Brief/Technical Note

Effect of Ethanol on Opioid Drug Permeability Through Caco-2 Cell Monolayers

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INTRODUCTION

Several articles have shown that alcohol can alter the pharmacokinetics and bioavailability of drugs through changes in gastric emptying, gastrointestinal motility, drug metabolism and solubility, and hepatic clearance (1–5). Acute and chronic ethanol exposure cause functional and morphological damage to the gastrointestinal mucosal surface through mucosal injury, epithelial membrane integrity, and disruption of the tight junction barrier (6–8). Co-ingestion of alcoholic beverages with certain extended-release drug product formulations may accelerate drug release and affect the rate of its gastrointestinal absorption (9). Also, ethanol may enhance the adverse effects of opioids (10).

When Opana® (oxymorphone) tablets were co-ingested with 4–40% ethanol, there were variable effects on the area under the concentration–time curve (AUC) and maximal plasma concentration ($C_{\max}$) (11). The increase in $C_{\max}$ due to ethanol was seen with another extended release oxymorphone product with no effect on AUC (12). Elevated $C_{\max}$ was seen with 14% and 40% ethanol when taken with oxycodone multiparticulate capsules (13). A minimal effect on overall exposure was seen with a controlled release OROS® hydromorphone formulation with 4–40% ethanol with an increase in $C_{\max}$ (9).

The objective of this project was to determine whether the presence of ethanol alters the *in vitro* permeability of model opioid drugs. Oxycodone and hydromorphone have moderate intestinal permeabilities with a bioavailability of 60–87% and 62%, respectively, whereas oxymorphone has a low bioavailability of 10% (11,14,15). Atenolol and metoprolol

experiments were carried out to evaluate alcohol effects on low and high permeability drugs, respectively (16).

MATERIALS AND METHODS

Oxycodone HCl, hydromorphone HCl, metoprolol tartrate, and atenolol were from Sigma Chemical Co. (St. Louis, MO). Oxymorphone HCl was from Mallinckrodt (Hazelwood, MO). Anhydrous absolute ethanol was from McCormick Distilling Co., Inc. (Weston, MO). All cell culture media, buffers and reagents were purchased from Life Technologies, Inc. (Grand Island, NY). Caco-2 cells were from American Type Culture Collection (Manassas, VA) and used at passage numbers 29–56. Transwell® polycarbonate filters (12 mm diameter, 0.4 μm pore) were from Corning Inc. (Corning, NY). High purity HPLC solvents were from Fisher Scientific (Fairlawn, NJ) or Burdick & Jackson (Muskegon, MI).

HPLC assays were conducted on an Agilent 1100 system (Wilmington, DE). Separation of the opioids was completed on a 4.6×250 mm Zorbax® Extend-C18 column (Agilent) with 5 μm particle size at 30°C. The mobile phase was composed of equal volumes of 20 mM ammonium bicarbonate (pH 10.5) and methanol delivered at a rate of 1.0 mL/min. Detection was at 238 nm with an injection volume of 50 μL . HPLC assays for atenolol and metoprolol were conducted with a 5 μm particle size 4.6×150 mm Luna® C8(2) column (Phenomenex, Torrance CA) (16). The mobile phase contained 10% acetonitrile in 10 mM phosphate buffer (pH 3.0) with flow maintained at 1 mL/min. The injection volume was 10 μL and monitored with a fluorescence detector at $\lambda_{\text{ex}}/\lambda_{\text{em}}$ of 228/320 nm.

The Caco-2 cells and monolayers were cultivated at 37°C in a humidified atmosphere with 5% CO₂. The culture media was comprised of Dulbecco's modified Eagle's medium, 0.1 mM nonessential amino acids, 1% penicillin–streptomycin solution, 10 mM sodium bicarbonate and 10% fetal bovine serum. The culture media on the cells and monolayers was replaced three times a week. The Transwell® filters were seeded at a density of approximately 84,000 cells and allowed to grow for 21 days (16).

The transport buffer was comprised of Hank's balanced salt solution, with the apical (AP, pH 6.8) and basolateral (BL,

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pH 7.4) buffers supplemented with 10 mM 2-(*N*-morpholino) ethane-sulfonic acid and 25 mM *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethane-sulfonic acid, respectively. For the transport study, the culture media was replaced with transport buffers and the monolayers incubated for 30–60 min at 37°C. Transepithelial electrical resistance (TEER) was then measured (Millipore Millicell®-ERS, Bedford, MA) in each monolayer.

The buffer in the AP chamber of each well was then replaced with 0.16 mg/mL (opioids) or 0.4 mg/mL (atenolol, metoprolol) drug solution in AP buffer along with 0%, 1%, 3% or 5% ethanol using five to six wells per group. The plates were returned to the 37°C incubator on a plate shaker for agitation during the transport experiment. At 15, 30, 60, 90, 120 and 180 min, the filter inserts were transferred to a new well containing pH 7.4 buffer. P_{app} ($\times 10^{-6}$ cm/sec) was calculated as follows:

$$P_{app} = \frac{V_R}{(A \times C_0)} \times \frac{dC}{dt}$$

where V_R is the receiver chamber volume, A is the filter surface area, C_0 is the initial donor concentration, and dC/dt is the slope of the linear portion of the concentration vs. time curve. Analysis of variance (ANOVA) with Tukey's Studentized range test was applied to the data using SAS® (v. 9.1, SAS Institute, Cary, NC). ANOVA assumptions of normality and homoscedasticity were verified by normal probability plots using Excel 2003 (Microsoft® Office, Redmond, WA) and Bartlett's Test using SAS®, respectively.

RESULTS AND DISCUSSION

The effect of ethanol on Caco-2 cell monolayer integrity was examined by measuring monolayer TEER values over a

Fig. 1. Effect of ethanol on Caco-2 cell monolayer TEER values. 0% (filled circles), 1% (hollow rectangles), 3% (filled triangles), 5% (filled diamonds), 10% (hollow circles), 20% (hollow triangles), 40% (filled rectangles). Mean $\pm$ SD of four wells

Fig. 2. Permeability of (a) oxycodone, (b) oxymorphone, (c) hydromorphone, (d) atenolol and (e) metoprolol through Caco-2 cell monolayers in the absence and presence of ethanol. Mean $\pm$ SD of five to six wells

3-hour period. There was a decrease on TEER over time in all the groups due to the presence of alcohol. (Fig. 1) A concentration-dependent decrease in TEER was also detected by Ma *et al.* and Yamashita *et al.* with 1–10% ethanol over 1–6 hours (8,17).

The addition of 3% ethanol significantly increased the permeability ($11.0 \pm 0.60 \times 10^{-6}$ cm/sec) of oxycodone ($p < 0.05$, Fig. 2A). Ethanol increased the P_{app} of oxymorphone ($1.43 \pm 0.08 \times 10^{-6}$ cm/sec) in a concentration-dependent manner ($p < 0.05$, Fig. 2B) The transport of hydromorphone ($31.2 \pm 1.6 \times 10^{-6}$ cm/sec) through the cell monolayers was not altered by the presence of 1–5% to 5% ethanol (Fig. 2C) The permeability of atenolol ($1.11 \pm 0.19 \times 10^{-6}$ cm/sec) through the monolayers was significantly enhanced with increasing ethanol amount ($p < 0.05$, Fig. 2D). Ethanol had no effect on the transport of metoprolol ($12.4 \pm 0.7 \times 10^{-6}$ cm/sec) through the monolayers. (Fig. 2E)

In the presence of 7.5% ethanol, the transport of low permeability mannitol through Caco-2 cell monolayers increased over 60 min (8). In contrast, ethanol (2–10%) had no significant effect on the P_{app} of highly permeable dexamethasone (17). Low, non-toxic doses of ethanol ($\leq 10\%$) can cause an opening of the tight junction barrier resulting in increased solute permeability through the gastrointestinal epithelium (9,18).

These *in vitro* results were consistent with those observed from clinical bioavailability studies. There was a dose-dependent increase in $C_{\max}$ for hydromorphone when co-ingested with 4–40% ethanol (11,12). In this permeability study, oxymorphone permeability increased as the ethanol increased. (Fig. 2B) A 13% increase in $C_{\max}$ was seen clinically with oxycodone in the presence of 5% ethanol with greater increases at 14% and 40% ethanol (13). *In vitro*, there was increased oxycodone permeability only with 3% ethanol. (Fig. 2A) Plasma hydromorphone concentrations were slightly higher after dosing with an OROS® formulation of hydromorphone with solutions of 4–40% alcohol with no effect on overall AUC (9). *In vitro* there were no significant effects on hydromorphone permeability in the presence of 1–5% ethanol. (Fig. 2C)

CONCLUSION

Ethanol significantly increased the permeability of oxycodone, oxymorphone and atenolol in a concentration-dependent (1–5%) manner. The permeability of hydromorphone and metoprolol were not altered by ethanol. These results suggest that alcohol increases the transport of low permeability drugs through Caco-2 cell monolayers, consistent with results from clinical bioavailability studies. Thus, cell permeability assay can assist in determining the influence of low ethanol concentrations on drug transport through the intestine.

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