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Regional permeability of coenzyme Q10 in isolated rat gastrointestinal tracts

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The objective of the study was to identify the region with the maximum permeability for low bioavailable coenzyme Q10 (CoQ) in the gastrointestinal tract. To evaluate the regional differences in permeability, male Sprague-Dawley rats, 250–300 g, were anesthetized and the gastrointestinal segments were isolated. Stomach, duodenum, jejunum, ileum and colon tissues were mounted on a Navicyte side-by-side diffusion apparatus. Radiolabeled CoQ (1 μM in DMEM, pH 7.4, 37 °C was added to the donor side and the samples withdrawn from the receiver compartment at predetermined time intervals were analyzed using a scintillation counter. Membrane integrity was monitored by ^{14}C-mannitol permeability. The apical to basal permeability coefficients (Papp $\times 10^{-6}$, cm/s) were 0.32 ± 0.13, 3.14 ± 0.89, 1.36 ± 1.4, 0.83 ± 0.40, and 1.59 ± 0.13, for CoQ through rat stomach, duodenum, jejunum, ileum, and colon tissues respectively. The basolateral to apical permeability coefficients (Papp $\times 10^{-6}$, cm/s) were 1.6 ± 0.2, 2.2 ± 1.2, 0.88 ± 0.12, 1.6 ± 0.42, and 1.9 ± 0.41 respectively. Therefore the region of maximum CoQ permeability is duodenum followed by colon and ileum. Jejunum and stomach regions also have fairly high permeability. Therefore CoQ formulations should be made with an aim to target the duodenum to get maximum dosage effect.

1. Introduction

Targeting of drugs to various sites of the gastrointestinal tract (GIT) requires preliminary studies on intestinal permeability and determination of the site of maximum drug permeability. Intestinal permeability is considered to play a crucial role in determining the overall absorption of orally administered drugs. Permeability variation in the penetration barriers to the drugs which have carrier-mediated permeability may be attributed to differential levels of expression of transporters in different GIT segments. Regional variations in expression of enzymes and transporters are reported to influence the absorption of drugs such as digoxin and paclitaxel (Stephens et al. 2002). For instance, changes in intestinal absorption of iron have been reported to correlate with alterations in expression of divalent-metal transporter 1 (DMT1) and ferroportin 1 (FP1) transmembrane transporters in the duodenum (Zoller et al. 2001). Variability in calcium absorption has been reported to be due to variable expression of calcium transport protein, CaT1 in duodenum (Barley et al. 2001).

Coenzyme Q_{10} (2,3 dimethoxy-5 methyl-6-decaprenyl benzoquinone), also known as ubidecarenone, is a highly lipophilic antioxidant with poor aqueous solubility and slow absorption leading to low and variable oral bioavailability (Folkers et al. 1995; Overad et al. 1999). It is currently being used as a nutritional supplement which has gained popularity for its beneficial effects in many cardiovascular neurodegenerative diseases (Sarter, 2002). The distribution of radiolabeled CoQ following intraperitonial administration in rats was reported by Bentinger et al. (2003). CoQ has low bioavailability following oral administration and distributes widely throughout the body. CoQ known to have antioxidant and membrane stabilizing properties is the only endogenously produced lipid with a redox function in mammals. All cells are capable of synthesizing CoQ and no redistribution between organs occurs through the blood. CoQ is necessary for adenosine triphosphate (ATP) production. Animal studies demonstrate a bioavailability of 2–3%. With high doses of dietary CoQ, the blood concentration in both rats and humans can be increased about 2- to 4-fold. Following absorption from the GI tract, CoQ is taken up by chylomicrons. The major portion of an exogenous dose of CoQ is deposited in the liver and packaged into VLDL lipoprotein. The studies on metabolism of exogenous CoQ and the effect of exogenous CoQ on the metabolism of endogenous CoQ have been reported by Nakamura et al. (1999). Preliminary studies in our laboratory indicated that CoQ is highly permeable through Caco-2 cell monolayers and its permeability involves multiple carrier-mediated transport mechanisms. CoQ with its low aqueous solubility and high permeability can be categorized into class-II of the biopharmaceutical classification scheme (BCS). Study of regional differences

in absorption to determine the maximal permeability region should be very helpful to improve the bioavailability of orally administered drugs. To know the absorption window region will be useful for designing a modified release dosage form that targets these specific region and so leads to higher bioavailability.

Rat intestinal segments are widely used as an *in vitro* model for absorption studies of drugs (Cong et al. 2001). NaviCyte diffusion assembly has been used for permeability studies using Caco-2 cell monolayers as well as for assessing permeability of drugs across skin and other tissues (Wu et al. 2000). In this work, the objective was to identify the region of maximum permeability for CoQ in the gastrointestinal tract using an *in vitro* model comprising of different schemes of the intestine mounted onto a Navicyte diffusion assembly.

2. Investigations, results and discussion

The two directional (apical to basal and basal to apical) permeability of CoQ from five different sites of the gastro-intestinal tract was studied. The Papp values obtained for apical to basal and basal to apical permeability of CoQ through these regions of rat GIT are depicted in the Figure 1. The permeability coefficients of CoQ through the duodenum region was found to be greater ($3.1 \pm 0.8 \times 10^{-6}$ cm/s) as compared to stomach ($0.3 \pm 0.1 \times 10^{-6}$ cm/s), jejunum ($1.3 \pm 0.4 \times 10^{-6}$ cm/s), ileum ($0.87 \pm 0.4 \times 10^{-6}$ cm/s) and colon ($1.5 \pm 0.1 \times 10^{-6}$ cm/s) segments of rat GIT. This suggests that there is a regional difference in CoQ permeation and hence absorption.

Prior experiments in our laboratory indicated that insulin has greater permeability from jejunum and ileum as compared to duodenum (Agarwal et al. 2001). However, in the present study, we found that CoQ permeation was maximum through the duodenum region. Different phenomena and factors may contribute to this high absorption in the duodenum region. One of the reasons may be that the duodenum region supposedly has the highest enzymatic activity; hence drugs with carrier-mediated transport may get absorbed to a greater extent through this region. Another reason for this high permeability may be due to the higher expression of numerous transporters such as divalent metal transporter, SGLT1, GLUT2, and GLUT5 hexose transporters and enzymes such as cytochrome P450s in this region as compared to other regions of the GIT (Lenzen et al. 1996). Preferential drug absorption at a specific location is usually attributed to physiological membrane phenomena or active transport sites. Besides these, the factors contributing to regional differences in drug absorption include regional differences in the composition and thickness of mucus, pH, surface area, and enzyme activity. For instance, the pH of the gastric environment is an important determinant for digestion of compounds with high lipophilicity. Stomach pH of < 2 is optimal for the partial digestion of proteins and carbohydrates, but not for fats and fat soluble compounds. Hence, the studies were carried out in buffer media. The pH duodenum is approximately 5.5–6.5 (neutral to basic pH), and bile salts, which are required to digest fats and fat soluble compounds, are secreted in the small intestine, starting from this region. CoQ is reported to be sequestered by chylomicrons and then distributes to the liver to be incorporated into very low density lipoproteins (VLDL), and excreted primarily through the biliary tract, and over 60% is recovered in the feces (Yamamura 1985; Lucker et al. 1984). Lucker et al. (1984) have reported that CoQ pharmacokinetics involves enterohepatic recycling, i.e. reabsorption at the ileal region. This may also contribute to the variable bioavailability of CoQ after oral administration. The targeting of CoQ delivery to the duodenum region using a modified drug delivery system, with a pH dependent release will provide a means for reducing the enterohepatic recycling of the drug. The absorptive surface area in the small intestine is high in the proximal small intestine due to the presence of villi and decreases in the more distal sections. For instance, the absorptive surface area of the total small intestine is roughly 250 m^2, which is divided into duodenum, jejunum and ileum regions as 117 cm^2, 1.177 cm^2 and 824 cm^2 respectively. However, the colon region has an absorptive surface area of only 3.0 cm^2 (Rigas et al. 1998). The volume of different regions is also a factor, as greater volume results in dilution of the substances, leading to lower concentrations at the site of absorption. For most orally administered compounds, the site of absorption is assumed to be the same throughout the gastrointestinal tract, with passive processes controlling the rate and extent of absorption. The different volumes of these regions have been reported to be 123, 1227, 1718 and 3018 mL for duodenum, jejunum, ileum and colon regions respectively (Rigas et al. 1998). If the area to size ratio is taken into consideration, the concentration of the drug in the duodenal region will be higher as compared to that of other parts in the GIT.

Therefore the maximal duodenal absorption of CoQ may be envisaged as complex process involving both active/facilitated process and passive diffusion. If drug release is not complete before the dosage form passes beyond the region of maximal absorption, efficient dosage utilization and bioavailability will be severely compromised. Considering the low transit time and low volume in the duodenal region, formulation of these compounds as rapid release dosage form designed to be released at duodenal pH 5.5–6.5 will have a significant impact on absorption and bioavailability. The mannitol permeability across these tissues <10 nm/s and hence, it can be concluded that the tissue remained viable for up to 120 min (Table). Mannitol Papp values of 20 nm/s are reported to be acceptable for permeability studies to consider the tissues to be intact and non-leaky (Hidalgo 2001).

In this study, the region with highest permeability of CoQ across GIT of the rats was determined. The maximal perme-

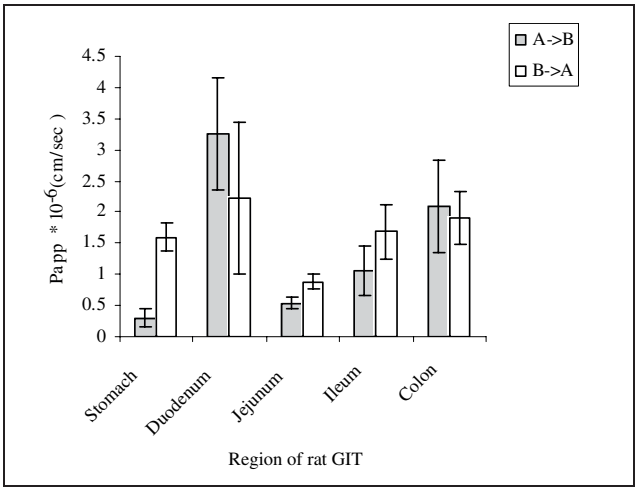

Fig: Bidirectional permeability (apical to basal vs. basal to apical) of CoQ through segments of rat GIT presented as Papp values in cm/sec on Y-axis and region of GIT on X-axis

Table: Papp (nm/s) values of mannitol through rat GIT

Direction	Stomach	Duodenum	Jejunum	Ileum	Colon
Apical to basal	0.597 ± 0.205	1.197 ± 0.291	3.304 ± 2.477	1.698 ± 0.893	3.604 ± 1.827
Basal to Apical	6.197 ± 3.201	6.048 ± 1.287	3.043 ± 1.290	1.989 ± 0.830	2.617 ± 0.209

ability was seen through the duodenum region followed by the colon. Jejunum and stomach regions also have substantial permeability. Hence targeting of the immediate release dosage form to these sites (duodenum and/or colon regions) for maximum bioavailability should be considered while designing a dosage form.

3. Experimental

3.1. Chemicals

CoQ was a generous gift from Kyowa Hakko USA (New York, NY). CoQ was custom radiolabeled by Perkin Elmer NEN life sciences (Boston, MA) and obtained as 0.680 mCi/ml solution in ethyl acetate. Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), penicillin, streptomycin and Phosphate Buffered Saline (PBS) were obtained from American Tissue Culture Collection (Rockville, MD). All other chemicals used were of reagent grade and used as received.

3.2. Transport studies through intestine

Male Sprague-Dawley rats, 250–300 g, were used for the permeability experiments. The rats were anesthetized using ketamine-xylazine solution and the GIT tissues were isolated. The proximal intestinal region, 13 cm starting from 2 cm below the pyloric sphincter was considered as duodenum and 13 cm distal intestinal region, 2 cm from the colon was taken as ileal segment. The remaining small intestine (central part) was considered as jejunum for the study. The colon region was removed following the caecum and was used for the permeability experiments as well. A side-by-side diffusion apparatus from Trega Biosciences (San Diego, CA) attached to a water bath for maintaining a temperature of 37 °C was used for all the experiments. Different tissues were mounted without stripping on a pre-heated acrylic half-cell and the cell assembly was then placed in a heated block after joining the other half-cell. The exposed surface area was 1.78 cm^2 and the reservoir volume was 6 mL. The donor and receiver compartments were immediately filled with pre-warmed oxygenated transport media. The buffer was circulated by a gas lift (95% O_2– 5% CO_2). The flow-rate of gas lift was adjusted to 10 ± 2 mL min^{-1} using a flow meter (ADM 1000, J & W Scientific, Folsom, CA). The tissues were equilibrated for 10 min before the radiolabled drug was added to the donor compartment. The quantification for the amount permeated was done by mixing test samples (10 μL) with scintillation cocktail (5 mL). ^{14}C-Mannitol and ^{3}H-CoQ dpms were counted with a Beckman LSC6000K liquid scintillation counter. The paracellular leakage through the tissues was determined by calculating the permeability coefficient (P_{app}) of mannitol.

3.3. Data analysis

Apparent permeability coefficients (P_{app}) of CoQ_{10}, and ^{14}C-mannitol were calculated using eq. (1).

$$P_{app} = \frac{dM}{dt} \cdot \frac{1}{AC_0} \quad (1)$$

where, dM/dt is the flux across the tissue (dpm's/min), A is the surface area of the membrane (1.78 cm^2) and C_0 is the initial drug concentration (1 μM). The results of experiments performed at least in triplicate are presented as mean ± SD.

3.4. Statistical data analysis

Statistical data analysis was performed using the Tukey-Kramer Multiple Comparison ANOVA with $P < 0.05$ as the minimal level of significance.

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