PHARMACOKINETICS, PHARMACODYNAMICS AND DRUG METABOLISM

Increased Paracellular Absorption by Bile Salts and P-Glycoprotein Stimulated Efflux of Otilonium Bromide in Caco-2 Cells Monolayers as a Model of Intestinal Barrier

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ABSTRACT: The present study investigates the intestinal permeability of otilonium bromide, a spasmolytic drug used to treat irritable bowel syndrome, across Caco-2 cell monolayers. The amount of otilonium bromide transported was determined by high-performance liquid chromatography-mass spectrometry. Epithelial barrier integrity was estimated by measuring transepithelial electrical resistance and the transport of reference compounds, P-glycoprotein activity by measuring rhodamine 123 efflux. Results showed that the apparent permeability of otilonium bromide was comparable to that of our zero permeability marker, inulin, in the apical-to-basal direction and similar to that of rhodamine 123 in the basal-to-apical direction. The P-glycoprotein substrate, verapamil, prevented otilonium bromide efflux and, conversely, otilonium bromide inhibited P-glycoprotein activity. Bile salts induced a transient opening of tight junctions, as measured by selective increase of paracellular transport, and significantly enhanced the absorption of otilonium bromide. In turn otilonium bromide potentiates the effect of bile salts on tight junctions without modifying their critical micellar concentration or altering cell viability. In conclusion, otilonium bromide is a paracellularly transported drug whose absorption, in amounts sufficient to exert a spasmolytic effect, is favoured by bile salts. P-glycoprotein, by stimulating efflux, contributes to remove excess compound, restraining its distribution and site of action to the intestinal wall. J Pharm Sci 97:4087–4100, 2008

Keywords: otilonium bromide; Caco-2 cells; bile salts; intestinal absorption; permeability; P-glycoprotein; paracellular transport

Abbreviations: ABC, ATP-binding cassette protein; CMC, critical micelle concentration; EDTA, disodium ethylenediaminetetra-acetate; EGTA, ethylene-glycol-*O*,*O*′-bis(2-aminoethyl)-*N*,*N*,*N*′,*N*′-tetraacetic acid; FCS, foetal calf serum; FITC, fluorescein isothiocyanate; HBSS, Hank's balanced salt solution; LDH, lactate dehydrogenase; P_{app}, apparent permeability coefficient; PBS, phosphate buffered saline; TEER, transepithelial electrical resistance.

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INTRODUCTION

Otilonium bromide is a quaternary ammonium salt,[1] possessing spasmolytic properties, that is used for the treatment of irritable bowel syndrome (IBS) because of its selectivity toward the colon.[1–3] Indeed, the colon is more sensitive than other gastrointestinal tracts to the relaxing action of otilonium bromide and rather than vascular or respiratory smooth muscle.[4,5] Otilonium bromide inhibits the contraction of intestinal smooth muscle regardless to the nature of the agonist that initiates the spasmogenic stimulus since it possesses a wide spectrum of action and exerts a potent local calcium blocking, antimuscarinic and tachykinin antagonist effect.[4,6–8] Otilonium bromide has been shown to inhibit the contractility of the colonic circular smooth muscle by interfering with the mobilisation of calcium from intra- and extracellular sources induced by three main receptors for excitatory transmitters, that is muscarinic and tachykinin NK_1 and NK_2 receptors.[8] Moreover otilonium bromide binds in a competitive manner and blocks voltage-operated calcium channels[4,7–9] and it may also modulate physiologically active calcium signalling pathways involved in the stimulated secretion of water and ions at colonic epithelium level.[10]

Both preclinical[4] and clinical studies,[1,11] have shown that, when orally administered at doses producing spasmolytic action, otilonium bromide lacks both central and peripheral systemic side effects typical of other antimuscarinic and calcium-blocking drugs. This may be explained by the poor systemic absorption of the drug as substantiated by two studies in man showing that otilonium bromide plasma levels after oral administration were undetectable.[11,12]

Indeed, *in vitro* and *in vivo* studies performed using the ^{14}C-labeled compound showed a selective distribution for the large intestine and poor systemic absorption after oral administration as witnessed by the radioactivity found almost exclusively in the gastrointestinal tract, in the inner layer of the colonic circular muscle and submucosa and by the very low values of total radioactivity in blood and plasma.[13,14]

The intestinal epithelium represents the principal barrier to the delivery of drugs into the systemic circulation and to their effective oral bioavailability. Drugs can cross this barrier essentially by diffusion either through the cell membranes via the transcellular route, for lipophilic molecules, or through the intercellular space via the paracellular route, for small hydrophilic ones. While specific carrier proteins present in the intestinal epithelium are responsible for the active absorption process of nutrients (e.g. amino acids, di/tripeptides, glucose, etc.), some drugs can be substrates for multidrug resistance proteins that actively secrete them into the intestinal lumen for elimination.[15]

The differentiated Caco-2 intestinal cell line, derived from a human colon carcinoma, is the most frequently used for drug absorption studies to evaluate the potential oral bioavailability of new synthetic molecules.[16] The popularity of this model is mainly due to its ability to undergo spontaneous enterocytic differentiation in culture and to display many structural and functional properties of mature human intestinal epithelial cells.[17–19] Differentiated Caco-2 cells form a highly polarised monolayer of cells exhibiting apical microvilli with brush border membrane and also tight junctions that made them impermeable to solutes. Moreover, they possess a wide panel of enzymes and transport proteins, specific to the enterocyte and differentially distributed at either the apical or the basolateral site of the cells, making them a valuable transport model system for the small intestinal epithelium. When cultured on filters separating two compartments, they form a monolayer simulating the intestinal barrier and allow rapid and mechanistic evaluation of drug absorption compatible with screening studies and for investigating the relationship between the molecular structure, physicochemical properties, and absorption potential of compounds in humans. Different groups have demonstrated its usefulness in mimicking intestinal absorption in humans as indicated by comparison studies between *in vivo* (human small intestine) and *in vitro* (Caco-2 cells) intestinal absorption of a variety of drugs.[20–22]

This study aimed to investigate the characteristics that allow otilonium bromide to exert full efficacy and selectivity as spasmolytic agent at intestinal level but apparently without systemic absorption, by using Caco-2 cells, a suitable *in vitro* model to investigate human drug intestinal permeability. To further characterise the transport of otilonium bromide through the intestinal epithelia we have evaluated the apparent permeability of the drug in more physiological conditions by including bile salts to the transport buffer.