in terms of target oral bioavailability and an acceptable inter-subject variability. R & D programmes to design such dosage forms will therefore be primarily product-specific rather than platform-based. While there are a plethora of GRAS-type excipients at preclinical stages of research, innovative formulation design will be the key to progression to the clinic.

Acknowledgements

This work was financially supported by Science Foundation Ireland (Strategic Research Cluster Grant 07/SRC/B1154). David Brayden is a consultant to Merrion Pharmaceuticals, Dublin, Ireland.
Table 1: Pharmacokinetics of FD4 (5mM) in rat colonic instillation following either co-administration with C<sub>10</sub> (100mM) at 0 min, or pre-treatment with C<sub>10</sub> for 10-60 min (* P = 0.002; ** P =0.001, ***P< 0.0001, compared with C<sub>10</sub> at 0 min)

<table>
<thead>
<tr>
<th>PK parameter</th>
<th>C&lt;sub&gt;10&lt;/sub&gt; (0 min)</th>
<th>C&lt;sub&gt;10&lt;/sub&gt; (-10 minutes)</th>
<th>C&lt;sub&gt;10&lt;/sub&gt; (-30 min)</th>
<th>C&lt;sub&gt;10&lt;/sub&gt; (-60 min)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (min)</td>
<td>5</td>
<td>60</td>
<td>120</td>
<td>120</td>
<td>60</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (μg/ml)</td>
<td>71.8 ± 8.3</td>
<td>14.1 ± 4.9</td>
<td>8.0 ± 3.4</td>
<td>8.5 ± 3.6</td>
<td>1.7 ± 0.8</td>
</tr>
<tr>
<td>AUC (μg/ml.min)</td>
<td>5146.3 ± 545.7</td>
<td>1362.97 ± 325.4</td>
<td>667.367 ± 210.7</td>
<td>631.4 ± 191.6</td>
<td>156.1 ± 76.6</td>
</tr>
<tr>
<td>F (%)</td>
<td>33 ± 3.5</td>
<td>8.7 ± 2.1</td>
<td>4.3 ± 1.4</td>
<td>4.0 ± 1.2</td>
<td>1.0 ± 0.5</td>
</tr>
<tr>
<td>Enhancement ratio</td>
<td>33</td>
<td>8.7*</td>
<td>4.3**</td>
<td>4**</td>
<td>1***</td>
</tr>
</tbody>
</table>
Figure Legends

**Fig. 1:** Effect of C₁₀ (100mM) pretreatment on the absorption of FD4 (5mM) following rat colonic instillations. (A) Plasma concentration of FD4 (µg/ml) following either co-administration (0 min) with, or pretreatment with C₁₀ for 10, 30 or 60 min and (B) log concentration of FD4 (µg/ml) in plasma. Each value represents the mean ± SEM of 3 independent experiments. Symbols: ○ FD4 alone; ■ FD4 and C₁₀ (co-administration); ◆ FD4 and C₁₀ (10 min pre-treatment); ▼ FD4 and C₁₀ (30 min pre-treatment); ▲ FD4 and C₁₀ (60 min pre-treatment).

**Fig. 2:** Light micrographs illustrating the effect of C₁₀ on morphology of the rat colonic epithelium following instillations of (A) saline control, (B) C₁₀ (100 mM) after 10 min (C) C₁₀ (100 mM) after 30 min, (D) and C₁₀ after 60 min. Horizontal bars = 250 µm.
Fig. 1
Fig. 2
References


8. McHugh C, Madigan K, Walsh A, Fox J, Leonard TW, Quint JB: Mer-101, a multicenter, Phase II study to compare Mer-101 20mg tablets to intravenous zoledronic acid 4mg in prostate cancer patients. *J. Clin. Oncol. 27* (15 suppl.) 5161 (2009). **Important clinical data showing that a GIPET-bisphosphonate formulation given once a week orally can achieve similar efficacy and safety profile as a monthly injection.**


23. Anderberg EK, Lindmark T, Artursson P: Sodium caprate elicits dilatations in human intestinal tight junctions and enhances drug absorption by the paracellular route. Pharm. Res. 10(6), 857-864 (1993). *One of the first papers looking at C_{10} in Caco-2 and making the link to tight junction opening in vitro.*


**Comprehensive review on mechanisms of intestinal recovery.**