Dynamic computed tomography with low- and high-molecular-mass contrast agents to assess microvascular permeability modifications in a model of liver fibrosis

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ABSTRACT

Interstitial collagen formation and transformation of the fenestrated hepatic sinusoids into continuous capillaries are major ultrastructural changes that occur in liver cirrhosis and fibrosis. These modifications lead to progressive restriction of blood–liver exchanges. The purpose of our study was to evaluate the permeability changes in a model of hepatic fibrosis by using dynamic computed tomography (CT) enhanced with contrast agents of different molecular masses. Dynamic single-section CT of the liver was performed after intravenous bolus administration of a low-molecular-mass contrast agent (iobitridol) and an experimental high-molecular-mass agent (P840) in normal control rabbits and in rabbits with hepatic fibrosis. Hepatic, aortic and portal venous time–density curves were fitted with a dual-input one-compartmental model to calculate the hepatic mean transit time and distribution volume of the contrast agents. In the rabbits with liver fibrosis, the mean transit time of the high-molecular-mass agent was shorter than that of the low-molecular-mass agent (10.0 ± 1.8 s and 12.0 ± 2.2 s respectively; \( P < 0.05 \)). The distribution volume accessible to the high-molecular-mass agent was also smaller (22.2 ± 4.8 % compared with 32.0 ± 6.7 %; \( P < 0.01 \)). In the normal rabbits, the mean transit times of the high- and low-molecular-mass agents did not differ significantly, and nor did their distribution volumes. Our results demonstrate decreased sinusoidal permeability for the high-molecular-mass agent P840 in a model of hepatic fibrosis. Non-invasive assessment of permeability changes in liver fibrosis can be performed with dynamic CT and contrast agents of different molecular masses.

INTRODUCTION

In the normal liver, the endothelial cells lining the sinusoids lack a basement membrane and are perforated by a multitude of fenestrae 50–200 nm in diameter. These features allow instantaneous free access of small and large molecules to the hepatocytes through the extravascular Disse’s spaces. In liver cirrhosis and fibrosis, the endothelial fenestrae disappear, continuous basement membranes develop, and extensive depositions of collagen fibres occur in the Disse’s spaces [1]. This so-called capillarization of the hepatic sinusoids has a profound effect on blood–hepatocyte exchange [2].

The use of contrast-enhanced computed tomography

Key words: computed tomography (CT), contrast enhancement, liver blood supply, liver CT, liver fibrosis, quantitative CT.

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(CT) and compartmental modelling for the non-invasive assessment of liver perfusion parameters has been previously described and validated [3,4]. Because these parameters can be influenced by the sinusoidal barrier, the purpose of the present work was to report the feasibility of using dynamic CT enhanced with low- and high-molecular-mass contrast agents to probe microvascular permeability modifications in a model of liver fibrosis.

MATERIALS AND METHODS

Animals
The study protocol was approved by the Ethics Committee on animal care at our institution. Experiments were performed on 14 male New Zealand White rabbits (Iffa Credo, Brussels, Belgium) weighing 3.6 ± 0.7 kg. The animals were divided into an experimental group (n = 8) and a control group (n = 6). Rabbits in the experimental group were fed on a diet supplemented with 2% (w/w) cholesterol (ICN Biomedicals, Asse, Belgium) and were given diethyl stilboestrol (10 mg, subcutaneous) twice weekly for 12 weeks [5]. At the end of the treatment, liver biopsies were performed to assess fibrosis and sinusoidal changes. Light microscopy was performed on liver specimens fixed in Bouin’s solution and embedded in paraffin. General histological features were assessed on haematoxylin/eosin-stained sections, and fibrosis was evaluated with Masson’s trichrome and Sirius Red stainings. For electron microscopy, specimens were fixed in 2.5% (w/v) glutaraldehyde solution, post-fixed in 1% (w/v) osmium tetroxide solution and embedded in Epon. Thin sections were contrasted with uranyl acetate and lead citrate.

CT scanning protocol
CT studies were performed on a Twin RTS scanner (Marconi Medical Systems, Cleveland, OH, U.S.A.). The rabbits were anaesthetized with ketamine and xylazine hydrochloride after an 18 h fast. A single-slice level that included the liver, aorta and portal vein was scanned continuously for 60 s after injection of contrast material, using the following parameters: 2 mm thickness, 180 mm field-of-view, 512 × 512 matrix, 120 kVp, 100 mA, 1 s scan time, 360° scan angle and 0.3 s reconstruction interval.

The iodinated contrast agents used in this study were a commercially available, low-molecular-mass agent, iobitridol (Xenetix®; Guerbet, Roissy, France), and an experimental monodisperse high-molecular-mass agent, P840 (Guerbet). The molecular mass of iobitridol is 835 Da and its apparent molecular diameter is 1.5 nm, while the molecular mass of P840 is 13736 Da, with an apparent diameter of 6.35 nm (C. Corot, unpublished work). The low-molecular-mass agent was injected first in all rabbits, and the high-molecular-mass agent was injected 60 min later. A dose of 350 mg of I/ml, i.e. 1 ml/kg iobitridol and 1.9 ml/kg P840, was injected at a rate of 1 ml/s, followed by 10 ml of saline solution. Injections were performed with an automated power injector (CT 9000 ADV; Liebel-Flarsheim, Cincinnati, #2002 The Biochemical Society and the Medical Research Society
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Image analysis
CT data processing was performed on a Silicon Graphics O2 workstation (Silicon Graphics Inc., Mountain View, CA, U.S.A.) using programs written in IDL (Research Systems Inc., Boulder, CO, U.S.A.), as described previously [4]. Briefly, regions of interest were placed in the aorta, the portal vein and the liver to generate time-density curves (Figure 1). These curves were analysed using a dual-input one-compartmental model. The general equation for this functional model is:

$$C_L(t) = \int_0^t k_{ls} C_a(t' - \tau_s) + k_{lp} C_p(t' - \tau_p) e^{-k_p(t'-t')/k_2} dt'$$

where $C_a(t)$, $C_p(t)$ and $C_L(t)$ represent the concentrations in the aortic plasma, the portal venous plasma and the liver respectively; $\tau_s$ and $\tau_p$ represent the transit times from the aorta and portal vein regions respectively to the liver region of interest; and $k_{ls}$ represents the aortic plasma inflow rate constant, $k_{lp}$ the portal venous inflow plasma rate constant and $k_2$ the outflow rate constant. The distribution volume (%) of the contrast agent through the liver compartment was calculated as $100 \cdot (k_{ls} + k_{lp})/k_2$, and the mean transit time (s) as $1/k_2$ [6]. Comparisons between the results obtained with the two contrast agents were performed by using the Wilcoxon signed-rank test. A $P$ value of < 0.05 was considered statistically significant.

RESULTS
Light microscopy showed centrolobular liver fibrosis in all animals (Figure 2). Electron microscopy revealed widened Disse’s spaces containing abundant collagen fibres and the development of basement membranes in

Figure 2  Light microscopic view of rabbit liver after 12 weeks’ administration of cholesterol and diethyl stilboestrol
Sirius Red staining shows irregular fibrosis that predominates in the centre of the hepatic lobules (arrows). Magnification $\times$ 15.

Figure 3  Electron microscopic view of rabbit liver after 12 weeks’ administration of cholesterol and diethyl stilboestrol
A basement membrane is observed adjacent to the hepatocyte plasma membrane (arrows). Hepatocytes show cytoplasmic degeneration with phagolysosomes. Original magnification $\times$ 32396.

The contrast agents did not provoke adverse reactions in the rabbits during or after CT scanning. In the experimental group, the mean transit time and the distribution volume for iobitridol were 12.0 $\pm$ 1.2 s and 32.0 $\pm$ 6.7% respectively. With P840, these parameters were significantly decreased. The mean transit time was 10.0 $\pm$ 1.8 s ($P < 0.05$ compared with iobitridol) and the distribution volume was 22.2 $\pm$ 4.8% ($P < 0.01$ compared with iobitridol). In the control group, the mean transit time and distribution volume with iobitridol were 9.1 $\pm$ 1.8 s and 25.0 $\pm$ 3.4% respectively. No significant differences were found for these parameters with P840 [mean transit time, 7.7 $\pm$ 1.9 s ($P > 0.2$); distribution volume, 23.3 $\pm$ 3.6% ($P = 0.16$)].

DISCUSSION
Our study shows that dynamic CT performed with contrast agents of different molecular masses can be used for the non-invasive assessment of microvascular permeability changes in rabbits with cholesterol/stilboestrol-induced liver fibrosis. This experimental model provides a valuable model of sinusoidal capillarization without small or large intrahepatic shunts [7]. In clinical practice, the permeability changes induced by capillarization are important, since they result in
impaired exchanges between sinusoidal blood and hepatocytes, decreasing drug elimination and impairing liver function [8].

The modifications to sinusoidal permeability that occur in liver fibrosis have been assessed previously by perfusing the liver with multiple radiolabelled indicators [2,9]. This multiple-indicator dilution technique, proposed initially by Goresky [10], is invasive, since catheterization of the hepatic artery or the portal vein and the hepatic veins is required in order to inject reference substances and collect serial blood samples. By using peripheral intravenous injections of iodinated contrast agents of different molecular masses, we observed that the distribution volume and the mean transit time of a high-molecular-mass agent in the liver were significantly decreased in comparison with those of a low-molecular-mass agent in rabbits with liver fibrosis. These differences indicate that transepithelial exchanges of large molecules are restricted under conditions of liver fibrosis by sinusoidal capillarization, as demonstrated by light microscopy and electron microscopy.

Our CT method gives results that are similar to those obtained using the multiple-indicator dilution method, without the need for multiple catheterizations. Therefore this CT method has the potential to assess non-invasively changes in sinusoidal permeability that occur in liver fibrosis in humans. The advantage of our new approach is that two contrast agents of different molecular masses were used in the same rabbits. Therefore diagnosis of permeability changes in hepatic fibrosis can be performed without comparing data obtained in rabbits with liver fibrosis with those from a control group or with pre-established cut-off values. Furthermore, follow-up studies can be performed by comparing the ratios of values obtained with the two contrast agents. Longitudinal comparisons may be useful to predict the benefit of therapy, but this should be assessed in further studies.

The high-molecular-mass iodinated agent used in our study is an experimental contrast material. Similar high-molecular-mass iodinated contrast agents have been used previously in animal studies [11], but extensive toxicological studies are needed before these agents can be used in humans. Dynamic CT scanning as performed in the present study has two main disadvantages: first, it requires ionizing radiation, and secondly only one section level of the liver can be studied. As mentioned previously [3], strategies to decrease the dose and the use of multirow detector CT scanners should be considered in the future.

In conclusion, quantification of liver perfusion parameters using dynamic CT and contrast agents of different molecular masses is a promising method for the assessment of changes in microvascular permeability in liver fibrosis.

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REFERENCES


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