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LETTER TO THE EDITOR

Absorption of Unconjugated Bile Acids from the Perfused Jejunum of the Anesthetized Rat: Structure–Activity Relationships and Rate-Limiting Steps

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Abbreviations

CA	cholic acid
CDCA	chenodeoxycholic acid
CDC-gly	glycochenodeoxycholate
CDC-tau	taurochenodeoxycholate
norCDCA	24nor-chenodeoxycholic acid
norUDCA	24nor-ursodeoxycholic acid
P_{app}	permeability observed experimentally
P_m	permeability coefficient for the membrane
P_{uwl}	permeability coefficient of the unstirred water layer
UCA	ursocholic acid
UDCA	ursodeoxycholic acid

Unconjugated bile acids are not only formed in the mammalian intestine by bacterial enzymes, but are also used as therapeutic agents for the treatment of cholestatic liver disease and for cholesterol gallstone prevention and dissolution. Ursodeoxycholic acid (UDCA) is used for the treatment of primary biliary cholangitis (formerly termed primary biliary cirrhosis) where it decreases the need for liver transplantation (Borg, 2006). UDCA is also used for the prevention of gallstone formation in obese patients who have rapid weight loss after bariatric surgery (Sugerman et al., 1995). Recently, 24-norUDCA (a homolog of UDCA with one less carbon atom in the side chain) has been shown to improve liver tests in primary sclerosing cholangitis (Fickert et al., 2017). A semisynthetic bile acid, obeticholic acid, is the 6α-ethyl derivative of CDCA and a potent agonist of the nuclear receptor FXR (Farnesoid X receptor). Obeticholic acid has been shown to improve liver tests in patients with primary biliary cholangitis who have not responded to treatment with UDCA (Nevens, 2016) and is being tested in other liver diseases.

Bile acids used for therapy are formulated in the form of protonated acid. This physical form has extremely low aqueous solubility (<100 μM), and crystal dissolution is generally considered to be rate limiting in their absorption. Nonetheless, it seems important to define the rate of uptake of unconjugated bile acids from an aqueous solution in which the bile acid is largely present in the form of the water-soluble bile acid anion. We report here the rate of passive intestinal absorption of six polyhydroxy unconjugated bile acids from a perfused jejunal segment of the anesthetized rat. We determined the influence of the unstirred water layer by including D-glucose in the perfusate, a solute whose rate of uptake is determined solely by the thickness of the unstirred water layer (Levitt, Fume, Strocchi, Anderson, & Levitt, 1990; Anderson, Levine, Levitt, Kneip, & Levitt, 1968).

Male Wister rats weighing 350–400 g were used. A jejunal segment of the anesthetized rat was perfused using a bidirectional pump to minimize the unstirred water layer (Doluisio, Billips, Dittert, Sugita, & Swintosky, 1969). The perfusate (pH 7.3) contained phosphate buffer, bile acid, glucose, and a polymeric dye (Polycyanine) as a

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nonabsorbable marker to correct for water movement into or out of the perfused segment (Dupas, Moreau, & Hofmann, 1985). Samples (25 μL) of the perfusate were obtained by means of a fine catheter inserted through the intestinal wall into the lumen, enabling calculation of the first-order disappearance rate.

Bile acids labeled with ^{14}C or ^{3}H were synthesized in this laboratory and were radio purified by zonal scanning. The bile acid concentration in perfusates was 0.1 mM, a concentration at which only bile acid monomers are present. At least four studies were conducted for each unconjugated bile acid and pairwise comparisons for rates of absorption were tested for statistical significance using Student's *t*-test. Concentrations of labeled bile acids were determined by liquid scintillation counting or in some instances by a sensitive enzymatic assay (Roda, Kricka, DeLuca, & Hofmann, 1982). This study was approved by the Committee on Animal Care and Protection of the University of California San Diego.

The influence of nuclear substitution on membrane permeability was determined for four natural C_{24} bile acids chenodeoxycholic acid (CDCA), UDCA, cholic acid (CA), and ursocholic acid (UCA). Their structure is given in Table 1. The influence of side-chain length was assessed using two C_{23} bile acids (24-norCDCA and 24-norUDCA) that have four rather than five carbon atoms in the side chain. The effect of conjugation on absorption was defined using the glycine and taurine conjugates of CDCA.

Calculated values for the permeability of the membrane and the lipid membrane were obtained using the equations shown in Box 1 (Ho & Higuchi, 1974), and are summarized in Table 1. Nuclear hydroxylation had a marked effect on membrane permeation as dihydroxy bile acids were absorbed more rapidly than trihydroxy bile acids (CDCA > CA; UDCA > UCA ($p < 0.05$ for each pair). In addition, changing the orientation of the hydroxyl group at C-7 from α to β greatly decreased the rate of absorption: CDCA > UDCA; CA > UCA) ($p < 0.05$ for all comparisons). Side-chain length had a variable effect (CDCA < norCDCA) but absorption of norUDCA did not differ from that of UDCA. Uptake of CDCA was so rapid that it was entirely controlled by the thickness of the unstirred water layer. The glycine and taurine conjugates of CDCA showed negligible absorption, in agreement with previous studies (Wilson, 1981). Based on the work of Marcus et al. (1991), it is reasonable to equate disappearance from the lumen as being equivalent to absorption from the small intestine. Anesthesia has been shown to increase the thickness of the unstirred layer (Levitt et al., 1990), so that the calculated P_{uwl} values are only valid for the experimental conditions of this study. The values of P_m (membrane permeability) should be rather similar across species as they are normalized to the epithelial area.

Our measurements of net absorption are in general agreement with previous studies of unconjugated bile acid absorption in humans (Krag & Phillips, 1974; van Berge Henegouwen & Hofmann, 1977), in the rabbit (Aldini et al., 1996), and in the rat (Schiff, Small, & Pietsch, 1972; Wilson, 1981), but provide new information on the effect of side-chain length. Such information may be of interest as norUDCA is now in clinical trials for the treatment of cholestatic liver disease (Fickert et al., 2017).

We conclude that the rate of unconjugated bile acid absorption from the jejunum is influenced markedly by the pattern of bile acid hydroxylation and to a lesser extent by the side-chain length. CDCA is absorbed so rapidly that its uptake is largely determined by the thickness of the unstirred water layer. Changing the C-7 hydroxy group from an α to a β configuration greatly decreases the rate of absorption. C_{23} 24-NorCDCA and 24-norUDCA were well

Table 1 Permeability coefficients of bile acids ($\times 10^4$) cm/s (mean ±standard deviation) in the perfused rat jejunum at pH 7.3[a]

Bile acid	Structure	P_{app}	P_{uwl}	P_m	% P_{uwl} controlled[b]
Unconjugated					
CDCA	C_{24}, 3αOH, 7αOH	1.03 ± 0.18	1.08 ± 0.19	22.2 ± 10.0	95
norCDCA	C_{23}, 3αOH, 7αOH	0.78 ± 0.19	0.91 ±0.13	5.46 ± 3.52	85
UDCA	C_{24}, 3αOH, 7βOH	0.67 ± 0.05	1.20 ± 0.52	1.52 ± 1.47	56
norUDCA	C_{23}, 3αOH, 7βOH	0.64 ± 0.15	0.99 ±0.14	1.81 ±0.82	65
CA	C_{24}, 3αOH, 7αOH, 12αOH	0.52 ± 0.24	0.68 ± 0.27	1.25 ± 0.48	76
UCA	C_{24}, 3αOH, 7βOH, 12αOH	0.24 ± 0.08	0.87 ± 0.06	0.33 ± 0.16	27
Conjugate					
CDC-tau	C_{24}–CDC-taurine	0.05		~0.05	<10
CDC-gly	C_{24}–CDC-glycine	0.1–0.2		~0.05	<10

[a]CDC-gly, glycochenodeoxycholate; CDC-tau, taurochenodeoxycholate; P_{app}, apparent permeability; P_m, permeability of the membrane; P_{uwl}, permeability of the unstirred water layer.

[b]Calculated as P_{app}/P_{uwl} (×100).

absorbed from the jejunum, presumably also by passive means.

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Authors' contributions

J.-L.D. performed the experiments and drafted the initial manuscript; N.H. provided the method for data analysis and generated the values in Box 1. A.H. planned the experimental design and revised the draft manuscript prepared by J.-L.D. All authors have read and approved this version of the manuscript.

Conflict of Interest

None of the authors have any conflict of interest.

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Box 1. Calculation of permeability coefficients
From the first equation:

$$K = \frac{A}{V} \cdot \frac{1}{\frac{1}{P_{\text{uwl}}} + \frac{1}{P_{\text{m}} + P_{\text{p}}}} \tag{1}$$

where K is the first-order rate constant given by the absorption of glucose or a given bile acid; A is the geometric area of the perfused jejunal segment of length L; V is the volume of solution in the perfused segment.

$$\frac{A}{V} = 2\sqrt{\frac{\pi L}{V.}}$$

P_{uwlB} is the undisturbed water layer permeability of bile acid; P_{uwlG} is the undisturbed water layer permeability of glucose; P_{mB} is membrane permeability of bile acid; P_{p} is pore permeability. In the case of bile acids P_{P} is the insignificant because the size of the bile acid molecules precludes paracellular absorption:

$$\frac{1}{P_{\text{uwl}}\text{B}} + \frac{1}{P_{\text{m}}\text{B}} = \frac{A}{V \cdot K_{\text{B}}} \tag{2}$$

and for glucose (unstirred water layer rate controlled) wherein $P_{\text{uwl}} \lll P_{\text{m}}$,

$$\frac{1}{P_{\text{uwl}}\text{G}} = \frac{A}{V \cdot K_{\text{G}}} \tag{3}$$

Aqueous diffusion relationships, accounting for the molecular sizes of glucose and the bile acids, leads to the following ratio:

$$\frac{P_{\text{uwl}}\text{B}}{P_{\text{uwl}}\text{G}} = \left(\frac{M_{\text{G}}}{M_{\text{B}}}\right)^{\frac{1}{3}} \tag{4}$$

where M is the molecular weight. From Equations 2–4 we arrive at the following equation.

$$P_{\text{mB}} = \frac{A}{V} \cdot \frac{1}{\frac{1}{K_{\text{B}}} - \frac{1}{K_{\text{G}}}\left(\frac{M_{\text{B}}}{M_{\text{G}}}\right)^{\frac{1}{3}}} = \frac{1}{\frac{1}{P_{\text{EXP}}\text{B}} - \frac{1}{P_{\text{uwl}}\text{B}}} \tag{5}$$

P_{mB} values were calculated for each experiment.