Mucosal Drug Delivery

Vincent H. L. Lee

This review focuses on epithelial drug transport mechanisms in mucosal drug delivery: the final step of a four-part process. Reference is made to the mucosa lining the oral cavity and the gastrointestinal tract, the two mucosae most often succumbing to the side effects of cytotoxic chemotherapeutic drugs. This review will be devoted to carrier-mediated transport, particularly as it relates to the intestinal dipeptide transporter PepT1. This transporter protein appears to be enriched in tumor epithelial cells, to be rather robust to the cytotoxic effects of chemotherapeutic drugs, and to lend itself to the molecular engineering of drugs that target this transporter in tumor epithelial cells. In contrast to the gastrointestinal tract, much less is known about the type and capacity of drug transport processes in the buccal epithelial cells and about how these processes may be altered in disease state (including cancer) and be manipulated pharmaceutically to optimize drug absorption. [J Natl Cancer Inst Monogr 2001;29:41–4]

Mucosal drug delivery is a multistep process. It comprises (a) targeting of the delivery system at a specific region or cell type in a mucosa, (b) retention of that delivery system where it is anchored, (c) drug release from the delivery system at a predeterminded pattern that is not necessarily constant, and (d) access of the drug to the drug-transport machinery in the epithelial cells. In the context of this conference, I will focus on the gastrointestinal tract and the oral cavity, the two mucosae that often succumbed to the side effects of chemotherapy. While targeting drug release to either the small or the large intestine can be achieved by exploiting the broad biochemical differences between those two regions in the gastrointestinal tract, such as pH and microbial enzyme content (1,2), we do not yet know the unique biochemical markers in each of the three parts of the small intestine to target drug release at a given part of this organ. The same can be said of the large intestine. In contrast, targeting drug release to a specific region in the oral cavity is relatively straightforward, given its accessibility. In light of the differences among the buccal, sublingual, gingival, and palatal tissues in the oral cavity, as shown in Table 1, it is expected that drug bioavailability would vary with the tissue of exposure.

Epithelial Drug Transport Mechanisms

However sophisticated the drug delivery system is with respect to targeting, retention, and pattern of drug release, there must be a match between the drug’s physicochemical characteristics and the epithelial cell’s endogenous transport mechanisms for drug uptake and transport to occur. These mechanisms fall into two categories: passive transport and active transport. Passive transport, in turn, comprises paracellular and transcellular transport, while active transport comprises carrier-mediated transport and endo-transcytosis. In general, the epithelial transport of any drug can be considered as the sum of all four transport processes, although in reality, only one, or at most two, of the four transport mechanisms would predominate. Transforming growth factor-β3 (TGF-β3), a 25-kd protein, apparently reduced the severity of oral mucositis induced in a hamster model (3) sufficiently enough to implicate a role of endocytosis in protein uptake by the buccal epithelial cells. Nevertheless, the possibility that this protein does not need to enter the cells to bring about a pharmacologic effect cannot be ruled out. In the study just cited, recombinant TGF-β3 (20 μg) was applied topically to the hamster cheek pouch as a 7.9-μM solution in 0.1 mL physiologic-buffered saline containing 0.01% Tween 20 and 1% ethanol four times daily over a 24-hour period before chemotherapy with an intraperitoneal dose of 5-fluorouracil (5-FU) at doses of 60–80 mg/kg on day 0 and 40–60 mg/kg on day 2.

The Case for Carrier-Mediated Transport

Carrier-mediated transport is an area of active research in drug delivery today. Carrier-mediated transport, either Na+- or H+-coupled, may be playing a more prominent role in drug transport than originally envisaged (4). For instance, benzoic acid, a weak acid that is predicted to be absorbed exclusively by transcellular passive transport in accordance with the pH-partition hypothesis, actually mainly relies on the H+-monocarboxylate transporter for absorption (5). The associated $K_m$ is 1.28 mM. It is interesting that a monocarboxylate transport system may also exist in cultured buccal epithelial cells in the rabbit (6). Uotoguchi et al. (7) subsequently verified the involvement of carrier-mediated transport in the uptake of salicylic acid from the hamster cheek pouch, followed by drug appearance in the systemic circulation. This finding sets the stage for searching for other drug transporters in the buccal epithelial cells that may be of utility in facilitating drug uptake.

The Intestinal Dipeptide Transporter PepT1 as an Illustrative Example

The intestinal dipeptide transporter PepT1 is perhaps the drug transporter that has captured the most attention toward mucosal drug delivery recently. This H+-coupled transporter has a rather wide substrate specificity (8–15), including dipeptides and tripeptides, β-lactam antibiotics, angiotensin-converting enzyme inhibitors, and bestatin and even compounds without an obvious peptide bond or equivalent, such as δ-aminoevulinic acid (16) and ω-amino fatty acids (ω-AFA) (17). In the small intestine, the population of this transporter increases from the duodenum to the ileum (18).

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Table 1. Oral epithelium characteristics

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Structure</th>
<th>Thickness, μm</th>
<th>Blood flow, mL min⁻¹ cm⁻²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buccal</td>
<td>Nonkeratinized</td>
<td>500–600</td>
<td>2.4</td>
</tr>
<tr>
<td>Sublingual</td>
<td>Nonkeratinized</td>
<td>100–200</td>
<td>0.97</td>
</tr>
<tr>
<td>Gingival</td>
<td>Keratinized</td>
<td>200</td>
<td>1.47</td>
</tr>
<tr>
<td>Palatal</td>
<td>Keratinized</td>
<td>250</td>
<td>0.89</td>
</tr>
</tbody>
</table>

*Adapted from (31).
†Thickness of human oral epithelium (32).
‡Blood flow in oral mucosa of the rhesus monkey (33).

Enrichment in Tumor Epithelial Cells

Nakanishi et al. (19) and Gonzalez et al. (20) demonstrated independently that PepT1 appeared to be enriched in cancer epithelial cells. Specifically, Nakanishi et al. (19) observed [¹⁴C]Gly-Sar uptake by the human fibrosarcoma cell line HT1080 but not by IMR-90 (a normal diploid cell line), with a $K_m$ of 11.4 ± 3.3 μM and a $V_{max}$ of 26.8 ± 4.0 nmol/15 minutes per milligram protein. Optimal dipeptide uptake was at pH 6 and was subject to competition by cefadroxil and bestatin. Similar observations were made by Gonzalez et al. (20) in two human pancreatic cancer cell lines—AsPc-1 and Capan-2. The $K_m$ was 0.80 ± 0.17 μM for AsPc-1 and 1.0 ± 0.2 μM for Capan-2, and the corresponding $V_{max}$, was 65 ± 4.4 nmol/10 minutes per milligram protein and 10.9 ± 1.0 nmol/10 minutes per milligram protein, respectively. The enrichment of PepT1 in cancerous epithelial cells represents a target of drug design for the specific delivery of peptidomimetic anticancer drugs into tumor cells. Such a strategy has been applied to improving the intestinal uptake of the nucleoside analogues acyclovir and zidovudine (AZT) by forming $\beta$-amino acid ester prodrugs (Fig. 1) (21). There was a threefold to 10-fold increase in intestinal permeability that was selective for the l-amino acid esters.

Resistance to Cytotoxic Effects of Chemotherapy

For reasons that are not immediately forthcoming, PepT1 appears to be more resilient to the cytotoxic effect of chemotherapy than do other membrane-bound proteins. Tanaka et al. (18) investigated the mechanism of the resistance of PepT1 to mucosal injury in the intestine of rats treated with an oral dose of 300 mg/kg 5-FU. These investigators attributed such a resistance to increased synthesis of PepT1 rather than to a change in the kinetic properties of the residual absorbing cells. Specifically, although the amount of sucrase and a Na⁺-dependent glucose transporter protein in intestinal vesicles decreased markedly after 5-FU treatment, the amount of PepT1 protein remained largely unaffected. Moreover, levels of amino acid, glucose, and phosphate transporter messenger RNAs (mRNAs) were profoundly depressed in 5-FU-treated animals, whereas the level of PepT1 mRNA conversely increased. Coincidentally, Ihara et al. (22) reported that PepT1 gene regulation was substantially enhanced under malnourishment in spite of atrophic changes of intestinal mucosa in Sprague-Dawley rats.

Subcellular Compartmentalization and Its Modulation

The apical plasma membrane is not the only subcellular component in which PepT1 is found. PepT1 also exists in the lysosomal membrane (23–25) (Fig. 2). This subcellular compart-mentalization of PepT1 raises the questions of whether the ratio of PepT1 population between the lysosomal and apical plasma membrane is static, how this ratio may be altered pharmacologically, and what role biopolymers may play in altering this ratio. We do not yet have information on whether the compartmentalization of PepT1 in the intestinal epithelial cells is static. Nevertheless, there is evidence for the pharmacologic alteration in the density of PepT1 at the apical plasma membrane.

The acute translocation of PepT1 from the intracellular PepT1 pool to the apical surface was reported by Thamotharan et al. (26) in 1999. They found that preincubation of Caco-2 cells with 5 nM insulin for 1 hour stimulated Gly-Gln uptake by 80% (Fig. 3), consistent with an elevation of the apical expression of PepT1 by the same magnitude. This effect manifested itself within 60 minutes. There was no change in the mRNA level PepT1. Moreover, disruption of the trans-Golgi network (TGN) with 5 μM brefeldin A, thereby halting the migration of newly synthesized PepT1 to the apical membrane, did not affect either the basal or insulin-stimulated dipeptide uptake. In contrast, 10 μM colchicine, which depolarized microtubules (MTs), abolished insulin-stimulated dipeptide uptake, even though it did not have any effect on basal dipeptide uptake. This finding suggests that insulin may stimulate the translocation of PepT1 to cell surface in an MT-dependent manner.

![Fig. 1. Chemical structures of acyclovir (ACV) and zidovudine (AZT) and their intestinal membrane permeabilities in comparison with their prodrugs in rats. Error bars denote mean ± standard error of the mean for n = 4–6. Adapted from (21).](image-url)
As another example of pharmacologic manipulation of the ratio of PepT1 in the apical plasma membrane and the intracellular pool, Fujita et al. (27) recently reported that a selective σ₁ ligand, (+)pentazocine, increased the uptake of Gly-Sar in Caco-2 cells in a concentration-dependent (0.001–10 μM) and time-dependent (1–24 hours) manner. A minimum of 2 hours of incubation was required, and the maximal increase in dipeptide uptake was 200%. Kinetically, this can be attributed entirely to an increase in the maximum velocity of dipeptide uptake. Semi-quantitative reverse transcription–polymerase chain reaction suggests that (+)-pentazocine up-regulates PepT1 in Caco-2 cells at the level of increased mRNA.

As an alternative to the use of drugs to manipulate the density of PepT1 at the apical plasma membrane, biopolymers may be considered. Such a speculative role for biopolymers is based on the observations that chitosan, as well as its derivative, enhances paracellular permeability in Caco-2 cells (28) and that chitosan enhances the transcytotic capacity of Calu-3 cells (29). As can be seen in Fig. 4, incubation of Caco-2 cell monolayers with N-trimethylchitosan (TMC), a water-soluble chitosan derivative, resulted in pronounced and immediate reduction in transepithelial electrical resistance (TEER) in a concentration-dependent manner over the 1.5%–2.5% range (30). Concentrations of 1% or less were ineffective. Reversibility of the TEER-lowering effect was evident at 1.5% and 2% on removing TMC from making contact with the cell monolayer. Monolayer exposed to 2.5% TMC was slower in recovery. Using a human airway epithelial cell line (Calu-3), Witschi and Mrsny (29) found that spray-dried chitosan microspheres (14%–17% acetylation, molecular weight 300 000), 2–4 μm in diameter, enhanced the transport of bovine serum albumin by 20 times over a 6-hour period. Induction of the release of cytokines, such as interleukin...
(IL)-6 and IL-8, was suggested to be the triggering factor. It would be interesting to evaluate whether chitosan also affects the trafficking of membrane-bound transporter proteins such as PepT1.

**CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH**

Clearly, there is effective transport machinery in the intestinal epithelial cells to facilitate the absorption of a diverse array of therapeutic molecules. By contrast, much less is known about the basal capacity of the various transport processes in the epithelial cells lining the various regions of the oral cavity and about how this capacity may be altered during oral mucositis. This subject deserves further study. It is equally important to investigate how these transport processes in both epithelia may be altered in patients undergoing chemotherapy and to determine whether such possible alteration may be exploited to protect those epithelial cells from further insult with the use of chemoprotective drugs that would seize on an altered transport pathway.

**REFERENCES**


**NOTE**

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