Evaluation of the hepatic structure in normal dogs and dogs with congenital portosystemic shunts

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Abstract

This study included five dogs with a congenital portosystemic shunt (CPSS), which had surgical attenuation of the shunt. Liver biopsies were taken for Electron Microscopy (EM) evaluation. The evaluation was focussed on the vasculature within the liver. The aspects of the liver samples were described with Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Light Microscopy (LM). The results were compared with liver biopsies of three dogs with normal liver function. In the liver of dogs with a shunt, the endothelial lining of the sinusoids was less porous, more extracellular matrix (ECM) was present, there was steatosis in the hepatocytes and the sinusoids were shorter, in comparison to the normal dog liver. To gain more understanding in why for some dogs surgical attenuation of the shunt leads to development of the liver vasculature and for others not, hepatic tissue should also be studied after the surgery, using the same techniques. Comparing these tissues will give information about differences in liver vasculature before and after surgery.

Keywords: congenital portosystemic shunt, dog, electron microscopy

Introduction

Congenital portosystemic shunt (CPSS) is a condition in dogs in which the portal bloodflow bypasses the liver and reaches the systemic venous circulation directly. For this study, liver biopsies of dogs with CPSS were taken for evaluation with the same method as described in a paper of Hunt et al (2004).[6] So far, little has been written about the EM appearance of the liver of dogs with a portosystemic shunt and normal dogs. The study was focussed on the differences in vascular structure seen between liver samples of CPSS dogs and normal dogs, in particular the sinusoids. The liver samples were evaluated with Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Light Microscopy (LM). The results were compared with liver biopsies of three dogs with normal liver function. This research may function as an aid to clarify the mechanisms involved in hepatic development after surgical attenuation and it may gain more

Table 1
Details of dogs included in this research

Patient	*Breed*	*Sex*	*Shunt*	*Age at surgery*
1 Mindy	Beagle	Female	Intrahepatic	7 months
2 Grace	Bichon Frise	Female	Extrahepatic	5 months
3 Daisy	Terrier	Female	Extrahepatic	13 months
4 Eddie	Jack Russel Terrier	Male	Extrahepatic	21 months
5 Galian	Affenpinscher	Male	Intrahepatic	30 months
6 Benny	Cattle Dog	Female	Control	2 months
7 Bella	Schnauzer	Female	Control	24 months
8 Fluffy	Border Collie	Female	Control	3 months

understanding why some livers proliferate after surgical attenuation of the shunt and why other livers do not. This report will describe the relevant differences between hepatic tissue of dogs with CPSS and normal dogs that were found using different techniques of imaging.

Materials and methods

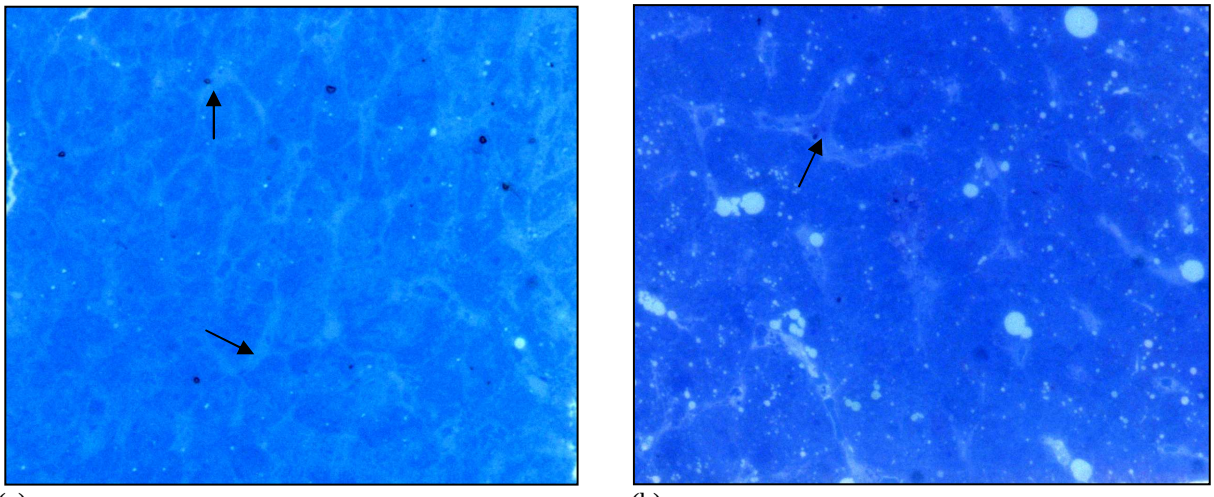
Animals and samples

Biopsies of the liver tissue were taken during surgery from five dogs with CPSS presented between March, 2007 and January, 2008 to the University Veterinary Centre, Sydney. Three dogs (Bichon Frise, Terrier, Jack Russel Terrier) were diagnosed with an extrahepatic shunt and two dogs (Beagle, Affenpins) with an intrahepatic shunt. The age of the dogs ranged between 5 and 30 months at the time of surgery (median 13 months). Two dogs were male and three were female dogs. They all underwent attenuation of the shunt through cellophane banding. [6] In the same period control liver biopsies were taken of three healthy dogs (Cattle Dog, Border Collie, Schnauzer) that underwent surgery unrelated to liver disease. The age of these healthy dogs ranged between 2 and 24 months (median 3 months) and they were all female. (Table 1)

Processing and examination of the samples

After biopsy, the liver tissue was immediately placed in a 2.5% glutaraldehyde in 0.1M phosphate buffer for primary fixation, which stabilizes the tissue by crosslinking the proteins of the cells and therefore the cells are quickly killed. They were kept in here until biopsies of all the patients were assembled. Then, the primary fixative was washed out with 0.1M phosphate buffer, three times for five minutes each. After that, the samples were left in a secondary fixative (1% OsO_4 in 0.1M phosphate buffer) for an hour. The osmium stabilises the membrane lipids of the cells and organelles. Dehydration in ethanol (30%-100%) was performed.

The samples for SEM where treated with Hexamethyldisilazane (HMDS) for three minutes and where put in the desiccator to dry. After that, they were snapped in liquid nitrogen and mounted on stubs and coated with gold by low vacuum sputter coating to increase the conduction and contrast and to prevent accumulation of electric charge on the specimen during the irradiation with electrons. Now the samples were assessed on the Scanning Electron Microscope (Philips SEM 505). This type of electron microscope images the surface of the sample by scanning it with a high-energy beam of electrons. The electrons interact with the atoms of the sample and these



(a) (b)

Fig. 1 (a) Normal canine liver. The sinusoids are seen as light tortuous lines. (400x enlarged). (b) Liver of a 13-months-old dog with an extrahepatic CPSS. Shorter sinusoids are seen. (400x enlarged)

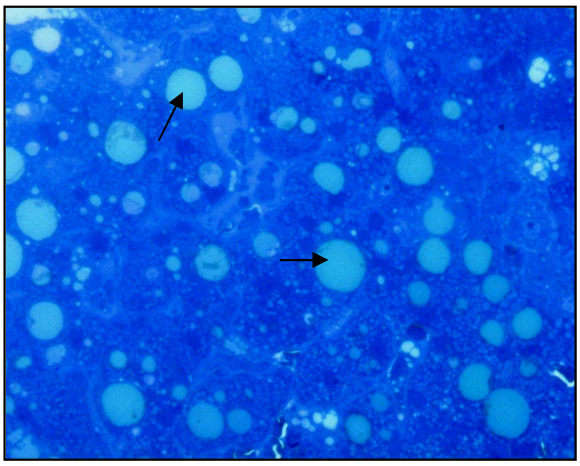


Fig. 2 Liver of a 30-month-old dog with an intrahepatic CPSS. There are many lipid vacuoles visible in the hepatocytes (arrows). (400x enlarged)

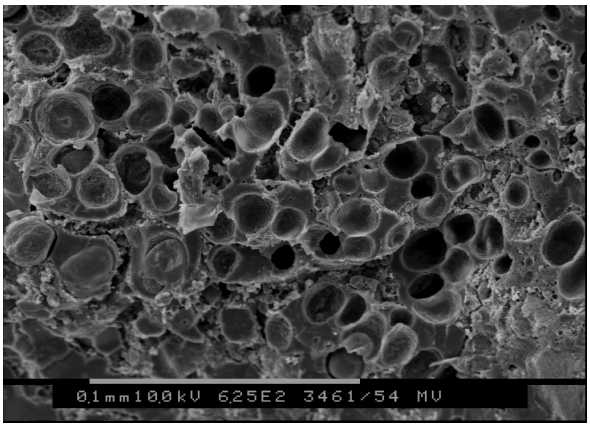


Fig. 3 SEM image of the liver of a 30-month-old dog with an intrahepatic CPSS. Many impressions of the tissue by fat droplets are seen. This indicates steatosis.

signals are translated by the computer to produce an image of the surface of the sample.

The TEM/LM samples where put in Spurr's resin to make blocks. After that they were cut in 0.3 µm semithin (LM) and 90 nm ultrathin (TEM) sections. The LM sections were coloured with Toluidine Blue, a basic dye that colours the nuclei and after that evaluated with the light microscope.

The TEM sections were put on a 200 mesh Cu grid and assessed on the Transmission Electron Microscope (Zeiss 902). They weren't coloured, as the contrast was sufficient. This microscopy technique produces an image by transmitting a beam of electrons through the ultrathin sample. The electrons interact with the sample and produce a cross-section image of it.

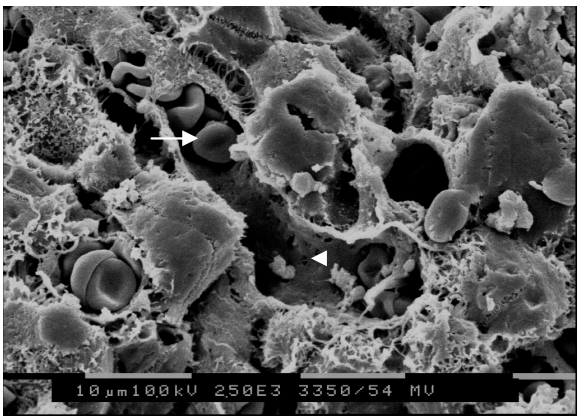
Results

Animals

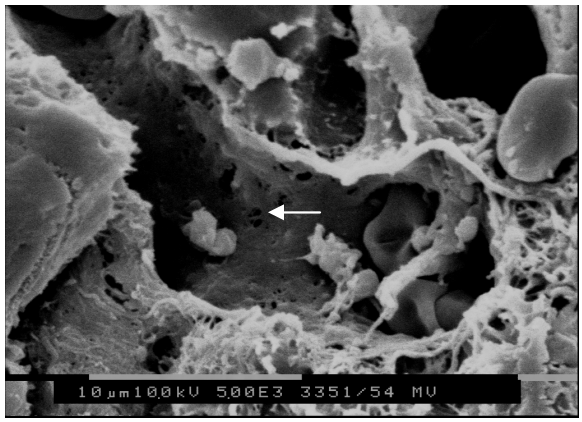
All five dogs were diagnosed with a congenital portosystemic shunt and had variable clinical signs associated with this disease. Table 1 shows the dogs included in the research with their breed, sex, shunt type and age at surgery.

Light Microscopical Characteristics

The abnormalities seen in the livers with CPSS were similar in all five cases, although the degree was slightly different. There were fewer venes and they were

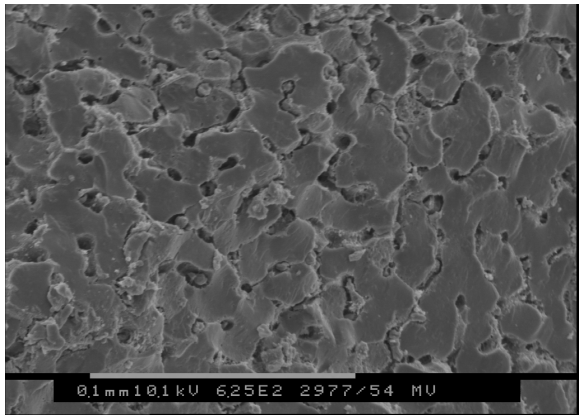


(a)

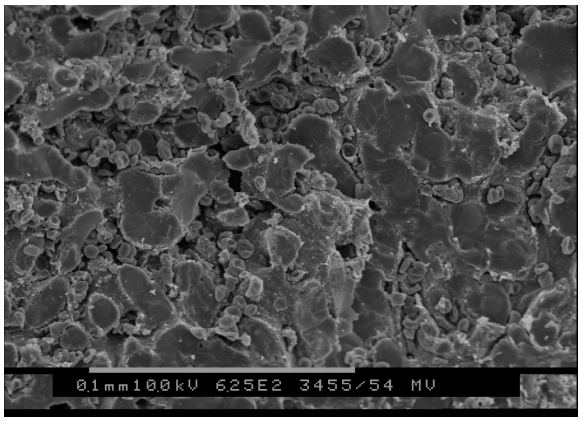


(b)

Fig. 4 SEM images of a liver from a 5-month-old dog with an extrahepatic CPSS. (a,b) A sinusoid is shown with erythrocytes (arrow). Also fenestrae in the endothelial wall (arrowhead) are visible. The image shows a lot of ECM. Image (b) shows an enlargement of the image. The fenestrae are more clearly seen. There are less fenestrae than in a healthy liver.



(a)



(b)

Fig. 5 (a) SEM image of a normal liver from a 24-month-old dog. The sinusoids are shown. (b) SEM image of the liver from a 30-month-old dog with an intrahepatic CPSS. The vessels are damaged and the sinusoids can barely be distinguished.

significantly shorter (figure 1). Also many vacuoles were seen in the hepatocytes, which is indicative for lipid accumulation *in vivo*. During dehydration of the tissue sample lipids are dissolved, so lipid droplets were seen as empty holes. In normal liver only one or two lipid vesicles are visible per hepatocyte, whereas in the CPSS liver significantly more lipid vesicles were visible (figure 2). There were only few erythrocytes in the sinusoids which means that the tissue was well fixated.

Scanning Electron Microscopical Characteristics of CPSS liver tissue

The SEM images showed a lot of extracellular matrix (ECM). Gaps and fenestrae could be seen in the endothelium and at some places the endothelial lining was discontinuous. On these images the impressions of the lipid droplets could be seen also (figure 3). The SEM images showed damaged endothelium and fibrous material in the extracellular space. The intact endothelium parts of the sinusoids are less porous (figure 4). There was a high vascularisation level and also there were

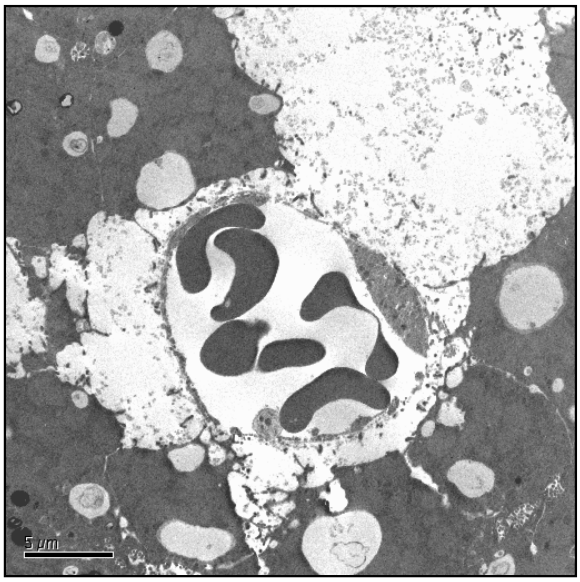


Fig. 6 TEM image of the liver of a 5-month-old dog with an extrahepatic CPSS. The image shows extremely damaged endothelium.

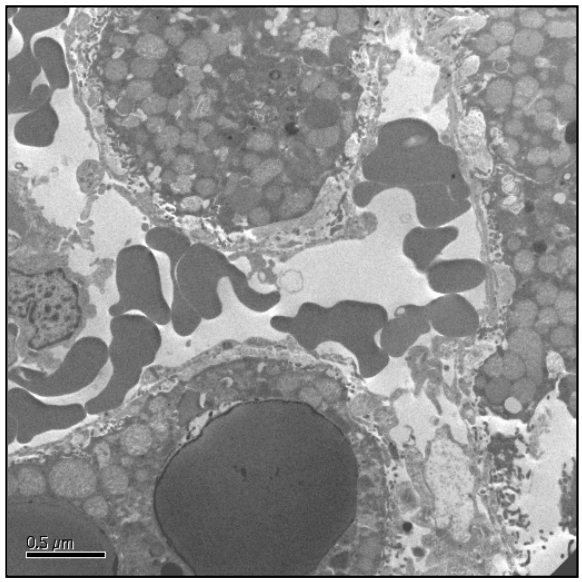


Fig. 7 TEM image of the liver of a 30-month-old dog with an intrahepatic CPSS. The image shows a sinusoid with erythrocytes. The endothelium of the sinusoid is thicker, with less pores, which means that there may be less exchange over the endothelium. There is also a large fat droplet visible.

short blood vessels visible, in particular the sinusoids (figure 5).

Transmission Electron Microscopical Characteristics of CPSS liver tissue

The TEM images showed that the endothelium was severely damaged, most likely due to the disease (figure 6). There was a lot of fat accumulation and an increased amount of extracellular matrix. There were fewer pores visible, which is called defenestration, and the endothelium of the sinusoids was thicker (figure 7). In the space of Disse there was more collagen and therefore, the endothelium has become less porous. This means there was less exchange between the parenchyma cells and the blood vessel in both directions and lipid accumulation possibly occurred.

In conclusion, three differences were found in shunt dogs compared to normal dogs:

1. The endothelial lining of the sinusoids of shunt dogs appeared to be less porous. This is called defenestration.
2. There was a lot of ECM present.
3. There was steatosis in the hepatocytes of the shunt dogs. This might have been the consequence of the restricted passage through the endothelium because of defenestration and ECM deposit.
4. The sinusoids were shorter.

Discussion

In this study, significant histological differences were found between livers of dogs who suffer from CPSS and normal dog livers. However, three comments can be made with respect to the results. First, the samples used in this research have been in the primary fixative for variable periods of time. This has a negative effect on the quality of the tissue, and therefore, the imaging. Secondly, the processing and imaging was done by newly trained students, which also has an effect on the images. However, this was a small effect as the students were trained intensively on the different techniques and the images were of very good quality according to an expert on liver electron microscopy. The third comment is that the number of dogs included in this research was limited; therefore more dogs have to be examined to confirm the conclusions.

The finding of histologic fat accumulation in CPSS liver tissue was similar to the reportings of Parker *et al*. (2008). They studied if preoperative histologic examination of PSS liver samples could be used to predict the long-term outcome in dogs after surgical attenuation of the shunt and found that there was no correlation between the severity of the hepatic histologic lesions prior to surgery and the prognosis after surgery.[9]

In a study of Isobe et al (2008), the incidence of lipogranulomas in the liver was compared between dogs with portosystemic shunts and normal dogs. Concluded was that the lipogranuloma density in the liver was significantly higher in the CPSS group. [7] This was similar to our findings.

In a review article, Braet (2004) discussed the ultrastructure of hepatic endothelial fenestrae. Special domains were found that are involved with *de novo* formation and disappearance of fenestrae. According to Braet, further insight in the functional and structural organization of the liver sieve can be achieved by the use of electron microscopic tomography. [2] In this report the fenestrae in the endothelial lining of the sinusoids where reduced in comparison with normal livers. Baade et al (2006) compared canine livers with CPSS before and after partial ligation of the shunt. Arteriolar and ductular proliferation, hypoplasia of the portal veins, and atrophy and steatosis were some of their findings in the shunt livers. After surgery, the same lesions were seen, though there were signs of resolution of hepatic changes. [1]

As there was no follow-up after ligation of the shunt in this research, little can be concluded about postoperative changes in the livers. To answer the question why some livers proliferate after ligation and

why others do not, follow-up after the surgery is essential for future investigations. Few papers have been written in which sinusoids were studied with electron microscopy. Several papers have been written about this subject in Japanese, but there is no English translation available. In one paper the micromorphological characteristics of hepatic sinusoidal endothelial cells of the dog have been studied by electron microscopy. The size of the endothelial fenestrae was also measured, using scanning electron microscopy. [4]

Electron microscopic descriptions on the sinusoidal endothelium of rats, goats, sheep and humans have been done. [3,5,10,11]

In a paper by Jackowiak, the normal microvascularization of the canine hepatic ducts has been studied using scanning electron microscopy. [8]

This research showed defenestration of the endothelial lining of the sinusoids, steatosis in the hepatocytes and shorter sinusoids in CPSS livers of dogs. These are all signs of impaired liver growth and function. To answer the question why some livers proliferate after surgery and others not, it is essential to assemble biopsies after ligation surgery and compare these liver samples with the ones taken before surgery. Also, the histological findings should be compared between the dogs with sufficient hepatic proliferation and the dogs without sufficient hepatic proliferation.

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