CORTICOSTEROID-INDUCED ALTERATION IN LIVER FUNCTION IN DOGS AND ITS DECREASE POSSIBILITIES

Jevgēnija Kondratjeva, Edīte Birģele
Latvia University of Agriculture
jkondratjeva@inbox.lv

Abstract
Nowadays excessively used corticosteroids in veterinary medicine induce steroid hepatopathy in dogs (*Canis lupus familiaris*). The objective of this study was to determine the possibility of the hepatoprotectants to decrease the corticosteroid-induced alteration in such dogs' blood serum enzymes as alaninaminotransferase (ALAT), gammaglutamyltranferase (GGT), alkaline phosphatase (AP) and corticosteroid-induced thermostable alkaline phosphatase (cAP). The study took place in private veterinary clinics in Riga, Latvia, during 2013, with the permission of dogs' owners. Twenty eight animals, which received corticosteroids due to present diagnosis, were divided into two groups. In the first group long-lasting corticosteroid methylprednisolone acetate injection was used once, while in the second group the hepatoprotectants were used after the injection of corticosteroids. It was discovered that after 14 and after 30 days of hepatoprotectants use, blood enzymes were significantly lower ($p<0.05$) than in dogs that did not receive hepatoprotectants. In both groups the enzyme values did not reach the reference limits. The study is set to investigate further if and when the values reach the reference limits.
Key words: dogs, corticosteroids, liver, blood serum enzymes, hepatoprotectants.

Introduction

Corticosteroids are widely used in veterinary medicine. These medications are used for animals with allergic and anaphylactic reactions, in shock, with autoimmune diseases or in other conditions (Badylak and van Vleet, 1981; Lucena et al., 1999; Abraham et al., 2006). Despite the fact that using corticosteroids can cause some complications such as osteoporosis, *diabetes mellitus*, hypertension, cataracts, iatrogenic hyperadrenocorticisms and others, they are used excessively and being overdosed (Levine et al., 2008).

Local and systemic corticosteroids are used in veterinary medicine, both causing changes in different organ systems of the animal, especially in the endocrine system and in the liver morphofunctional condition (Badylak and van Vleet., 1981; Abraham et al., 2006). It is proved that corticosteroids can cause changes in 2-3 days after the beginning of the therapy. Injectable and long-acting corticosteroids cause more complicated changes in comparison to orally used and local corticosteroids (Dillon et al., 1980). Corticosteroid-induced alteration in the liver morphofunctional condition is called steroid hepatopathy. This is a specific pathology only in dogs (*Canis lupus familiaris*) (Fittschen and Bellamy, 1984). It is known that some blood serum enzymes indirectly reflect liver morphofunctional condition, but histological findings of the liver biopsy reflect it directly. Increased values of such blood serum enzymes as alaninaminotransferase (ALAT), aspartataminotransferase (ASAT), gammaglutamyltranferase (GGT), alkaline phosphatase (AP) and corticosteroid-induced thermostable alkaline phosphatase (cAP) are specific for the steroid hepatopathy. The alkaline phosphatase value increases in the serum because of glycogen raised deposit in the liver and vacuolization of hepatocytes (Badylak and van Vleet, 1981; Lucena et al., 1999). This process is caused by corticosteroids – an isoenzyme of alkaline phosphatase that is specific only to dogs, produced at hepatocytes (Dillon et al., 1980). Increased AP value is one of the most used biochemical indicators in the diagnostics of the liver disease (Center et al., 1992). The possibility to distinguish corticosteroid-induced AP increase from liver pathology induced AP increase has greater differential diagnostic value. The safe differential method is thermic processing of AP isoenzymes – corticosteroid-induced AP isoenzyme is thermostable (Teske, 1999; Feldman and Nelson, 2004).

Different solutions have proved capable of protecting the dog's body and especially its liver against the negative corticosteroid alteration. There exists a description of the evaluation of the aminoacid S-adenosylmethionine's influence on systemic and hepatic effects on prednisolone in dogs (Center et al., 2005), the efficiency of a butafosfan and vitamin B12 ('Catosal') on biochemical and hematological blood parameters in dogs treated with dexamethasone (Deniz et al., 2009) and others. Even though the hepatoprotectants are included in various health supplements, their efficiency towards protecting or reversing corticosteroid-induced changes in dog liver are insufficiently investigated.

The aim of the study is to investigate the corticosteroid-induced alteration in the liver function in the dogs and the possibility to decrease liver alterations by the use of hepatoprotectants.

Materials and Methods

The study took place in private veterinary clinics in Riga, Latvia, during 2013, with the permission of dogs'

owners. Twenty eight dogs of various age, weight, breed and gender were used in the present study. All dogs had a confirmed disease and were treated with corticosteroids. The animals were divided into two groups conditionally: in the first group dogs received only an injection of corticosteroids, in the second dogs received hepatoprotectants after the injection. For this study we selected a long-acting corticosteroid – methylprednisolone acetate - 40 mg mL^{-1} in intramuscular route once on the first day of the study in a dose of 0.1 mg kg^{-1}, but as hepatoprotectective agent – 'GlutaMax', which contains the essence of silymarin (*Silybum marianum*) – 133 mg in each pill, meant for 15 kg of bodyweight; the essence of curcuma (*Curcuma longa*) – 33 mg for each 15 kg of bodyweight; and the essence of artichoke (*Cynara scolymus*) – 66.6 mg, choline, lecithin, B group vitamins, zinc. These were used once per day *per os* from the first day of study for 30 days.

To estimate the hepatoprotectant influence on dog's condition and the possibility to protect the liver against corticosteroids impact, the following blood serum enzymes were determined – alaninaminotransferase (ALAT), gammaglutamyltransferase (GGT), alkaline phosphatase (AP) and corticosteroid-induced thermostable alkaline phosphatase (cAP) values (Hoffmann et al., 1977). The concentration of the enzymes increases because of the hepatotoxic drug influence on hepatocytes (Fittschen and Bellamy, 1984).

The hepatoprotectant 'GlutaMax' was used because of its unique composition. Silymarin has antioxidant, hepatoprotective, antifibrotic, and anti-inflammatory effects (Flatland, 2003; Johnson, 2008). The essence of this plant is used in the treatment of experimentally induced mushroom hepatotoxicity (Vogel, 1984). Silymarin is used in human medicine as additional treatment of acute viral hepatitis, alcoholic liver disease and toxin or drug-induced hepatitis (Johnson, 2008). The essences of curcuma and artichoke have antioxidant and anti-inflammatory effects and are also used for liver detoxication. Choline regulates the metabolism of fats, works against fatty degeneration of liver. N-acetylcysteine provides a ready source of glutathione to detoxify toxic intermediates. N-acetylcysteine should also be considered for the treatment of any severe toxinrelated hepatic injury (Johnson, 2008). Lecithin decreases the necrosis of hepatocytes. Zinc is used to decrease hepatic copper content in breeds with hepatic copper accumulation and chronic liver diseases. In other canine hepatobiliary disorders zinc is suggested because of its antioxidant and antifibrotic effects (Johnson, 2008).

A day before corticosteroid use blood samples were collected from each animal *v. jugularis*. To separate the serum we centrifuged the blood on 1,300 rounds per minute for 10 minutes (Gulbis, 2011). We analyzed the serum not more than 15 minutes after the separation. GGT, ALAT and AP were determined in serum by biochemical analyzer 'MINDRAY BS-120'. Corticosteroid-induced enzyme thermostable alkaline phosphatase was determined by Teske method (Teske, 1999), the blood serum was handled for 15 minutes in temperature 60 °C, therefore only thermostable isoform will be presented into blood serum.

To analyze the data the programs MS Excel and '*RStudio*' were used. P-values less than 0.05 were considered to be statistically significant. For the comparison of blood serum enzymes values T-test was used.

Results and Discussion

Before the study (day 0) blood serum enzyme values were defined for each dog. It was found that serum enzymes as ALAT, GGT, AP and cAP values were similar to each other ($p>0.05$) and were within reference limits (Table 1).

Table 1

The values of some blood serum enzymes in dogs during the study

Parameters	Reference limits (Geffre et al., 2009)	Groups	Using of hepato protectant	Day 0	Day 14	Increase from Day 0 (times)	Day 30	Increase from Day 0 (times)
ALAT	10 – 100 U L^{-1}	1.	Not used	69.2±8.0	206.4±28.8	3.0	177.6±19.5	2.6
		2.	Used	66.3±6.7	157.5±8.6	2.3	141.3±8.6	2.0
GGT	0 – 9.6 U L^{-1}	1.	Not used	5.0±0.7	19.6±4.4	3.9	17.8±3.5	3.6
		2.	Used	4.6±0.8	12.3±1.7	2.7	11.0±1.3	2.4
AP	23 – 212 U L^{-1}	1.	Not used	59.2±5.5	412.8±75.3	7.0	329.2±46.7	5.6
		2.	Used	63.2±6.1	274.0±20.5	4.0	221.2±18.1	3.5
cAP	-*	1.	Not used	12.5±2.2	265.1±41.4	21.0	210.6±32.7	16.8
		2.	Used	13.4±2.1	205.2±22.5	15.0	158.4±17.1	11.8

*Corticosteroid-induced alkaline phosphatase (cAP) does not have reference limits, because in healthy body it could not exist.

Fourteen days after the injection of long-lasting corticosteroid methylprednisolone acetate all dogs from the group one had a very high enzyme activity. The mean value of ALAT (206.4 ± 28.8 U L^{-1}) was approximately three times higher than on day 0 (69.2 ± 8.0 U L^{-1}); the mean value of GGT increased 3.9 times (19.6 ± 4.4 U L^{-1} versus 5.0 ± 0.7 U L^{-1}); the mean value of AP increased approximately seven times from the day 0 (412.8 ± 75.3 U L^{-1} versus 59.2 ± 5.5 U L^{-1}), but cAP mean value in serum was 21 times higher than on the day 0 (265.1 ± 41.4 U L^{-1} and 12.5 ± 2.2 U L^{-1}, respectively) (see Table 1).

The second purpose of this study was to find out how effectively the hepatoprotectant 'GlutaMax' can decrease the alteration in the biochemical parameters on the serum, which was caused by methylprednisolone acetate. It became apparent that all the investigated enzyme values in blood serum obtained from the dogs from group two, which were using hepatoprotective agents after the long-lasting corticosteroid injection were significantly lower ($p<0.05$) than these in the dogs from group one (see Table 1).

The mean value of ALAT in dogs from the group two on day 14 increased to 157.5 ± 8.6 U L^{-1}, which was only 2.3 times higher than ALAT value on day 0 and significantly lower than the value from the animals of the group one – 206.4 ± 28.8 U L^{-1} ($p<0.05$) (see Table 1). The mean value of GGT in the group two increased to 12.3 ± 1.7 U L^{-1},compared with 19.6 ± 4.4 U L^{-1}, as seen in group one (see Table 1 and Figure 2). The increase of the mean value of AP was 274.0 ± 20.5 U L^{-1}, and this was only four times higher than in the same group on day 0 in comparison with group one where the increase was 7 times higher (see Table 1). Corticosteroid-induced AP (cAP) mean value in group two on the fourteenth day was also high – 205.2 ± 22.5 U L^{-1}, that was 15 times higher than on day 0, but in group one the same value was 21 times higher than on day 0 (see Table 1).

The mean value of ALAT in dogs from group one serum on **day 30** increased to 177.6 ± 19.5 U L^{-1}, which is only 2.6 times higher than on day 0 (not 3 times as it was in the same animals' serum on day 14). The mean value of GGT in the first group on day 30 was 3.6 times higher (17.8 ± 3.5 U L^{-1}) than in the same animals' serum on the day 0, which was significantly higher ($p<0.05$), but lower than on day 14 (see Table 1). The mean value of AP on the day 30 in dogs from the group one was 5.6 times higher than on day 0 and it was 329.2 ± 46.7 U L^{-1}. But this increase was significantly lower ($p<0.05$) than the increase on day 14 (7 times) (see Table 1). The cAP mean value on day 30 in dogs' from the group one had increased 16.8 times, compared with 21 times on day 14 (see Table 1).

It should be noted that corticosteroid-induced thermostable AP in serum was found in small amounts (12.5 ± 2.2 U L^{-1}) even on the day 0. This can be explained with stress condition because of the veterinarian presence and blood collecting from the animal. It is acknowledged that it is higher glucocorticoid, e.g. cortisol level in blood when animal is in stress (Feldmann et al., 1994). It is experimentally proven that there are small amounts of thermostable alkaline phosphatase even in healthy dogs' blood as the authors describe it with being in stress condition (Hoffmann et al., 1977; Fukui et al., 2006).

It should be noted that the mean values of ALAT, GGT, AP and cAP in the serum of the dogs from the second group on day 14 and also on day 30 because of influence of hepatoprotectant 'GlutaMax' were significantly lower. That indicates that the negative effect of long-acting methylprednisolone acetate is decreased. Every enzyme mean value was lower compared to the same mean values from the animals of the first group, but none of the enzymes achieved the reference limits (see Table 1).

It can be concluded that the hepatoprotectant 'GlutaMax' used for 30 days, and even for 14 days, after one injection of long-lasting methylprednisolone acetate in dosage of 0.1 mg kg^{-1} bodyweight in dogs, significantly decreases corticosteroid-induced great increase of value of such enzymes as ALAT, GGT, AP, cAP in dogs serum.

In regard to these enzyme values on the day 30, it should be noted that all of them had the tendency to decrease in comparison to the fourteenth day of the study (see Table 1).

The results of our study prove the fact of corticosteroid-induced negative effects on liver functional condition (Badylak and van Vleet, 1981; Lucena et al., 1999; Abraham et al., 2006). These negative effects have been reflected by enzymes ALAT, GGT, AP and especially cAP significant increase in blood serum. The hepatoprotectant 'GlutaMax' could not completely prevent these functional failures of liver during this study. The question consists of how long time do we need to reverse the values of these enzymes in dogs' serum to reference limits in order to show that the negative effects of corticosteroids are completely gone. It would be desirable to confirm complete reversing by histological and immunohistochemical findings. The investigations on this direction are ongoing.

Conclusions

1. The corticosteroid methylprednisolone acetate statistically significantly increases the values of alaninaminotransferase (ALAT), gammaglutamyltranferase (GGT), alkaline

phosphatase (AP) and corticosteroid-induced thermostable alkaline phosphatase (cAP) in dogs' blood serum.

2. The hepatoprotectant 'GlutaMax' in dosage of 1 pill for 15 kg of bodyweight, used 30 days after the one injection of long-lasting methylprednisolone acetate for dogs, significantly decreases the values of alaninaminotransferase (ALAT), gammaglutamyltranferase (GGT), alkaline phosphatase (AP) and corticosteroid-induced thermostable alkaline phosphatase (cAP) in dogs' blood serum.

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