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SPONTANEOUSLY ARISING DISEASE

Distribution of Connective Tissue in the Male and Female Porcine Liver: Histological Mapping and Recommendations for Sampling

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Summary

The pig is a large animal model that is often used in experimental medicine. The aim of this study was to assess, in normal pig livers, sexual dimorphism in the normal fraction of hepatic interlobular and intralobular connective tissue (CT) in six hepatic lobes and in three macroscopical regions of interest (ROIs) with different positions relative to the liver vasculature. Using stereological point grids, the fractions of CT were quantified in histological sections stained with aniline blue and nuclear fast red. Samples (415 tissue blocks) were collected from healthy piglets, representing paracaval, paraportal and peripheral ROIs. There was considerable variability in the CT fraction at all sampling levels. In males the mean fraction of interlobular CT was 4.7 ± 2.4% (mean ± SD) and ranged from 0% to 11.4%. In females the mean fraction of the interlobular CT was 3.6 ± 2.2% and ranged from 0% to 12.3%. The mean fraction of intralobular (perisinusoidal summed with pericentral) CT was <0.2% in both sexes. The interlobular CT represented >99.8% of the total hepatic CT and the fractions were highly correlated (Spearman $r = 0.998$, $P < 0.05$). The smallest CT fraction was observed in the left medial lobe and in the paracaval ROI and the largest CT fraction was detected in the quadrate lobe and in the peripheral ROI. For planning experiments involving the histological quantification of liver fibrosis and requiring comparison between the liver lobes, these data facilitate the power analysis for sample size needed to detect the expected relative increase or decrease in the fraction of CT.

Keywords: connective tissue; histology; liver; pig

Introduction

Both small and large animals are used to study the mechanisms of the origin and spread of liver fibrosis, often together with the regenerative capacity and healing of the liver. Fibroses of different aetiologies have been studied predominantly in mice (George *et al.*, 2003; Anstee and Goldin, 2006; Machado *et al.*, 2015) and rats (Yi *et al.*, 2012; Nowatzky *et al.*, 2013; Fakhoury-Sayegh *et al.*, 2015). However, small animal models of liver fibrosis have several limitations due to the small organ size (Lossi *et al.*, 2016). Therefore, it is usually not possible to study phenomena such as portocentral and portoportal bridging fibrosis, modelling of the biomechanics of trauma, lobar resection and regeneration or surgical

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techniques. Large animal models are needed for the improved translation of experimental work into human medicine. Apart from sheep liver (Ghodsizad *et al.*, 2012), the porcine liver is the most widely used large animal model (Avritscher *et al.*, 2011; Kawamura *et al.*, 2014; Bruha *et al.*, 2015; Nygård *et al.*, 2015; Wang *et al.*, 2015) to study improvements in invasive (Croome *et al.*, 2015) and non-invasive methods of liver disease management (Gnutzmann *et al.*, 2015), to interpret animal experiments and to translate the results of animal models to human medicine (Arkadopoulos *et al.*, 2011; Watson *et al.*, 2016; Budai *et al.*, 2017). Chen *et al.* (2013) showed that histological assessment of liver fibrosis in the pig correlates with non-invasive splenic magnetic resonance imaging. Combining the data obtained for connective tissue (CT) with data on the microvascular bed of the porcine liver (Eberlova *et al.*, 2016, 2017) would improve existing models of human liver perfusion. Moreover, fibrosis is an important part of porcine liver diseases, such as in pigs suffering from biliary and peribiliary cysts (Komine *et al.*, 2008) or swine hepatitis E (Lee *et al.*, 2010).

Summarizing the present literature, porcine liver fibrosis and cirrhosis of different aetiologies can be used as a model for human liver fibrosis and cirrhosis (Avritscher *et al.*, 2011). In the porcine liver, fibrosis is usually induced by CCl_4 (Zhang *et al.*, 2009), by alcohol (Lee *et al.*, 2017), by a high-fat diet or by a Western-style diet (Panasevich *et al.*, 2018) or by using pentoxifylline (Peterson and Neumeister, 1996). It has been proposed that porcine liver fibrosis may be staged according to human standards using the Metavir scoring system (Ishak *et al.*, 1995; Zhou *et al.*, 2014; Yin *et al.*, 2017); however, this required several modifications (Huang *et al.*, 2014, 2017).

The major fibrogenic cells in the liver are the hepatic stellate cells, portal fibroblasts, fibrocytes, bone marrow-derived cells that are activated and transdifferentiated into hepatic myofibroblasts and possibly hepatocytes and cholangiocytes that transition to myofibroblasts (Forbes and Parola, 2011; Zhao *et al.*, 2016; Kisseleva, 2017). Histological assessment of the location of fibrosis and identification of the source of fibrogenic cells is necessary when assessing the severity of the liver disease and the patient's prognosis (Takahashi and Fukusato, 2014; Stasi and Milani, 2016). Biopsy samples are usually scored in terms of their grade (Scheuer, 1991) and stage (Saxena, 2011). Six specific foci of liver fibrogenesis have been proposed for scoring, namely portal, pericellular (i.e. perisinusoidal), pericentral (i.e. perivenular), centrilobular, ductal (i.e. periductal) and ductular (Batts and Ludwig, 1995; Gohlke *et al.*, 1996; Brunt *et al.*, 1999; Sakhuja, 2014; Takahashi and Fukusato, 2014).

During chronic hepatitis, fibrosis starts and spreads from portal regions, forming stellate periportal scars or enlarging the portal tracts (Lefkowitch, 2007). Steatofibrosis in alcoholic liver disease begins in the pericentral region and extends in a perisinusoidal pattern, where it is more pronounced than in hepatitis C infection (Zaitoun *et al.*, 2001). This phenomenon leads to centroportal and portoportal bridging and, together with the regenerating nodular parenchyma, results in cirrhosis (Theise, 2013). Similar histological findings have been reported in non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatohepatitis (NASH), but lack the pericentral origin of fibrosis (Brunt *et al.*, 1999; Kleiner *et al.*, 2005; Takahashi and Fukusato, 2014). Primary biliary cirrhosis involves fibrosis of small intrahepatic bile ducts (Lindor *et al.*, 2009; Working Subgroup for Clinical Practice Guidelines for Primary Biliary Cirrhosis, 2014). Central hepatic veins are often retained in their centrilobular location, even in cirrhosis. Sclerosing cholangitis shows bile duct scarring biliary fibrosis, leading eventually to cirrhosis (Hirschfield *et al.*, 2013; de Vries *et al.*, 2015).

The amount of CT in the human liver is usually estimated during routine analysis of liver biopsy samples, according to widely used scoring systems (Scheuer, 1991; Ishak *et al.*, 1995; Bedossa and Poynard, 1996). However, Standish *et al.* (2006) highlighted several limitations of subjective or semiquantitative scoring as both the interobserver and intraobserver variability might disqualify the data generated from comparative studies or from evaluations of non-invasive methods of liver fibrosis. Therefore, the need for an objective, reproducible measure, preferably generating continuous data, has been articulated (Saxena, 2011).

To the best of our knowledge, no published data are available for continuous quantitative histological parameters that demonstrate the normal intersexual and interindividual variability in the fraction of CT in various macroscopical regions of porcine liver lobes. Therefore, the aim of the present study was to assess the content and distribution of normal hepatic CT in the domestic pig and to provide sampling recommendations for further histopathological studies. The following null hypotheses were tested: (1) the volume fraction of CT in the liver is the same in male and in female pigs, (2) the volume fraction of CT in the liver is the same in all hepatic lobes and (3) the volume fraction of CT in the liver is the same in three macroscopical regions with different positions related to the liver vasculature (regions of interest; ROIs): the peripheral regions of the liver lobes, the regions near