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D-penicillamine treatment of copper-associated hepatitis in Labrador retrievers

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ABSTRACT

D-penicillamine is effectively used in the lifelong treatment of copper toxicosis in Bedlington terriers and Wilson's disease in humans. A complex form of copper-associated hepatitis has recently been characterized in the Labrador retriever. The aims of this study were to evaluate the effectiveness of D-penicillamine treatment for copper-associated hepatitis in this breed, to study the effects on hepatic copper, iron and zinc concentrations, and to evaluate parameters to predict optimal duration of treatment. Forty-three client owned Labrador retrievers that were diagnosed with increased hepatic copper were treated with D-penicillamine and underwent at least one follow-up examination including a liver biopsy for histopathological scoring of inflammatory lesions. Hepatic copper, iron and zinc concentrations were determined in the initial and follow-up biopsies by instrumental neutron activation analysis. The influence of initial hepatic copper concentration, sex, age, D-penicillamine formulation and the occurrence of side effects were investigated for their influence on hepatic copper concentration after a certain period of treatment by generalized mixed modelling.

D-penicillamine proved to be effective in reducing hepatic copper concentration and associated inflammatory lesions. Parameters derived from the model can be used to estimate the necessary duration of D-penicillamine treatment for Labrador retrievers with increased hepatic copper concentration. Continuous, lifelong D-penicillamine treatment is not recommended in this breed, as there may be a risk for hepatic copper and zinc deficiency.

Introduction

Hereditary copper toxicosis in dogs has been identified in a number of purebred dog populations, including the Bedlington terrier (Twedt et al., 1979), West Highland White terrier (Thornburg et al., 1986), Skye terrier (Haywood et al., 1988), Dalmatian (Webb et al., 2002), Doberman (Mandigers et al., 2004), and most recently the Labrador retriever (Hoffmann et al., 2006). In Bedlington terriers a deletion of exon 2 of the *COMMD1* gene leads to extreme accumulation of copper in the liver (Van de Sluis et al., 2002). In the other breeds, hepatic copper accumulation does not reach the very high concentrations reported in the Bedlington terrier and appears to have a more complex genetic background with an important role for environmental factors in the pathogenesis (Fieten et al., 2012b).

In the Labrador retriever there is a strong female predisposition and the disease is often characterized by a long subclinical phase. Nutrition seems to be an important environmental factor in the aetiology (Fieten et al., 2012a; Hoffmann et al., 2006, 2009). In affected Labrador retrievers, copper accumulation in the liver continues over time (Hoffmann et al., 2009) and, without treatment, eventually causes hepatitis.

The best studied human form of copper toxicosis is Wilson's disease. This autosomal recessive disease is caused by mutations in the gene coding for the copper transporter ATP7B (Bull et al., 1993; Tanzi et al., 1993) and is characterized by accumulation of copper in the liver, brain and cornea (Gitlin, 2003). The overall therapeutic approach for copper toxicosis in humans and animals is to create a negative copper balance. This can be achieved by using the copper chelator D-penicillamine (DPA). This highly soluble degradation product of penicillin binds copper at its SH-group and promotes urinary copper excretion (Walshe, 1956).

Since its discovery, DPA has become the most widely used copper chelator in the treatment of Wilson's disease in humans (Weiss and Stremmel, 2012) and has been shown to be effective for the treatment of copper toxicosis in dogs as well (Hoffmann et al., 2006; Mandigers et al., 2005; Twedt et al., 1979). Treatment monitoring relies on the evaluation of repeated liver biopsies. However,

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no evidence-based data exist on the rate of decrease in hepatic copper content, nor is there information available on the optimal interval for recheck biopsies in dogs with complex forms of copper toxicosis.

In addition, no information is available about factors that may influence the rate of copper decrease such as sex, age, hepatic copper concentration before start of therapy, occurrence of side effects and DPA formulation. DPA forms relatively stable chelates with all biologically active trace metals, including iron and zinc (Lenz and Martell, 1964), and could promote urinary excretion of these metals as well. The influence of DPA treatment on hepatic iron and zinc concentrations in dogs has not yet been studied. Besides the evaluation of metal chelation, the effects of DPA treatment on the activity and stage of copper-associated hepatitis are important aims for successful treatment.

The present study investigated the effects of DPA in Labrador retrievers with increased hepatic copper: (1) to establish a model to predict the necessary duration of treatment to reach a normal hepatic copper concentration; (2) to evaluate the effect of DPA treatment on the activity and stage of copper-associated hepatitis; and (3) to determine the effect of DPA treatment on hepatic iron and zinc concentrations. The results of this study contribute to the development of an evidence based treatment protocol for Labrador retrievers with copper toxicosis.

Materials and methods

Animals

The Labrador retrievers used in this retrospective study were referred to the Department of Clinical Sciences of Companion Animals, Utrecht University between 2003 and 2010. Diagnosis was established through histological assessment of liver biopsy specimens and quantitative copper determination in liver tissue. All affected dogs were treated with DPA capsules produced at our veterinary pharmacy or with Metalcaptase (Heyl) (enteric coated penicillamine tablets intended for human use). All dogs were evaluated using at least one follow-up biopsy.

Data on signalment, type and duration of treatment, plasma levels of alanine aminotransferase (ALT) and alkaline phosphatase (AP) and the occurrence of side effects during treatment were collected from the medical records. Side effects were scored on a 0–2 scale (0 = no side effects; 1 = mild side effects [decrease in appetite, occasional vomiting] with no necessity to cease therapy; 2 = severe side effects [anorexia, severe vomiting] with necessity to temporarily cease therapy). All dogs were client owned and data were collected after obtaining the informed consent of their owners. All procedures were approved by the University of Utrecht's Ethical Committee on Animal Experiments as required under Dutch legislation.

Assessment of liver biopsies

At least three liver biopsies were collected from the left lateral liver lobe with a 14 G needle using a Tru-cut device under ultrasound guidance. Two biopsy specimens were fixed in 4% neutral buffered formalin and embedded in paraffin. Paraffin sections of liver biopsies were stained with rubeanic acid, haematoxylin and eosin and according to Gordon and Sweet's staining protocol for reticulin. Histology was evaluated by one Board-certified veterinary pathologist (TSGAMvdI). Grading (necroinflammatory activity) and staging (fibrosis/nodular transformation) of hepatitis was based on a published system (Ishak et al., 1995; Ishak, 2000).

A separate biopsy specimen of at least 5 mg was collected in a metal free container and freeze dried prior to quantitative metal determination by instrumental neutron activation analysis (Bode, 1990). Dogs were considered to have normal hepatic copper when copper concentrations were <400 mg/kg dry weight liver (dwl) (Puls, 1994). Hepatic metal concentrations are reported in mg/kg dwl.

Statistics

All data were analysed using R statistics package 2.14.0 (R Development Core Team, 2011). A mixed model was used to assess the duration of treatment required for a decrease in hepatic copper concentration and to investigate the factors influencing this process. The analysis was performed with the R package 'nlme'. Restricted maximum likelihood was used to estimate the best fitting model. Maximum likelihood estimation was used to estimate the fixed effects of the parameters in the model. DogID was added as random effect to take into account the correlation between observations within a dog. The outcome was defined as hepatic copper concentration at a certain time of treatment in months (CuQy), wherein CuQ is the hepatic copper concentration in mg/kg dwl and y is the time of the control biopsy in months after initiation of therapy. Explaining parameters under investigation were sex (male, female), age at start of therapy (years), time of therapy until control biopsy (months), occurrence of side effects (0–1–2) and therapy type (compounded DPA or Metalcaptase (Heyl)). The best fitting model for the data was determined with a stepwise forward model using Akaike's information criterion. The validity of the final model was checked by studying the residuals on normality and constancy of variance.

A Wilcoxon signed rank test was used to compare hepatic grading and staging scores, and ALT and AP activity before and after therapy. The association between ALT and AP and hepatic copper and grade of hepatitis before and after treatment was tested with Pearson's product moment correlation. A paired t test was used to compare hepatic copper, iron and zinc concentrations before and after DPA therapy. $P < 0.05$ was considered statistically significant. Normally distributed data were summarized as mean ± standard deviation (SD) and non-normally distributed continuous data or count data were presented as median and range.

Results

Animals

The study population consisted of 43 Labrador retrievers of which 12 were male and 31 were female. The mean age at the start of therapy was 6.4 ± 2.2 years. Labrador retrievers were treated for a median duration of 4.8 months (range: 1.8–15.7). One dog was treated with compounded DPA capsules at age 4.7 years for 6.8 months. At age 8.7 years hepatic copper had re-accumulated and this dog was therefore treated again with Metalcaptase (Heyl) for 6.3 months.

A decrease in hepatic copper below 400 mg/kg dwl occurred in 21 dogs, while 23 dogs still had hepatic copper values above 400 mg/kg dwl (Fig. 1). Twenty-eight dogs were treated with compounded DPA capsules and 16 dogs with enteric coated tablets of the authorized human medicine Metalcaptase (Heyl). All dogs received their medication in a dose of 10 mg/kg orally twice daily, 30 min before a meal. Side effects were scored as 0, 1, and 2 in 12, 16 and 3 dogs, respectively. For 12 dogs no data regarding side effects were available from the medical records.

Factors influencing necessary treatment duration and modelling of copper decrease

Sex, age, therapy type and side effects did not significantly influence CuQy. A shorter therapy duration and higher hepatic copper concentrations before initiation of therapy both resulted in higher copper concentrations at time y. The relation between hepatic copper concentration and therapy duration was non-linear due to an interaction between copper concentrations before therapy onset and therapy duration and followed the equation:

$$\mathrm{CuQ}y = -81.85 + 0.99\mathrm{CuQ0} + 51.0T1 - 3.92T1^2 - 0.16\mathrm{CuQ0} * T1 + 0.92 \times 10^{-2}\mathrm{CuQ0} * T1^2$$

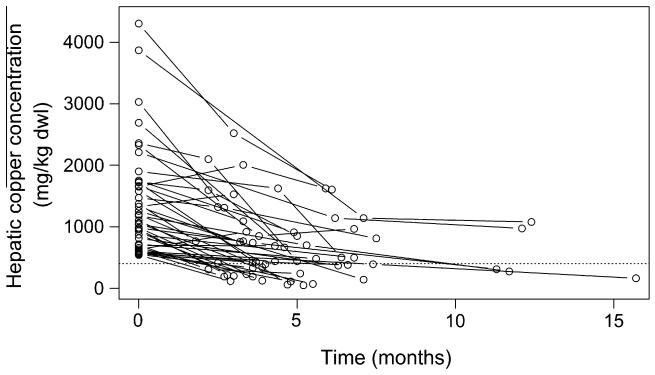

Fig. 1. Decline of hepatic copper concentrations (mg/kg) in Labrador retrievers during D-penicillamine treatment.