

# ILV-Bestellung vom 02.02.2015

U 499

<b>Von:</b> 578/2 Charité - Universitätsmedizin Berlin, Campus Charite Mitte	<b>An:</b> 578/m : Charité Universitätsmedizin Berlin, Magazin	<b>Signatur</b> Z 15 /034
<b>Titel der Zeitschrift:</b> Pharmacology		<b>Besteller:</b> Matthias König Doktorand - CVK Reinickendorfer Str. 61 13347 Berlin Computational Systems Biochemistry Haus 10
<b>ISSN (optional):</b> 0031-7012	<b>PMID (optional):</b> 392549	
<b>Jahr:</b> 1979	<b>Band/Jahrgang:</b> 19	<b>Heft:</b> 3
<b>Seiten:</b> 105-10		<b>Klinik/Institut/Abteilung:</b>
<b>Verfasser des Aufsatzes:</b> Keiding, S		<b>Telefon:</b> (030) 450 576104/317 <b>Fax:</b> (030) 450 576 920 <b>Post bitte an:</b> Charité - Universitätsmedizin Berlin, Campus Charite Mitte Medizinische Bibliothek Fernleihe 10098 Berlin Deutschland
<b>Titel des Aufsatzes (gekürzt):</b> Hepatic clearance measurements and pharmacokinetics		
<b>Bearbeitungsvermerke der liefernden Bibliothek:</b>		<b>Vermerke der bestellenden Bibliothek:</b>

## Current Review

Pharmacology 19: 105–110 (1979)

# Hepatic Clearance Measurements and Pharmacokinetics

S. Keiding and P. Buch Andreasen

Medical Department A, Division of Hepatology, Rigshospitalet, Copenhagen

**Key Words.** Drug metabolism · Hepatic clearance · Liver circulation · Liver cirrhosis ·

Pharmacokinetics

**Abstract.** This review emphasizes the need for well-defined models for hepatic pharmacokinetics and discusses the interpretation of hepatic clearance measurements. Such recent approaches are subjected to theoretical and experimental comparisons. The pharmacokinetic consequences, including a classification of drugs according to hepatic clearance, are outlined.

Measurements of hepatic clearance may be useful for prediction of dosage in clinical pharmacology and for quantitative evaluation of liver function in the clinical management of patients with liver diseases. In this review we will focus on some recently suggested pharmacokinetic interpretations of hepatic clearance measurements.

### Hepatic Saturation Kinetics

Many hepatic processes are enzymatic reactions and it has been shown for many such reactions *in vitro* that the conversion rate ( $v$ ) is related to the concentration ( $c$ ) by Michaelis-Menten kinetics:

$$v = \frac{V_{\max} \cdot c}{K_m + c}, \quad (1)$$

where  $V_{\max}$  is the maximal conversion rate and  $K_m$  the half saturation concentration.

It is often assumed that at drug concentrations attained under usual therapeutic conditions, the hepatic elimination of drugs follows first-order kinetics. For most drugs, however, this simplification will probably not hold true when a detailed analysis of the elimination kinetics is performed. Rather the kinetics often follows saturation kinetics, where  $V_{\max}$  and  $K_m$  are 'apparent' values in the sense that they describe the processes at the complex situation in the intact liver with several rate-limiting enzymatic processes, metabolite production, membrane-bound enzymes, varying metabolic conditions, protein binding, impairment during liver disease, and — as discussed below — directional sinusoidal perfusion. For example, it has already been demonstrated that first-order ki-

netics does not give a satisfactory description of the elimination of diphenylhydantoin (2), acetylsalicylic acid (15), and dicoumarol (16) in man.

### Blood Flow and Pharmacokinetics in the Intact Liver

In contrast to the *in vitro* situation the intact liver is a structured organ, and this has to be taken into account in quantitative evaluation of hepatic pharmacokinetics. In the test tube the drug concentration can be uniform, whereas in the intact liver the elimination (or production) of substances from (or into) the blood creates a concentration gradient from the inflow ( $c_i$ ) to the outflow ( $c_o$ ). At steady-state with no time-dependent change in blood concentration,  $dc/dt = 0$ , we have

$$v = F \cdot (c_i - c_o) \quad (2)$$

where  $v$  is the elimination rate and  $F$  the flow rate.

Two simple approaches to mathematical models of hepatic steady-state elimination kinetics have recently been proposed. Winkler *et al.* (24) in 1974 proposed a so-called sinusoidal perfusion model, which describes the decreasing sinusoidal concentration as an effect of elimination in hepatocytes lining directionally perfused sinusoidal tubes. The mathematical treatment of this idea gives a Michaelis-Menten relation with the logarithmic average concentration  $\hat{c}$  as 'operative' sinusoidal concentration (3):

$$v = \frac{V_{max} \cdot \hat{c}}{K_m + \hat{c}} \quad (3)$$

where  $\hat{c} = (c_i - c_o)/\ln(c_i/c_o)$ . At high blood concentrations where the elimination becomes more and more saturated, the sinusoidal concentration gradient approximates a straight line

and  $\hat{c}$  approximates  $(c_i + c_o)/2$ . At very low concentrations, the concentration gradient becomes exponential. The model has been used to describe the elimination of galactose (13), ethanol (12), and antipyrine (1) in the intact liver.

In practice it is usual to determine a so-called clearance ( $v/c$ ) from an exponential drug concentration time curve after a bolus injection. This presumes first-order kinetics, i.e. proportionality between  $v$  and  $c$ . For reactions following Michaelis-Menten kinetics this is the case for concentrations much less than  $K_m$ . In the usual terminology  $v/c_i$  is often called 'systemic' clearance (the concentration being measured in the peripheral blood) and  $v/c_o$  'intrinsic' clearance (the concentration measured in the hepatic veins). Both of these clearance measurements, however, depend on flow according to the sinusoidal perfusion model (see fig. 1, 2). A flow-independent clearance can be estimated from the model, namely  $v/\hat{c}$ , called 'true' clearance. It equals  $V_{max}/K_m$ , i.e. the slope of the Michaelis-Menten relation (equation 3) for the concentrations approximating zero.

Gillette (9) in 1971 proposed that the hepatic outflow concentration be used as an approximation of the sinusoidal concentration. This presumes that the drug concentration in the hepatic veins is equal to the concentration at the location where elimination actually takes place. The corresponding Michaelis-Menten relation is:

$$v = \frac{V_{max} \cdot c_o}{K_m + c_o} \quad (4)$$

Rowland *et al.* (22) consequently suggested the use of 'intrinsic' clearance =  $v/c_o$  as a flow-independent clearance measurement. This venous equilibration model, however, does not take into account the effect of the blood

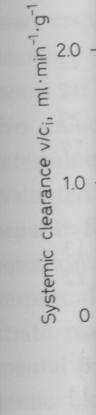
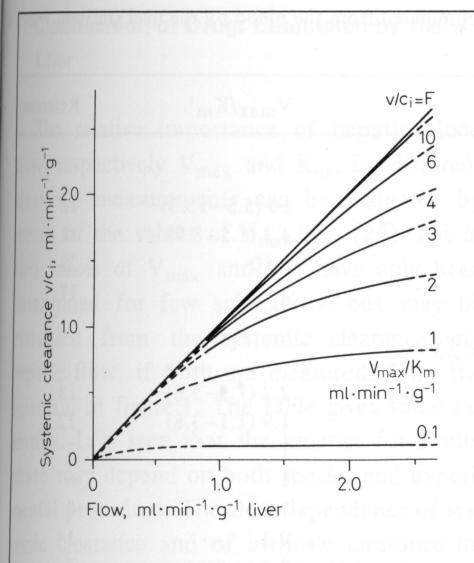


Fig. 1.  
=  $F(1-e^{-kt})$   
= elimination  
= hepatic  
rate;  $K_m$  =

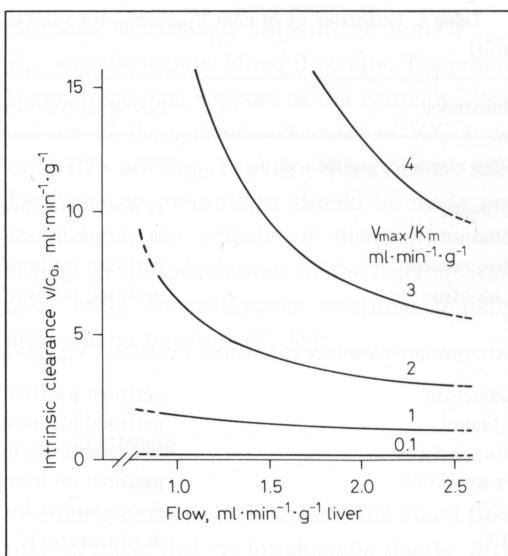
concentrations  
this ob-  
interpret  
in phar-  
discuss  
perimen-  
possible  
kinetics  
one of

Expe-  
Mod-

It  
models  
under  
differ-  
Experi-



**Fig. 1.** Flow-dependence of systemic clearance  $v/c_i = F(1 - e^{-V_{max}/FK_m})$ . The term  $v$  indicates hepatic elimination rate;  $c_i$  = hepatic inflow concentration;  $F$  = hepatic blood flow;  $V_{max}$  = maximal elimination rate;  $K_m$  = saturation concentration (24).



**Fig. 2.** Flow-dependence of intrinsic clearance  $v/c_o = F(e^{V_{max}/FK_m} - 1)$ . The term  $v$  indicates hepatic elimination rate;  $c_o$  = hepatic outflow concentration;  $F$  = hepatic blood flow;  $V_{max}$  = maximal elimination rate;  $K_m$  = half saturation concentration.

concentration gradient in the sinusoids. Despite this obvious weakness as regards biological interpretation, this model has been widely used in pharmacokinetics. In the following we will discuss some of the problems concerning experimental comparison of the two models and possible consequences for human pharmacokinetics and drug administration of accepting one of the two models as working hypothesis.

#### Experimental Comparison of the Two Models

It may be difficult to compare the two models because  $\hat{c}$  and  $c_o$ , or  $v/\hat{c}$  and  $v/c_o$  may under many conditions be so similar that the difference is concealed by experimental errors. Experiments, therefore, must be designed with

the purpose of maximizing the difference, while keeping all other variables constant. The experimental procedure (e.g. liver perfusion) must be of high quality, and the metabolic stability must be controlled in each experiment.

Only few studies, designed to compare the two models, have been published. The prediction of flow-independence of  $\hat{c}$ , respectively  $c_o$  at a given  $v$  (equation 3 and 4, respectively) was tested by means of galactose elimination in recirculating perfused rat livers (11). The results were consistent with the sinusoidal perfusion model and did not agree with the venous equilibration model. Another study (19) reaches the opposite conclusion: changes of  $c_o$  for lidocaine in once-through perfused rat livers at first-order conditions following flow changes were better predicted by the venous equilibration model than by the sinusoidal perfusion

**Table I.** Estimates of *in vivo*  $V_{max}/K_m$  for substances eliminated from the blood by the liver ( $\text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$  liver)

Substance	Species, procedure	$V_{max}/K_m^1$	Reference
<i>High clearance substances, i.e. <math>V_{max}/FK_m \geq 2.3</math></i>			
Ethanol	<i>in situ</i> human liver <sup>2</sup>	10 (5.5–13.3)	12
Ethanol	<i>in situ</i> pig liver <sup>2</sup>	4.3 (1.5–8.2)	12
Propranolol	perfused rat liver <sup>4</sup>	>10	23
Lidocaine	perfused rat liver <sup>4</sup>	5	17
<i>Intermediate clearance substances, i.e. <math>0.06 &lt; V_{max}/FK_m &lt; 2.3</math></i>			
Galactose	perfused pig liver <sup>2</sup>	2.2 (1.3–3.6)	13
Ethanol	perfused pig liver <sup>2</sup>	1.9 (1.1–3.8)	12
Propranolol	<i>in situ</i> monkey liver <sup>3</sup>	1	7
Propranolol	perfused rat liver <sup>3</sup>	1	6
ICG	<i>in situ</i> human liver <sup>4</sup>	1.0	8
ICG	<i>in situ</i> cat liver <sup>4</sup>	0.3	14
Antipyrine	<i>in situ</i> monkey liver <sup>3</sup>	0.3	7
<i>Low clearance substances, i.e. <math>V_{max}/FK_m \leq 0.06</math></i>			
Antipyrine	perfused pig liver <sup>2</sup>	0.05 (0.03–0.08)	1

<sup>1</sup> Mean (range).

<sup>2</sup> Estimates of  $V_{max}$  and  $K_m$  from sets of steady state values of  $c_i$ ,  $c_o$  and  $F$  (or  $v$ ), equation 2.

<sup>3</sup> Approximate estimate of  $V_{max}/K_m$  from systemic clearance ( $v/c_i$ ) and  $F$ , equation in figure 1.

<sup>4</sup> Approximate estimate of  $v/\hat{c}$  ( $= V_{max}/K_m$ ) from infusion(s) at first-order kinetics from  $c_i$ ,  $c_o$  and  $F$  (or  $v$ ).

model. However, the requirement for stability of the perfusions may be questioned (17). Two studies using metabolite production of either lidocaine (20) or phenacetin (18) were not able to distinguish between the two models.

### Pharmacokinetic Consequences

The consequences of using either model in pharmacology is most strikingly demonstrated by the change in drug concentration after oral administration if the hepatic blood flow is altered, e.g. due to heart disease or treatment with a  $\beta$ -blocking agent. If hepatic blood flow decreases from 1.2 to 1.0  $\text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$  liver the

oral dose for a drug with elimination kinetics like lidocaine,  $V_{max}/K_m$  about  $5 \text{ ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$  liver (see table I), should be about doubled to obtain the same peripheral blood concentration according to the sinusoidal perfusion model (cf. fig. 2), whereas it should not be changed according to the venous equilibration model.

Since the sinusoidal perfusion model takes the effect of the unidirectional flow in the intact liver into consideration, we suggest this model be used as the working hypothesis at present. As the experimental accuracy improves it will be possible to extend the present model to take into account, for example, the variation, within the liver, of enzyme content and flow per sinusoid (4).

### Classification Liver

The relative flow, respective clearance means of the *in vivo* values of determined from evaluated from hepatic flow, equation in examples. It is strate may de mental pro temic clearances with fig. 1, 2).

For high clearance ma hepatic bloo clearance sub used as an provided th pyrine and purpose. Fo however, sy hepatic blo substances hepatic blo

The earl on the ex ratio of  $V_{max}$  extraction *in vitro* va reflect th discussed a

The h green (IC tions, has classified (see table

	r (ml·min <sup>-1</sup> ·g <sup>-1</sup> )
Reference	
12	
12	
23	
17	
13	
12	
7	
6	
8	
14	
7	
1	

and F (or v).

on kinetics  
min<sup>-1</sup>·g<sup>-1</sup>  
doubled to  
centration  
ion model  
be changed  
model.  
odel takes  
ow in the  
uggest this  
othesis at  
improves  
ent model  
the varia-  
ntent and

### Classification of Drugs Eliminated by the Liver

The relative importance of hepatic blood flow, respectively  $V_{max}$  and  $K_m$ , for different clearance measurements can be evaluated by means of the values of  $V_{max}/K_m$  (25). The *in vivo* values of  $V_{max}$  and  $K_m$  have only been determined for few substances, but may be evaluated from the systemic clearance and hepatic flow, if both are measured, from the equation in figure 1. The table gives some examples. It is seen that the kinetics for a substrate may depend on both species and experimental procedure. The flow-dependence of systemic clearance and of intrinsic clearance increases with higher values of  $V_{max}/FK_m$  (see fig. 1, 2).

For high clearance substances, systemic clearance may be used as an estimate of the hepatic blood flow (cf. table I, fig. 1). For low clearance substances, systemic clearance may be used as an estimate of liver function ( $V_{max}$ ), provided that  $K_m$  is constant. Recently, amino-pyrine and antipyrine have been used for this purpose. For intermediate clearance substances, however, systemic clearance depends on both hepatic blood flow and liver function, and these substances are not clinically useful to evaluate hepatic blood flow or liver function.

The earlier proposals of classifications based on the extraction ratio, respectively *in vitro* ratio of  $V_{max}/K_m$  (21) are not useful, since the extraction ratio is flow-dependent (10) and the *in vitro* values for enzymatic reactions may not reflect the complex reactions *in vitro*, as discussed above.

The hepatic elimination of indocyanine green (ICG) in man, during steady-state conditions, has a  $V_{max}/K_m$  ratio about unity, and is classified as an intermediate clearance substance (see table I). As shown in figure 1 the systemic

clearance accordingly depends on both  $V_{max}/K_m$  and the hepatic blood flow rate. Thus there is no theoretical support of the common clinical use of the systemic clearance of ICG as an estimate of the hepatic blood flow rate. Furthermore precautions should be made not to include the effects of distribution and storage in the calculations of the hepatic clearance using concentrations measured initially after a bolus injection (5, 26).

### Liver Disease

During cirrhosis effective hepatic blood flow may decrease due to intrahepatic shunts. Also  $V_{max}$  often decreases in liver disease, but it has not been investigated yet if  $K_m$  values change as well. The conditions of using clearance measurements with respect to first-order kinetics may even no longer be fulfilled during liver disease (25).

### References

- 1 Andreasen, P.B.; Tønnesen, K.; Rabøl, A., and Keiding, S.: Michealis-Menten kinetics of phenazone elimination in the perfused pig liver. *Acta pharmac. tox.* 40: 1–13 (1977).
- 2 Arnold, K. and Gerber, N.: The rate of decline of diphenylhydantoin in human plasma. *Clin. Pharmac. Ther.* 11: 121–134 (1970).
- 3 Bass, L.; Keiding, S.; Winkler, K., and Tygstrup, N.: Enzymatic elimination of substrates flowing through the liver. *J. theor. Biol.* 60: 393–409 (1976).
- 4 Bass, L.; Robinson, P., and Bracken, A.J.: Hepatic elimination of flowing substrates. The distributed model. *J. theor. Biol.* 72: 161–184 (1978).
- 5 Branch, R.A.; James, J.A., and Read, A.E.: The clearance of antipyrine and indocyanine green in normal subjects and in patients with chronic liver disease. *Clin. Pharmac. Ther.* 20: 81–89 (1976).

- 6 Branch, R.A.; Nies, A.S., and Shand, D.G.: The disposition of propranolol. General implications of the effects of liver blood flow on elimination from the perfused rat liver. *Drug Metab. Disposit.* 1: 687-690 (1973).
- 7 Branch, R.A.; Shand, D.G.; Wilkinson, G.R., and Nies, A.S.: Increased clearance of antipyrine and *d*-propranolol after phenobarbital treatment in the monkey. *J. clin. Invest.* 53: 1101-1107 (1974).
- 8 Caesar, J.; Shaldon, S.; Chiandussi, L.; Guevara, L., and Sherlock, S.: The use of indocyanine green in the measurement of hepatic blood flow and as a test of hepatic function. *Clin. Sci.* 21: 43-57 (1961).
- 9 Gillette, J.R.: Factors affecting drug metabolism. *Ann. N.Y. Acad. Sci.* 174: 43-66 (1971).
- 10 Keiding, S.: Hepatic elimination kinetics. The influence of hepatic blood flow on clearance determinations. *Scand. J. clin. Lab. Invest.* 36: 113-117 (1976).
- 11 Keiding, S. and Chiarantini, E.: Effect of sinusoidal perfusion on galactose elimination kinetics in perfused rat liver. *J. Pharmac. exp. Ther.* 205: 465-470 (1978).
- 12 Keiding, S.; Johansen, S.; Midtbøll, I.; Rabøl, A., and Christiansen, L.: Ethanol elimination kinetics in human liver and pig liver *in vivo* (to be published).
- 13 Keiding, S.; Johansen, S.; Winkler, K.; Tønnesen, K., and Tygstrup, N.: Michaelis-Menten kinetics of galactose elimination by the isolated perfused pig liver. *Am. J. Physiol.* 230: 1302-1313 (1976).
- 14 Krarup, N. and Larsen, J.A.: The influence of dye infusion rate and hepatic plasma flow on indocyanine green clearance. *Scand. J. clin. Lab. Invest.* 36: 183-188 (1976).
- 15 Levy, G.: Pharmacokinetics of salicylate elimination in man. *J. pharm. Sci.* 54: 959-967 (1965).
- 16 O'Reilly, R.A.; Aggeler, P.M., and Leong, L.S.: Studies on the coumarin anticoagulant drugs: a comparison of the pharmacodynamics of dicoumarol and warfarin in man. *Thromb. Diath. haemorrh.* 11: 1-22 (1964).
- 17 Pang, K.S.: Hepatic clearance of drugs. Discrimination between two models and implications in pharmacokinetics and therapeutics; thesis, University of California (1976).
- 18 Pang, K.S. and Gillette, J.R.: Kinetics of metabolite formation and elimination in the perfused rat liver preparation. Differences between the elimination of preformed acetaminophen and acetaminophen formed from phenacetin. *J. Pharmac. exp. Ther.* 207: 178-194 (1978).
- 19 Pang, K.S. and Rowland, M.: Hepatic clearance of drugs. II. Experimental evidence for acceptance of the 'well-stirred' model over the 'parallel tube' model using lidocaine in the perfused rat liver *in situ* preparation. *J. Pharmacokinet. Biopharm.* 5: 655-679 (1977).
- 20 Pang, K.S. and Rowland, M.: Hepatic clearance of drugs. III. Additional experimental evidence supporting the 'well-stirred' model, using metabolite (MEGX) generated from lidocaine under varying hepatic blood flow rates and linear conditions in the perfused rat liver *in situ* preparation. *J. Pharmacokinet. Biopharm.* 5: 681-699 (1977).
- 21 Rane, A.; Wilkinson, G.R., and Shand, D.G.: Production of hepatic extraction ratio from *in vitro* measurement of intrinsic clearance. *J. Pharmac. exp. Ther.* 200: 420-424 (1977).
- 22 Rowland, M.; Benet, L.Z., and Graham, G.G.: Clearance concepts in pharmacokinetics. *J. Pharmacokinet. Biopharm.* 1: 123-136 (1973).
- 23 Shand, D.G.; Rangno, R.E., and Evans, G.H.: The disposition of propranolol. II. Hepatic elimination in the rat. *Pharmacology* 8: 344-352 (1972).
- 24 Winkler, K.; Bass, L.; Keiding, S., and Tygstrup, N.: The effect of hepatic perfusion on assessment of kinetic constants; in Lundquist and Tygstrup, Regulation of hepatic metabolism. 6th Alfred Benzon Symp., Munksgaard, Copenhagen, pp. 797-807 (1974).
- 25 Winkler, K.; Bass, L.; Keiding, S., and Tygstrup, N.: The physiologic basis for clearance measurements in hepatology (to be published).
- 26 Zito, R.A. and Reid, P.R.: Lidocaine kinetics predicted by indocyanine green clearance. *New Engl. J. Med.* 298: 1160-1163 (1978).

Received: January 26, 1979

Accepted: January 29, 1979

S. Keiding, Medical Department A 2151,  
Rigshospitalet, Copenhagen (Denmark)