Portal hypertension associated with end-stage liver disease and microRNA analysis

In association with

University College Dublin Veterinary Hospital is investigating the potential use of microRNAs as biomarkers to aid decision making in cases of canine liver disease with portal hypertension

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It is hoped that this study's findings will help in decisions around the treatment of canine liver disease.

Portal hypertension (PH) is caused by increased resistance, increased blood flow or both within the portal circulation. PH can be classified as pre-hepatic (caused by increased resistance in the extra hepatic portal vein), hepatic (which can be further classified into presinusoidal, sinusoidal and postsinusoidal) and post-hepatic (obstruction of the larger hepatic veins, post-hepatic vena cava or the right atrium) in origin (Buob *et al*, 2011). Portal hypertension can be associated with end-stage liver disease in dogs, such as can be seen with hepatic fibrosis or cirrhosis, which is also a major cause of PH in humans (Jansen *et al*, 2014).

The consequences of PH are many, including ascites, multiple acquired portosystemic shunts (MAPSS) and hepatic encephalopathy. Ascites develops because of increased portal venous pressure. Concurrently, splanchnic vasodilation develops leading to pooling of blood within the abdomen. As a consequence, there is a decreased circulating blood volume and as liver dysfunction progresses further, vasodilators that escape liver degradation are released into the systemic circulation which cause further problems with systemic blood pressure. It has previously been shown that ascites is a negative prognostic indicator in dogs (Raffan *et al*, 2009). As portal pressure increases, MAPSS can develop. These represent the opening of embryonic vessels between the portal circulation and the vena cava with the aim of decompressing the portal circulation. As a result of these vessels, neurotoxins – including ammonia – can bypass the liver, reaching the systemic circulation and as a result the brain, causing hepatic encephalopathy (HE).

It is commonly reported that dogs with portal hypertension have a poor outcome with short survival time. This perceived poor prognosis can lead to early euthanasia. Routine blood tests and imaging studies often do not help to differentiate those cases that can do better than others and having additional biomarkers could make this decision-making process easier.

MicroRNAs are noncoding RNAs that play an important role in various cell processes and have been investigated in humans as non-invasive biomarkers of liver inflammation, fibrosis and cancer (Arrese *et al*, 2015; Enache *et al*, 2014; Roderburg *et al*, 2014). MicroRNAs appear to be organ specific and are relatively stable in blood (Oosthuyzen *et al*, 2018). They have been shown to be more sensitive than ALT assessment and potentially could represent the biomarker(s) required to aid decision making in PH cases. They have also been shown to be useful for monitoring the response to treatment and progression of canine chronic hepatitis (Sakai *et al*, 2018).

MicroRNAs have not been assessed in dogs with portal hypertension associated with intrinsic liver disease. This study could help us determine if microRNA changes are present in PH cases and if they are analogous to those identified in humans. From a clinical viewpoint, this could help determine prognosis in individual cases and may also help guide treatment.

STUDY AIMS

The aims of this study are: to characterise microRNAs in dogs with end-stage liver disease with and without portal hypertension at the time

of diagnosis and potentially throughout treatment; and to determine if these microRNA profiles are prognostic/can help guide treatment.

STUDY DESIGN

Dogs will be recruited from the UCD veterinary hospital and from other tertiary referral centres in the UK.

- Dogs with end-stage liver disease will be identified, characterised by biochemical liver disease and/or imaging evidence of liver disease.
- These dogs will be divided into two groups, those with and those without portal hypertension. Dogs with portal hypertension will be defined by the presence of ascites and a serum albumin >15g/L or by the presence of multiple acquired portosystemic shunts.
- Samples will be collected at baseline: routine CBC, biochemistry, imaging data. MicroRNA assessment (blood and potentially free abdominal fluid) will be performed on residual samples.
- Standard treatment will be given to each group.
- MicroRNA analysis (again on residual samples) and routine blood parameters will be reassessed at two weeks, one month, three months, six months and one year as part of standard care for hepatopathy cases.
- Survival analysis will be determined.
- MicroRNAs will be assessed using Nanostring technology. UCD is one of only a few centres with this technology available for veterinary clinical studies.

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