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Functional assessment of hepatobiliary secretion by ^{11}C-cholylsarcosine positron emission tomography

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ABSTRACT

Positron emission tomography (PET) with ^{11}C-cholylsarcosine (^{11}C-CSar), a radiolabelled synthetic *N*-methylglycine (sarcosine) conjugate of cholic acid, is a novel molecular imaging technique that enables quantitative assessment of the individual transport steps involved in hepatic secretion of conjugated bile acids. Here, we present the method and discuss its potential clinical and scientific applications based on findings in the first human study of healthy subjects and patients with cholestasis. We also present a clinical example of a patient studied during and six months after an episode of drug-induced cholestatic liver injury.

1. Introduction

Formation of bile is a vital liver function and cholestasis is defined as impaired bile formation and/or bile flow [1–2]. The transport of conjugated bile acids from sinusoidal blood to hepatocytes is facilitated by the Na^+-taurocholate co-transporting polypeptide and the organic anion transporting polypeptides [3], backflux to blood across the basolateral membrane by the multidrug resistance-associated proteins 3 and 4 and the heteromeric organic solute transporter α-β [3–5], and secretion into bile across the apical hepatocytes membrane by the bile salt export pump (BSEP) [6]. Because these transport proteins are affected differently depending on the underlying cholestatic disorder [1–3,7], there is a clinical and scientific need for methods that enable *in vivo* quantification of the transport of conjugated bile acids between blood, hepatocytes and bile.

2. Quantification of hepatobiliary secretion

Hepatic clearance of intravenously administered bile acids, calculated from the plasma disappearance curve, has been suggested as a measure of hepatobiliary secretory function in man [8–10]. However, the systemic clearance of bile acids is very efficient with plasma half-times < 3 min even in patients with mild cholestasis [8–10] and increased extrahepatic distribution in patients with cholestasis [10]. Plasma clearance of bile acids thus depends not only on hepatic uptake and secretion but also on hepatic blood flow and extrahepatic distribution [11]. Moreover, systemic measurements do not provide information on the individual transport steps involved in hepatobiliary secretion from blood to bile.

For a more detailed kinetic analysis of hepatobiliary secretion, hepatoscintigraphy with ^{75}Se-labelled homocholic acid taurine (^{75}Se-SeHCAT) was used in patients with primary biliary cholangitis and primary sclerosing cholangitis [12]. The authors found unaffected hepatic uptake of ^{75}Se-SeHCAT from blood but increased hepatic retention, which was interpreted as impaired secretion into bile. However, the kinetic analysis did not include possible backflux from hepatocytes to blood and could not take into account the rapid transport of bile acids between blood, hepatocytes and bile compartments.

Other imaging methods for characterization of cholestasis and quantification of hepatobiliary secretion include ^{99}Tc-mebrofenin scintigraphy or SPECT [13–14], Gb-EOB-DTPA MRI [15–16] and (15*R*)-[^{11}C]-TIC-Me PET [17–18], but these imaging agents are not derived from bile acids and are accordingly transported by different hepatic transport proteins. Consequently, while useful for other purposes, they cannot be used to study the hepatic secretion of bile acids.

3. Functional assessment using ^{11}C-CSar PET/CT

Positron emission tomography (PET) with ^{11}C-cholylsarcosine (^{11}C-CSar), a radiolabelled synthetic *N*-methylglycine (sarcosine) conjugate of cholic acid, is a novel molecular imaging technique that enables quantitative assessment of the individual transport steps involved in

Abbreviations: ^{75}Se-SeHCAT, ^{75}Se-labelled homocholic acid taurine; PET, positron emission tomography; ^{11}C-CSar, [*N*-methyl-^{11}C]cholylsarcosine; CT, computed tomography; PS_{mem}, permeability surface area product for ^{11}C-CSar from blood to hepatocytes; Cl_{int}, hepatic intrinsic clearance of ^{11}C-CSar

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hepatic secretion of conjugated bile acids [19–21]. Unlabelled cholylsarcosine has been studied extensively in man and has physiochemical and physiological properties similar to endogenous cholylglycine and cholyltaurine [22–24].

The ^{11}C-CSar PET/CT method comprises intravenous administration of ^{11}C-CSar and measurement of tracer concentration in liver tissue by a dynamic PET scan combined with measurements of arterial and hepatic venous concentrations of ^{11}C-CSar in blood samples. In the first human study [21], hepatic blood flow was calculated using a constant infusion of indocyanine green (ICG) and Fick's principle [21]. Potential competitive inhibition between ICG and ^{11}C-CSar [25] was excluded in a subgroup of the subjects [21].

In the human ^{11}C-CSar PET/CT study, nine healthy subjects and eight patients with cholestasis of different aetiology were examined [21]. The cholestatic disorders were primary biliary cholangitis, primary sclerosing cholangitis, autoimmune hepatitis, alcoholic cirrhosis, and alcoholic hepatitis. Hepatic extraction fractions and clearance values of ^{11}C-CSar were calculated from blood concentrations of ^{11}C-CSar. Specific rate constants for the transport of ^{11}C-CSar between blood, hepatocytes and bile compartments and for bile flow were estimated by fitting the model in Fig. 1 to PET and blood data. The estimated parameters enabled calculation of blood-to-hepatocytes and hepatocytes-to-bile concentration gradients of ^{11}C-CSar as well as the mean hepatic residence time, which is the average time that ^{11}C-CSar molecules spend in the hepatocyte before being either transported into bile or back to blood [21].

Table 1 presents the rate constants for exchange of ^{11}C-CSar between blood, hepatocytes and bile (K_1, k_2, k_3) and bile flow (k_5) as well as the mean residence time and non-flow-dependent clearance from blood to hepatocytes (PS_{mem}) and from blood to bile (Cl_{int}). Data from healthy subjects were normally distributed and, as shown in Table 1, with narrow 95% confidence intervals which implies a low variation in normal subjects and is in line with a low intra-individual variation [21]. Data from patients depended on aetiology and the degree of cholestasis and are therefore presented as median (range). In the control subjects, the rate constant for secretion from hepatocytes to bile (k_3) was much higher than the rate constant for backflux (k_2) in accordance with efficient hepatocellular secretion of conjugated bile acids into bile. The mean residence time was < 3 min and both PS_{mem} and Cl_{int} were high (Table 1). Because of the efficient transhepatic clearance, the concentration gradient between hepatocytes and blood was only 2.6 but 1400 between intrahepatic bile and hepatocytes. In the patients with cholestatic liver disease, both k_3 and Cl_{int} were reduced and the individual mean residence time was increased up to 24 min. These alterations correlated to the degree of cholestasis as measured by plasma concentration of bile acids in peripheral blood. Another interesting finding was that the PS_{mem} for ^{11}C-CSar from blood to hepatocytes was reduced in the group of patients with alcoholic liver injury (both acute and chronic) when compared to patients with other causes of cholestasis; in the latter group of subjects, PS_{mem} was similar to the values in the control subjects. Although the number of patients examined was small, this finding was statistically highly significant and could be the result of an overall impaired liver function. In support of this, rat studies have shown that both cholestasis and inflammation impairs hepatobiliary secretion of taurocholate [26–27]. It may not be surprising that cholestatic conditions that include parenchymal damage also affect uptake of conjugated bile acids across the basolateral hepatocyte membrane, but ^{11}C-CSar PET/CT provides a detailed *in vivo* assessment of how much the individual steps contribute to the observed cholestasis.

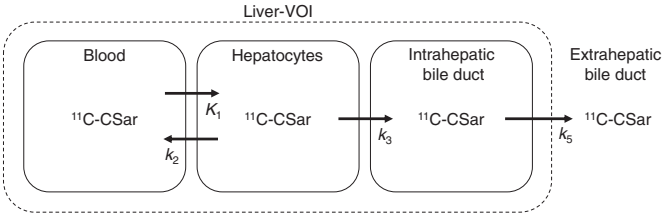

Fig. 1. Kinetic model of the transport of ^{11}C-CSar from blood to bile. The rate constants K_1 (mL blood/min/mL liver tissue), k_2 (min^{-1}), k_3 (min^{-1}), and k_5 (min^{-1}) describe the transport between the compartments. The figure is reprinted from [21] with permission from Elsevier.

4. Drug-induced liver injury investigated by ^{11}C-CSar PET/CT

The following previously unpublished case report illustrates another potential application of ^{11}C-CSar PET/CT, namely evaluation of patients with drug-induced liver injury (DILI). A 57-year-old previously healthy woman was referred to our department with pruritus, jaundice, and biochemical signs of severe cholestasis without parenchymal liver injury (Table 2A). Clinical work-up did not reveal any autoimmune or viral cause and the intra- and extra-hepatic bile ducts were normally calibrated on abdominal ultrasound. The patient had been treated for pneumonia with amoxicillin and clavulanic acid approximately one month prior to debut of the cholestatic symptoms and our conclusion was that she suffered from DILI, a diagnosis that was supported by a liver biopsy. To explore the cholestasis in detail, she was examined by a 60-min dynamic ^{11}C-CSar PET/CT scan during the acute phase and again six months later when she felt fully recovered without having received any specific treatment. Because ICG was unavailable for the first scan, the hepatic blood perfusion was calculated as the ratio between the unidirectional clearance from blood to hepatocytes, K_1, and the initial hepatic extraction fraction [11].

The ^{11}C-CSar PET/CT scan performed during the acute phase (Fig. 2A and Fig. 3A and B) demonstrated a reduced hepatic uptake of ^{11}C-CSar from blood with PS_{mem} reduced to 2/3 of the lower limit of normal and a severe apical secretory dysfunction in terms of reduced k_3 (Table 2B). While the rate constant for backflux from hepatocytes to blood (k_2) and the concentration ratio between intrahepatic bile and hepatocytes (900 *versus* a lower limit of normal of 633 [21]) were within normal range, the mean hepatic residence time was significantly increased to 5.1 min compared to an upper limit of normal of 2.8 min. Cl_{int} was significantly reduced to 0.24 mL blood/min/mL liver tissue compared to a lower limit of normal of 1.31 mL blood/min/mL liver tissue. We attributed the reduction in PS_{mem} to an overall impaired liver function due to inflammation and the decreased k_3 and Cl_{int} to impaired function of BSEP. Six months after the acute phase (Figs. 2B and 3C and D), k_3 was higher than normal values while PS_{mem} and Cl_{int} were now within the normal range (Table 2B). This case thus shows that the hepatocytes were able to more than double the transport efficacy of bile acids from hepatocytes to bile when no longer subject to the acute event of DILI. Although the ^{11}C-CSar PET/CT examination did not lead to a change in treatment of the patient, it did provide a unique insight into the pathophysiology of this case of DILI and how the liver adapted after the acute event.

5. Perspectives

At the current stage, it is unknown how treatment strategies modulate individual steps in the hepatobiliary transport of conjugated bile acids. This may improve with increasing knowledge of the signalling network that governs hepatic bile acid homeostasis and transport. ^{11}C-CSar PET/CT could become a useful tool for evaluation of the direct effects of specific treatment strategies and proof-of-concept studies of novel therapeutics in humans.

The distinction between biliary atresia and other causes of cholestasis in infants is challenging and often requires an extensive clinical work-up including abdominal ultrasound, biopsy and ^{99m}Tc-mebrofenin scintigraphy [28]. With regards to ^{99m}Tc-mebrofenin scintigraphy, the sensitivity is close to 100% with a strong positive predictive value, but the specificity is only around 70% as ^{99m}Tc-mebrofenin scintigraphy