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A physiologically based model of hepatic ICG clearance: Interplay between sinusoidal uptake and biliary excretion

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ABSTRACT

Although indocyanine green (ICG) has long been used for the assessment of liver function, the respective roles of sinusoidal uptake and canalicular excretion in determining hepatic ICG clearance remain unclear. Here this issue was addressed by incorporating a liver model into a minimal physiological model of ICG disposition that accounts of the early distribution phase after bolus injection. Arterial ICG concentration–time data from awake dogs under control conditions and from the same dogs while anesthetized with 3.5% isoflurane were subjected to population analysis. The results suggest that ICG elimination in dogs is uptake limited since it depends on hepatocellular uptake capacity and on biliary excretion but not on hepatic blood flow. Isoflurane caused a 63% reduction in cardiac output and a 33% decrease in the ICG biliary excretion rate constant (resulting in a 26% reduction in elimination clearance) while leaving unchanged the sinusoidal uptake rate. The terminal slope of the concentration–time curve, *K*, correlated significantly with elimination clearance. The model could be useful for assessing the functions of sinusoidal and canalicular ICG transporters.

1. Introduction

Indocyanine green (ICG) is an organic anion dye that is eliminated exclusively by the liver. It is widely used to assess hepatic function, e.g., in hepatic injury and septic states (Kortgen et al., 2009), and to determine donor liver function after transplantation (Niemann et al., 2002; Hori et al., 2006). From studies in rats, it is known that hepatic ICG clearance is determined by two processes, sinusoidal uptake and canalicular excretion (Sathirakul et al., 1993). There is evidence that in human liver sinusoidal transport is mainly mediated by the organic anion transporting polypeptide (OATP), whereas the multi-drug resistance associated protein (MRP2) and the multi-drug resistance P-glycoprotein (MDR3) may be involved in canalicular efflux of organic anions (for a review, see Kusuhara and Sugiyama, 2010). However, under *in vivo* conditions where only plasma ICG concentrations are measured, the roles of these processes are poorly understood. It appears that hepatocellular uptake and not hepatic blood flow may be rate limiting in dogs (Ketterer et al., 1960), but there is little quantitative information on the relative contribution of each process to ICG elimination. Thus, the goal of the present study was to determine whether modeling of ICG disposition curve could reveal the interplay between sinusoidal uptake and biliary excretion in determining hepatic ICG clearance (i.e., when the time profile of biliary excretion is not available). To this end, we applied a novel pharmacokinetic model to ICG plasma concentration–time data obtained using frequent arterial sampling in awake and isoflurane anesthetized dogs (Avram et al., 2000).

Estimation of hepatocellular uptake clearance and biliary excretion rate constant of ICG was based on a circulatory model. Lumping the systemic organs into two subsystems, hepatosplanchnic and non-splanchnic circulation, the approach is similar to that used to describe [^{13}N]ammonia kinetics (Weiss et al., 2002) and is an extension of the circulatory model of ICG disposition (Weiss et al., 2006, 2007). Thus, in contrast to previous approaches based on compartmental modeling (for a review, see Ott, 1998), we have adopted a minimal physiological model of ICG whole body pharmacokinetics that includes a space-distributed liver model (Weiss and Roberts, 1996). This model has been used to analyze data obtained in the isolated perfused rat liver (Weiss et al., 2000; Hung et al., 2002). The difficulty with a more complex model lies in the large number of model parameters relative to the available data. Prior information obtained on the system without liver (Weiss et al., 2006) was incorporated in parameter estimation using a population approach (D'Argenio et al., 2009) to facilitate parameter identifiability. The fact that isoflurane anesthesia decreased cardiac output to about 37% of that in the awake state (Avram et al., 2000) allowed an examination of the effect of liver blood flow.

The advantages of physiologically-based pharmacokinetic (PBPK) modeling have been reviewed recently (Rowland et al.,

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2011). Although PBPK models are normally based on a compartmental structure (differential equations), in the present approach the subsystems of the body were modeled by transit time density (TTD) functions for at least two reasons. First, since ICG distributes within the vascular system, we are also aiming to describe the initial phase (the first minutes) of the disposition curve to estimate cardiac output as a main determinant of the mixing process (Weiss, 2009). This would be not possible using a conventional compartmental model for the lung. The initial peak of the arterial concentration time curve mainly represents the TTD through the lung. That the latter can be described by the inverse Gaussian density has been shown in tracer kinetics (Sheppard et al., 1968). The usefulness of the inverse Gaussian TTD in pharmacokinetics can be explained by the facts that it represents the first passage time distribution of a random walk process with drift (Seshadri, 1999) and that it is the solution to the convection–dispersion organ model (Roberts et al., 2000). Second, in a PBPK model based on differential equations it would not be possible to integrate a space-distributed liver model. Furthermore, while due to their structural complexity, PBPK models are typically used for simulation, this study is an attempt to develop a minimal PBPK model for ICG that can be identified using plasma concentration data. This method may lead to a better understanding of the processes determining ICG clearance *in vivo*, e.g., for characterizing the function of transporters involved in sinusoidal uptake and biliary excretion of ICG in normal and disease states. The model was also used to examine the role of the terminal slope of the ICG concentration–time curve (blood disappearance rate, K), that is routinely used as a marker of liver function, as a surrogate for hepatic clearance.

2. Methods

2.1. Data

The data were obtained from a previous study of ICG disposition in dogs (Avram et al., 2000). Four dogs (body weight 28.4 ± 5.9 kg) were studied while awake and again while anesthetized with 3.5% isoflurane (2.3 minimum alveolar concentration, MAC). Briefly stated, at time $t = 0$ min, 5 mg of ICG in 1 ml of ICG diluent was flushed into the right atrium within 4 s using 10 ml of a 0.9% saline solution. Arterial blood samples were collected via an indwelling iliac artery catheter every 1.8 s for the first 28.8 s and every 3.6 s for the next 32.4 s using a roller pump and fraction collector. Subsequently, 18 3-ml arterial blood samples were drawn manually at 12 s intervals to 2 min, at 30 s intervals to 4 min, at 5 and 6 min, and every 2 min to 20 min. Plasma ICG concentrations were measured by high-performance liquid chromatography and plasma concentrations were converted to blood concentrations by multiplying them by one minus the hematocrit. For further details on study design, protocol, and measurements, please see Avram et al. (2000).

2.2. Model

To develop a circulatory model of ICG disposition that can be identified solely on the basis of arterial blood concentration–time data, its structural complexity must be reduced to a minimum. The most rigorous structural simplification is given in terms of the pulmonary and systemic circulation, both of which are characterized by transit time density (TTD) functions of ICG molecules (Weiss et al., 2006). The pulmonary (p), or central, circulation is located between the points of injection and arterial sampling. Here the systemic circulation is split into two subsystems arranged in parallel, the hepatosplanchnic circulation and rest of the systemic circulation (*rs*) (i.e., extrasplanchnic vascular beds) (Fig. 1). The hepatosplanchnic circulation consists of two organs in series, the gut and the liver. When the TTD of the subsystems are non-exponential (no well mixed compartments), the equation for the arterial blood ICG concentration, $C(t)$, after bolus venous injection (dose, D_{iv}) in a recirculatory system is only available in the Laplace domain. Denoting the Laplace transform of a function $f(t)$ by $\hat{f}(s) = L[f(t)]$, the model consists of the pulmonary and systemic subsystem with TTDs, $\hat{f}_p(s)$ and $\hat{f}_s(s)$. Since the TTDs of two subsystems connected in series is the product, $\hat{f}(s) = \hat{f}_1(s)\hat{f}_2(s)$, and the input to the pulmonary circulation is given by $\hat{C}_{pul,in} = \hat{C}(s)\hat{f}_s(s) + D_{iv}/Q$, i.e., the output of the systemic circulation plus contribution of bolus dose (Q denotes cardiac output). From $\hat{C}(s) = \hat{C}_{pul,out} = \hat{C}_{pul,in}(s)\hat{f}_p(s)$, we finally obtain:

$$\hat{C}(s) = \frac{D_{iv}}{Q}\frac{\hat{f}_p(s)}{1 - \hat{f}_s(s)\hat{f}_p(s)} \tag{1}$$

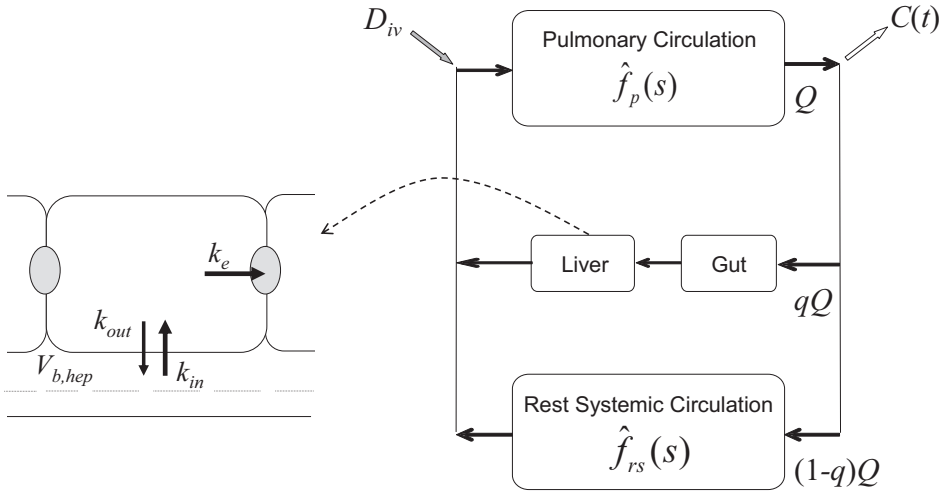

Fig. 1. Circulatory model of hepatic ICG elimination kinetics consisting of heterogeneous subsystems, the pulmonary and systemic circulation, in which the latter is split into two parallel subsystems, the hepatosplanchnic bed (gut and liver in series) and the rest of the systemic circulation, with blood flows qQ and $(1 - q)Q$, respectively (Q is cardiac output). All subsystems are characterized by transit time density functions in the Laplace domain. The pulmonary circulation and the rest of the systemic circulation as well as the gut are characterized by inverse Gaussian transit time density functions denoted by $\hat{f}_i(s)$. In the distributed model of hepatic ICG elimination, $V_{b,hep}$ is the extracellular liver volume, k_{in} is the sinusoidal uptake rate constant (which is determined by the uptake clearance and extracellular liver volume, $k_{in} = CL_{uptake}/V_{b,hep}$), k_{out} is the rate constant of back-transport, and k_e the canalicular excretion rate constant.