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New Clearance Evaluation Method for Hepatological Diagnostics

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Summary

Pulse dye densitometry (PDD) enables the evaluation of hemodynamic state as well as liver function. A repeated examination, even after a short pause (or under stress condition), enables to follow safely the dynamics of liver pathology. From presented parameters we have evaluated as reliable the *C5*-clearance, an expression of equilibrium state in the two compartment liver system. Furthermore, *T*-index expresses ratio of *C5* value to cardiac output, it is a sensitive indicator of the blood pole, i.e. sinusoidal uptake, which is in very good correlation with staging of hepatopathies. The isolated *h* constant in correlation to *T*-index is valuable For functional grading. The Japanese automatic analyzer of indocyanine green (ICG) dilution and elimination curves, after incorporation of a two compartment mathematical model, becomes more useful for complex hepatological diagnostics. Non-invasive PDD is becoming of uppermost importance to clinical interest, yielding comparable results as other complicated and invasive examinations and may be, therefore, repeated in short time intervals for different indications with minimal stress of examined patient.

Key words

Pharmacokinetics • Steatofibrosis hepatis (SFH) • Indocyanine green • Two-compartment model • Non-linear regression analysis • Clearance value • *T*-index

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Introduction

Non-invasive pulse dye densitometry (PDD) is becoming of uppermost importance to clinical interest, to comparable results from other complicated and invasive examinations (Haruna *et al*. 1998) and may be, therefore, repeated in short time intervals for different indications (Imai *et al*. 1998) with minimal stress of examined patient (Mitchell *et al.* 1995a, Su *et al.* 1999, Tichý and Loučka 2000).

The aim of this work was the analysis of dilution and elimination curves after single bolus injections of indocyanine green (ICG) (Iijima *et al.* 1998, Yang *et al.* 1972). We have focused our attention on the most sensitive clearance parameters for hepatological diagnostics (Shimizu *et al.* 1995, Su *et al.* 1999) and on the relationship between simultaneously determined hemodynamic and clearance values. Further, using our mathematical model (Komenda and Tichý 1965, Tichý and Komenda 1965, Tsukada *et al.* 1996) we have compared it with ICG kinetic analysis implemented in Japanese automatic PDD analyzer (NIHON-COHDEN).

Methods

PDD is carried out on bed-ridden patients with an empty stomach. Dilution and elimination curves describe the dependence of ICG concentration (mg/dm^3) on time (t). The state of circulation is usually simultaneously evaluated by bioimpedance measurement by using cardiodynamic data processing system (CDDPS) (Tichý 1995), with monitoring systemic blood pressure such as mean arterial pressure. Intravenous bolus in a dose *25-50 mg* (average *38 mg*) was applied in less than *20 s*. ICG concentration is monitored by nose or ear recorder (Mitchell *et al.* 1995b, Tichý and Loučka 2000) and determined values are compared with values estimated using direct photometry of at least two blood

samples obtained during elimination phase in a precisely determined time.

Dilution curve is classically examined according to Hamilton (Tichý *et al.* 2005, Yang *et al.* 1972) and enables determination of cardiac output (*CO*) (Folkow and Neil 1971, Yang *et al.* 1972) or, in relation to body surface area, cardiac index (*CI*). Correlation between hemodynamic parameters, determined by ICG dilution curves and by CDDPS is very satisfactory (Tsukada *et al.* 1996), however, in this work, it is not analyzed in detail. Appearance time (*AT*) is time interval between bolus application and appearance of curves ascendant branch, expression of circulatory velocity (Yang *et al.* 1972).

(1)

$$c(t) = \frac{g(t)}{\int_0^\infty g(t)dt}$$

Dilution curve *c(t)* is proportional to circulation concentration of tracer, further treated by numerical integration according to Simpson's rule. The integral corresponds to area under this curve.

CO is determined from amount of given indicator (I_0), average concentration (*mP*) and mean transit time (*MTT*) (Folkow and Neil 1971)

(2)

$$CO = \frac{I_0}{mP \cdot MTT} \cdot 60$$

Dilution curves are monitored in one or two recirculatory peaks (Fig. 1). Pathological pattern of deformed curves in presence of intracardial shunts was not analyzed. Concentration signal may be, with satisfactory precision approximation, considered as rectangular pulse (Jaroš 1998, McBean and Rovers 1998).

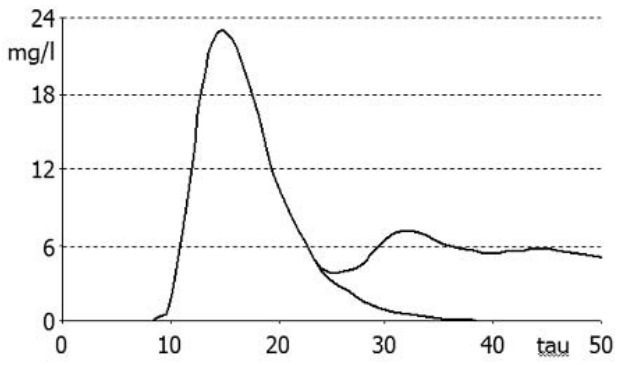

Fig. 1. Dilution curve.

Elimination curve (Fig. 2a) is given by gradual disappearance of ICG from blood, selectively through liver (Haruna *et al.* 1998, Iijima *et al.* 1998, Tichý and Loučka 2000). This process can be monitored from the 3rd to 45th min. The initial phase, after ICG application, may be analyzed by extrapolation or by calculation and virtual concentration *P(t)* in time *t = 0* may be defined. The *P(0)* in ratio to the given dose of ICG (I_0) permits the calculation of *PV* (plasma volume) or *BV* (blood volume) (Iijima *et al.* 1998, Schad *et al.* 1987). In our examination the mean value of *PV*, in relation to body weight 55 ± 11 ml/kg, is considered to be physiological and is comparable with other determination methods (Haruna *et al.* 1998).

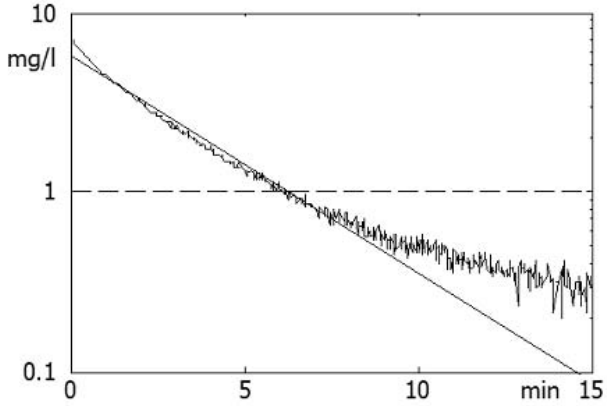

Fig. 2a. Elimination curve.

Elimination curves analysis

a) One parameter model:

The concept of a one-parameter model for ICG elimination is still being applied, including automatic analyzer NIHON-COHDEN. This issues from the differential equation describing the first order kinetics.

(3)

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

For initial condition *t = 0, I(t) = I_0* we obtain the following solution:

(4)

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

assuming the complete distribution of given amount in

plasma volume (*PV*) in a concentration *P(0)*. The only diagnostic quantity is *k* constant here.

b) Two parameter model:

The process between blood and liver (on sinusoidal membrane) and that between liver cells and bile (on canalicular membrane) are taken into account (Komenda and Tichý 1965, Tichý 1969). The agreement of digitalized curves (Fig. 2b) and their prediction, during utilization of a two parameter model, is very good (Tichý and Komenda 1965).

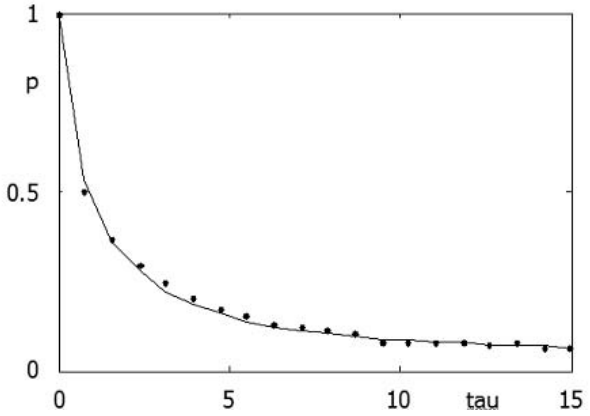

Fig. 2b. Digitalized elimination curve.

Differential equation, describing this proposed model (Tichý and Loučka 2000, Tichý *et al.* 2005), has the following form:

(5)

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

Bernoulli equation of the second order is under consideration where for initial condition $t = 0$, $I(t) = I_0$, it holds:

(6)

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

When parameters are introduced it follows:

(7)

$$h = \frac{H_0}{I_0}, \quad \varphi = k\frac{h-1}{h}$$

then two parameter model is obtained in the following form:

(8)

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

where parameters are evaluated by non-linear regression. It is the way how to obtain two independent characteristics *k* and *h* from data illustrated in Figure 2b and to use whole part of curve for treatment.

Estimated values and indexes were statistically evaluated in relation to hepatological diagnosis, based on complex clinical and laboratory examination, including liver biopsy. This complex enables staging (*S*) and grading (*G*) of liver diseases (Lata *et al.* 2000, Ludwig 1995).

The clearance parameters according to following equations were calculated and their diagnostic sensibility was tested. For calculation of constants *k* and *h* the non-linear regression according to formula (8) Marquardt-Lenvenberg algorithm was used. From individual values *k* and *h* constants the diagnostic clearance parameters were calculated according to the following equations (9) to (15):

(9)

$$C_1 = kI_m \frac{h \ln h}{h-1}$$

(10)

$$C_2 = \frac{2kI_m}{1-h+\sqrt{1+h^2}}$$

(11)

$$C_3 = 2kI_m$$

(12)

$$C_4 = \sqrt{2}kI_m$$

(13)

$$C_5 = \frac{kI_m h^2 \ln h}{h^2-1} = \frac{2C_1C_6}{C_3}$$

(14)

$$C_6 = kI_m \frac{h}{h+1} = C_3 \frac{h}{2(h+1)}$$

(15)

$$T = \frac{C5}{CO}$$

Subjects

The studied groups consisted from 31 males and 3 females (average age 55.4 years; range 20-79 years). Four patients were first examined while lying down and for the second time with equivalent ICG dose under continual stress of 60 to 70 W in unchanged bed-ridden position for a period of 30 min.

Group 0) Physiological liver, without evidence of liver disease (n=6).

Group 1) Damage of liver (non-cirrhotic) was defined by different degree of steatosis, fibrotization of periportal fields, eventually by portoportal septal formation. Different cellularization in portobilliar fields and alteration of lobular structure (alcoholic n=12 and non-alcoholic n=11) were found. This concerns mainly patients with metabolic syndromes of diabetes type II, with insulin resistance, disorder of lipid metabolism, gout and morbid obesity (BMI ≥ 40) (n=23).

Group 2) Cirrhotic liver with evolution of portal hypertension was classified according to Child-Pugh's score (Ludwig 1995) in group A, B, and C (n=5).

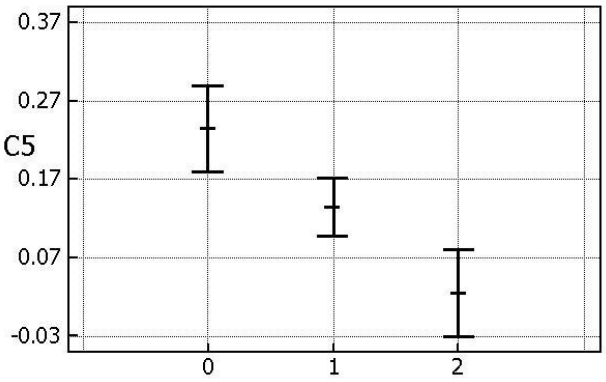

Fig. 3. 95 % confidence intervals of C5.

The activity of hepatocellular damage was evaluated and was taken into account with type and size of parenchymal cells necrosis in grading, including the laboratory findings, especially aminotransferase levels and hepatitis markers. Analysis of variance (Jaroš 1998), namely one-way ANOVA and Kruskall-Wallis test (McBean and Rovers 1998), was used for statistical treatment. The same method was used for the statistical evaluation of the complex clinical state worth of all determined clearance parameters (see eqs. (9) to (15)). The proper *C5* value (equation (13)) has a great differential diagnostic ability, as follows from (Fig. 3) and is statistically defined by 95 % confidence interval.

All given confidence intervals of mean values are disjunctive, differing three groups of hepatopathies presented above. The quantity k calculated from model represented by eq. (4) is not able to differentiate reliably these three groups of hepatopathies (Fig. 4).

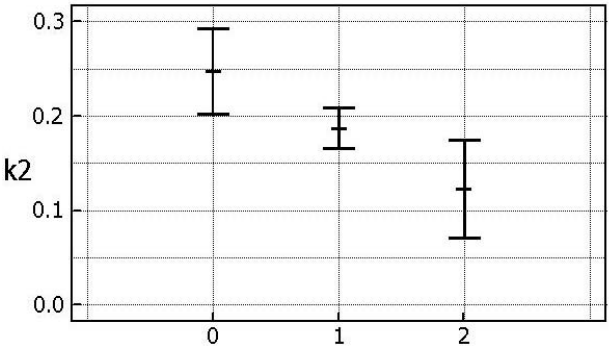

Fig. 4. 95 % confidence intervals for k_2 value.

T-index eq. (15) is also considered to be clinically very important because it reliably distinguishes this relatively heterogeneous non-cirrhotic group from cirrhotic patients, mainly A type (four-times) and C type (once).

The discrimination ability of *T*-index is also apparent during evaluation of the group (0), without conclusive hepatopathy (bioptically not verified), as compared to alcoholic and non-alcoholic steatosis/ steatofibrosis (group 1) (mostly bioptically verified) as it is shown in Figure 5.

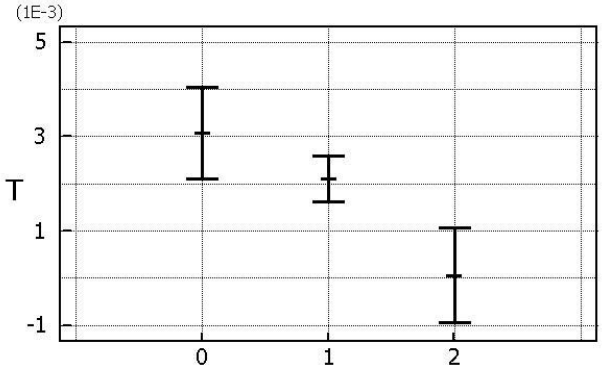

Fig. 5. 95 % confidence intervals for *T* value.

Table 1. *C5* boundaries determined for classification.

Clinical diagnosis	Assignment in Figure	*C5* confidence intervals 95 %
Healthy	0	<0.17989 ; 0.29189>
Steatosis and/or fibrosis	1	<0.11192 ; 0.17041>
Cirrhosis	2	<0.00000 ; 0.09337>

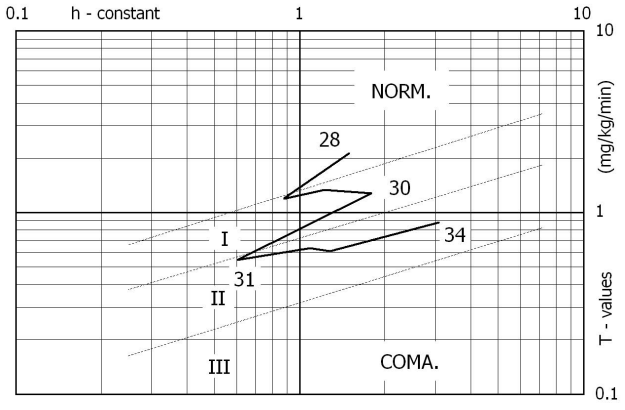

Fig. 6. The relationship of isolated h constant to *C5* or *T*-values. 28, 30, 31, 34 indicate the age of patient, I, II, III represent the degrees according to Child classification.

Results and Discussion

Priority is given to ICG clearance before the previously widely used bromsulphoftalein (BSP) (Komenda and Tichý 1965, Tichý and Komenda 1965), because ICG does not cause anaphylactic or other allergic reactions. Unfortunately, the availability of ICG is limited in our country. The more complex is the physiopathological knowledge of pharmacokinetics of used indicators, the richer is the clinical experience, the more increases its diagnostic worth. ICG, but also BSP (when used for many years), enables to determine liver damage, its severity even though it has different etiology (Lata *et al.* 2000). This was interpreted as functional blood pool failure of liver (on sinusoidal membrane), in close relation to staging of liver diseases. For the activity evaluation, i.e. grading, the correlation with presented clearance and *T*-index parameters is of low significance. For grading (functional state of canalicular membrane) is better expressed by isolated *h* constant in relation to *C5* or *T*-values (Fig. 6).

List of symbols

C	clearance values	[6 . 10^{-5}.s^{-1}, 6 kmol.kg^{-1}.s^{-1}]
h	relative liver capacity	[1]
$H(t)$	amount of tracer in the liver space	[mmol], [mg]
H_0	absolute liver capacity	[mmol], [mg]
$I(t)$	rest amount in blood	[mmol], [mg]
I_0	given dose of indicator	[mmol], [mg]
I_m	given dose related to body weight	[mmol/kg, mg/kg]
k	relative elimination rate	[(60.s) $^{-1}$]
$p(t)$	relative plasma concentration	[1]
t	time	[60.s]
PV	plasmatic volume	[ml]
CO	cardiac volume	[ml.s^{-1}]
T	index	[1]
$C5$	clearance	[6 . 10^{-5}.s^{-1}, 6 kmol.kg^{-1}.s^{-1}]

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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