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A REVERSIBLE CLEARANCE MODEL FOR THE ENTEROHEPATIC CIRCULATION OF DRUG AND CONJUGATE METABOLITE PAIR

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ABSTRACT:

An analytic expression for the plasma clearance of a drug, which undergoes enterohepatic circulation (EHC) in intact form and in the form of a hydrolyzable conjugate metabolite, was derived based on a four-compartment model that features the three successive steps of the recycling cascade: biliary excretion, intestinal hydrolysis, and reabsorption. The kinetic equation consists of irreversible and partially reversible clearance terms. The irreversible terms represent the removal of drug from the systemic circulation in a unidirectional fashion, such as renal clearance and extraconjugative biotransformation pathways. The reversible terms represent the two recycle pathways: biliary excretion of the parent compound, and the formation of a conjugate metabolite and its subsequent excretion into bile. Mathematically, the reversible clearance terms can be resolved into the product of a net recycled fraction and an irreversible clearance estimate for either biliary excretion or conjugate formation. The net recycled fractions are, in turn, a function of the competitive kinetics of drug or drug conjugate at each step of the EHC cascade. The derived clearance equation provides a useful conceptual framework in the kinetic analysis of factors controlling the reversibility of plasma drug clearance as a result of EHC. Analysis of the model also points to the development of new experimental strategies in elucidating the EHC of xenobiotics.

Various mathematical models have been developed to investigate the influence of enterohepatic circulation (EHC)[1] on the disposition kinetics of drugs. The effect of EHC on specific pharmacokinetic parameters such as area under the plasma concentration time curve (1, 2), systemic availability (3–6), and elimination half-life (7–11) have been examined. All of the theoretical studies reported to date have focused on the enterohepatic recycling of parent drug. Indeed, a number of drugs are excreted intact into bile and subsequently reabsorbed from the intestinal lumen, *e.g.* digoxin (12), digitoxin (13), doxycycline (14), cimetidine (3), and ampicillin (13). However, there are many more examples of drugs that undergo enterohepatic cycling primarily in the form of a conjugate metabolite. The most notable examples are compounds subject to extensive glucuronidation, such as etorphine (15), oxazepam (16), morphine (17), diethylstilbestrol (18), valproic acid (19), phenolphthalein (20), carprofen (21), diflunisal (22) and lorazepam (23). The glucuronic acid conjugates of these drugs are readily excreted into bile. Upon reaching the intestine, the glucuronide metabolite can be hydrolyzed by bacterial β-glucuronidase present in the intestinal microflora, and the liberated aglycone is then reabsorbed into the portal circulation. Enterohepatic cycling is known to occur also *via* other hydrolyzable forms of drug conjugates (*e.g.* ethereal sulfates, glycine conjugates). A few of the previously proposed EHC models did include recycling *via* a drug conjugate (1, 5, 6). However, there was always the stipulation that the drug conjugate is eliminated exclusively by excretion into bile (*i.e.* no extrabiliary excretion). It was also assumed that the conjugate metabolite is totally hydrolyzed in the gut, followed by complete absorption of the aglycone. In effect, only the limiting situation of a completely recoverable EHC was considered. Consequently, the earlier models do not permit an investigation of how extrabiliary clearance, gut bacterial hydrolysis, and intestinal reabsorption controls the extent of EHC of a drug conjugate. The purpose of this commentary is to present a theoretical analysis of the factors governing the recycling kinetics of both parent drug and conjugate metabolite, and the impact of these factors on the area under the plasma drug concentration time curve or on the clearance of the parent compound.

We begin by introducing a simple compartmental model which provides explicit accounting of the cascade of events involved in the EHC of the drug and conjugate metabolite pair. Based on this model, a general clearance equation is derived which delineates the modulating influence of each of the kinetic steps involved in the enterohepatic recycling of a drug and drug conjugate. A systematic analysis of the model equation is presented to illustrate its applicability in the interpretation of drug clearance data in situations where EHC is mediated predominantly by a conjugate metabolite. Finally, experimental strategies that can be employed to elucidate the kinetics of drug conjugate recycling are discussed.

Enterohepatic Circulation Model

Fig. 1 shows the kinetic model used to represent the joint EHC of a drug and its conjugate metabolite. A glossary of terms used in the figure and the following equations is presented in table 1. Distribution of the drug and its conjugate in the body is represented in each case by a two-compartment model composed of the systemic region and the gastrointestinal tract. These two compartmental units are then linked in a parallel fashion to make up the final model. All of the rate processes depicted in this model are assumed to follow first order or linear kinetics.

The drug is introduced as a bolus injection into the systemic compartment for the parent compound (D_S). Elimination of the

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[1] Abbreviation used is: EHC, enterohepatic circulation.

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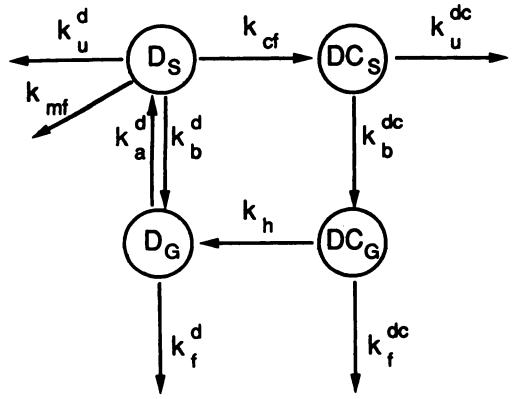

FIG. 1. *A compartmental model for the joint EHC of parent drug and its conjugate metabolite.*

Table 1 contains the corresponding glossary of terms.

parent drug from the systemic compartment occurs *via* four parallel pathways:[2] conjugate formation (k_{cf}), extraconjugative metabolic pathways (represented by a sum rate constant of k_{mf}), biliary excretion (k_b^d), and renal excretion (k_u^d). Once formed, the conjugate metabolite is assumed to distribute readily throughout the systemic compartment as represented by the compartment DC_S.[3] The metabolite is eliminated from the systemic compartment by two competing pathways: excretion into urine (k_u^{dc}) or into bile (k_b^{dc}). Biliary excretion of drug and drug conjugate is assumed to be a continuous first-order process.[4] Drug conjugate that reaches the gut compartment (DC_G) is either cleaved by hydrolytic enzymes in the intestinal lumen (k_h) or excreted in the feces (k_f^{dc}). Reabsorption of the conjugate metabolite itself is assumed to be negligible. The rate term for fecal excretion is a function of the transit time of the conjugate metabolite in the intestine. The parent drug in the gut compartment (D_G), which is derived either directly from biliary excretion or indirectly from enzymatic hydrolysis of the conjugate metabolite, may be absorbed and reintroduced into the systemic compartment (k_a^d) or cleared from the intestinal lumen (k_f^d).

The following set of differential equations describes the compartmental mass transfer for the EHC model shown in fig. 1.

$$\frac{dD_S}{dt} = k_a^d \cdot D_G - k_u^d \cdot D_S - k_{mf} \cdot D_S - k_{cf} \cdot D_S - k_b^d \cdot D_S \quad (1)$$

$$\frac{dDC_S}{dt} = k_{cf} \cdot D_S - k_u^{dc} \cdot DC_S - k_b^{dc} \cdot DC_S \quad (2)$$

$$\frac{dDC_G}{dt} = k_b^{dc} \cdot DC_S - k_h \cdot DC_G - k_f^{dc} \cdot DC_G \quad (3)$$

$$\frac{dD_G}{dt} = k_b^d \cdot D_S + k_h \cdot DC_G - k_a^d \cdot D_G - k_f^d \cdot D_G \quad (4)$$

[2] The present model is limited to drugs (and conjugate metabolites) with low extraction ratio, in which case hepatic clearance is solely a function of the activities of the various drug-metabolizing enzymes and the biliary transport system.

[3] This implies that a rate-limiting, diffusional barrier does not exist at the sinusoidal membrane of the hepatocyte. The conjugate metabolite equilibrates rapidly between liver and blood.

[4] The kinetic complexities of gallbladder emptying were ignored in order to facilitate a mathematical derivation for the model. However, it has been shown that, while the inclusion of a more complex time delay function to represent the rhythmic emptying of bile into the intestinal lumen leads to a more realistic prediction of multiple peaking in the plasma concentration time profile, the area integral and, hence, the blood clearance estimate is not altered (2).

TABLE 1

Glossary of terms

D_S	Amount of drug in the systemic compartment (units of mass)
DC_S	Amount of drug conjugate in the systemic compartment
DC_G	Amount of drug conjugate in the gut compartment
D_G	Amount of drug in gut compartment
k_{cf}	Rate constant for drug conjugate formation (units of time $^{-1}$)
k_{mf}	Sum of rate constants for formation of other metabolites
k_u^d	Rate constant for drug excretion into urine
k_b^d	Rate constant for drug excretion into bile
k_f^d	Rate constant for drug excretion into feces
k_a^d	Rate constant for drug reabsorption from gut compartment
k_u^{dc}	Rate constant for drug conjugate excretion into urine
k_b^{dc}	Rate constant for drug conjugate excretion into bile
k_f^{dc}	Rate constant for drug conjugate excretion into feces
k_h	Rate constant for hydrolysis of drug conjugate to parent drug
AUAC	Area under the amount time curve for parent drug in the systemic compartment
AUC	Area under the blood/plasma concentration time curve for parent drug
V_S	Apparent volume of distribution of parent drug in the systemic compartment
Cl_S = Dose/AUC	Apparent systemic clearance of drug (units of volume/time)
$Cl_r^d = k_u^d \cdot V_S$	Renal clearance of drug
$Cl_{mf} = k_{mf} \cdot V_S$	Sum of all other metabolite formation clearances
$Cl_b^d = k_b^d \cdot V_S$	Biliary clearance of drug
$Cl_{cf} = k_{cf} \cdot V_S$	Conjugate formation clearance
f_a^d	Fraction of drug reabsorbed from gut compartment
f_b^{dc}	Fraction of drug conjugate in systemic compartment that is excreted into bile
f_h	Fraction of drug conjugate in gut that undergoes hydrolysis back to parent drug
F_{cf}	Fraction of drug conjugate formed from drug in the systemic compartment
F_b^d	Fraction of drug in the systemic compartment that is excreted into bile

The modeling objective was to derive an explicit equation for plasma clearance of parent drug in terms of the various kinetic parameters which describe the individual steps involved in the enterohepatic recycling of drug and drug conjugate.

Transforming eqs. 1 to 4 into the Laplace domain yields the corresponding eqs. 5 to 8:

$$s \cdot \overline{D_S} - D_S(0) = k_a^d \cdot \overline{D_G} - k_u^d \cdot \overline{D_S} - k_{mf} \cdot \overline{D_S} - k_{cf} \cdot \overline{D_S} - k_b^d \cdot \overline{D_S} \quad (5)$$

$$s \cdot \overline{DC_S} - DC_S(0) = k_{cf} \cdot \overline{D_S} - k_u^{dc} \cdot \overline{DC_S} - k_b^{dc} \cdot \overline{DC_S} \quad (6)$$

$$s \cdot \overline{DC_G} - DC_G(0) = k_b^{dc} \cdot \overline{DC_S} - k_h \cdot \overline{DC_G} - k_f^{dc} \cdot \overline{DC_G} \quad (7)$$

$$s \cdot \overline{D_G} - D_G(0) = k_b^d \cdot \overline{D_S} + k_h \cdot \overline{DC_G} - k_a^d \cdot \overline{D_G} - k_f^d \cdot \overline{D_G} \quad (8)$$

The initial amount of drug in the systemic compartment equals

the dose (*i.e.* $D_S(0)$ = Dose). The amount of drug in the gut compartment ($D_G(0)$) as well as the amount of drug conjugate in the systemic and gut compartments ($DC_S(0)$ and $DC_G(0)$) are set to zero at time = 0. The system of linear equations can be solved algebraically for $\overline{D_S}$ in terms of the transfer rate constants as shown in the next equation.

$$\overline{D_S} = \text{Dose} \Big/ \left[k_u^d + k_{mf} + k_{cf} + k_b^d - \frac{k_a^d \cdot k_b^d}{s + k_a^d + k_f^d} - \frac{k_b^{dc} \cdot k_h \cdot k_a^d \cdot k_{cf}}{(s + k_b^{dc} + k_u^{dc}) \cdot (s + k_h + k_f^{dc}) \cdot (s + k_a^d + k_f^d)} \right] \quad (9)$$

The limit of $\overline{D_S}$ as the Laplace operator approaches zero equals the area under the amount *vs.* time curves (AUAC) for drug in the systemic compartment, *i.e.* $\lim_{s \to 0} \overline{D_S}$ = AUAC.

$$\text{AUAC} = \text{Dose} \Big/ \left[k_u^d + k_{mf} + k_{cf} + k_b^d - \frac{k_a^d \cdot k_b^d}{k_a^d + k_f^d} - \frac{k_b^{dc} \cdot k_h \cdot k_a^d \cdot k_{cf}}{(k_b^{dc} + k_u^{dc}) \cdot (k_h + k_f^{dc}) \cdot (k_a^d + k_f^d)} \right] \quad (10)$$

AUAC divided by the apparent volume of distribution of drug in the systemic compartment (V_S) gives the area under the plasma concentration time curve (AUC).

$$\text{AUC} = \text{AUAC}/V_S \quad (11)$$

Substituting eq. 10 in eq. 11 and solving for plasma drug clearance (Cl_S) or Dose/AUC yields:

$$\text{Dose/AUC} = k_u^d \cdot V_S + k_{mf} \cdot V_S + k_b^d \cdot V_S - \left[\frac{k_a^d}{(k_a^d + k_f^d)} \right] \cdot k_b^d \cdot V_S + k_{cf} \cdot V_S - \left[\frac{k_b^{dc}}{k_b^{dc} + k_u^{dc}} \cdot \frac{k_h}{k_h + k_f^{dc}} \cdot \frac{k_a^d}{k_a^d + k_f^d} \right] \cdot k_{cf} \cdot V_S \quad (12)$$

Eq. 12 can be simplified by substituting the products of the exit rate constants from the systemic compartment and the apparent volume of distribution for the corresponding clearance terms (see table 1). It is also evident that the quotient terms in the right side of the equation can be defined as the following fractions:

$$f_b^{dc} = \frac{k_b^{dc}}{k_b^{dc} + k_u^{dc}} \quad (13)$$

i.e. fraction of drug conjugate in the systemic compartment that is excreted into bile;

$$f_h = \frac{k_h}{k_h + k_f^{dc}} \quad (14)$$

i.e. fraction of drug conjugate in the gut compartment that undergoes enzymatic hydrolysis to yield the aglycone; and

$$f_a^d = \frac{k_a^d}{k_a^d + k_f^d} \quad (15)$$

i.e. fraction of drug reabsorbed from the gut compartment. Hence, the final clearance equation[5] can be expressed as follows:

$$Cl_S = Cl_r^d + Cl_{mf} + Cl_b^d - f_a^d \cdot Cl_b^d + Cl_{cf} - f_b^{dc} \cdot f_h \cdot f_a^d \cdot Cl_{cf} \quad (16)$$

or in the rearranged form,

$$Cl_S = Cl_r^d + Cl_{mf} + (1 - f_a^d) \cdot Cl_b^d + (1 - f_b^{dc} \cdot f_h \cdot f_a^d) \cdot Cl_{cf} \quad (17)$$

[5] The same equations apply to systemic clearance during steady-state iv drug infusion.

Results and Discussion

Theoretical Considerations. *Model Features.* The clearance equation derived in the preceding section reveals several fundamental characteristics of the EHC model. The equation features a combination of *irreversible* and *partially reversible* clearance terms. The terms Cl_r^d and Cl_{mf} represent the irreversible removal of parent drug from the systemic compartment by renal excretion and biotransformation pathways other than conjugation. In comparison, the terms for biliary clearance and conjugate formation clearance are considered to be partially reversible by virtue of the fact that a given fraction of the amount of drug eliminated *via* these pathways is returned to the systemic circulation. Thus, each of the two latter clearance estimates, Cl_b^d and Cl_{cf}, are modified by a parenthetical term denoting the net amount of drug or drug conjugate ultimately excreted into the feces (*i.e.* prevented from being recycled). The *net removal fraction* for the biliary clearance of the intact drug ($1 - f_a^d$) or the *net recycled fraction* (f_a^d) reflects the competitive kinetics of the two parallel processes of intestinal absorption and fecal excretion. In the case of clearance due to conjugate formation, the net removal fraction or the net recycled fraction for the formation clearance of conjugate metabolite is a complex function of the extent to which drug conjugate is excreted *via* the biliary route, the degree of intestinal deconjugation, and the bioavailability of the liberated aglycone. In fact, the net recycled fraction, mathematically equal to the product of the fractional terms (f_b^{dc}, f_h, and f_a^d), represents the concerted effects of the three respective components involved in the recycling cascade. Each of the aforementioned fractions can be expressed in terms of the rate constants for the competing first order kinetic processes involved (see eqs. 13 to 15).

In essence, the derived clearance equation expresses the modulating effect of two parallel reversible processes on the systemic drug clearance—namely, the reabsorption of intact drug and the regeneration of parent drug from the conjugate metabolite. Since each of the rate events involved in the recycling of the drug and its conjugates is explicitly recognized, the equation provides a convenient analytical tool to predict changes in blood clearance in the event of perturbation in any of the metabolic and distributional processes.[6] The remainder of this section is devoted to a component analysis of eq. 17.

Limiting Cases. An inspection of eq. 17 reveals several limiting cases with respect to the net recycled fractions. The recycled fraction for drug conjugate ($f_b^{dc} \cdot f_h \cdot f_a^d$) equals unity when the derived drug conjugate is excreted exclusively into bile (*i.e.* $f_b^{dc} = 1$), when it is followed by its complete hydrolysis in the gut lumen ($f_h = 1$), and when the liberated aglycone is completely reabsorbed ($f_a^d = 1$). In such a case, the conjugate formation clearance term (Cl_{cf}) in effect disappears from eq. 17.

$$Cl_S = Cl_r^d + Cl_{mf} \quad (18)$$

In a kinetic sense, the full reversibility of the drug conjugate formation is kinetically equivalent to the distribution of the

[6] It should be noted that eq. 17 (as well as eq. 29 in a later part of the text) does not apply to drugs or conjugate metabolites exhibiting high extraction characteristics. Also, the consideration of a diffusional barrier for the conjugate between liver and blood will introduce further complexities. In these instances, a physiologically based model is required. More complex forms of the equations involving blood flow and diffusional parameters can be derived.

parent drug into a non-eliminating compartment. This is often referred to as futile cycling. One practical implication of futile cycling is an apparent lack of effect on systemic drug clearance in the event of a change in conjugate formation clearance, such as inhibition or induction of the conjugation pathway. Also, the recycled conjugate metabolite will not be found in the excreta, *e.g.* urine and feces. *In vivo* evidence of conjugation can only be ascertained by the presence of conjugate metabolite in blood circulation and tissues. It also becomes obvious that in the general case of *partial* reversibility (*i.e.* $0 < f_b^{dc} \cdot f_h \cdot f_a^d < 1$), the cumulative amount of drug conjugate recovered in the excreta does not reflect the full extent of systemic conjugation.

Consider the other limiting case when the conjugate recycled fraction approaches zero. This latter scenario can occur after blockage of biliary excretion of drug conjugate or inhibition of conjugate hydrolysis. In either instance, the drug conjugate formation term is no longer modified by the recycled fractions.

$$Cl_S = Cl_r^d + Cl_{mf} + (1 - f_a^d) \cdot Cl_b^d + Cl_{cf} \qquad (19)$$

Clearance due to formation of drug conjugate now becomes an irreversible pathway of drug elimination. Analogous limiting cases exist with the recycling term for the parent drug. If the recycled fraction of drug (f_a^d) is 1 (*i.e.* 100% of the drug is reabsorbed), biliary excretion of drug no longer contributes to systemic clearance.

$$Cl_S = Cl_r^d + Cl_{mf} + (1 - f_b^{dc} \cdot f_h) \cdot Cl_{cf} \qquad (20)$$

Finally, in the event no drug reabsorption takes place, both net recycled fractions for the drug and drug conjugate are reduced to zero; systemic drug clearance is maximized in that all the component clearance terms represent irreversible removal of drug from the systemic circulation.

$$Cl_S = Cl_r^d + Cl_{mf} + Cl_b^d + Cl_{cf} \qquad (21)$$

A summary of the limiting cases for eq. 17 is presented in table 2.

According to the previous definitions, the fraction of drug conjugate excreted into bile (f_b^{dc}), the fraction of conjugate hydrolyzed by intestinal bacteria (f_h), and the fraction of drug reabsorbed from the intestine (f_a^d) are fundamentally related to the rate constants representing the competition between reentry and elimination pathways for the drug conjugate or the parent drug at each level of the recycling cascade. We will now examine in detail how perturbations in these competitive processes affect systemic drug clearance. To focus our attention on the role of the drug conjugate, we will assume for the remainder of this section that a negligible amount of intact drug is excreted into bile. In other words, recycling occurs exclusively *via* the drug conjugate. The various cases considered are summarized in table 3.

Fraction of Drug Conjugate Excreted into Bile (f_b^{dc}). The fraction of drug conjugate in the systemic compartment that is excreted into bile is governed by the relative magnitudes of biliary and renal clearance of the conjugate metabolite. An increase in the biliary clearance of drug conjugate (holding f_a^d and f_h constant, case A.1.a in table 3) would result in an increase in the fraction of drug conjugate entering the bile. This increase in f_b^{dc} would promote the recirculation of the conjugate species, eventually leading to a decrease in the systemic clearance of drug. In contrast, inhibiting the biliary excretion of drug conjugate (case A.1.b) results in a decrease in f_b^{dc} and recycling of drug conjugate. This effectively increases the apparent drug clearance. An example of the latter case can be found in a pharmacokinetic study on the glucuronidation of morphine in dogs reported by Jacqz *et al.* (24). Morphine is excreted in bile predominantly in the form of glucuronic acid conjugates. The effect on plasma morphine clearance due to obstruction of biliary excretion of the glucuronide metabolites (*i.e.* $Cl_b^{dc} \rightarrow 0$) was studied in bile duct-ligated dogs.[7] A 2-fold increase in the plasma clearance of morphine, from 51.5 ± 30 to 94.5 ± 21 ml/min/kg, was observed, presumably due to interruption of the recycling of morphine.

An alternate means by which f_b^{dc} can be altered is through changes in the renal clearance of the conjugate metabolite, especially when renal excretion contributes significantly to the systemic clearance of the drug conjugate. In theory, an increase in the renal clearance of a drug conjugate (case A.2.a) would decrease f_b^{dc} and thereby increase systemic drug clearance, although there is no known example in the literature. A more frequently encountered situation is a decrease in renal conjugate clearance (case A.2.b). The resulting increase in f_b^{dc} with all else being constant would enhance recycling of drug conjugate and decrease systemic drug clearance. This scenario offers an attractive explanation for some puzzling findings with the interaction between indomethacin and probenecid reported by Baber *et al.* (25). In that study, co-administration of probenecid was shown to decrease the systemic clearance of indomethacin from 174 ± 21 to 107 ± 14 ml/hr/kg. Probenecid did not alter the renal clearance of intact indomethacin; therefore, the decrease in systemic clearance was primarily due to a decrease in the nonrenal clearance of indomethacin, from 168 ± 24 to 104 ± 15 ml/hr/kg. Inhibition of indomethacin metabolism by probenecid was postulated as the causal mechanism. However, subsequent animal studies have failed to demonstrate any inhibition of indomethacin metabolism in the presence of probenecid (26). It is known that indomethacin is enterohepatically recycled as a glucuronide conjugate in animals and that orally administered indomethacin is well absorbed (*i.e.* $f_a^d = 1$) in humans (27). Under the circumstances, the nonrenal clearance of indomethacin can be represented by a modification of eq. 17.

$$Cl_S - Cl_r^d = Cl_{mf} + (1 - f_b^{dc} \cdot f_h) \cdot Cl_{cf} \qquad (22)$$

Since Cl_{mf} and Cl_{cf} are not affected by probenecid in humans, the decrease in nonrenal clearance of indomethacin in the presence of probenecid may be explained by an increase in either the f_b^{dc} or f_h. Indeed, Baber *et al.* (25) observed a pronounced inhibition of the renal clearance of indomethacin glucuronide (from 271 ± 48 to 126 ± 57 ml/min) by probenecid. Thus, a decrease in the renal clearance of indomethacin glucuronide probably diverted the conjugate metabolite into bile, which led indirectly to a decrease in the systemic clearance of drug as a result of more extensive enterohepatic circulation.

A similar situation could exist during renal failure. Although retention of acyl glucuronides and regeneration of parent drug from the conjugate metabolite is a well recognized event in the anephric state (28), the site(s) of deconjugation has rarely been elucidated, *i.e.* whether it be systemic or during enterohepatic circulation. Nonetheless, in either case the underlying principle is the same.

[7] The ureters of these animals were also ligated to prevent the rapid loss of circulating morphine glucuronides through renal excretion. Ureter ligation was not expected to affect morphine clearance since very little morphine is eliminated by renal clearance.

TABLE 2

Limiting cases of eq. 17 for Cl_s

Recycling of Drug Only:	$Cl_r^d + Cl_{mf} + (1 - f_a^d) \cdot Cl_b^d$
A. Complete recycling ($f_a^d \to 1$):	$Cl_r^d + Cl_{mf}$
B. No recycling ($f_a^d \to 0$):	$Cl_r^d + Cl_{mf} + Cl_b^d$
Recycling of Drug Conjugate Only:	$Cl_r^d + Cl_{mf} + (1 - f_b^{dc} \cdot f_h \cdot f_a^d) \cdot Cl_{cf}$
C. Complete recycling ($f_b^{dc} \to 1, f_h \to 1, f_a^d \to 1$):	$Cl_r^d + Cl_{mf}$
D. No recycling ($f_b^{dc} \to 0, f_h \to 0$, or $f_a^d \to 0$):	$Cl_r^d + Cl_{mf} + Cl_{cf}$
E. Partial recycling	
E.1. Conjugate eliminated exclusively by bilary excretion ($f_b^{dc} \to 1$):	$Cl_r^d + Cl_{mf} + (1 - f_h \cdot f_a^d) \cdot Cl_{cf}$
E.2. Complete drug conjugate hydrolysis ($f_h \to 1$):	$Cl_r^d + Cl_{mf} + (1 - f_b^{dc} \cdot f_a^d) \cdot Cl_{cf}$
E.3. Complete aglycone reabsorption ($f_a^d \to 1$):	
E.4. Elimination of drug conjugate exclusively in bile and complete drug conjugate hydrolysis ($f_b^{dc} \to 1, f_h \to 1$):	$Cl_r^d + Cl_{mf} + (1 - f_a^d) \cdot Cl_{cf}$
E.5. Elimination of drug conjugate exclusively *via* bile and complete aglycone reabsorption ($f_b^{dc} \to 1, f_a^d \to 1$):	$Cl_r^d + Cl_{mf} + (1 - f_h) \cdot Cl_{cf}$
E.6. Complete conjugate hydrolysis and complete drug reabsorption ($f_h \to 1, f_a^d \to 1$):	$Cl_r^d + Cl_{mf} + (1 - f_b^{dc}) \cdot Cl_{cf}$

TABLE 3

Alteration in systemic clearance (Cl_s) due to perturbations at each step of the recycling cascade

Step	Perturbation	Consequences
A. Fraction of conjugate metabolite excreted into bile, f_b^{dc}	1. Conjugate biliary clearance, k_b^{dc}	
	a. increase	$\uparrow f_b^{dc} \to \downarrow Cl_s$
	b. decrease	$\downarrow f_b^{dc} \to \uparrow Cl_s$
	2. Conjugate renal clearance, k_u^{dc}	
	a. increase	$\downarrow f_b^{dc} \to \uparrow Cl_s$
	b. decrease	$\uparrow f_b^{dc} \to \downarrow Cl_s$
B. Fraction of conjugate hydrolyzed in gut, f_h	1. Conjugate hydrolysis, k_h	
	a. decrease	$\downarrow f_h \to \uparrow Cl_s$
	2. Conjugate fecal excretion, k_f^{dc}	
	a. increase	$\downarrow f_h \to \uparrow Cl_s$
	b. decrease	$\uparrow f_h \to \downarrow Cl_s$
C. Fraction of liberated aglycone reabsorbed, f_a^d	1. Reabsorption rate of drug, k_a^d	
	a. decrease	$\downarrow f_a^d \to \uparrow Cl_s$
	2. Fecal excretion of drug, k_f^d	
	a. increase	$\downarrow f_a^d \to \uparrow Cl_s$
	b. decrease	$\uparrow f_a^d \to \downarrow Cl_s$

Fraction of Drug Conjugate Hydrolyzed in Gut (f_h). A second set of kinetic parameters controlling the recycling of drug conjugate is the fraction of conjugate hydrolyzed in the gut compartment. This fraction reflects a balance of the rate of bacterial hydrolysis and the transit rate of the conjugate metabolite through the intestine. Gut bacterial β-glucuronidase activity is related to the composition and abundance of the microflora, which to a large extent is influenced by the diet. It is known that many orally administered broad spectrum antibiotics can suppress a number of bacterial groups in the intestine that possess β-glucuronidase enzyme (*e.g. Escherichia coli* and *E. bacteroides*). Also, gut bacterial β-glucuronidase activity can be inhibited with a specific inhibitor such as D-glucaro-1,4-lactone (29). In general, inhibition of conjugate hydrolysis lowers the amount of aglycone released and effectively increases the systemic drug clearance (case B.1.a).

As was pointed out earlier, the fecal excretion rate constant is, in effect, a measure of intestinal transit time of the conjugate. An increase in gut motility could increase the extent of fecal excretion and, at the same time, diminish the extent of hydrolysis, resulting in an increase in systemic drug clearance (case B.2.a). Similarly, an adsorbant that selectively binds polar drug conjugate could decrease the availability of the conjugate metabolite for bacterial hydrolysis and increase systemic clearance. Even though, in principle, drug detoxification can be accelerated by promoting the fecal excretion of its recycled drug conjugates, there is no example of such an application in clinical toxicology.

Fraction of Drug Reabsorbed from the Gut (f_a^d). The third fractional parameter that affects the reversibility of the conjugate formation (as well as biliary clearance of the parent drug) relates to the extent of intestinal reabsorption (cases C.1 and C.2). The importance of this final step of the EHC cascade is well appre-

ciated. The use of cholestyramine and other anion exchange resins to interrupt EHC in the treatment of digitalis intoxication serves as an excellent illustration of the critical role of aglycone reabsorption in the recycling cascade.

Experimental Considerations. Although the reversible pharmacokinetics of EHC have been the subject of a number of theoretical studies, relatively little attention has been directed toward establishing appropriate experimental strategies for a quantitative or kinetic characterization of the various steps involved in the EHC cascade. This is especially the case with drugs that undergo EHC in the form of conjugate metabolites. Based on the preceding analysis of our EHC model, several useful experimental approaches can be suggested.

Recycling Index. Colburn (4) had suggested earlier that the difference in the area under the plasma drug concentration time curves under intact and bile exteriorized conditions reflects the degree of enterohepatic recycling. A similar qualitative comparison of AUC was applied to a recent analysis of lorazepam pharmacokinetics in ponies with intact and interrupted EHC (23). Within the framework of the EHC model developed herein, a kinetic relationship of the EHC parameters to the AUC ratio between bile-intact and bile-shunted animals can be derived.

When EHC is intact, the AUC (AUC_i) is given by the following equation, which is a rearrangement of eq. 16.

$$\text{Dose}/AUC_i = Cl_r^d + Cl_{mf} + Cl_b^d + Cl_{cf} - (f_a^d \cdot Cl_b^d + f_b^{dc} \cdot f_h \cdot f_a^d \cdot Cl_{cf}) \quad (23)$$

When EHC is interrupted by bile exteriorization, the AUC (AUC_{bc}) equation can be reduced to an expression equivalent to eq. 21.

$$\text{Dose}/AUC_{bc} = Cl_r^d + Cl_{mf} + Cl_b^d + Cl_{cf} \quad (24)$$

From eqs. 23 and 24, an expression for AUC ratio can be derived.

$$\frac{AUC_{bc}}{AUC_i} = 1 - \frac{f_a^d \cdot Cl_b^d + f_b^{dc} \cdot f_h \cdot f_a^d \cdot Cl_{cf}}{Cl_r^d + Cl_{mf} + Cl_b^d + Cl_{cf}} \quad (25)$$

Let

$$F_{cf} = \frac{Cl_{cf}}{Cl_r^d + Cl_{mf} + Cl_b^d + Cl_{cf}} \quad (26)$$

$$F_b^d = \frac{Cl_b^d}{Cl_r^d + Cl_{mf} + Cl^d + Cl_{cf}} \quad (27)$$

Note that the above fractions denote the respective clearance contribution of conjugate formation and biliary excretion to the total sum of irreversible clearances for the parent drug, *i.e.* the fraction of dose which is converted to the conjugate metabolite and that which is excreted into bile in intact form *in the absence of EHC.* Substitution of eqs. 26 and 27 in eq. 25 and subsequent rearrangement leads to the following expression for the area ratio:

$$\frac{AUC_{bc}}{AUC_i} = 1 - f_a^d \cdot F_b^d - f_b^{dc} \cdot f_h \cdot f_a^d \cdot F_{cf} \quad (28)$$

Eq. 28 can be further rearranged to yield the *recycling index* (RCI).

$$RCI = 1 - \frac{AUC_{bc}}{AUC_i} = f_a^d \cdot F_b^d + (f_b^{dc} \cdot f_h \cdot f_a^d) \cdot F_{cf} \quad (29)$$

RCI provides a convenient experimental measure of the overall extent of enterohepatic recycling *in vivo* and is theoretically appealing in that the index is a simple function of the net recycled fractions (see footnote 6). In essence, RCI represents the fraction of drug cleared from the systemic circulation that will eventually be returned to the systemic compartment during a given cycle.

Further elucidation of the factors controlling the extent of EHC for a particular compound and its conjugate metabolite would require an estimate of the constituent fractions for each of the net recycled fraction terms in eq. 29. Very rarely have attempts been made to delineate the individual steps of EHC in a quantitative fashion, especially for a drug that recycles *via* its conjugate metabolite. Part of the reason may be related to the fact that a systemic kinetic approach to the problem has never been developed. Several possible experimental approaches to the determination of the component fractions are considered below. The discussion is focused on the case when only drug conjugate is recycled (*i.e.* $f_a^d \cdot F_b^d = 0$).

Estimating the Fraction of Administered Dose Excreted into Bile as the Conjugate Metabolite ($f_b^{dc} \cdot F_{cf}$). The most direct approach to determining this fraction is by iv administration of the parent compound to a bile duct-cannulated animal. The cumulative amount of drug or the conjugate metabolite excreted in bile as a fraction of the administered dose is measured. Since the experiment is carried out under a nonrecycling condition, the implicit assumption is that these fractions remain constant during repeated biliary recycling.

It should be noted that the fraction of dose excreted in bile as the conjugate metabolite depends on both the extent to which drug is cleared from the systemic circulation by conjugation (*i.e.* F_{cf}) and the extent of biliary excretion of the conjugate metabolite (*i.e.* f_b^{dc}). In many instances, the conjugate metabolite is eliminated entirely by renal and biliary clearance. Hence, both F_{cf} and f_b^{dc} can simply be estimated from cumulative recovery of derived drug conjugate in urine and bile after parent-drug administration in a bile duct-cannulated animal.

A more general approach to the estimation of F_{cf}, as proposed by Pang (30), would entail a comparison of the AUCs of drug conjugate after separate iv administration of equimolar doses of parent drug and synthetic drug conjugate in a bile-exteriorized animal. The fraction of the conjugate dose excreted into bile (*i.e.* f_b^{dc}) can be measured in the same experiment. However, experiments should be performed to assure that the disposition kinetics of the derived and synthetic conjugate metabolite are identical, in that a diffusional barrier does not exist between liver and blood (31), and there is no hepatic first-pass conjugation. If deviations from these conditions are found, a more elaborate model will be required to describe the enterohepatic cycling kinetics.

Estimating the Fraction of Drug Conjugate in the Gut That is Hydrolyzed and Reabsorbed as Parent Drug ($f_h \cdot f_a^d$). An estimate of this fraction can be obtained by rearranging eq. 29 when only drug conjugate is recycled (*i.e.* $f_a^d \cdot F_b^d = 0$).

$$f_h \cdot f_a^d = \frac{1 - (AUC_{bc}/AUC_i)}{f_b^{dc} \cdot F_{cf}} = \frac{RCI}{f_b^{dc} \cdot F_{cf}} \quad (30)$$

A practical application of the derived equation would involve parallel clearance experiments with intact and bile duct-exteriorized animals. In both sets of studies, serial blood sampling is required to determine the AUC ratio of parent drug. As was discussed in the preceding section, the product $f_b^{dc} \cdot F_{cf}$ or the fraction of dose excreted in the bile as the conjugate metabolite

can be estimated from cumulative biliary excretion of drug conjugate in the bile duct-cannulated animals.

A more traditional method of estimating the extent of intestinal hydrolysis and reabsorption involves the "linked" animal model (32, 33). Following drug administration, the bile obtained from a donor animal is administered intraduodenally to a recipient animal also with a bile fistula, and the excreta from both animals are collected and assayed. Although the linked animal model provides a direct assessment of $f_h \cdot f_a^d$, the animals are subject to extensive abdominal surgery which may disturb normal gastrointestinal and liver physiology. Moreover, the extent of recycling may be dependent upon the rate of intraduodenal infusion of the donor bile, in the event the capacity of the gut flora to hydrolyze the glucuronide conjugate is saturable and rate limiting, as has been demonstrated with phenolphthalein glucuronide (34). Hence, the presently proposed pharmacokinetic approach in an intact animal may be a more convenient and, possibly, a more physiologically realistic assessment of $f_h \cdot f_a^d$. Further experiments will be required to validate the proposed method.

A further resolution of the drug conjugate recycled fraction into the component fractions, f_h and f_a^d, is fraught with experimental difficulties. Analysis of drug content in feces collected from intact animals may provide some clues. For example, the absence of any conjugate product in feces may indicate complete intestinal hydrolysis of the conjugate (*i.e.* $f_h = 1$). In a similar manner, the absence of free drug in feces may lead to the conclusion that the liberated aglycone was completely absorbed. However, these observations are valid only if gut bacterial metabolism does not continue *ex vivo* (requiring special care in handling of fecal samples) or the aglycone is not subject either to further bacterial metabolism or to spontaneous chemical degradation in the intestine.

One experimental approach that has been used to estimate f_h is to infuse bile from a donor animal containing known quantities of drug conjugate into the duodenum of either the control recipient animal or animals pretreated with antibiotics to suppress intestinal microflora (34). The amount of aglycone absorbed by one group (as assessed by AUC measurements) is compared with the amount absorbed by the other. However, complete abolition of gut bacterial metabolism is never assured. Also, additional control experiments must be conducted to verify whether antibiotic pretreatment would interfere with the absorption of the parent compound.

It has been suggested that intraduodenal infusion of the parent drug (preferably in a "bile" vehicle) would allow a direct assessment of the extent of intestinal reabsorption (f_a^d). Again, it may be difficult, if not impossible, to simulate the concentration gradient profile of the liberated aglycone along the length of the intestines during normal transit of the drug conjugate. The degree of reabsorption may be critically dependent upon the aglycone gradient if the mucosal permeability or transport characteristics of the drug vary between different regions of the intestine.

It is clear from the preceding discussion that a number of methodological issues remain unresolved with respect to quantitative resolution of the EHC kinetics of a drug substrate. Future efforts in this area should be directed toward a critical evaluation of the existing experimental approaches.

Summary

An equation which accounts for the effects of EHC of a drug and conjugate metabolite pair on the plasma clearance of the parent drug was developed. The model equation provides for the first time a conceptual framework for a comprehensive kinetic analysis of the consecutive steps involved in EHC of a drug and conjugate metabolite pair. Our analysis of several literature reports pointed to extrabiliary clearance of the conjugate metabolite as an important, but often overlooked, determinant of the extent of EHC. Finally, the theoretical analysis also revealed means by which the fractional parameters for each discrete step in the EHC cascade can be determined experimentally. This should lead to improvements in experimental strategies leading toward a more complete characterization of the EHC of drugs in the future.

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