Computers in Biology and Medicine 73 (2016) 108–118

Contents lists available at ScienceDirect

Computers in Biology and Medicine

journal homepage: www.elsevier.com/locate/cbm

Zonated quantification of steatosis in an entire mouse liver ☆

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ARTICLE INFO

Article history:
Received 22 February 2016
Received in revised form
6 April 2016
Accepted 9 April 2016

Keywords:
Histological serial sections
Whole-slide scans
Zonation
Steatosis
Dextromethorphan
Quantitative image analysis
Pharmacokinetics simulations
Virtual Liver

ABSTRACT

Many physiological processes and pathological conditions in livers are spatially heterogeneous, forming patterns at the lobular length scale or varying across the organ. Steatosis, a common liver disease characterized by lipids accumulating in hepatocytes, exhibits heterogeneity at both these spatial scales. The main goal of the present study was to provide a method for zonated quantification of the steatosis patterns found in an entire mouse liver. As an example application, the results were employed in a pharmacokinetics simulation.

For the analysis, an automatic detection of the lipid vacuoles was used in multiple slides of histological serial sections covering an entire mouse liver. Lobuli were determined semi-automatically and zones were defined within the lobuli. Subsequently, the lipid content of each zone was computed. The steatosis patterns were found to be predominantly periportal, with a notable organ-scale heterogeneity.

The analysis provides a quantitative description of the extent of steatosis in unprecedented detail. The resulting steatosis patterns were successfully used as a perturbation to the liver as part of an exemplary whole-body pharmacokinetics simulation for the antitussive drug dextromethorphan. The zonated quantification is also applicable to other pathological conditions that can be detected in histological images. Besides being a descriptive research tool, this quantification could perspectively complement diagnosis based on visual assessment of histological images.

1. Introduction

Many physiological processes and pathological conditions in human and animal livers exhibit spatial heterogeneity. Livers are anatomically structured in lobes [1], the liver tissue is structured in lobuli [1], which are in turn subdivided into zones [2,3]. Typically, metabolic or clearance processes take place in zonated form [4]. This zonation is mainly due to zonated gene/enzyme expression [5,6]. The size of the respective periportal or pericentral zone depends on the specific phenomenon. In particular for pathological conditions, the sizes may additionally vary over time, e.g., for necrosis and regeneration after administration of carbon tetrachloride [7]. To allow describing the interaction of physiological processes and pathological states with zonation of different size, the classical geometric notion of lobuli divided into three zones [8] was refined to a larger number of zones in this study. Heterogeneity across the organ may be present as well, mainly due to pathological conditions varying at this coarser length scale, e.g.,

Abbreviations: CV, central vein; DXM, dextromethorphan; EvG, Elastica-van Gieson; GS, glutamine synthetase; HE, hematoxylin & eosin; PF, portal field; PK, pharmacokinetics

☆Preliminary results of this study were presented at the Virtual Physiological Human Conference 2014 (VPH 2014): L.O. Schwen, A. Homeyer, M. Schwier, F. Gremse, A. Schenk, L. Küpfer, U. Dahmen, O. Dirsch, T. Preusser: *Multiscale Simulation of Zonated Metabolism in Steatotic Livers*, available at http://seek.virtual-liver.de/presentations/703.

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http://dx.doi.org/10.1016/j.compbiomed.2016.04.004
0010-4825/

fibrosis [9,10], cirrhosis [11], or carcinoma [12,13].

Zonation of pathological conditions is relevant due to their interaction with various metabolic or clearance processes taking place in zonated form [4]. Quantitative data describing the extent of a pathological condition depending on the zonal position can thus provide data complementary to a diagnosis typically based on visual assessment of histological images. Quantifying additionally the organ-scale heterogeneity can help estimate the impact of the disease on overall organ function. Spatially resolved quantitative data describing a liver disease is indispensable for accurate simulations of physiological processes, e.g., [14,7,15].

In the present study, steatosis is considered as one example of a pathological condition which is known to occur in spatially heterogeneous form. Steatosis is a common human liver disease frequently caused by dietary misconduct or alcohol abuse [16]. Steatotic livers are characterized by lipids accumulating in the hepatocytes [17]. Steatosis is most typically located in the pericentral region, but it may also occur in periportal form, see [18] and the references therein. In addition, heterogeneity can also be observed across the whole organ [19]. Both zonation and organ-scale heterogeneity were present in the mouse liver considered in this study, see Fig. 1. The main goal of the present study was to quantify these heterogeneities, in order to allow using the results for a pharmacokinetics (PK) simulation.

Clinical diagnosis of steatosis is based on histological assessment of liver biopsies, representing only a tiny fraction of the whole organ. For this purpose, e.g., hematoxylin & eosin (HE) or oil Red-O staining [20] is used, providing a distinction of micro- and macro-vesicular steatosis as well as a zonation [21]. Clearly, this only provides local data and does not permit examining the whole organ. Zonation of steatosis is easily assessed qualitatively by experienced pathologists, but is typically not quantified. At the organ scale, steatosis can be quantified using ultrasound and computed tomography [22] as well as magnetic resonance imaging [23], all of which are non-invasive, but do not reveal all features accessible by histology. Experimentally, lipid contents can be determined using biochemical measurements, which can be compared to averaged results obtained by other approaches [24]. This approach, however, lacks spatial resolution.

Detecting lobuli is the basis for zonated quantification. In most species, their boundaries are not determined by anatomical structures, so they need to be inferred from typically optical image data of sufficiently high resolution. A manual approach for determining lobular shapes [25,26] is of prohibitive workload for more than a few lobuli, so a semi-automatic procedure with an algorithmic component similar to [27] was employed here.

Recent advances in histological whole-slide imaging and image processing [28] permit quantifying steatosis using serial sections of entire organs of small animals such as mice. Besides the detection of lipid vacuoles, steatosis can be quantified as average values per lobe or on smaller tiles, typically squares that are not physiologically motivated. To the best of our knowledge, this kind of analysis has neither previously been performed in 3D to cover a whole organ, nor have zonated distributions and their heterogeneity been quantified at this scale.

Previous own studies [15,29] showed the influence of spatially resolved pathological perturbations in PK modeling, in particular in combination with zonated metabolism. The present study now offered the opportunity to use actual measured steatosis patterns for the whole organ as opposed to the synthetic spatial patterns used previously [15,29].

In the present paper, an approach is presented for zonated quantification of steatosis in a mouse liver. For this purpose, histological whole-slide scan images of serial sections of a whole mouse liver with different stainings were used. An image registration was performed for two purposes: for being able to combine information made visible by different stainings [30] and for obtaining 3D analysis results [31]. In our case, portal fields (PFs) and central veins (CVs) were detected using glutamine synthetase (GS) staining in order to determine lobuli and zones within them. In neighboring HE-stained slides, lipid vacuoles indicating macrovesicular steatosis were detected by an automatic procedure in a set of slides covering an entire liver. This combined analysis was used for zonated quantification of steatosis for the entire liver. Finally, the analysis results were used in a multiscale whole-body PK simulation, in which the liver model represented both organ-scale heterogeneity and zonation.

2. Material and methods

In this section, the workflow from creating specimens via image registration, annotation of PFs and CVs, tessellation in lobuli and zones, detection of lipid vacuoles, and zonated quantification is presented. The in silico part of this workflow is illustrated in Fig. 2. Finally, a modeling approach is described where the analysis result was used as simulation input.

2.1. Histological imaging of a steatotic mouse liver

Steatosis in a male C57/BL6N mouse (Charles River, Sulzfeld, Germany) was induced by feeding a methionine/choline-deficient high fat diet (E15652-94 EF R/M, high fat MCD mod. low methionine and choline experimental diet [32]; ssniff, Sulzfeld,

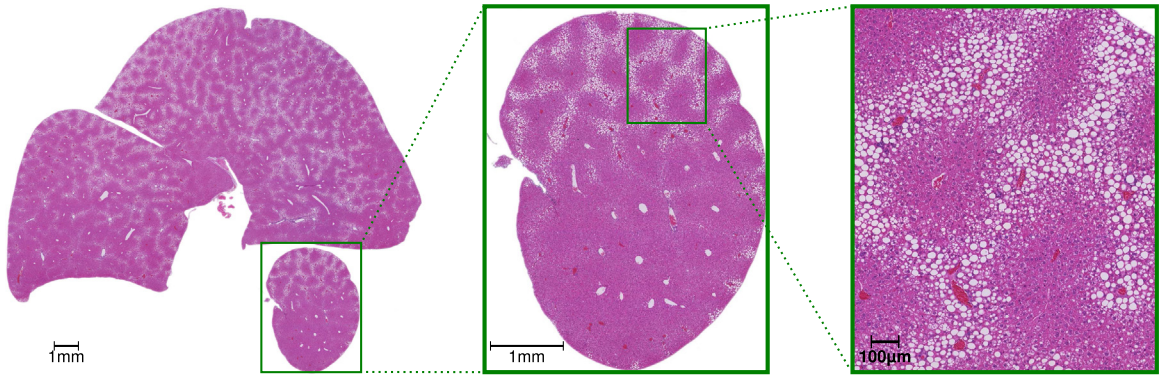

Fig. 1. *Heterogeneity of steatosis*: Large vacuoles in hepatocytes, indicating steatosis, are visible as bright spots in this histological whole-slide scan of a mouse liver shown in three different magnifications (from left to right: whole slide, one lobe, detailed view). The steatosis forms distinct zonal patterns, in this case periportal ones, and occurs spatially heterogeneous across the organ. The main goal of this study was to quantify these heterogeneities. The images in this figure were manipulated for clarity (removal of background, contrast adjustments).