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Carrier-mediated Transport of Riboflavin in the Rat Colon

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ABSTRACT: Carriers involved in riboflavin transport have generally been presumed to be localized in the upper small intestine. However, using a closed loop technique, we found that in the rat colon the absorption of riboflavin could be significantly reduced by raising the concentration from 0.1 to 200 μM and by adding lumiflavin, an analogue of riboflavin. These results suggest that saturable transport by the carrier that is specific for riboflavin and analogues may also be involved in riboflavin absorption in the colon. At the lower concentration of 0.1 μM, carrier-mediated transport was suggested to prevail, compared with passive transport, both in the colon and the small intestine. Furthermore, carrier-mediated transport in the colon was comparable with that in the small intestine. This study is the first to suggest carrier-mediated riboflavin transport in the colon. Although the riboflavin transport system in the colon needs to be subjected to more detailed investigation of its transport functions and role in riboflavin absorption after oral administration, it would be of interest to explore potential use of this carrier as a system for drug delivery. Copyright © 2000 John Wiley & Sons, Ltd.

Key words: riboflavin; colon; small intestine; carrier-mediated transport; rat

Introduction

In an early human study by Levy and Jusko it was found that the gastrointestinal absorption of riboflavin (vitamin B_2) was greater and less saturable in unfasted subjects than in fasted ones [1]. To explain this observation, they hypothesized that riboflavin absorption is limited by site-specific saturable transport by the carrier and that reduced gastrointestinal motility in unfasted subjects, by reducing the rate of riboflavin delivery to the site of absorption and consequently keeping its concentration lower, may result in more efficient and less saturable absorption. Since then, although the site of riboflavin absorption has remained undetermined, it has generally been presumed that riboflavin carriers may be localized in the upper small intestine. However, in contradiction of this hypothesis, a more recent study in rats suggested that riboflavin carriers may be distributed throughout the small intestine [2]. It has also been suggested that the absorption of riboflavin produced by colonic bacteria may occur in the colon [3], although the transport mechanism is still unclear. Thus, the localization of riboflavin carriers awaits clarification, although it may be that riboflavin carriers are distributed not only in the upper small intestine but also further down in the colon.

The colon has so far attracted much less attention than the small intestine as a site of drug absorption. However, recent advances in controlled-release techniques have allowed the delivery of drugs to this lower part of gastrointestinal tract following oral administration, increasing the general interest in colonic drug absorption. Although little effort has been made yet to find carriers that can transport drugs in the colon, it is of interest to discover if any of the transport systems that are known to transport drugs in the small intestine may be involved and to explore their potential use as

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targets for drug design strategies to produce agents with improved absorption in the colon, as well as in the small intestine.

Therefore, we have focused on the riboflavin carrier in the present study and examined riboflavin absorption in the rat colon, comparing it with that in the small intestine, to see if carrier-mediated transport might be involved.

Materials and Methods

Chemicals

[^{3}H]Riboflavin (1.0 GBq mmol^{-1}) and [1,2-^{14}C]polyethylene glycol (PEG) 4000 (0.481 GBq g^{-1}) were purchased from Moravek Biochemicals, Inc. (Brea, CA, USA) and DuPont-NEN Co. (Boston, MA, USA), respectively. Unlabelled riboflavin was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other reagents were of analytical grade and commercially obtained.

Absorption from the Intestinal Loop

Male Wistar rats, weighing about 300 g, were used without fasting prior to the experiments. Each rat was anesthetized with urethane (1.125 g kg^{-1}, i.p.) and the absorption of riboflavin was evaluated as described previously [4]. Briefly, the abdomen of each rat was cut open, and a 5-cm closed loop was prepared by ligating both ends of the selected segment after internally washing with saline, either in the small intestine (midgut) at about 20 cm below the duodeno-jejunal flexure or in the colon immediately below the ileo-cecal junction. After introducing 0.5 mL riboflavin solution (0.1 or 200 μM), which was prepared in phosphate buffer (20.1 mM $Na_2HPO_4 \cdot 12H_2O$, 47.0 mM KH_2PO_4, 101.0 mM NaCl, pH 6.4) and containing trace amounts of [^{3}H]riboflavin and [^{14}C]PEG 4000 (nonabsorbable marker), the intestinal loop was returned to the abdominal cavity, and the abdomen was closed by sutures. The rectal temperature was monitored and maintained at 37°C, using a heat lamp, until one hour later the loop was isolated to obtain the luminal solution. A portion of the luminal solution (50 μL) was placed in a counting vial and mixed with 5 mL of Scintisol EX-H (Dojindo Lab., Kumamoto, Japan), a scintillation fluid, to determine the radioactivity in a liquid scintillation counter (LSC-5100, Aloka Co., Tokyo, Japan). The fraction of the dose of riboflavin absorbed was estimated as the fraction that disappeared from the loop after correcting for a minor volume change (about 3% on average) based on the change in PEG 4000 concentration.

Experiments were also conducted using 0.1 μM riboflavin solution containing 200 μM lumiflavin to examine the effects of lumiflavin on riboflavin absorption.

In some experiments to examine if riboflavin might be degraded, a portion of luminal solution obtained at the end of each experiment was filtered through a disposable filter (Dismic-25CS 0.45 μm; Advantec Co., Tokyo, Japan) and then subjected (200 μL) to HPLC (LC-10A; Shimadzu Co., Kyoto, Japan) equipped with a radio-detector (RLC-700; Aloka Co., Tokyo, Japan): column, μBondapak C_{18} (3.9 mm i.d. × 300 mm; Waters, Milford, MA, USA); mobile phase, 90% 5 mM tetrabutylammonium phosphate and 10 mM ammonium phosphate in 20% methanol, and 10% acetonitrile; flow rate, 1.0 mL min^{-1}.

Systemic Absorption and Urinary Excretion of [^{3}H]Riboflavin-Derived Radioactivity

Each rat had a cannula inserted into the right jugular vein under light ether anesthesia, and [^{3}H]riboflavin solution (0.1 or 200 μM) was administered into the colonic loop as described above. The rat, which regained consciousness shortly after administration, was unrestrained in a metabolic cage and, periodically, blood samples were obtained via the jugular cannula. Each blood sample (250 μL) was centrifuged for 3 min in a Microfuge E (Beckman Instruments, Palo Alto, CA, USA) to obtain plasma. Urine was collected throughout the experimental period and, at the end of experiment, the rat was forced to smell ether soaked into adsorbent cotton to induce urination. A portion (50 μL) of each plasma and urine sample was placed in a counting vial and the radioactivity was determined as described above.

Intravenous administration experiments were conducted for comparison, using the same solutions and doses, 0.05 or 100 nmol (0.5 mL of 0.1 or 200 μM solutions), as those for intraintestinal administration but using different rats.

Statistical Analysis

Levels of statistical significance were assessed using Student's *t*-test.

Results

As shown in Figure 1, when the concentration of riboflavin was raised from 0.1 to 200 μM, the fraction absorbed was markedly reduced in the colon (by about 90%) as well as in the small intestine (by about 80%). Riboflavin absorption was also reduced by lumiflavin, an analogue of riboflavin, in both sites. These results suggest that saturable transport by the carrier specific for riboflavin and its analogues may be involved in riboflavin absorption, not only in the small intestine but also in the colon.

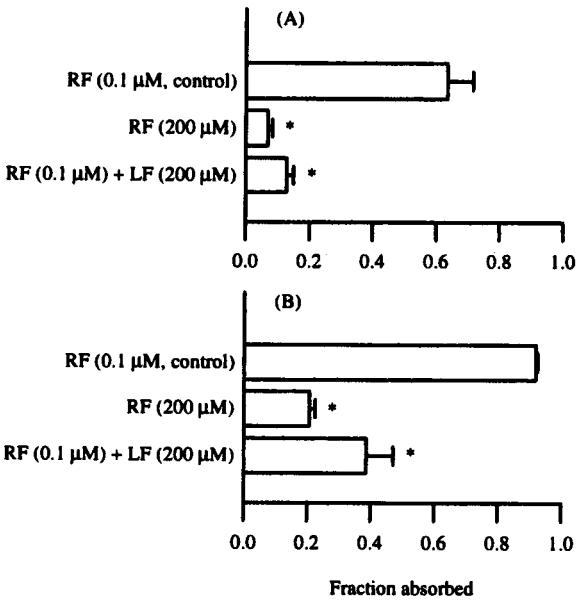

Figure 1. Effects of concentration and lumiflavin (LF) on riboflavin (RF) absorption from closed rat intestine loop. Results are the mean ± S.E. ($n = 3$). Fraction absorbed was evaluated 60 min after administration of [^{3}H]riboflavin solution (0.5 mL for a 5-cm segment) to the colon (A) or small intestine (B). *$p < 0.01$ compared with the control

HPLC analysis was used to see if riboflavin might be degraded in the intestinal lumen and additional experiments were conducted using 1.5 μM riboflavin solution ($n = 1$ each of the colon and small intestine). Although HPLC analysis was not performed in the experiments using the lower concentration of 0.1 μM, because of insufficient assay sensitivity, the fractions absorbed in these experiments, 46 and 86%, respectively, in the colon and small intestine, were closer to the respective values for 0.1 μM than for 200 μM, suggesting that carrier-mediated transport makes a significant contribution. Because no degraded riboflavin was detected in both colon and small intestine (data not shown), as evaluated at 60 min after administration, the increased fractional absorption at the lower concentrations is more likely attributable to the carrier-mediated transport of riboflavin than to the absorption of degradation products with higher permeability.

Focusing on the issue of the concentration-dependence, colonic riboflavin absorption was evaluated in terms of absorption into the systemic circulation and subsequent urinary excretion to obtain further evidence for carrier-mediated transport. The plasma concentrations of [^{3}H]riboflavin-derived radioactivity after colonic administration were, when normalized by dose, significantly higher for the lower dose (0.1 μM solution) than for the higher dose (200 μM solution) throughout the experimental period of 60 min, while those after intravenous administration were independent of dose (Figure 2). As shown in Table 1, the area under the plasma concentration versus time curve (AUC), normalized by dose and calculated for the experimental period of 60 min, for colonic administration was about three times greater for the lower dose after further normalization with regard to that for intravenous administration. The urinary excretion (% of dose) after colonic administration was also greater for the lower dose, while that after intravenous administration was greater for the higher dose (Table 1). The increased excretion at the higher dose in the intravenous administration experiments suggests saturable renal tubular reabsorption of riboflavin and its radiolabelled derivatives, as reported for humans [5]. Although the difference observed in

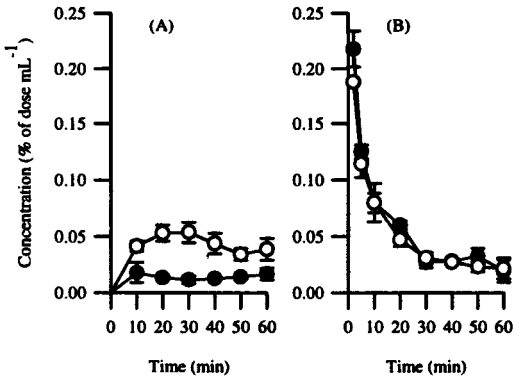

Figure 2. Plasma concentrations of [^{3}H]riboflavin-derived radioactivity in the rat. Results are the mean ± S.E. ($n = 3$). [^{3}H]Riboflavin solution (0.5 mL), 0.1 μM (○) or 200 μM (●), was administered to the colonic loop (A) or intravenously (B)

the colonic administration experiments did not reach statistical significance, after normalization by the urinary excretion after intravenous administration, the urinary excretion after colonic administration was about ten times greater for the lower dose. Thus, this shows that the appearance of [^{3}H]riboflavin-derived radioactivity in the systemic circulation and its subsequent urinary excretion after colonic administration are, after appropriate normalization, consistently greater for the lower dose, reflecting the results of riboflavin disappearance from the lumen (Figure 1). Although the detailed disposition characteristics of riboflavin and its metabolites were not addressed in this study, the results for the total radioactivity derived from [^{3}H]riboflavin strongly support the suggestion of saturable riboflavin transport by the carrier in the colon.

Discussion

According to an earlier study in the perfused rat small intestine *in situ* [6], the absorption rate (J) versus concentration (C) profile of riboflavin conformed to the following equation which assumes a Michaelis–Menten type carrier-mediated transport component and a passive transport component:

$$J = \left(\frac{J_{max}}{K_m + C} + P \right) \cdot C$$

where the maximum transport rate (J_{max}), apparent Michaelis constant (K_m) and passive transport coefficient (P) were 12.0 pmol min^{-1} (g tissue)$^{-1}$, 0.38 μM and 6.4 μL min^{-1} (g tissue)$^{-1}$, respectively. The equation and parameters predict that the apparent transport coefficient of J/C would decline by about 80% following an increase in concentration from 0.1 to 200 μM, as a result of the saturation of carrier-mediated transport, and that riboflavin would be primarily transported passively, with a negligible contribution from carrier-mediated transport, at the higher concentration of 200 μM, while carrier-mediated transport would prevail at the lower concentration of 0.1 μM. The observed extensive reduction, by about 80%, in the fraction absorbed in the small intestine in the present study is consistent with the prediction for the apparent transport coefficient and, hence, the residual transport component observed at the higher concentration may represent a passive transport component as predicted. The extensive inhibition of riboflavin absorption by lumiflavin

Table 1. Systemic absorption and urinary excretion of radioactivity after administration of [^{3}H]riboflavin to a rat colonic loop

C	AUC (% of dose min mL^{-1})		Urinary excretion (% of dose)	
(μM)	Colon	iv	Colon	iv
0.1	2.46 ± 0.36 (0.78)	3.16 ± 0.02	3.1 ± 0.6 (0.525)	5.9 ± 0.9
200	0.79 ± 0.18** (0.23)	3.45 ± 0.26	1.4 ± 0.6 (0.047)	30.0 ± 3.3*

Data are the mean ± S.E. ($n = 3$). *$p < 0.01$ compared with the value for 0.1 μM; **$p < 0.02$. Values in parentheses represent the ratio of the colon: iv value. C, riboflavin concentration in the dosing solution; AUC, area under the plasma concentration versus time curve estimated by the trapezoidal method. The AUC and urinary excretion were evaluated over the experimental period of 60 min.

(200 μM) is also in agreement with earlier reports that lumiflavin can, with the inhibition constant of about 1.4 μM, almost completely inhibit carrier-mediated riboflavin transport at concentrations above 20 μM [2,7].

Although carrier-mediated riboflavin transport in the small intestine has been suggested to be Na^+-dependent secondary active transport, some recent studies have suggested that it might not be, strictly, Na^+-dependent, requiring some other factors in addition to, or instead of, Na^+ [8,9]. Thus, the issue of Na^+-dependence seems still to be inconclusive. As far as substrate specificity is concerned, a tricyclic-like structure analogous to the isoalloxazine ring in the molecules of riboflavin and lumiflavin seems to be required for substrates, as suggested by the inhibition of riboflavin transport by lumiflavin in this and some other studies [2,7–12]. D-Ribose, which forms the side-chain attached to the isoalloxazine ring in riboflavin, reportedly does not inhibit riboflavin transport, suggesting that it does not play a major role in substrate recognition by the carrier [8,9].

Our findings in the colon constitute the first report suggesting carrier-mediated riboflavin transport in the lower part of the gastrointestinal tract. Although riboflavin absorption in the colon has yet to be subjected to detailed kinetic analysis, absorption at the higher concentration of 200 μM may represent a passive transport component, as in the small intestine, while carrier-mediated transport would prevailingly account for about 90% of riboflavin transport at the lower concentration of 0.1 μM. It should be noted that the suggested carrier-mediated transport in the colon is comparable with that in the small intestine at the lower concentration. Although the molecular nature of the carriers has not been identified, neither in the colon nor in the small intestine, and the structural requirements of substrates are still largely unknown, designing drug molecules targeted to the carriers would be of great pharmaceutical interest as a strategy for producing drugs with improved absorption in the colon as well as in the small intestine.

A slightly acidic solution (pH 6.4) was used for the colon as well as for the small intestine to maintain the same pH conditions, while the luminal pH could be slightly higher in the colon [13]. Although carrier-mediated riboflavin transport may also be Na^+-dependent in the colon, as suggested in the small intestine, the effect of pH on transport functions may also need to be examined in the future.

To date, the carrier-mediated transport of organic nutrients and drugs in the colon has not been a subject of extensive investigation, with the exception of monocarboxylates [14–16], dicarboxylates [17], folate [18,19] and biotin [20]. The small intestine, with a large surface area and abundant carriers, when available, is no doubt the major site for the absorption of nutrients and drugs following oral administration. However, for those nutrients which have been suggested to be absorbed by carriers in the colon, it has been suggested that carriers may assist in the efficient uptake of low levels of nutrients produced by luminal bacteria for localized nutrition of colonocytes. This may also be true for riboflavin.

In conclusion, evidence is presented for the first time in rats *in situ* that suggests carrier-mediated riboflavin transport in the colon. It will be of great pharmaceutical interest to further clarify such transport, exploring the potential use of the carrier as a target for drug design strategies to produce agents with improved absorption in the colon, as well as in the small intestine. The suggestion of efficient colonic riboflavin absorption by the carrier is inconsistent with an earlier suggestion in humans that riboflavin carriers may be localized in the upper small intestine [1]. Although it is not easy to resolve this apparent discrepancy, the factors involved in processing riboflavin after entry into the intestinal epithelia may need to be examined in more detail in the future, since the earlier suggestion in humans is based on the measurement of riboflavin excreted in urine.

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