The New Clearance Method for Hepatic Diagnostics

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Received May 24, 2005, Accepted October 4, 2005

Key words: Pharmacokinetics – Hepatoselective substances – Two-compartment model – Non-linear multiparametric analysis – Liver diseases staging

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Abstract: New multiparametric analysis and method of their evaluation were derived. The bolus dye injection is used for evaluation of ICG (indicyaningreen) and BSP (bromsulphophtalein) kinetics. This arrangement is a form of spectrophotometric measurement of BSP in plasma and/or pulse dye densitometry of ICG. Clearance values enable to differentiate liver diseases and to add their clinical staging. Pathophysiology of dye kinetics is discussed from two points of view i.e. sinusoidal and canalicular membrane transfer.

Introduction and Critical Review of Mathematical Treatment

Models of tracer elimination from the blood stream were verified more than fifty years ago [7] .The mathematical formula of these models was generally a set of ordinary differential equations well-known from classical pharmacokinetics [9, 18]. In most models the solution is expressed by an exponential relationship [6], describing Arrhenius' kinetics of monomolecular chemical reactions. This concept is unfortunately valid only in special cases, therefore many other model constructions have been carried out e.g. a nonlinear [2, 5] model, and that with more than one parameter.

The frequently used model for clearance diagnostics is based on enzymatic kinetics according to Michaelis-Menten [4]. The infusion arrangement used for clinical liver diagnostics and investigation [11, 12, 15] yields intrinsic clearance as a global function parameter.

$$(1) \quad C = V / P_L = V_{max} / k_m$$

where P_L is the logarithmic mean of serum concentrations in the peripheral circulation (P_A) and in blood samples from liver veins (P_V). In condition of estimation it follows

$$(2) \quad P_L = \frac{P_A - P_V}{\ln \frac{P_A}{P_V}}$$

The theoretical background for intrinsic clearance (C) in detail presumes a highly skilled medical staff as well as certain inconvenience for patients. The evaluation of equilibrium by means P_A – and especially P_V – values is difficult.

The alternative dynamic procedure is represented by a single injection of hepatoselective substantives, e.g. bromsulphofthalein (BSP) or cardiogreen (ICG) as a tracer and, consequently, sample analysis in the defined time intervals. The elimination curve can be constructed i.e. time dependency of tracer concentration changes in digitized form [23, 21]. These courses are treated by means of a regression method application, mostly nonlinear, giving model parameters. In papers [16, 25, 26] the nonlinear differential equation is described as a model. The graphic-mathematical evaluation of data is presented to obtain basic parameters k- and h-constants. The above mentioned process

became recently measurable [22, 10] by direct ICG spectrophotometry (finger or auricular piece or nose [17] with the advantage of non-invasive clinical examination.

This diagnostic procedure is logically completed by on-line evaluation in real time, which means a useful extension of these examinations.

Material and Methods

Mathematical Model

The theoretical background of the bolus injection method is the nonlinear mathematical model [16, 23, 25]. The analogy of chemical kinetics of the second order is utilized for mass transfer through the cell membrane from the blood transport system into the hepatocyt and from there into the bile [9, 28, 30].

The nature of the process is complex. Haemodynamic mass transfer and biochemical components are involved. The mathematical description is based on abstraction so that in its derivation the details of real mechanism are omitted.

For this purpose we substitute the phenomenological model using the classical transport equations by concept of two parallel phenomena.

First of them is the distribution of colour indicator in blood plasma. We can consider the dispersion of this substance on the particle containing such amount of effective agent, which can be totally eliminated just by one hepatic cell. The probability of contact of the particle defined above and hepatic cell we assign as P(A), simply probability of elementary random event A. We will call it the "offer of eliminated agent".

The second step involving the studied complex of biochemical and haemodynamic components could be approximately described as absorption of a tracer particle mentioned above by hepatic cell fulfilling simultaneously two conditions: It is healthful and does not participate on the elimination process yet. We suppose in correspondence with physiological experience, that hepatic cells participating in the elimination process need relatively long time period for their regeneration. That is why they do not participate on the continuing clearance. The event of presence such cell we assign as B with probability P(B). If we consider these two events as independent in statistical sense and the rate of elimination prepositional of their simultaneous occurrence these we can write:

$$(3) \quad \frac{dI}{dt} = \alpha . P(A) . P(B)$$

This formula can be rewritten to differential equation describing time-concentration profile if we consider that probability P(A) is proportional of indicator actual concentration in blood plasma. The number of cells holding the capacity is similarly dependent on the concentration difference between hepatic capacity and present amount of tracer eliminated from blood plasma. If we take this relationship as linear, then we get system of two equations

(4) $P(A) = \beta\, I(t)$
$P(B) = \gamma\, (Ho - H(t))$

than we can introduce new constant by relation where negative value of k is connected with decreasing concentration of tracer. The relationship -$k = \alpha.\ \beta.\ \gamma$ leads mediatory after supply (4) to eq. (3) to

(5) $$\frac{dI(t)}{dt} = -kI(t)(H_0 - H(t))/H_0$$

that is the mathematical description dynamic equilibrium process explaining condition. Parameter k is the relative rate of elimination and *Ho* the liver capacity, *H(t)* amount of tracer transported to the liver. In addition, we can consider that for the amount injected (I_0) the relationship is given by:

(6) $$I_0 = H(t) + I(t)$$

and from the substitution to eq. (5) it follows that:

(7) $$\frac{dI(t)}{dt} = -kI(t)\left(1 - \frac{I_0}{H_0}\right) - \frac{k}{H_0} I^2(t)$$

Eq. (7) is the differential equation of a Bernoulli type and for initial conditions $t = 0$ $I(t) = I_0$ there exist a solution

(8) $$\frac{I(t)}{I_0} = \frac{\left(1 - \frac{I_0}{H_0}\right) e^{-k\left(1 - \frac{I_0}{H_0}\right)t}}{1 - e^{-k\left(1 - \frac{I_0}{H_0}\right)t}}$$

We introduce the relative constant $h = \frac{H_0}{I_0}$ and $\varphi = k\frac{(h-1)}{h}$ and thus eq. (8) could be formally simplified

(9) $$p(t) = \frac{I(t)}{I_0} = \frac{(h-1)e^{-\varphi t}}{h - e^{-\varphi t}} = \frac{h-1}{he^{\varphi t} - 1}$$

This relation is a decreasing function. The limit values of parameter *h (h = 1 or h = 0)* are from a physical and physiological view unacceptable, because for each value of *t p = 0* or *p = 1* respectively. There are two limit cases on the right side of eq. (7). For $H_0 \to \infty$ it follows:

(10) $$\frac{dI(t)}{dt} = -kI(t)$$

(11) $$\frac{I(t)}{I_0} = e^{-kt}$$

For the second alternative $I_0 \to H_0$ (*h=1*) in analogy

$$(12)\quad \frac{d\,I(t)}{d\,t} = -\frac{k}{I_0} I^2(t)$$

with solution

$$(13)\quad p(t) = \frac{I(t)}{I_0} = \frac{1}{1+kt}$$

The sense of these two limits is rather illustrative, but they could play a role in the system behaviour towards parameter h and its diagnostic validity.

The courses of the function given by eq. (8) for different parameters h and k are illustrated on Figure 1 together with limit cases given by eqs. (11) and (13) (dashed and dotted line).

The k and h evaluation from experimental curves enables the use of algorithms of nonlinear regression, where set of normal equations is solved with Newton-

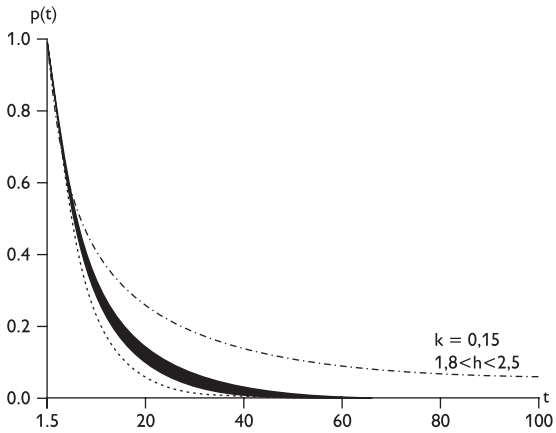


Figure 1 – The typical course of time profile according to eqs. (9), (11) and (13). When initial condition t = 0; I = Io is valid, we obtain.

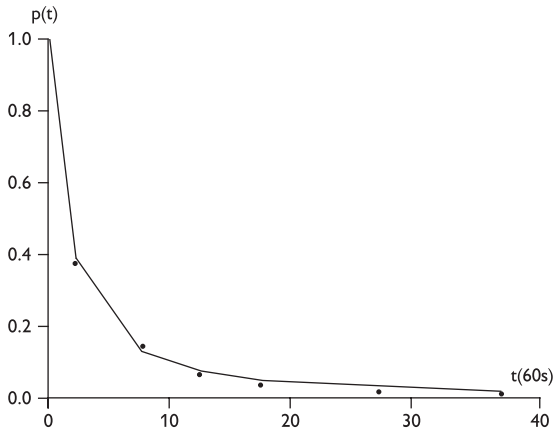


Figure 2 – Nonlinear regression curve for dynamic model given by eq. (5).

Raphson method. It appears that this procedure gives in a small amount (from five to eight) iterations results with a regular precision. Non-stability occurs especially under circumstances near to limit value of parameter h. The typical course of elimination curve obtained after adequate dose of indicator application is drawn on the Figure 2.

Experimental Results

Global process characteristics

For the evaluation of results of diagnostic procedures using bolus injection of indicator in stationary arrangement it is necessary to determine a significant parameter which can differentiate liver diseases and the state of disease with respect to other clinical and laboratory findings. The authors of the static method [12, 15] are proposing the intrinsic clearance (eq. (1)) as satisfactory for these requirements. Even the dynamic process yields an analogous parameter. Rigorous application of the Michaelis-Menten model leads to the relation

$$(14)\quad C_2 = \frac{2 k I_m}{(1-h) + \sqrt{1+h^2}}$$

which is obtained on the basis, that elimination rate is maximal at the beginning of the process and its value is $k.I_0$ .The relationship of elimination rate on the $I(t)$ has a parabolic shape, as can be seen from eq. (16). This follows from eq. (7) and reaction rate definition.

$$(15)\quad |V| = \frac{d\,H(t)}{d\,t} = -\frac{d\,I(t)}{d\,t}$$

$$(16)\quad |V| = k\left[I(t)\left(1-\frac{1}{h}\right) + \frac{I^2(t)}{H_0}\right]$$

From (15) it is easy to show that quadratic parabola has the following formula

$$(17)\quad w = \frac{|V|}{k\,I_0} = p\,\left(1-\frac{1}{h}\right) + \frac{1}{h}\,p^2$$

Only the positive root of this eq. holds for $w = 1/2$

$$(18)\quad p_L = \frac{(1-h) + \sqrt{h^2+1}}{2}$$

If we return to dimension quantities, then the relations analogical to enzyme kinetics of Michaelis-Menten [18] $w_{max} = 1$, $V_{max} = k\,I_0$ give the formula for C_2 eq. (13). Limit case for $h \to 0$ leads to

$$(19)\quad C_3 = 2 k I_m$$

and for $h \to 1$

(20) $C_4 = \sqrt{2}\, k\, I_m$

These characteristics correspond with limit cases discussed in detail in the preceding paragraph. They are linearly dependent on each other. Additionally we can obtain other formulas for C-intrinsic hepatic clearance according to conception of analogy with infusion method [12]. Let us to introduce moment t_e

(21) $$\frac{s(t_e)}{p(t_e)} = \frac{P_V}{P_A} = h$$

and if this moment exists the state is correspondent to the equilibrium state known from the infusion method conception. Reform to dimensionless quantities, then the set of equations is as follows

(22) $s(t_e) + p(t_e) = 1$

(23) $$P_A = \frac{h-1}{h\, e^{\varphi t} - 1}$$

(24) $$\frac{s(t_e)}{p(t_e)} = h$$

is satisfied only if the relationship

(25) $$t_e = \frac{1}{k} \frac{\ln h}{1 - \frac{1}{h}}$$

is valid. For the moment defined above we get from eq. (23)

(26) $$p(t_e) = \frac{1}{h+1}$$

and for dimensionless reaction rate it is

(27) $$w = \frac{h}{(h+1)^2}$$

Using the same procedure as in preceding cases we get

(28) $$C_6 = I_m k \frac{h}{h+1} = C_3 \frac{h}{2(h+1)}$$

in which the affect of parameter h is incorporated. This quantity is not linearly dependent on the proceeding ones. In the moment t_e the relation is kept:

(29) $$\frac{P_V}{P_A} = \frac{H(t_e)}{I(t_e)} = \frac{H_0}{I_0} = h$$

and if simultaneously logarithmic mean of concentration P_A and P_V could be written as:

$$(30)\quad P_L = \frac{P_V - P_A}{\ln \frac{P_V}{P_A}} = P_V \frac{h-1}{h \ln h}$$

Then it will be also the logarithmic mean of concentrations H_0 and I_0:

$$(31)\quad I_L = H_0 \frac{h-1}{h \ln h}$$

and finally in a dimensionless form

$$(32)\quad p_L = \frac{h-1}{\ln h}$$

When using reaction rate V_{max} ; $w = 1$ and K_m analogy as the fraction p_L/h then

$$(33)\quad C_1 = \frac{V_{\max} h}{p_L m} = \frac{k I_0}{m} \frac{h \ln h}{h-1} = k I_m \frac{h \ln h}{h-1}$$

or according to eq. (26) when

$$(34)\quad |V| = \frac{k I_0 h}{h+1}$$

we get the last characteristics

$$(35)\quad C_5 = \frac{k I_m h^2 \ln h}{h^2 - 1} = \frac{2 C_1 C_6}{C_3}$$

where C_1 and C_6 are linearly independent on each other and their sensibility with respect to inlet parameters k and h is necessary to compare with other characteristics given by eqs. (14, 19, 20, 28 and 35).

Discussion of Results

Clearance (C) values were calculated from elimination curves constructed from direct or indirect spectrophotometry versus time. Every direct curve (auricular or finger piece) was corrected by ICG concentration estimated from minimally one direct measurement in blood sample at the 15th minute after injection. Data before and after the orthopedic operation under a halotan narcosis were re-evaluated in 13 patients presented in [27]. In patients with liver disease the Child staging is based on bioptic findings mostly by laparoscopic assessment, completed by biochemical and immunological examination [26]. Normal C-values represent 11 measurements in healthy medical students. All global characteristics of elimination curves in digitized form were determined from eqs. (14), (19), (20), (28), (33) and (35). Almost every data set displays evidently positive agreement with the model equation (after fitting procedure).

In the group of patients we recognized the diseases and linked it up with the assignment (see Table 1. below). From figures 3 and 4 it is clear that practically every characteristic could be used for differential diagnostic purposes.

The mean values of these characteristics are given in the Table 2. We can see, that in each group of people (I, II, III) or (IV, V) values of all computed quantities C1, C2, C3, C4, C5 a C6 are different and the lower they are, the more serious disease is present. In Figures 3 and 4 these results are illustrated in a graphic form by "Box-and-Whisker plot" method. The one-way ANOVA and Kruskall-Wallis test show significant effect of both factors (I, II, III) and (IV, V). On the other side we have to admit, that our results cannot unmistakably distinguish between steatosis and cirrhosis, because their confidence intervals is not disjunctive. It could be explained by relatively small number of people in the each group. Despite this fact we consider this method being promising for the clinical use.

Conclusions

1. The two-compartment model is based on the concept of stochastic behaviour of BSP and ICG elimination curves. The probability theory yields the non-linear differential equation with two parameters. The estimated clearance values are helpful for differential diagnostics and classification of liver diseases. This concept differs substantially from other models discussed earlier inclusive the infusion method. It appears that presented clearance characteristics can recognise different liver diseases, the functional state and namely their staging.

Table 1 – Description of patients investigated

ID	Number of patients	Classification of disease
I	11	Healthy students
II	7	Hepatitis chronica (steatofibrosis, without or with mild activity)
III	10	Cirrhosis hepatis 7 – compensated Child A + B 1 – Child C 2 – primary biliary
IV	13	Orthopaedic operations, before
V	13	After and under halotan narcosis

Table 2 – The mean values of intrinsic clearance. Characteristic for each group of examined persons

ID	Number of patients	Mean value					
		C1	C2	C3	C4	C5	C6
I	11	2.187	2.621	3.310	2.341	1.409	1.058
II	7	0.4308	0.5631	0.7531	0.5325	0.2454	0.2136
III	10	0.3533	0.4875	0.6791	0.4802	0.1844	0.1759
IV	13	2.698	1.246	1.444	1.021	2.483	0.5505
V	13	1.128	1.002	1.317	0.9311	0.8286	0.3888

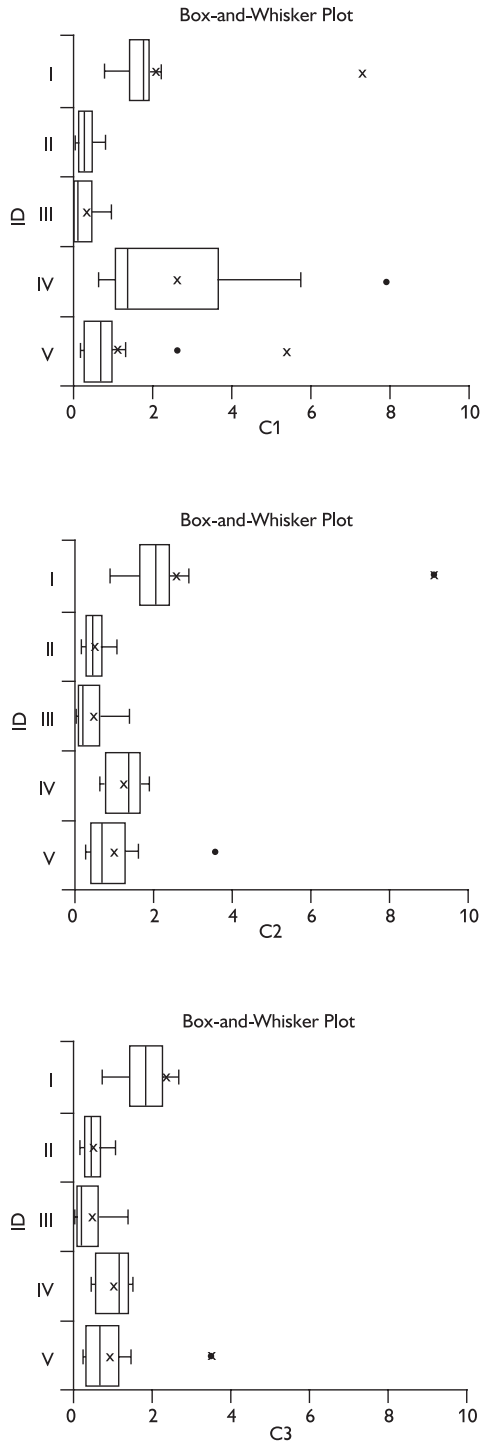
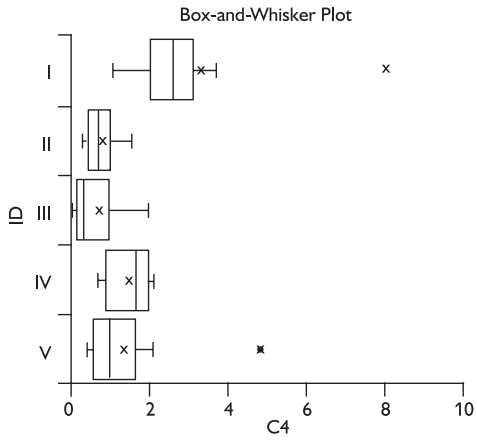
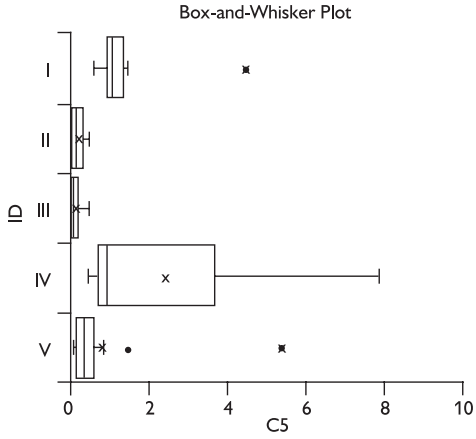


Figure 3 – Clearance characteristics comparison for C1, C2 and C3.





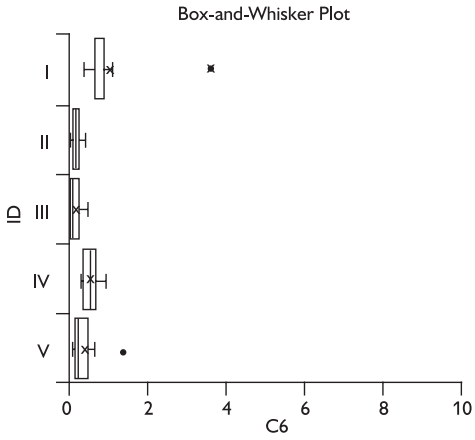


Figure 4 – Clearance characteristics comparison for C4, C5 and C6.

2. For model identification the nonlinear method of least squares was utilized with application of Newton-Raphson algorithm for set of nonlinear equations solution. The agreement with our data appears to be positive without regard to the measurement performed using classical or new method of blood clearance estimation.
3. Presented clinical results confirm their validity in clinical practice as well as for pathophysiological studies. A completion of material by means PDD of ICG is prepared for presentation.

List of symbols

C	clearance	[ml/(60.s^{-1})]
h	relative hepatic capacity	[*1*]
H(t)	extracted amount	[mmol], [mg]
H_0	absolute liver capacity	[mmol], [mg]
I(t)	residual amount in blood	[mmol], [mg]
I_0	injected dose	[mmol], [mg]
I_m	dose to body weight	[mmol/kg, mg/kg]
k	relative elimination rate	[(60.s^{-1})]
K_m	Michaelis constant	[mmol/l, mg/l]
p(t)	relative plasma concentration, retention	[*1*]
P(t)	plasma concentration	[mmol/l, mg/1]
E(t)	relative extracted amount	[*1*]
t	time	[(60.s^{-1})]
V	absolute elimination rate	[mmol/60.s^{-1}], [mg/ 60.s^{-1}]
w	rate defined by eq. (17)	[*1*]

Subscripts

0	initial value of time or amount
1, 2, 3 4, 5, 6	clearance according to eqs. (33), (14), (19), (20), (35), (28)
A	arterial or peripheral plasma dye concentration
V	hepatic vein plasma dye concentration
e	time of equilibration
max	maximal value
L	logarithmic mean

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