Prednisone Treatment Alters the Serum Amylase and Lipase Activities in Normal Dogs without Causing Pancreatitis

C. Fittschen and J.E.C. Bellamy*

ABSTRACT

In order to test the hypothesis that treatment with glucocorticoids causes pancreatitis in dogs, 18 mongrel dogs were divided into three groups of six individuals, each group receiving prednisone at different doses orally or intramuscularly for two weeks. Two groups consisting of six dogs each served as controls. Treatment for two weeks with oral prednisone at 1.2 mg/kg body weight or at 4 mg/kg body weight daily decreased the serum amylase activities, but increased the serum lipase activities. Postmortem examinations revealed microscopic evidence of mild pancreatitis in only one dog given prednisone, that clinically appeared normal. It was concluded that daily doses of 4 mg prednisone/kg body weight or less given orally or intramuscularly for two weeks do not cause pancreatitis in dogs.

Key words: Glucocorticoids, prednisone, canine pancreatic enzymes, serum amylase, serum lipase.

RÉSUMÉ

Cette expérience consistait à vérifier l'hypothèse selon laquelle l'utilisation de glucocorticoïdes chez le chien cause une pancréatite. Les auteurs utilisèrent à cette fin trois groupes de chiens bâtards qui en comptaient chacun six et ils leurs administrèrent diverses doses buccales ou intramusculaires de prednisone, pendant deux semaines. Deux autres groupes de chiens, qui en comptaient chacun six, servirent de témoins. L'administration buccale quotidienne de prednisone, à raison de 1,2 ou 4 mg/kg de poids corporel, pendant deux semaines, diminua l'activité de l'amylase sérique, mais elle intensifia celle de la lipase sérique. L'histopathologie révéla une légère pancréatite chez seulement un des chiens qui avaient reçu de la prednisone, indice que des doses quotidiennes orales ou intramusculaires de prednisone, à raison de 4 mg/kg de poids corporel, ou moins, pendant deux semaines, ne causent pas de pancréatite canine.

Mots clés: glucocorticoïdes, prednisone, enzymes pancréatiques canins, amylase sérique, lipase sérique.

INTRODUCTION

Glucocorticoid treatment has been suggested as a cause of pancreatitis in humans (1,2) in dogs (3) and in laboratory rabbits. Clear morphological and serochemical evidence for an association between cortisone treatment and pancreatitis has been presented for rabbits (4,5), whereas in man, the relationship is more ambiguous; in spite of the extensive use of glucocorticoids, the incidence of steroid related pancreatitis is low in humans and is related neither to the dose, nor to the duration of steroid therapy (1). In the dog, no case reports of steroid related pancreatitis have been published. Controlled studies of glucocorticoid effects on dogs have revealed neither morphological evidence (6), nor serochemical evidence of pancreatitis (7). The extensive use of prednisone in small animal medicine makes the risk to induce pancreatitis an important consideration. In the present study, we investigated the effects of frequently used doses of oral and intramuscular prednisone on the serum amylase and lipase activities, which are the common measurements used to support the clinical diagnosis of pancreatitis in man and in dogs (3,8). The serochemical data and the postmortem findings suggest that treatments for two weeks with the doses of prednisone employed in this study do not cause pancreatitis in the dog.

MATERIALS AND METHODS

Thirty mongrel dogs ranging from one and one-half to four years of age, and weighing 11-26 kg, were vaccinated against canine distemper and canine hepatitis. During a four week period of acclimatization to life in confinement they showed no sign of disease. A commercial dog food (Purina Dog Chow, Ralston Purina Canada Inc., Mississauga, Ontario) was given twice a day throughout the preconditioning and the experimental period, and water was provided four times daily ad libitum. Each dog was housed singly in an indoor cage. The dogs were arbitrarily divided into five groups of six individuals each: The oral control (OC) group received no medication; the low oral (LO) group

*Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0. Present address of senior author: Department of Pathology and Parasitology, College of Veterinary Medicine, Auburn University, Auburn, Alabama 36849.

Reprint requests to J.E.C. Bellamy.

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received 0.6 mg prednisone (Deltasone, Upjohn Co. of Canada, Don Mills, Ontario)/kg body weight twice daily orally; the high oral (HO) group received 2 mg prednisone/kg body weight twice daily orally; the intramuscular control (IMC) group received daily intramuscular injections of the vehicle of the commercial product used in the intramuscular group; the intramuscular (IM) group received 4 mg prednisone (Meticorten®, 40 mg/mL, Schering Corporation, Kenilworth, New Jersey)/kg body weight intramuscularly once daily. The OC group consisted of two females and four males. All other groups had equal numbers of both sexes. For logistic reasons, the experiment was subdivided into five trials using six animals each time. In the first three trials, two dogs each from groups OC, LO and HO were used. Three dogs each from groups IMC and IM were taken for trials 4 and 5. The dogs were kept in the same set of kennels and the same room during each trial. It was assumed that the time staggering did not influence the experimental results. The prednisone was given for 15 days. Specimens for hematological and serochemical studies were taken four days before the start of the trial and on days 6, 11 and 15. On day 15, the dogs were euthanized by injection of pentobarbital and a necropsy was performed.

A complete blood cell count was determined on EDTA-treated blood with an automated cell counter (Model S, Coulter Electronics, Hialeah, Florida). The white cell differential was calculated from the composition of cell types in 100 leukocytes on blood smears stained with Wright's Giemsa stain.

Serum amylase activity was estimated with a new enzymatic method (Amylase-DS, Beckman Instruments Inc., Carlsbad, California) which was adapted to use on an automated batch analyser (ABA 100, Abbott Laboratories, Mississauga, Ontario). Lipase activity was measured after incubation of serum for six hours with an olive oil substrate (Sigma Chemical Co., St. Louis, Missouri) by titration of the fatty acids released. The serum urea nitrogen (SUN) concentration was determined on serum with an automated analyzer (BUN Analyser II, Beckman, Edmonton, Alberta).

At necropsy, five sections of each pancreas were taken for histological studies: two from the left lobe and three from the right lobe (one from the corpus and two from the head). After fixation in 10% neutral buffered formalin, the specimens were embedded in paraffin, sectioned at 6 μm and stained with hematoxylin-eosin (HE) and by the Periodic-Acid Schiff reaction (PAS).

Pieces of tissue measuring less than 1 mm^3 were fixed with 2% glutaraldehyde in 0.1 M Na-cacodylate buffer, postfixed with 1% osmium tetraoxide in 0.1 M Na-cacodylate buffer, dehydrated in a series of alcohols and embedded in epon. Thin sections of 90-100 nm were stained with uranyl acetate-lead citrate.

The serum amylase values had a normal distribution and were analysed using analysis of variance and the means were compared with Student-Newman-Keul's multiple range test (9). Due to the skewed distribution of the serum lipase values, the lipase activities were compared by the Wilcoxon rank sum test for comparison of two independent groups (10). In the multiple range test as well as in the rank sum test the level of significance applied was 5%.

RESULTS

Clinically, the control dogs remained in good condition. All animals treated with prednisone showed polydipsia and polyuria. The dogs in the oral groups remained clinically normal, whereas those in the IM group became less active in the second half of the study, developing generalized muscle atrophy and a pendulous abdomen.

Controls showed no hematological changes throughout the study, whereas the leukograms of all dogs receiving prednisone were characterized by neutrophilia, monocytosis, lymphopenia, and eosinopenia that were most severe in the IM group. One dog in the IM group had a degenerative left shift (more band neutrophils than segmented neutrophils) presumably due to a focal bronchopneumonia and an abscess on the injection site in the thigh musculature found at postmortem examination.

Changes in the serum amylase and serum lipase activities are summarized in Table I. In all medicated groups, the amylase activity was decreased significantly on day 6 and remained low throughout the experiment. The lipase activity was increased significantly only in the IM group and only on day 6. No changes in the SUN concentrations were noted in any treatment group. On postmortem examination none of the dogs treated with prednisone had gross pancreatic abnormalities. Histological changes of the pancreatic acini or the duct system on HE or PAS stained sections were found only in one animal in the IM group which had a few interstitial foci or fat necrosis surrounded by a minimal neutrophil infiltrate. No ultrastructural alterations of the pancreas were observed in dogs given prednisone.

TABLE I. Group Changes Of The Serum Amylase and Lipase Activities in Controls and Dogs Treated with Prednisone

Variable	Group	Time (days) 0	6	11	15
Amylase (SI Units/L) mean (+ SD)	OC[a]	2309 (± 1321)	2141 (± 439)	2117 (± 512)	2655 (± 587)
	LO	2114 (± 730)	1076 (± 430)[s+]	915 (± 170)[s]	1166 (± 340)[s]
	HO	2141 (± 443)	910 (± 236)[s]	800 (± 192)[s]	988 (± 243)[s]
	IMC	1890 (± 152)	1775 (± 315)	1830 (± 428)	1954 (± 331)
	IM	2357 (± 450)	966 (± 430)[s]	1007 (± 475)[s]	920 (± 355)[s]
Lipase (SI Units/L) median (range)	OC	25 (0-190)	15 (0-80)	10 (0-70)	15 (0-180)
	LO	15 (0-50)	20 (0-120)	30 (0-120)	50 (0-80)
	HO	10 (0-40)	10 (0-60)	35 (20-60)	25 (0-80)
	IMC	40 (10-60)	40 (0-80)	15 (0-60)	25 (20-60)
	IM	15 (0-20)	80 (0-160)[s]	75 (20-200)	60 (20-160)

[a]OC — oral controls
LO — low oral treatment group
HO — high oral treatment group
IMC — intramuscular controls
IM — intramuscular treatment group
[+s] — indicates a significant difference from controls (p = 5%)

DISCUSSION

An etiological relationship between glucocorticoid treatment and pancreatitis has been proposed in human patients (1,2) and substantiated experimentally in rabbits, in which a high incidence of cortisone-induced pancreatitis was shown after experimental medication with corticosteroids (4,5). In contrast, the clinical, histological, and serochemical data presented here indicate that treatment with prednisone does not cause pancreatitis in the dog. Clinical signs in canine pancreatitis are variable, and include vomiting and, occasionally, diarrhea. The abdomen may be painful on palpation, but this is not consistently found (3,11). As the symptoms of pancreatitis resemble those of other disorders, the determination of serum lipase and amylase activities are commonly used to support or rule out the diagnosis of pancreatitis (3,11,12). The increase of serum amylase and lipase activities in acute inflammation of the pancreas frequently, but not consistently, parallel each other (3,11,12). Hyperlipemia is commonly (3) but not always (13) found in canine pancreatitis.

In man, the diagnosis of pancreatic inflammation on the basis of serum enzyme levels has been problematic even in conjunction with suggestive clinical signs, as the amylase activity is frequently increased in a variety of disorders involving the abdomen and other parts of the body (14), and little is known about lipase activites in health or diseases other than pancreatitis. Thus, some disorders clinically diagnosed as pancreatitis, but not confirmed by laparotomy or necropsy may have been due to other causes. In normal humans, amylase passes the glomerular filter due to its small size (MW 50,000) (14) and 75% is reabsorbed in the distal tubules (8,15). The enzyme can form larger conglomerates (macroamylase) (16), or adhere to plasma proteins such as immune globulins (14) and thus escape glomerular filtration. In contrast, cleavage of the amylase molecule into smaller particles with retained enzyme activity was observed in the serum and urine of patients suffering from pancreatitis. The smaller molecular size was thought (17) to contribute to the enhanced amylase clearance observed in this disease (18). Five isoamylases are known in man: two of pancreatic origin (p-type) and three of nonpancreatic origin (s-type) (19). In normal human serum, the s-types predominate slightly over the p-types. Renal excretion of the pancreatic isoenzymes is faster than that of the nonpancreatic ones (20).

In dogs, one electrophoretically distinct isoenzyme each has been associated mainly with the pancreas and with the duodenum, respectively (12,21); two additional activity peaks were identified, but could not be attributed to a tissue of origin (22). Interestingly, normal canine serum contained mostly amylase activity with electrophoretic properties similar to the duodenal isoenzyme, whereas only a small fraction of activity migrated with pancreatic amylase (21,22). Similar to man, dogs can eliminate amylase in the urine (23,24,25). A separation of isoenzymes in the urine has not been described in the dog.

The decrease in serum amylase activities observed in this study is consistent with the findings of Parent, who used a different (amyloclastic) method to determine amylase activity in dogs treated with dexamethasone (7). Previous findings of increased serum amylase activity in dogs injected with cortisone (26) appear to conflict with our results, unless this difference is due to the different drug used. Since an amylase isoenzyme separation was not attempted, it is not known whether the decrease in amylase activity was due to a reduction of the pancreatic or of the intestinal isoenzyme. Maltase has been reported to contribute to the serum amylolytic activity in some diagnostic tests (27); however, this enzyme did not appear to interfere with the assay employed in the present study, since addition of maltase (Sigma Chemical Co., St. Louis, Missouri) to serum did not affect the readings of amylase activity (unpublished results). Thus, the decreased serum amylase was not likely caused by a reduction in maltase activity. No reports could be found supporting a glucocorticoid induced decrease in total pancreatic amylase production which could account for the decreased serum amylase levels. While macroamylasemia (16) or complex formation with serum proteins (14) could conceivably lead to loss of enzyme activity, they have not been associated with low serum amylase activity. Both conditions have been described only in man and were not related to glucocorticoid treatment. Hypoamylasemia could be explained by enhanced enzyme catabolism, although it is difficult to assess this possibility because the site of amylase degradation and inactivation has not been identified. Warshaw *et al* described a circulating amylase inhibiting factor associated with hyperlipemia in human pancreatitis, but not in lipemia of other causes (28). In the present study, the serum lipid content was not biochemically analysed, but gross hyperlipemia was not observed and only one of the 18 dogs given prednisone showed histological evidence of mild pancreatitis. Recently, Dillon *et al* found no evidence of pancreatitis and measured normal serum triglyceride levels in dogs after four weeks of prednisolone medication (6).

Urinary amylase clearance was not estimated. It seems conceivable, however, that the increased glomerular filtration rate induced by glucocorticoids (29) could lead to enhanced renal excretion of amylase. In pancreatitis of man, an inhibition of amylase reabsorption in the lower nephron is thought to enhance the renal amylase clearance (15,18). An analogous block in the lower nephron induced by glucocorticoids would be consistent with the changes in serum amylase activity observed in this study.

The increase in serum lipase activity observed in the IM group is consistent with previous findings (7) in dogs treated with dexamethasone. It would be misleading, however, to associate all increases in serum lipase activities with pancreatic disease, because little is known about the enzymes, which constitute the lipolytic activity of serum in health and nonpancreatic disease; their origin is uncertain and it is not known if they are true lipases or actually esterases. Among the hydrolases, lipases (EC 3.1.1.3.) are characterized by a specifically high hydrolytic activity on triglycerides (30). Although esterases also have preferential substrates, some of them lack absolute specificity and their activity overlaps with that of lipases and vice versa (31). An isoenzyme separation of

serum lipases has, to our knowledge, not been reported.

While total serum lipase determination has been a useful aid to the diagnosis of pancreatitis (3,11,12), the lack of knowledge about the tissues of origin of this enzyme activity, other than the pancreas, complicates its interpretation. The gastrointestinal epithelium contains enzymes with lipolytic activity (32,33) but its contribution to the serum lipase activity is unknown. The endothelial lipoprotein lipases of muscle and adipose tissue and hepatic lipase are normally accessible to the circulation since they can be released into the blood stream by heparin injection (34,35). Glucocorticoids may indirectly stimulate adipose tissue lipoprotein lipase by raising serum insulin levels (34,35,36,37). Due to the short half-life of lipoprotein lipase (35) and its rapid inactivation *in vitro* at 37° C (38), this enzyme is not likely to significantly increase the serum lipase activity in the lipase assay used in this study. As glucocorticoid treatment causes pronounced morphological liver changes (39,40,41) and human and rat liver contains lipolytic activity (42,43,44), hepatic lipase possibly could be responsible for the increased serum lipase activity observed. However, this question remains to be resolved. Interestingly, oral contraceptive steroids decrease the serum activity of hepatic lipase in man (44).

Increases in serum lipase levels were observed in several dogs of the IM group, whereas mild focal pancreatitis was noted in only one dog on histological examination suggesting that this finding was not related to the prednisone treatment. At no time did any of the animals show symptoms supportive of pancreatitis, such as vomiting, diarrhea, abdominal pain or anorexia. Similarly, dogs treated with high doses of dexamethasone, which had even higher and more persistently elevated serum lipase activities than observed in this study, developed no pancreatitis (7). It seems, therefore, that glucocorticoid medication, when given in high doses, can increase serum lipase levels without causing pancreatic acinar necrosis. If the origin of the lipase activity was the pancreas, an enzyme leakage due to sublethal injury would have to be assumed. Leakage, however, would presumably be nonselective. Therefore, leakage is difficult to reconcile with findings of decreased serum amylase and increased serum lipase activities, unless an enhanced escape of both lipase and amylase into the serum were associated with a predominantly enhanced removal of amylase.

The present study showed that a two week treatment with prednisone at the dosages given here does not cause pancreatitis in normal dogs. Canine disorders, in which glucocorticoid treatment may increase the risk of pancreatitis, remain to be identified.

The changes in serum amylase and lipase activities described here suggest that treatment with prednisone may interfere with the diagnosis of pancreatitis, as a steroid induced decrease in serum amylase activity could mask an existing pancreatitis, whereas an increase in lipase activity might be mistaken as an indication for pancreatitis.

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