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Hepatic stellate cells in the liver of dogs with steroid-induced hepatopathy

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Abstract

Morphological lesions in hepatic stellate cells caused by the immunosuppressive doses of dexamethasone were investigated in dogs. The archival samples of liver collected during a surgical biopsy were examined. The samples were fixed in 10% buffered formalin or Carnoy's solution and then stained with routine histochemical methods. The lesions were also investigated under electron microscope. It was demonstrated that the number of stellate cells significantly increased ($P = 0.0277$), yet the size of cytoplasmic lipid droplets significantly decreased ($P = 0.0001$). Even though steroid-induced hepatopathy is considered to be a reversible pathology, and the lesions in hepatocytes under the influence of dexamethasone occur in a short period, it was found that hepatic stellate cells proliferated and underwent activation. This resulted in collagen accumulation in the hepatic sinuses. The functional and morphological disturbances in the canine liver in the course of steroid-induced hepatopathy are initially subclinical, but the changes in the structure and function of hepatic stellate cells may become a cause of lesions in the wall of hepatic sinusoidal vessels, which may induce additional functional pathologies unrelated to the damage to hepatocytes.

Key words: dog, hepatic stellate cells, hepatopathy, dexamethasone.

Introduction

Steroid-induced hepatopathy, also referred to as glucocorticoid hepatopathy, is an example of advanced vacuolar lesions in canine hepatocytes (9). Most often it develops as a result of hyper-adrenocorticism due to adrenal or pituitary tumours. It also results from therapeutic use of glucocorticoids. Rarely, it may be induced by other steroid hormones used in therapy, such as progestins or aldosterone, as well as other drugs, for instance, D-penicillamine (12).

Steroid-induced hepatopathy is a reversible pathology (12) during which hepatocytes become swollen, increasing in size from 2 to 10 times (9). A subtle reticulum and spaces of different sizes and dimly outlined margins become visible in their cytoplasm. The nuclei of hepatocytes may be dislocated to the margins of cells. Excessive and uneven accumulation of glycogen is observed in the cytoplasm of hepatocytes. These lesions may be organised in zones or diffused (12). In addition, small necrotic focal areas or necrosis of single hepatocytes may occur. Small groups of neutrophils in the parenchyma (9) and focal myelopoesis (12) are also visible.

Laboratory blood test results are usually within reference values (9), though sometimes an increase in the activity of serum alkaline phosphatase is reported with the normal activity (3-50 U/l) of alanine aminotransferase.

Damage to the liver, *i.e.* hepatopathy, due to glucocorticoids, may be endogenous or iatrogenic caused by steroid therapy. Dexamethasone, a synthetic glucocorticoid with potent anti-inflammatory properties, is one of the drugs that induce hepatopathy. This agent does not have the activity of mineral corticoid and therefore is commonly used in

the cases that require potent anti-inflammatory and/or immunosuppressive action. The indications include autoimmune diseases or hypersensitivity reactions. This drug is also used as an inhibitor of the pituitary-adrenal axis in the dexamethasone inhibition test in the diagnosis of Cushing disease. Such wide use of the drug makes its adverse effects particularly important, especially these affecting the liver, but they have not been fully elucidated so far.

Hepatic stellate cells, Ito cells, lipocytes, or fat-storing cells, are situated in the perisinusoidal Disse spaces (10); pericites, which due to their processes, have contact with the hepatocytes and endothelial cells within the sinusoidal vessels (7). Their origin is still being discussed. By the expression of the same cytokeratines as in the hepatoblasts, they are thought to be the cells originating from the endoderm (1). They also have markers typical for cells originating from the mesoderm (smooth-muscle actin) and for stem cells (stem cell marker CD133) (1). In the healthy liver, their role involves the accumulation of vitamin A and regulation of blood flow through the sinuses (5, 9), as well as the production of collagen (10) and immunological reactions (1). They become activated after damage to the liver, resulting in the change in their phenotype (8) and intensification of collagen production (10).

Hepatic stellate cells are thought to be the most important cells involved in hepatic fibrogenesis (4). Their proliferation and differentiation into myofibroblast-type cells are associated with the progression of hepatic fibrosis; whereas, the expression of α-actin is regarded as a marker of their activation (4). As reported in the literature, hepatic stellate cells undergoing activation have smaller vitamin A-containing lipid droplets in the cytoplasm (8, 11). In humans, lipid droplets constitute 20.5% of the total hepatic stellate cell volume in a healthy liver (8). In addition, hepatic stellate cells are assumed to be transitional in humans when lipid droplets constitute less than 20% of the cytoplasm (5). Retinoids found in lipid droplets in hepatic stellate cells are responsible for the "dormant state" of these cells (5) and inhibition of their proliferation and collagen synthesis. In animals, the pathogenesis of these lesions has not been explained yet.

It is thought that the structure, size, and probably functions of hepatic stellate cells differ depending on the zone within the lobules: in the peripheral area of hepatic lobules they have shorter processes and smaller vitamin A-containing lipid droplets (5). After the liver damage, hepatic stellate cells are activated and stimulated to proliferate (5).

The current study aimed to determine the impact of lesions in the hepatocytes caused by immuno-suppressive doses of dexamethasone on the number and morphology of hepatic stellate cell in the canine liver, which may extend the knowledge of steroid-induced hepatopathy.

Material and Methods

The study was conducted with archival paraffin and Epon blocks with embedded liver specimens sampled in accordance with the liver biopsy procedure from six clinically healthy dogs aged 2–4 years and from the same dogs following the administration of dexamethasone (Dexaven Polfa, Poland) at immuno-suppressive doses of 1 mg/kg b.w. i.m. for four consecutive days (on the 5^{th} d after the first administration). Each sample was divided into three parts and fixed in 10% buffered formalin and Carnoy's solution for microscopic examination, and in 2.5% glutaraldehyde in cacodyl buffer at pH 7.4 for electron microscope analysis.

The specimens fixed in formalin were dehydrated in a series of alcohols and embedded in paraffin. The paraffin sections were stained with haematoxylin-eosin (HE) and Masson procedure for the presence of connective tissue (2).

The specimens fixed in Carnoy's solution were embedded in paraffin and stained for the presence of glycogen according to the PAS – dimedon method by McManus (2).

The sample for electron microscope examination was embedded in Epon 812. The half-thin sections were stained with the method by Levis and Knight, whereas the ultra-thin sections were contrasted with uranyl acetate. The sample was analysed under the Opton 900 PC TEM (Germany) transmission electron microscope and photographic documentation was collected.

The number of hepatic stellate cells was determined on the HE stained slides. They were counted in accordance with the method proposed by Sztark (5) for the number of hepatic stellate cells per 100 hepatocytes. Moreover, the volume and diameter of lipid droplets in a hepatic stellate cell were assessed using a computerised image analysis system LUCIA 4.21. Nikon.

Hepatic stellate cell count, as well as diameter and volume of lipid droplets were expressed as medians and quartile deviation (Me ± QD). Statistical analysis was conducted with non-parametric Wilcoxon signed-rank test using Statistica 10 (StatSoft Inc.). P value of ≤ 0.05 was considered statistically significant.

Results

In the clinically healthy dogs, the histopathological and electron microscopic examinations revealed only mild parenchymatous degeneration of hepatocytes around the portal veins. Furthermore, lipid vacuoles were observed in single hepatocytes in three dogs. These lesions were insignificant. Glycogen was evenly distributed in hepatocytes in all

three zones of the hepatic lobes in all examined animals.

The average hepatic stellate cell count per 100 hepatocytes was 5.41 ± 0.35. The cells had one to four clearly visible lipid droplets in the cytoplasm (Fig. 1) with the average dimension of 2.52 ± 0.38 µm and average volume of 8.35 ± 2.76 µL.

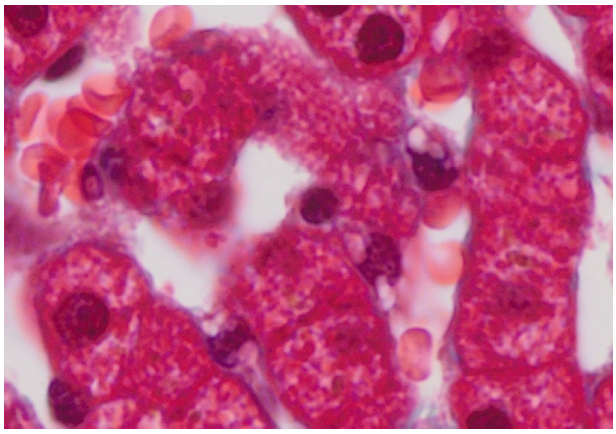

Fig. 1. Hepatic stellate cells in the perisinusoidal spaces in the liver in a clinically healthy dog. Large and regular lipid droplets are visible in the cytoplasm (staining with the Masson's method; 100×)

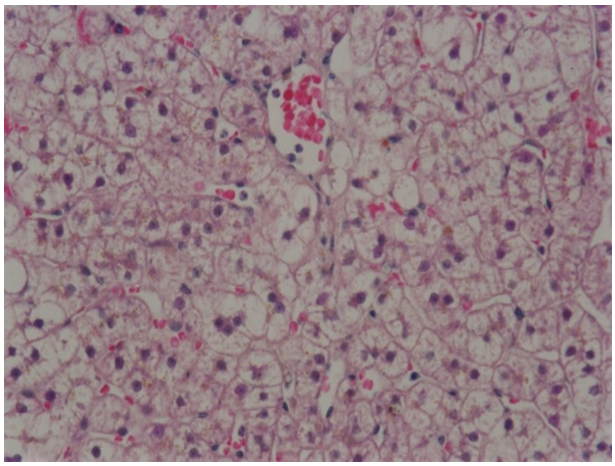

Fig. 2. Steroid-induced hepatopathy: swelling of hepatocytes, margination of neutrophils in the central vein (HE, 10×)

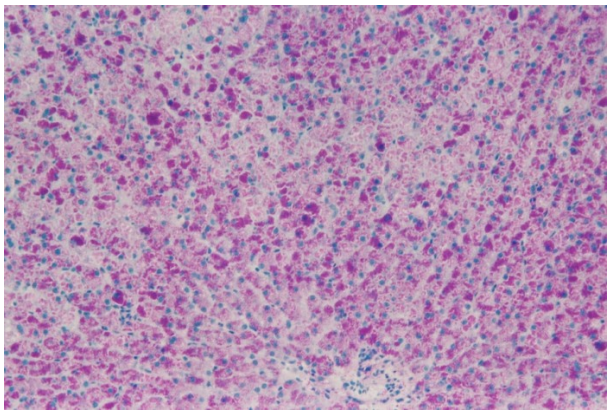

Fig. 3. Steroid-induced hepatopathy: irregular accumulation of glycogen in the cytoplasm of hepatocytes (PAS-dimedon staining; 5×)

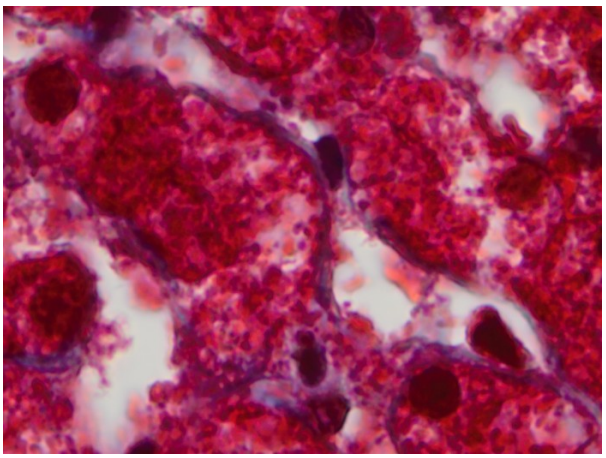

Fig. 4. Hepatic stellate cells in the perisinusoidal space in a dog with steroid-induced hepatopathy. Collagen fibres are also visible (Masson's staining; 100×)

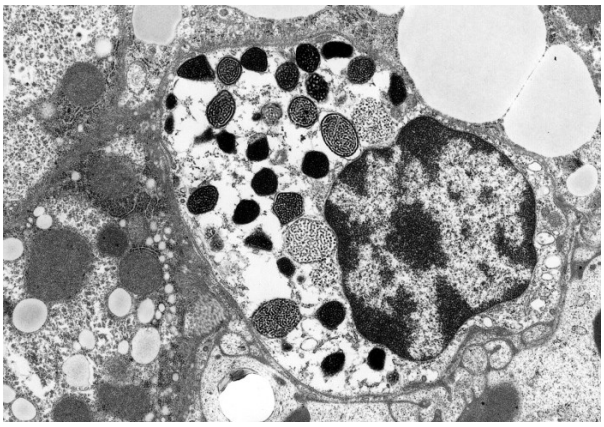

Fig. 5. Ultrastructural pattern of hepatic stellate cell in the liver; the cytoplasm with numerous small lipid droplets (10 000×)

Steroid-induced hepatopathy was reported in all examined animals after dexamethasone administration. The histopathological examination revealed the presence of hepatocytes of different size, generally substantially larger than normal (Fig. 2) due to their swelling. The nuclei of hepatocytes were situated centrally and translocated to the margin in some cells. PAS-dimedon staining revealed hepatocellular, irregular glycogen accumulation (Fig. 3).

Lipid vacuoles in hepatocytes were observed in one dog. In another individual, single hepatocytes that underwent coagulative necrosis were reported. Marginated neutrophils were observed in some small blood vessels but extramedullary myelopoesis was not detected. In two dogs, mild pericellular fibrosis and collagen fibres in the extrasinusoidal space were visualised with Masson's staining (Fig. 4).

The ultrastructural examination of the liver revealed that numerous and (most commonly) small lipid droplets, vacuoles, and sometimes myelin-like structures were frequently found in hepatocytes' cytoplasm. An increase in the content of glycogen in hepatocyte cytoplasm was also observed. In mitochondria, degradation of their cristae and rarefaction of their matrix (both of diverse intensity) were observed and these organelles were multiplied or sometimes hypertrophied. Sporadically, the extension

of rough endoplasmic reticulum canals was noted. Some hepatocytes were partly or entirely necrotic. The hepatocytes with double nuclei were quite frequently found. The examination under the electron microscope showed the same pattern (Fig. 5).

The average hepatic stellate cell count per 100 hepatocytes was 6.60 ± 0.52. The cells had numerous lipid droplets in the cytoplasm with the average dimension of 1.76 ± 0.16 μm and the average volume of 2.84 ± 0.62 μL.

The average hepatic stellate cell count per 100 hepatocytes was significantly higher after the administration of immunosuppressive dexamethasone dose ($P = 0.0277$). In turn, the average dimension and average volume of lipid droplets were significantly lower ($P < 0.0001$).

Discussion

Dexamethasone administered at the immuno-suppressive doses for a short period, *i.e.* four days, induced severe and advanced structural lesions, identified as steroid-induced hepatopathy. Necrosis of single hepatocytes was also observed.

Based on morphology and evaluation of the ultrastructure of hepatic stellate cells, it was found that hepatic stellate cell underwent activation and proliferated in response to the damage to hepatocytes, which was morphologically seen as a reduction of lipid droplets in the cytoplasm. Moreover, the number of hepatic stellate cells per 100 hepatocytes increased. Thus, it is supposed that simultaneously with the damage to hepatocytes (even if the lesions are described in the literature as reversible), there is a reaction in hepatic stellate cells, and, as a result, the extracellular matrix, including collagen, may accumulate. Such rapid reaction to the damage allows us to assume that in the case of subclinical lesions, which are indefinable in the blood test and functional liver tests, hepatic fibrosis may start at an initial stage of the damage. It is consistent with the observations by Budny *et al.* (3), who have reported that hepatic stellate cells are activated as early as on day 3 from the beginning of hepatic regeneration. According to Kmieć (8), the changes in activated hepatic stellate cells play an important role in the induction of a pro-fibrinogenic response in the damaged liver. An increase in the number of hepatic stellate cells is a prognostic of hepatic fibrosis (13).

Glucocorticoids are thought to inhibit the production of collagen by fibroblasts (5), but not by hepatic stellate cells and, therefore, the presence of collagen fibres in the extrasinusoidal spaces in dogs with steroid-induced hepatopathy may indicate that the changes resulting in capillarisation of sinusoidal vessels (*i.e.* the formation of the basal membranes in the vascular endothelium) started as early as on 5th d following the first administration of dexamethasone. In the case of progressive parenchymatous damage, capillarisation of the sinusoidal vessels causes additional dysfunction of the liver, creating a barrier between hepatocytes and blood.

To conclude, it is thought that hepatic stellate cells in the liver in dogs with steroid-induced hepatopathy undergo rapid morphological and, probably, functional changes, which lead to an increase in their number and stimulate the production of collagen. Functional and morphological disturbances in the liver induced by dexamethasone are initially subclinical, but lesions in the structure and function of hepatic stellate cells may cause lesions in the walls of sinusoid vessels. In the case of prolonged administration or immuno-suppressive doses of dexamethasone in dogs, it may cause additional hepatic disorders unrelated directly to the damage of hepatocytes.

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