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Hepatic clearance and liver blood flow

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The principle of using clearance measurements to estimate liver blood flow was suggested more than 40 years ago by Bradley et al. [1]. The application to clinical practice is, however, still a challenge to hepatologists. New procedures are currently being proposed – not always with sufficient acknowledgement of the limitations of the procedures.

The liver blood flow rate can be measured directly by Fick's principle using, e.g., indocyanine green (ICG) as a test substance [2]. Fick's principle is based on a constant intravenous infusion of a test substance which is solely eliminated by the liver, and measurement of the concentration in arterial (A) and hepatic venous blood (V). In the steady-state the flow rate Q is calculated according to simple mass conservation as

$$Q = v/(A - V) \quad (1)$$

where v is the hepatic elimination rate of the substrate. ICG has been shown to be a suitable test substance for this procedure, with negligible extrahepatic elimination [2], minimal correction for non-steady-state if the infusion dose is kept reasonably low [3], and good agreement with directly measured flow rates in isolated perfused pig livers [3]. It should be noted that in the present context the 'liver' blood flow is synonymous with the 'splanchnic' blood flow, since the arterial blood concentration is assumed to be identical with that of the hepatic inlet to the sinusoids.

A clearance measurement, where only peripheral blood samples are required, is less invasive than the use of Fick's principle and much easier to perform in clinical practice. This advantage, however, is gained at the expense of the introduction of a number of theoretical and practical limitations. Clearance is calculated as

$$Cl = v/A \quad (2)$$

with v and A as above. By comparing equations 1 and 2 it is seen that one requirement of using Cl as a flow measurement is that V is small compared to A, i.e. the hepatic extraction fraction

$$E = (A - V)/A \quad (3)$$

is close to 1.0. The degree of approximation of Cl to Q is equal to E, since from equations 1, 2, and 3, we have

$$Cl/Q = E \quad (4)$$

It may be noted that Fick's principle gives an estimate of the total liver blood flow, since the hepatic

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venous concentration measurement takes place in mixed blood, including outlet from functioning sinusoids as well as outlet from possible intrahepatic vascular shunts. In contrast to this, clearance of a suitable test substance gives the blood flow rate through functioning sinusoids.

A survey of the most commonly used test substances for clearance measurement and their applications may illustrate some of the problems.

Examples of clinical applications

Indocyanine green clearance is often used as a measure of liver flow. Repeated studies have demonstrated, however, that the hepatic extraction fraction is around 2/3 in subjects with no liver disease and around 1/3 in subjects with liver disease, with a substantial scatter [3]. The approximation to liver flow is correspondingly low and variable; cf. equation 4. Thus ICG clearance is of limited value as an estimate of the hepatic flow rate.

The use of galactose clearance as a flow measurement, first proposed by Tygstrup and Winkler in 1958 [4] and recently taken up by Henderson et al. [5], exemplifies additional aspects of the clearance-flow problem. Henderson et al. [5] found that the hepatic extraction fraction of galactose was relatively high but decreased when the infusion rate was increased. Thus, first-order kinetics, i.e. proportionality between elimination rate and blood concentration or concentration-independence of the extraction fraction, may not have been obtained as required (cf. equation 2). This might be due to partially saturated hepatic galactose elimination [6], and in that case lowering of the galactose infusion rate would improve the approximation to the flow rate. In subjects with severe liver impairment, however, a low hepatic galactose elimination capacity may determine clearance rather than Q, even if first-order kinetics is ensured [7].

Subjects with no liver disease, however, have a hepatic galactose extraction fraction of around 0.90 [5,8]. To see whether the galactose clearance consequently could be used as a 90% approximative measure of the hepatic flow in such subjects, galactose clearance was recently compared to the flow rate measured by ICG infusion and Fick's principle (S. Keiding, unpublished data). It was found to be systematically and significantly about 50% higher than the flow rate. The difference may be explained by a galactose uptake outside the splanchnic area (urinary excretion was negligible). This will overestimate the hepatic galactose elimination rate, assumed equal to the infusion rate (cf. equation 2).

Nitroglycerin has been proposed as a test substance for measuring hepatic vascular shunts [8]. The hepatic extraction fraction was close to 1.0 in subjects with no liver disease. Assuming a similarly high extraction fraction in subjects with liver disease, a peripheral concentration measurement following oral intake of nitroglycerin was interpreted to reflect the degree of porto-systemic vascular shunts. Increased peripheral blood concentrations of nitroglycerin during liver disease could, however, equally well be explained by a decreased hepatic extraction due to liver cell impairment. Further studies are required to clarify the influence of altered blood flow and shunts, respectively, on decreased liver function, before the nitroglycerin test can be accepted in clinical practice.

These examples demonstrate the need for a definition of the conditions during which substances can be employed for clearance measurements.

General clearance conditions

Extrahepatic elimination, apart from a possible renal excretion which may be measured, must be negligible. The galactose clearance example demonstrates the need for experimental examination of this for possible new test substances.

Secondly, the hepatic extraction fraction should be close to 1.0, since the extraction fraction directly gives the clearance/flow ratio. The conditions during which this is the case depend on the elimination kinetics of the various test substrates.

Enzymatic elimination in the intact liver

Most processes in the liver are enzymatic reactions (or active transport of a similar nature). Such processes often obey Michaelis-Menten kinetics, which describes the relation between the conversion rate of

the substrate (v) and the substrate concentration rate (C) in terms of the maximal conversion rate (V_{max}) and the half-saturation concentration (K_m):

$$v = \frac{V_{max} \cdot C}{K_m + C} \tag{5}$$

The elimination rate v approximates V_{max} when the concentration is increased and becomes large compared to K_m. At low concentractions the elimination approximates first-order kinetics, with v being proportional to the concentration. The Michaelis-Menten relation describes in vitro conditions, with the enzyme molecules and the substrate molecules being completely mixed at identical substrate concentrations everywhere in the solution. In the intact liver, however, the elimination of the substrate by the liver cells lining the sinusoid creates a continuously decreasing sinusoidal blood substrate concentration in the flow direction. The rate-limiting process of the elimination in the liver cell is assumed to follow Michaelis-Menten kinetics at the local blood substrate concentration at each place along the sinusoid [9]. This presumes diffusional equilibration of the substrate in each cross-section that readily keeps up with the elimination. The mathematical treatment of this idea gives [9] the following relationship between the substrate concentration in the sinusoidal inlet (A) and outlet (V), the substrate elimination rate (v) and the hepatic blood flow Q

$$v = V_{max} + QK_m \ln(V/A) \tag{6}$$

V_{max} is the maximal elimination rate for the whole liver, and K_m the half-saturation concentration for the enzymatic elimination in the liver cells. Together with Fick's relation (equation 1), equation 6 gives a complete description of the sinusoidal perfusion model by Bass et al. [9].

Since the present discussion considers the relationship between v and A, we can eliminate V from equation 6 using equation 1. This gives

$$v = Q \cdot A \cdot \left(1 - e^{-\frac{V_{max} - v}{QK_m}}\right) \tag{7}$$

This relation is valid for A varying from zero to infinity, viz. from first-order kinetics to saturation, as illustrated in Fig. 1. Reformulation of equation 7 gives

$$v/A = Q \cdot \left(1 - e^{-\frac{V_{max}}{QK_m}\left(1 - \frac{v}{V_{max}}\right)}\right) \tag{8}$$

Based on this equation, we will now examine the conditions under which clearance is a good or a bad estimate of the hepatic blood flow.

Flow-determined clearance

First-order kinetics with v/A being independent of variations in A (and in v) is in the simplest case obtained for $v \ll V_{max}$. Clearance will approach Q from below as the exponential factor in equation (8) is diminished, or in other words when the fraction V_{max}/QK_m is increased. (It may be noted that V_{max}/QK_m is dimensionless.) As illustrated in Fig. 2, clearance is a good estimate of Q at high V_{max}/K_m values, i.e. conditions where the enzymatic capacity of the liver is abundant compared to the amount of substrate offered by the blood flow. Clearance is a bad estimate of Q at low V_{max}/K_m values, where the enzymatic capacity becomes rate-limiting – as exemplified by galactose clearance in liver patients [6].

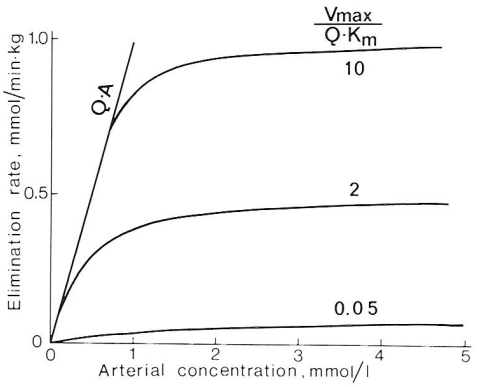

Fig. 1. Relation of hepatic elimination rate and arterial substrate concentration for three substrates with different values of V_{max}/QK_m as indicated. The straight line QA shows the amount of substrate offered to the liver by the blood flow. The curves illustrate the difference in flow-dependence of clearance v/A depending on the V_{max}/QK_m values.

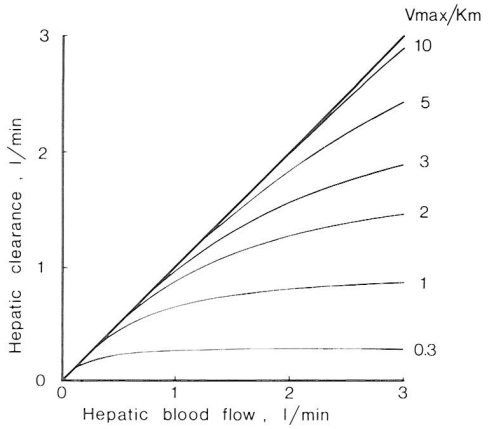

Fig. 2. Relation of hepatic clearance to liver blood flow for different values of V_{max}/K_m.

Clearance will be >95% of Q if V_{max}/QK_m is >3, and <80% of Q when V_{max}/QK_m is <1.5.

On the other hand, under special conditions, clearance may approximate Q, even for v comparable with V_{max}, with many enzymes (periportal hepatocytes) operating at near-saturation. This only takes place for substances where V_{max}/QK_m is very high, i.e. 10 or higher. The hepatic ethanol clearance in subjects with no liver disease is an example of this. V_{max}/K_m is about 10 l/min, and the hepatic extraction fraction is larger than 0.95 at hepatic ethanol elimination rates up to 60% of V_{max} [10]. The corresponding flow-determined clearance range is illustrated in Fig. 1.

Clearance in liver disease

Chronic liver disease causes a number of changes in liver structure and function, being of importance in the present context. V_{max} diminishes, both for enzymatic processes in the liver cells and for biliary excretion processes. The structure of the liver may be changed with 'capillarization' of the sinusoids, with a reduced number of endothelial pores. Thus substrates in the blood are not so readily available for the liver cells as during normal conditions, and radial diffusion may become rate-determining. This may be the reason for the marked reduction of the hepatic extraction of ICG found in some patients with cirrhosis [11].

Development of intrahepatic vascular shunts during liver disease may lead to a diminished hepatic extraction fraction in spite of a normal enzymatic capacity of the liver. If V_{max}/K_m in such a case is high enough compared to Q to ensure a high extraction fraction in functioning sinusoids, the measured hepatic extraction fraction could be used to estimate the fraction of the total hepatic blood flow which is being 'shunted' through the liver. This is the principle behind the nitroglycerin test. It is, however, difficult in practice to obtain data precise enough to allow a quantitative evaluation of the relative importance of these factors.

Other clearance measurements

This section gives a brief discussion of some other clearance values being relevant in the present context.

If V_{max}/QK_m is small, equation 8 reduces to [9]

$$v/A = V_{max}/K_m \tag{9}$$

In this case clearance can be used as an indirect measure of V_{max}, provided that K_m is not influenced by the disease. For V_{max}/QK_m being less than 0.05, v/A approximates V_{max}/K_m by 90% or more. This is the basis for using the antipyrine clearance as a quantitative measure of liver function [12].

Another example of a clearance value is that determined in respect to the substrate concentration in the hepatic outlet, i.e. v/V. It depends on both Q and V_{max}/K_m in a complex way [13]. In the limiting case with a very low value of V_{max}/QK_m, the arterio-venous concentration difference across the liver becomes small, and v/V approximates v/A and V_{max}/K_m. There has been some discussion in the literature as to whether this clearance could also be used as a flow-independent measure of V_{max}/K_m for substances with high values of V_{max}/QK_m. Experimentally induced flow changes, however, have confirmed the flow-dependence of v/V predicted by the sinusoidal perfusion model [13].

It should be noted that it is possible to obtain a direct measure of V_{max}/K_m if both A and V are measured. By eliminating Q from equations 1 and 6, we

obtain

$$v/\hat{c} = V_{max}/K_m \quad (10)$$

where $\hat{c} = (A - V)/\ln(A/V)$.

This clearance gives a direct and flow-independent estimate of V_{max}/K_m (i.e. the so-called 'intrinsic clearance'), with no approximations involved and with no limitations concerning the values of V_{max}/K_m or Q. This principle has recently been utilized to determine V_{max}/K_m for ICG elimination in subjects with and without liver disease [14]. It is positively correlated to the galactose elimination capacity (S. Keiding, unpublished data). It probably could be useful as a quantitative measure of the hepatic excretory function, but requires blood sampling from the hepatic vein.

Hepatic extraction and liver blood flow

In many studies, the hepatic extraction fraction E is used as a measure of the liver function. It is true that a large extraction fraction generally indicates good function, a small one indicating bad function. At first-order conditions ($v << V_{max}$), we have, from equation 7

$$E = 1 - e^{-\frac{V_{max}}{QK_m}} \quad (11)$$

This shows that E depends not only on V_{max}/K_m (being smaller, the smaller the V_{max}/K_m value) but also on Q (being smaller, the larger the Q value). Thus, the hepatic extraction fraction does not give an unambiguous measure of the liver function; on the contrary, it varies with variations in the hepatic flow rate.

Classification of clearance test substances

It appears from the above discussion that the relative importance of the hepatic blood flow for possible clearance test substances can be evaluated by means of the V_{max}/K_m value relative to Q. Accordingly a classification based on the V_{max}/K_m values has been proposed [15]. Only a substrate with a high V_{max}/K_m ratio ('intrinsic' clearance) during liver disease should be considered as a possible test substance for a clearance measurement of the hepatic flow rate.

In conclusion, the ideal test substrate for a clearance measurement of the functional hepatic blood flow has not yet been found. Quantitative measurements of the V_{max} values of the cystolic metabolic processes, as assessed by the galactose elimination capacity [16], and of the microsomal processes, as assessed by the antipyrine clearance [12], are available. If such measurements were combined with a clearance flow-measurement and a measure of the total hepatic blood flow (by means of Fick's principle), a nearly complete quantitative evaluation of the interplay of structural and functional changes during liver disease could be obtained. This may be of importance not only for medical treatment and drug administration, but also for prognostic evaluation of patients with liver disease.

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