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Expression of Dystrophin-associated Glycoproteins in Ito Cells of Healthy and Diseased Livers in Dogs and Cats

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Abstract

Activation of Ito cells and their metamorphosis into myofibroblasts is the primary process in fibrotic remodelling in chronic liver disease. The dystrophin-associated glycoprotein complex (DAGPC) is part of the cytoskeleton of muscle cells and is also expressed in other tissues. Because of its differential expression in muscle degeneration, we investigated this complex in normal healthy liver tissue and tissue with chronic liver degeneration in dogs and cats to gain information about cell alterations in chronic liver disease. In normal liver tissue from both species, we found mild expression of dystrophin 1 and β-dystroglycan, especially in Ito cells. Dystrophin 2 and y-sarcoglycan showed no expression. In chronic degenerative liver diseases, we found increased expression of dystrophin 1 and β-dystroglycan in Ito cells in dogs and cats. We suggest that this increased protein expression is an early sign of the metamorphosis of Ito cells in the beginning of chronic degenerative liver disease.

Keywords: Dog; cat; liver degeneration; dystrophin-associated glycoprotein complex

Introduction

The dystrophin-associated glycoprotein complex (DAGPC) is part of the cytoskeleton. Dustin and Brion (1988) distinguished microfilaments, intermediate filaments, and microtubules as cytoskeletal components. The functions of these proteins include cell contractility, organisation of cell shape, and intracellular transport (Denk *et al*., 1990). The DAGPC is part of the microfilaments. It combines the inner cytoskeleton with the extracellular matrix (Worton, 1995). The complex includes dystrophin, α-, β-, and y-sarcoglycan and α- and β-dystroglycan. Dystrophin connects actin filaments in the cytoplasm with α-, β-, and y-sarcoglycan and β-dystroglycan in the basal lamina. These proteins and laminin in the extracellular matrix are supported by α-dystroglycan (Kreis and Vale, 1999).

The dystrophin-associated glycoprotein complex are expressed in different tissues, such as the brain, kidneys, and liver (Renley *et al*., 1998; Rafael *et al*., 1999; Loh *et al*., 2001). There is much information about this complex in striated muscle cells (Ervasti and Campbell, 1991; Ervasti and Campbell 1993; Ervasti and Cambell, 1993). Different muscle diseases result in varying expression of the proteins of this complex (Dubowitz, 1992; McNally *et al*., 1996; Duclos *et al*., 1998; Duggan *et al*., 1998; Hack *et al*., 1989).

The most important disease of this complex is Duchenne muscular dystrophy. It is a disease with decreased expression of dystrophin. Duchenne muscular dystrophy is a severe form of the disease. A milder form is Becker muscular dystrophy, which is also a consequence of decreased expression of dystrophin, but not to such a low level. The diseases illustrate that not all proteins of the DAGPC are expressed equally; indeed, differential expression is responsible for different diseases.

Some data exist about cytoskeletal proteins in the liver. Loh *et al*., (2001) detected increased expression of dystrophin-associated protein in brain, kidney, and liver. Bedossa *et al*. (2002) investigated dystroglycan expression in hepatic stellate cells

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(also known as Ito cells). They found deposits of β-dystroglycan in hepatic stellate cells, but not on hepatocytes. Maher *et al.* (1988) detected expression of laminin in rat liver lipocytes. Gesemann *et al.* (1998) found low expression of dystroglycan in liver cells.

There are only few reports about DAGPC proteins and liver diseases (Denk and Lackinger, 1986; Denk *et al*.,1986; Denk *et al*., 1990). Currently, there are no data available about the expression of DAGPC in liver cells in dogs and cats.

Here, we report the expression of dystrophin-associated glycoproteins in normal liver tissue and chronic degenerative liver diseases in dogs and cats. The aim of the study was to describe Ito cell metamorphosis, an early and important step in the fibrotic remodelling process of chronic liver diseases. First, activated Ito cells transform into myofibroblasts and later to fibroblasts. Myofibroblasts are contractile and need cytoskeleton proteins for their function (Gressner, 1991; Bachem *et al*., 1992).

Materials and methods

From patients treated at the Small Animal Clinic, Institute of Veterinary Medicine of the University of Goettingen, liver biopsies were taken. We collected samples from two healthy dogs and two healthy cats and eight dogs and eight cats with chronic degenerative liver diseases. The diagnoses of liver diseases were made from clinical symptoms, clinical biochemistry, and histopathology.

Biopsies were taken under general anesthesia (cats, ketamine and acepromacine; dogs, levomethadon and acepromacine) using "truecut" biopsy needles (Surgivet) or during a laparatomy. One part of each biopsy was taken for further diagnosis and another was snap-frozen in liquid nitrogen for immunohistochemistry.

Using a cryostat (Microm Company), 8-10 µm sections were prepared from the biopsies. Immunohistochemistry was performed automatically in a Ventana NexES ICH staining module using the Ventana Basic DAB detection kit. The following monoclonal primary antibodies (Novocastra Company) were used: y-sarcoglycan (NCL-g-SARC, dilution 1:50), β-dystroglycan (NCL-b-DG, dilution 1:100), dystrophin 1-rod domain (NCL-DYS1, dilution 1:20), and dystrophin 2–C-terminus (NCL-DYS2, dilution 1:20). Subsequently, the sections were evaluated semi-quantitatively by light microscopy.

Results

We investigated normal liver tissue with immunohistochemistry for expression of y-sarcoglycan, β--dystroglycan, dystrophin 1 (rod domain) and dystrophin 2 (C terminus). All immunohistochemistry was done with antibodies that are commonly used for the detection of these proteins in dogs. There is no report about the expression of these proteins in cats. Thus, we investigated the antibodies in normal muscle tissue from cats. We found that the antibodies (from Novocastra) against y-sarcoglycan (at 1:50), β-dystroglycan (at 1:100), dystrophin 1 (at 1:20), and dystrophin 2 (at 1:20) were able to detect these proteins in cat muscle cells (Table 1).

Table 1. Usefulness of antibodies for normal muscle tissue in cats.

Antibody	Name	Dilution	Reaction in cats
γ-sarcoglycan	Novocastra NCL-G-SARC	1:50	Mild positive
β-dystroglycan	Novocastra NCL-B-DG	1:100	Positive
dystrophin 1	Novocastra NCL-DYS1	1:20	Positive
dystrophin 2	Novocastra NCL-DYS2	1:20	Positive

For the detection of the proteins in normal liver tissue, we investigated two cats and two dogs. The results from normal liver tissue in dogs and cats are shown in Table 2. In healthy cats, we found no expression of dystrophin 2 or y-sarcoglycan in liver. Expression of β-dystroglycan and dystrophin 1 was mild.

Table 2. Antibody reaction in normal liver tissue in dogs and cats.

Antibody	Reaction in dogs	Reaction in cats
γ-sarcoglycan	Negative	Negative
β-dystroglycan	Mild positive	Mild positive
Dystrophin 1	Mild positive	Mild positive
Dystrophin 2	Negative	Negative

In total, we investigated eight cases of liver disease in cats. In all cases, the pathological diagnosis was chronic degenerative liver disease. The diagnosis of degenerative liver disease was made if the histological picture showed hydropic swelling, vacuolization, intracellular lipid accumulation, and

signs of necrosis and fibrosis in liver tissue. We found no expression of y-sarcoglycan or dystrophin 2 in any case. In all cases, we found increased expression of β-dystroglycan and dystrophin 1. The expression of these proteins was found on some hepatocytes, but especially on Ito cells in the perisinusoidal space (Table 3; Fig. 1, 2). There was no apparent correlation between the degree of liver degeneration and the degree of protein expression.

In healthy dogs, we found only a mild reaction for dystrophin 1 and β-dystroglycan. Dystrophin 2 and y-sarcoglycan showed no reaction. Eight samples of diseased dog livers were investigated. The pathological diagnosis in each case was chronic degenerative liver disease. The criteria for the diagnosis were the same as in cats. There was no detectable expression of y-sarcoglycan or dystrophin 2 in any case. On the other hand, β-dystroglycan and dystrophin 1 expression was increased on the surface of some degenerative liver cells, but mostly on the surface of Ito cells. Similar to the results in cats, there was no apparent correlation between degree of liver degeneration and degree of protein expression.

Table 3. Results of immunohistochemistry (β-dystroglycan, dystrophin1) in chronic degenerated liver disease in dogs and cats

Species	Degree of the degeneration	β- DG	Dys 1
Mixed breed, female 7 years	moderate	+	++
Mixed breed, male, 6 years	moderate	+	+
Dachshund, male, 15 years	severe	+	+
Fox terrier, male, 12 years	severe	++	++
Dachshund, female, 7 years	moderate	+	++
Cocker spaniel, male, 5 years	moderate	++	++
German shepherd, female, 4 years	severe	+	++
Mixed breed, male, 13 years	severe	+	++
Domestic shorthair, male, 7 years	severe	++	++
British shorthair, female, 8 years	moderate	+	++
Domestic shorthair, female, 12 years	severe	++	++
Domestic shorthair, female, 4 years	moderate	+	++
Domestic shorthair, female, 10 years	severe	+	+
Siamese, male 10 years	severe	++	+++
Domestic shorthair, male, 6 years	moderate	++	++
Domestic shorthair, female, 8 years	severe	++	++

+ = mild elevated to normal, ++ = marked elevated to normal, +++ = severe elevated to normal

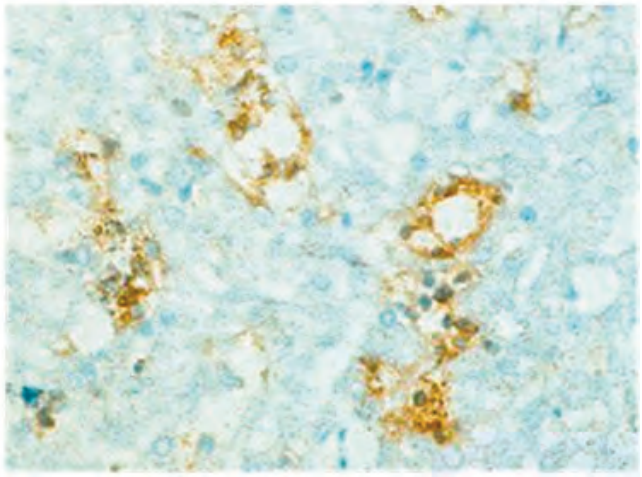

Fig.1. High expression of dystrophin 1 in cat liver tissue with marked liver cell degeneration.

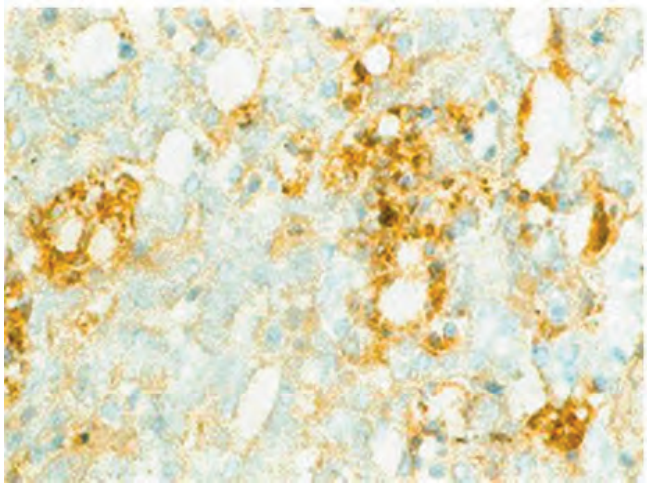

Fig. 2. High expression of β-dystroglycan in cat liver tissue with marked liver cell degeneration.

Discussion

Cytoskeletal proteins have gained increasing importance since some investigators found associations between the expression of these proteins and diseases. In Duchenne muscular dystrophy, for example, a direct association has been shown between the expression of dystrophin and the degree of the disease (Dubowitz, 1992). Altered expression of cytoskeleton proteins has also been associated with diseases in other organs (Denk and Lackinger, 1985; Kunze and Rustow, 1993; Cadrin and Martinoli, 1995; Bedossa *et al*., 2002). In our investigation, we analysed changes in the expression of dystrophin-associated glycoproteins in chronic degenerative liver tissue. The expression of DAGP in normal murine liver cells has been described by Durbeej *et al*. (1998). They found α/β-dystroglycan at the sinusoidal face of hepatocytes in healthy adult mice. In healthy dogs and cats, we found only infrequent mild expression of β-dystroglycan on hepatocytes and on Ito cells. α-Dystroglycan could not be examined in dogs or cats, because there is no known cross-reactivity with other species.

The role of dystrophin in non-muscle tissue was described by Tokarz *et al*. (1998) and Rafael *et al*. (1999). In these reports, dystrophin expression was investigated in non-muscle tissues of healthy mice and detected in cardiac, kidney, and liver tissue. In our investigation, we found expression of dystrophin 1 in normal liver tissue of dogs and cats, whereas dystrophin 2 was not detectable. We were unable to detect expression of y-sarcoglycan in healthy liver tissues of dogs or cats. In contrast to our observations, Noguchi *et al.* (2001) described y-sarcoglycan expression in non-muscle tissues in transgenic mice.

In chronic degenerative liver tissue, we found altered expression of β-dystroglycan and dystrophin 1, compared with normal liver tissue. Bedossa *et al*. (2002) described similar results, when they investigated hepatic stellate cells. They found increased expression of β-dystroglycan in cases of liver fibrosis. We found increased expression of β-dystroglycan and dystrophin 1 on liver cell surfaces and on perisinusoidal Ito cells. Hepatic stellate cells or Ito cells are located perisinusoidally and transform into fibroblastic cells in cases of liver fibrosis (Schmitt-Graff *et al*., 1993).

Liver fibrosis is a pathological process following chronic liver disease. Many investigators have described the role of Ito cells in the development of liver fibrosis (Tanaka *et al*., 1991; Tang *et al.*, 1994; Nanni *et al*., 1995). Our results indicate that the increased expression of β-dystroglycan and dystrophin 1 could be a sign of stellate cell activation or cell metamorphosis in cases of early fibrosis. Currently, there is no explanation for the absence of y-sarcoglycan and dystrophin 2 expression. We also found no correlation between the degree of degeneration and the degree of protein expression in this study. Because of a small number of animals investigated, more should be investigated in future studies to confirm these results.

In conclusion, we found mild expression of some DAGP in normal liver tissue in dogs and cats. In chronic degenerative liver disease, the expression of some DAGP (β dystroglycan and dystrophin 1) was increased. We suggest that this cell activation is the first step in liver fibrosis as a consequence of degenerative liver disease in dogs and cats. Further investigation is necessary to explain the steps between degenerative liver disease and liver fibrosis.

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