Transl Clin Pharmacol. 2021 Jun;29(2):78-87
https://doi.org/10.12793/tcp.2021.29.e12
pISSN 2289-0882·eISSN 2383-5427

Translational and Clinical Pharmacology TCP

Tutorial

Predicting human pharmacokinetics from preclinical data: clearance

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OPEN ACCESS

Received: Jun 18, 2021
Accepted: Jun 18, 2021

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Funding
This research was supported by the Technology Innovation Program (or Industrial Strategic Technology Development Program-Development of disease models based on 3D microenvironmental platform mimicking multiple organs and evaluation of drug efficacy) (20008413) funded By the Ministry of Trade, Industry & Energy (MOTIE, Korea).

Conflict of Interest
- Authors: Nothing to declare
- Editors: Nothing to declare

ABSTRACT

We have streamlined known *in vitro* methods used to predict the clearance (CL) of small molecules in humans in this tutorial. There have been many publications on *in vitro* methods that are used at different steps of human CL prediction. The steps from initial intrinsic CL measurement *in vitro* to the final application of the well-stirred model to obtain predicted hepatic CL (CL_H) are somewhat complicated. Except for the experts on drug metabolism and PBPK, many drug development scientists found it hard to figure out the entire picture of human CL prediction. To help readers overcome this barrier, we introduce each method briefly and demonstrate its usage in the chain of related equations destined to the CL_H. Despite efforts in the laboratory steps, huge *in vitro* (predicted CL_H)-*in vivo* (observed CL_H) discrepancy is not rare. A simple remedy to this discrepancy is to correct human predicted CL_H using the ratio of *in vitro-in vivo* CL_H obtained from animal species.

Keywords: Clearance; Translation; Human Prediction; Small Molecule

INTRODUCTION

Elimination of drugs from the human body occurs via metabolism and excretion. Metabolism of drugs is dependent on enzymatic biotransformation by drug-metabolizing enzymes. The metabolites may be further metabolized or excreted from the body via biliary or renal routes. Drugs that are not metabolized are also excreted.

Clearance (CL) is used to describe the capacity of the human body to eliminate drugs in terms of the volume of plasma or blood. It is defined as “volume of fluid (plasma or blood) cleared of drug per unit time.” CL is the crucial parameter to predict human pharmacokinetics (PK) from *in vitro* and animal PK data in drug development. Approaches to predict human CL may be categorized into allometry and physiologically based pharmacokinetic (PBPK) methods. The allometry-based approach is recommended to predict renal CL (CL_r) of drugs because renal excretion is dependent on the glomerular filtration rate of animal species, which is a physiological parameter well correlated with the body size (weight or surface area). In CL_H, mechanistic PBPK approaches using various *in*

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Author Contributions

Conceptualization: Yim DS; Data curation: Bae SH, Choi S; Funding acquisition: Yim DS; Methodology: Bae SH, Choi S; Supervision: Yim DS; Writing - original draft: Yim DS; Writing - review & editing: Yim DS, Bae SH, Choi S.

GLOSSARY

Caco-2	human colorectal adenocarcinoma cell
CL_H	hepatic clearance
CL_{int}	intrinsic clearance
CL_{int, CYP_i}	intrinsic clearance via i-th CYP isozyme
$CL_{int,bile}$	biliary intrinsic clearance
$CL_{int,efflux}$	active (transporter-mediated) sinusoidal efflux clearance
$CL_{int,met}$	metabolic intrinsic clearance
$CL_{int,pass}$	intrinsic passive diffusion clearance across the hepatocyte cell membrane
$CL_{int,uptake}$	active (transporter-mediated) sinusoidal influx clearance
CL_r	renal clearance
$CL_{r(dog)}$	dog renal clearance
$CL_{r(rat)}$	rat renal clearance
CL_{total}	total clearance
$CL_{u,int}$	unbound intrinsic clearance
$CL_{u,int,H}$	unbound intrinsic metabolic clearance in liver
$CL_{u,int,other}$	unbound intrinsic clearance via enzymes other than CYPs
C_{prot}	protein concentration in incubation system
Cu	unbound plasma concentration
CYP	cytochrome P450
DDI	drug-drug interaction
f_m	the fraction of drug metabolized by an enzyme
$f_{u,incubation}$	the fraction of unbound drug in in vitro incubation system
f_{uB}	unbound fraction in blood
HLM	human liver microsome
HPGL	hepatocytes/g liver
ISEF	Intersystem extrapolation factor
Km	Michaelis-Menten constant
logD	log of the octanol to water distribution coefficient
logP	log of the octanol to water partition coefficient
MDCK-II	Madin-Darby canine kidney y-II
MPPGL	microsomal protein (mg)/g liver
P_{app}	apparent permeability
Q_{HB}	hepatic blood flow
RAF	Relative activity factor
rhCYP	recombinant human CYP
SA_{HHEP}	the surface area of one million hepatocytes

vitro experimental data on drug metabolism are usually applied to predict human CL, but allometric approaches may also be used.

Thanks to several key articles on the *in vitro* methods to predict human CL_H published mainly in the 2000s, human CL_H may be predicted step by step from the hepatocellular or microsomal levels to the whole body of a 60 or 70 kg human. Equations quoted from the *in vitro* method articles are accordingly applied at each step to reach the predicted whole body CL_H, the final goal. Although those articles carry essential methods used in calculating human CL_H, the acronyms, and units used in the equations differ by the article because the authors of those articles are from different research teams. Some of the equations are inadvertently hidden in the appendix part of the articles. Moreover, detailed explanations of the logic or the origin of the equations are not given in some articles. Thus, even readers who have some experience in *in vitro* metabolism studies may be frustrated by the confusing mixture of acronyms and equations quoted without clarifying the reason or context when they read the papers to figure out the sequentially applied methods used to predict human CL_H. In order to help the students and researchers working on human PK prediction, we have streamlined those sequentially applied equations while introducing each method used to produce the corresponding equation.

LABORATORY METHODS AND SYSTEMS

Human hepatocytes, microsome, or rhCYP are the three popular laboratory materials used to measure the CL_{int} of drugs. Regardless of using any of them, researchers may measure the decrease in the substrate concentration only (substrate depletion method) or measure metabolite concentration when possible (metabolite measuring method). In this tutorial, we assumed the use of the substrate depletion method for hepatocytes and microsome (initiated at steps ❶ and ❷ in **Fig. 1**) and the metabolite measuring method for the rhCYP (initiated at steps ❸ and ❹ in **Fig. 1**) for a simple explanation.

STEPS TO PREDICT CL_H

To predict human CL_{total}, we need CL_H and CL_r. Prediction of human CL_r is typically estimated from single-species animal CL_r data. In the case of CL_H, it needs many steps initiated at the cellular or microsomal experiments. (**Fig.1**) Readers will trace the steps one by one in this tutorial. For simplicity of explanation, all the laboratory steps described hereafter were limited to the hepatic metabolism occurring by CYP isozymes only. Hepatic metabolism occurring via pathways other than CYP (⓬ $CL_{u,int,other}$) may be added later as step ⓭ in **Fig. 1**.

Steps to estimate ⓬ $CL_{u,int,other}$ were not reviewed in this tutorial because the basic principle is identical to that illustrated in **Fig. 1**. For example, the $CL_{u,int}$ by UGT-mediated metabolism as a part of $CL_{u,int,other}$ may also be obtained through the same pathway composed of steps ❶ ~ ⓫.

Step ❶ CL_{int}(μl·min^{-1}·10^{6}hepatocytes^{-1}) and ❷ $CL_{int,met}$(μl·min^{-1}·mg$_{protein}^{-1}$)

When the metabolites formed by different drug-metabolizing enzymes are not known, intrinsic CL of the drug is measured using the simple substrate depletion method in human hepatocytes (❶ CL_{int}), or liver microsome (HLM ❷ $CL_{int,met}$). Typical laboratory data from the substrate depletion studies appear like plots in **Fig. 2**. The concentration of the test article (candidate molecule) used is set to be much lower than K_m (0.1~1 μM ≪ K_m).

https://doi.org/10.12793/tcp.2021.29.e12

UGT	uridine 5'-diphospho-glucuronosyltransferase
V_{max}	maximum rate of metabolite formation
$V_{max,HLM}$	maximum rate of metabolite formation in human liver microsome
$V_{max,rhCYP}$	maximum rate of metabolite formation in human recombinant CYP

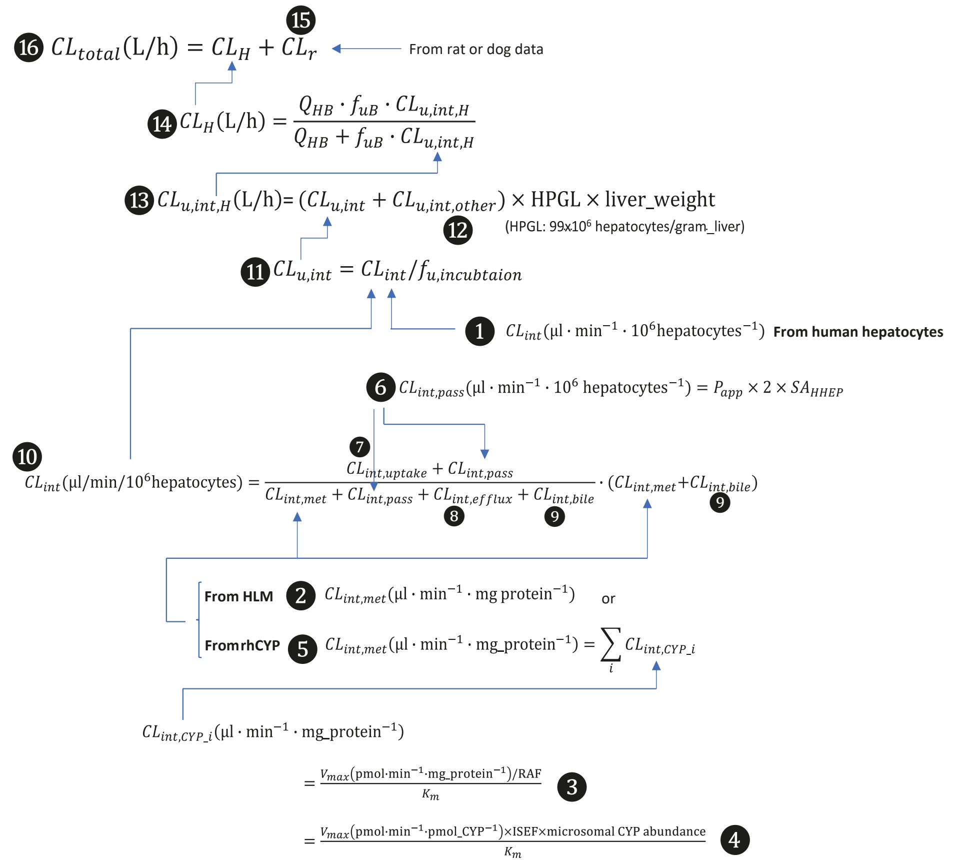

Figure 1. Sequential steps of the methods to predict human CL_H of small molecules from *in vitro* data. Units of the variables or steps should be appropriately converted to be inputted into the next steps. The units of each step shown are just examples of frequently used ones.
CL_{total}, total clearance; CL_H, hepatic clearance; CL_r, renal clearance; Q_{HB}, hepatic blood flow; f_{uB}, unbound fraction in blood; $CL_{u,int,H}$, unbound intrinsic metabolic clearance in liver; $CL_{u,int}$, unbound intrinsic clearance; $CL_{u,int,other}$, unbound intrinsic clearance via enzymes other than CYPs; HPGL, 99 × 10^6 hepatocytes/gram liver; $f_{u,incubation}$, the fraction of unbound drug in *in vitro* incubation system; CL_{int}, intrinsic clearance; $CL_{int,pass}$, intrinsic passive diffusion clearance across the hepatocyte cell membrane; P_{app}, apparent permeability; SA_{HHEP}, the surface area of one million hepatocytes; $CL_{int,uptake}$, active (transporter-mediated) sinusoidal influx clearance; $CL_{int,pass}$, intrinsic passive diffusion clearance across the hepatocyte cell membrane; $CL_{int,met}$, metabolic intrinsic clearance; $CL_{int,efflux}$, active (transporter-mediated) sinusoidal efflux clearance; $CL_{int,bile}$, biliary intrinsic clearance; HLM, human liver microsome; CYP, cytochrome P450; rhCYP, recombinant human cytochrome P450; CL_{int,CYP_i}, intrinsic clearance via *i*-th CYP isozyme; V_{max}, maximum rate of metabolite formation; RAF, relative activity factor; ISEF, intersystem extrapolation factor; Km, Michaeliis-Menten constant.

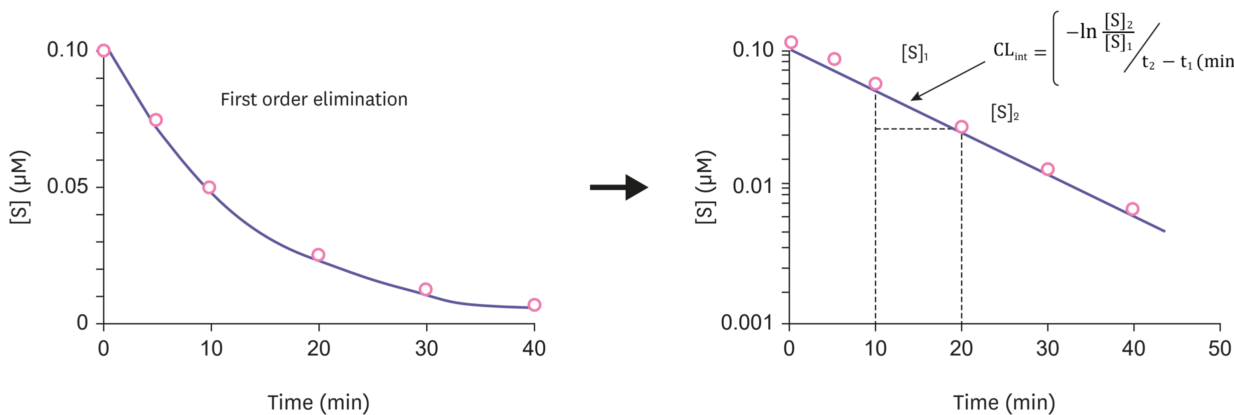

Figure 2. An example of data obtained using the substrate depletion method.

However, the simple substrate depletion approaches using HLM and hepatocytes do not propose any clue on the CYP isozymes metabolizing the drug because the effect by each isozyme is not separately measured. The rhCYP system can also be used in a substrate depletion approach to determine which CYP isozymes are responsible for drug metabolism. In the case of using hepatocytes (❶), the acronym CL_{int} did not include "met" (metabolism) in the subscript because the efflux, uptake, and diffusion over the plasma membrane and biliary excretion, if any, have contributed as well as the hepatic metabolism. (Read steps ❻~❾). For the same reason, step ❶ is directly connected to step ⓫ without being incorporated into step ❿. Its explanation is given at step ❿ in more detail.

Step ❸ $CL_{int,CYP_i}(\mu l \cdot min^{-1} \cdot mg_protein^{-1}) = \frac{V_{max}(pmol \cdot min^{-1}/mg_protein)/RAF}{K_m}$

Steps ❸ and ❹ in the **Fig. 1** measure each CYP (rhCYP) contribution using metabolite-formation approaches when metabolites are known. The results are corrected with RAF (step ❸) or ISEF (step ❹) [1], and the corrected result (CL_{int,CYP_i}) from each CYP is then inputted into step ❺ to sum up. Although the simple substrate depletion method may be helpful enough to predict human CL, information on the contribution by each CYP is a necessity to make critical decisions regarding drug metabolism and drug-drug interaction. Because the equations of RAF and ISEF are hard to understand for beginners, we briefly introduce the composition of their equations in this section.

When using rhCYP, the CL_{int} is calculated using Michaelis-Menten kinetics. The v, V_{max}, and K_m are estimated with the rate of metabolite formation by the CYP at various substrate concentrations.

$$CL_{int(M-M)} = \frac{v}{C} = \frac{V_{max}}{K_m + C} = \frac{V_{max}}{K_m} \; (when\ K_m \gg C) \qquad \text{Eq. 1}$$

However, the rate of metabolism by the rhCYP should be corrected by the amount of the specific CYP isozyme existing in the human microsome. The RAF used in step ❸ means the ratio of V_{max} values of the corresponding probe substrate measured with the rhCYP (e.g., midazolam for CYP3A4) and measured with the human liver microsome. If the test article were investigated with rhCYP3A4, the researcher should do the same thing on midazolam, the CYP3A4 probe to estimate its $V_{max,rhCYP}$. The $V_{max,HLM}$ of midazolam is also needed. A human microsomal study on midazolam has to be done for this purpose. When the study cannot be performed, published data on the probe substrate may be used instead. The RAF is calculated as in Eq. 2.

$$RAF = \frac{V_{max,rhCYP}(pmol \cdot min^{-1}/mg_protein)\ \text{of probe substrate}}{V_{max,HLM}(pmol \cdot min^{-1}/mg_protein)\ \text{of probe substrate}} \qquad \text{Eq. 2}$$

As written in Eq. 2, the V_{max} is expressed by the unit containing 1/mg protein. All the commercial rhCYP products are delivered with certificates where the protein content (unit: mg/mL) of the batch is written. The meaning of RAF can be easily understood by an example case of RAF = 2: This implies that the CYP isozyme activity of the rhCYP batch purchased by the laboratory was double the activity measured in the human liver microsome. Thus, the $CL_{int(M-M)}$ (Eq. 1) overestimated by the rhCYP batch should be corrected by dividing by 2, the RAF as indicated at step ❸ (**Fig. 1**).

 https://doi.org/10.12793/tcp.2021.29.e12

Step ❹ $CL_{int,CYP_i}(\mu L \cdot min^{-1}/mg_protein) = \frac{V_{max}(pmol \cdot min^{-1}/pmol_CYP) \times ISEF \times microsomal\ CYP\ abundance}{K_m}$

Another method to correct the inter-batch or inter-vendor variation of rhCYP is using the ISEF [1] instead of the RAF. The correction logic is similar, but the ISEF (Eq. 3) takes the CYP isozyme content into account. Both the RAF and ISEF use the V_{max} (or CL_{int}) of a probe substrate measured in rhCYP. However, the units are different, and its position in the equation (numerator or denominator) is opposite to that of the RAF. Readers may notice the difference by comparing Eq. 2 and 3.

$$ISEF = \frac{V_{max,HLM}(pmol \cdot min^{-1}/mg_protein)\ of\ probe\ substrate/[microsomal\ CYP\ abundance\left(\frac{pmol_CYP}{mg_protein}\right)]}{V_{max,rhCYP}(pmol \cdot min^{-1}/pmol_CYP)\ of\ probe\ substrate} \quad \text{Eq. 3}$$

In the denominator of Eq. 3, the $V_{max,rhCYP}$ has the unit of $pmol \cdot min^{-1}/pmol_CYP$, unlike the $V_{max,HLM}$ in the numerator of the Eq. 3 ($pmol \cdot min^{-1}/mg_protein$). Certificates inserted in the rhCYP products, the CYP content (pmol/mL) is also written together with the protein content (mg/mL) mentioned in step ❸. This information is converted to the CYP content (pmol/mg_protein) in Eq. 3. Like in the RAF, V_{max} of a probe substrate is measured in microsome and rhCYP, but the $V_{max,HLM}$ in the numerator of Eq. 3 is further divided by microsomal CYP abundance (the amount of CYP contained per 1 mg of microsomal protein, and the unit is: $\frac{pmol_CYP}{mg_protein}$). The microsomal CYP abundance has been reported for each CYP isozyme in HLM [2]. Dividing with this makes the ISEF in Eq. 3 unitless, as in the case of the RAF. The ISEF is then multiplied (not divided) in step ❹. The V_{max} and K_m in step ❹ are those of the test article measured using the rhCYP product. Also, the microsomal CYP abundance should be multiplied so that the $CL_{int_CYP_i}$, as the final result of step ❹, uses a unit including /mg_protein, not /pmol_CYP. This microsomal CYP abundance is identical to the one used in Eq. 3. Obtaining the ISEF parameters (Eq. 3) of a probe substance as a part of laboratory works on the test article will be an ideal approach, but the ISEF and microsomal CYP abundance values available in the literature [2] are frequently used to save time and resources.

The difference between RAF and ISEF is that the ISEF method is corrected for the CYP abundance. The ISEF method has been advocated over the RAF method by some researchers [3], but regulatory authorities do not recommend one method over the other.

Step ❺ $CL_{int,met}$ $(\mu L \cdot min^{-1} \cdot mg_protein^{-1}) = \Sigma_i CL_{int,CYP_i}$

The CL_{int,CYP_i} value of each CYP isozyme obtained at step ❸ or ❹ are summed up with their fractions in metabolism. According to the FDA guidance [4], sponsors should use both HLM treated with specific inhibitors and rhCYP to identify the enzymes metabolizing the test article (reaction phenotyping). The use of human liver tissues (freshly prepared or cryopreserved hepatocytes) is also an option in that guidance. However, it is not popular because the metabolizing enzymes to be inhibited are too many, and the diffusion of inhibitors beyond the hepatocyte cell membrane may also influence the assay.

The f_m (Eq. 4) by each CYP is calculated by dividing the $CL_{int_CYP_i}$ of the i-th isozyme with the sum obtained for all the isozymes tested ($\sum_i CL_{int,CYP_i} = CL_{int,met}$) or the $CL_{int,met}$ estimated using HLM. It will be used for predicting the magnitude of the drug-drug interaction (DDI) caused by inhibition of a CYP isozyme.

$$f_m = \frac{CL_{int_CYP_i}}{\sum_i CL_{int_CYP_i}} \quad \text{Eq. 4}$$

Step ❻ $CL_{int,pass}$ (μL·min^{-1}·10^{6}hepatocyte^{-1}) = P_{app} × 2 × SA_{HHEP}

Steps ❻–❾ are incorporated into step ❿. We explain the components of the step ❿ one by one. The $CL_{int,pass}$, in other words, $CL_{int,diffusion}$ is a parameter on passive diffusion of the test article into the cytoplasm of the hepatocyte. However high the $CL_{int,CYP}$ may be, the test article cannot be metabolized *in vivo* unless it passes the plasma membrane of the hepatocyte. The formula in step ❻ was first introduced in the report by Li et al. [5]. The P_{app} (unit: 10^{-6} cm/s) is the permeability obtained using the MDCK-II cell. The SA_{HHEP} is the surface area of one million hepatocytes ($4\pi r^2 \cdot 10^6$, where r is the average diameter, 13.52 μm, of the human hepatocyte assumed to be spherical). The logic behind "2×" of the SA_{HHEP} may be explained by the laboratory method measuring P_{app}. Regardless of using the Caco-2 or MDCK-II, P_{app} is measured using the speed of drugs to penetrate across the cell monolayer to move from apical to basolateral (or in the opposite direction). In order to move over the cell monolayer, the drug molecules must pass the plasma membrane twice: first from the apical solute to the cytoplasm, second from the cytoplasm to the basolateral solute. Unlike the experimental P_{app} measuring conditions, the drug molecules cross the plasma membrane only once to enter the hepatic cytoplasm [5]. Thus, the speed (permeability) entering the hepatocyte would be double the speed of passing the cellular monolayer in the P_{app} measuring chamber.

As for the P_{app}, the MDCK-II permeability is recommended over Caco-2 because the MDCK-II cells express few efflux transporters, unlike Caco-2. The known correlation between logD (at pH 7.4) and P_{app} [5] may be used when the MDCK-II permeability data is not available, as shown in Eq. 5 (the logD means $\log_{10}$D).

$$\log_{10}(P_{app}) = \text{logD}_{7.4} \times 0.4773 - 5.843 \quad \text{Eq. 5}$$

Finally, the $CL_{int,pass}$ (μL/min/10^6 hepatocytes) is obtained as "P_{app}×2×SA_{HHEP}" after appropriate unit conversion.

Steps ❼ ($CL_{int,uptake}$), ❽ ($CL_{int,efflux}$), and ❾ ($CL_{int,bile}$)

Besides the passive diffusion, drug molecules also pass the plasma membrane via uptake transporters or efflux transporters. Their influences are to be incorporated. Likewise, elimination via biliary excretion as well as metabolism also contributes to CL_{int}. We may measure $CL_{int,uptake}$, $CL_{inf,efflux}$, and $CL_{int,pass}$ using other *in vitro* experimental systems. The $CL_{int,bile}$ is measured in sandwich-cultured human hepatocytes. Because these methods are not routinely employed in conventional studies, they may be fixed to 0 when the data are unavailable.

Step ❿ CL_{int}(μL/min/10^{6}hepatocytes) =

$$\frac{CL_{int,uptake} + CL_{int,pass}}{CL_{int,met} + CL_{int,pass} + CL_{int,efflux} + CL_{int,bile}} \cdot (CL_{int,met} + CL_{int,bile})$$

The results from the previous steps ❷ or ❺ and ❻–❾ are incorporated in the equation for CL_{int} in this step. The equation for overall hepatic CL_{int} (step ❿) is derived from the dynamic relation of the intra-hepatocyte drug amount with its uptake, efflux, passive diffusion, metabolic elimination, and biliary excretion [6] that is illustrated in **Fig. 3**. Units of CL terms used are to be converted accordingly so that the final CL_{int} has the unit of μL/min/10^6 hepatocytes.

https://doi.org/10.12793/tcp.2021.29.e12

$$CL_{int} = \frac{CL_{int,uptake} + CL_{int,pass}}{CL_{int,met} + CL_{int,pass} + CL_{int,effluc} + CL_{int,bile}} \cdot \left(CL_{int,met} + CL_{int,bile}\right)$$

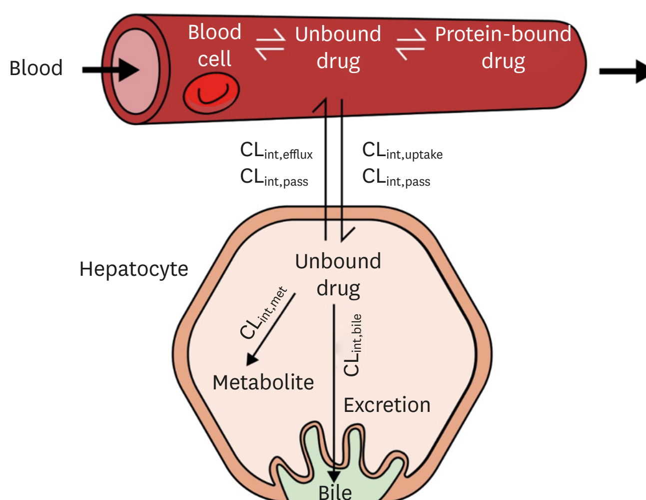

Figure 3. Schematic view of the CL terms involved in the estimation of overall intrinsic hepatic CL.
CL_{int}, intrinsic clearance; $CL_{int,uptake}$, active (transporter-mediated) sinusoidal influx clearance; $CL_{int,pass}$, intrinsic passive diffusion clearance across the hepatocyte cell membrane; $CL_{int,met}$, metabolic intrinsic clearance; $CL_{int,efflux}$, active (transporter-mediated) sinusoidal efflux clearance; $CL_{int,bile}$, biliary intrinsic clearance.

However, when the CL_{int} data is measured in whole hepatocytes (step ❶), step ❿ is not necessary. Because the test article must penetrate the plasma membrane of hepatocytes, the influences by $CL_{int,uptake}$, $CL_{inf,efflux}$ and $CL_{int,pass}$ were already incorporated in the measured results of the CL_{int} that makes the conversion at step ❿ redundant. When the test article is excreted to bile canaliculi, the $CL_{int,bile}$ would also contribute to the CL_{int} measured in the *in vitro* whole hepatocyte system if the biliary excretion mechanism in the hepatocytes functions appropriately despite the destructed canaliculi structure. Thus, the result of step ❶ is directly inputted into step ⓫ without undergoing step ❿.

Step ⓫ $CL_{u,int} = CL_{int}/f_{u,incubation}$

The CL_{int} in the previous step is then converted to $CL_{u,int}$ by dividing with $f_{u,incubation}$. Because only the unbound drug molecules interact with metabolizing enzymes in hepatocytes, we need to know $CL_{u,int}$, rather than the CL_{int} of the metabolizing enzymes (microsomal enzymes). Thus, the unbound drug fraction in the *in vitro* incubation buffer used for metabolic activity studies should be known. Although plasma protein binding is not likely in the incubation buffer, drug molecules may nonspecifically bind to microsomal protein, whose extent determines the $f_{u,incubation}$. Rather than measuring the $f_{u,incubation}$ experimentally, researchers use equations predicting the $f_{u,incubation}$ from the logP or logD values of the molecules. This approach was reported by Austin et al. [7,8], and Hallifax and Houston [9] proposed a revised equation (Eq. 6) with the non-linear relationship that shows improved performance.

$$f_{u,incubation} = \frac{1}{1 + C_{prot} \times 10^{0.072\times(\log P \text{ or } \log D)^2 + 0.067\times(\log P \text{ or } \log D) - 1.126}} \qquad \text{Eq. 6}$$

C_{prot} is the protein concentration in the incubation system.

When whole hepatocytes are used, another empirical equation (Eq. 7) was proposed [10]. In both equations, logD is used for acidic or neutral drugs and logP for basic drugs.

https://doi.org/10.12793/tcp.2021.29.e12

$$f_{u,incubation} = \frac{1}{1 + 125 \cdot V_R \times 10^{0.072\times(\text{logP or logD})^2+0.067\times(\text{logP or logD})-1.126}} \quad \text{Eq. 7}$$

and V_R is the ratio of cell volume/incubation volume, that is 0.005 at the cell concentration of 10^6 cells/ml.

Steps ⑫ and ⑬ $CL_{u,int,H}$(L/H) = ($CL_{u,int}$ + $CL_{u,int,other}$) × HPGL × liver_weight

$CL_{u,int,other}$ is the CL_{int} by enzymes other than CYP (e.g., phase II drug-metabolizing enzymes such as UGT or other enzymes). Although this is not commonly done, *in vitro* studies to obtain $CL_{u,int,other}$ may be performed if the test article is known to be substantially metabolized by hepatic enzymes other than CYP.

Now, the unit of $CL_{u,int}$ obtained in step ❿ is to be converted to the unit representing the whole liver of a human adult. The HPGL is known as 99 × 10^6 hepatocytes/gram liver [11], and the liver weight in adults is about 1,200–1,600 g, positively correlated with the body size [12]. If the /mg protein instead of the /10^6 hepatocyte were used to describe $CL_{u,int}$, the MPPGL (32 mg/g liver) [11] would be used instead of HPGL.

Steps ⑭, ⑮, and ⑯

The $CL_{u,int,H}$ is then incorporated into the well-stirred model. It should be kept in mind that the Q_{HB} and f_{uB} are those for blood, not plasma. Human CL_r predicted from direct correlation to $CL_{r(rat)}$, or $CL_{r(dog)}$ was better than the prediction from allometric scaling with multi-species data [13]. By summing up the CL_H and CL_r, we get the CL_{total} of the test article at last.

CL OF MONOCLONAL ANTIBODIES AND OTHER BIOLOGICS

Unlike small molecules, monoclonal antibodies are nonspecifically eliminated in the reticuloendothelial system and target cells if the antibody-target complex is internalized into the cells (target-mediated disposition). It is well known that their human CL is best predicted by applying the allometric exponent (about 0.8~0.9) on monkey's CL (single species) rather than on multiple species' CL [14]. The target-mediated drug disposition model with various disposition parameters incorporated is frequently used to predict human PK of monoclonal antibodies. CYP does not metabolize biologics, including antibodies. Their elimination may be closely related to their mechanisms of action. Detailed review on the CL of biologics is not in the scope of this tutorial.

COMPENSATION OF *IN VITRO-IN VIVO* DISCREPANCY

This tutorial tried to introduce the chain of methods currently used to predict human CL_H of small molecules. However, CL prediction of the current method has not been much satisfactory because of the huge *in vitro-in vivo* discrepancies. Specifically, the vast interlaboratory or inter-batch variation in microsomal or rhCYP-measured CL_{int} values are the main culprits of the tremendous discrepancies (sometimes in double digits). A frequently used method to deal with this issue is to correct with the ratio of *in vitro*-predicted CL_H (the value obtained at step ⓮ in this tutorial) and *in vivo*-observed CL_H obtained from animal PK studies (most commonly rats): i.e., when the predicted rat CL_H calculated from the rat

microsomal study is 5 times the observed rat *in vivo* CL_H, the predicted human CL_H should be divided by 5 to correct the *in vitro-in vivo* discrepancy. This correction method is possible only when the animal *in vitro* study (microsome or hepatocyte) is done together with the human *in vitro* study at the same laboratory.

FUTURE OF HUMAN CL PREDICTION

Many kinds of *in vitro* systems may mimic the live human liver better than the conventional culture method. The 3-D culture, bioprinting, and single or multi-organ chips are such examples. Because the technical advance is so fast, we cannot assert that one specific method or tool will be the future standard. If any of those approaches improve the performance, time, or budget of human CL prediction significantly, this tutorial may have to be rewritten thoroughly.

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