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Superficial Necrolytic Dermatitis in 11 Dogs with a History of Phenobarbital Administration (1995–2002)

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The clinical records of 11 dogs with histologically confirmed superficial necrolytic dermatitis (SND) and a history of phenobarbital (PB) administration (SND/PB) were evaluated retrospectively (1995–2002). Historical, clinical, clinicopathologic, ultrasonographic, and pathologic findings were compared with those in dogs with SND without prior PB exposure (SND/No PB; n = 9) and with those dogs with PB-associated hepatotoxicity without skin disease (PB/hepatotoxicity). Dogs in the SND/PB group accounted for 44% of all histologically confirmed cases of SND that were evaluated at The Ohio State University Veterinary Teaching Hospital between 1995 and 2002. Median age of dogs in the SND/PB group was 10 years, and median duration of PB therapy was 6 years. Mean alanine aminotransferase (ALT) activity was 239 U/L, and median duration of abnormally high ALT activity was 6.25 months before SND diagnosis. Plasma amino acid concentrations measured in 1 dog were severely decreased. Ultrasonographic findings of hypoechoic nodules with hyperechoic borders corresponded to pathologic findings of nodular areas of normal hepatic tissue surrounded by zones of collapsed parenchyma with vacuolated hepatocytes. Clinical, clinicopathologic, ultrasonographic, and pathologic features of SND/PB and SND/No PB were similar. PB-associated cirrhosis and overt hepatic failure were not features of SND/PB. Different pathogenic mechanisms might induce SND in dogs. Chronic administration of PB requires further examination as a potential risk factor for the development of SND.

Key words: Amino acids; Canine; Cirrhosis; Hepatocutaneous syndrome; Hepatopathy.

Canine superficial necrolytic dermatitis (SND) is a progressive, debilitating, cutaneous disorder commonly associated with hepatic abnormalities.[1–4] The term hepatocutaneous syndrome (HCS) describes the condition in which a concurrent hepatopathy is present,[2,3] and the terms necrolytic migratory erythema (NME) and metabolic epidermal necrosis (MEN) describe the cutaneous disease.[4–9] SND is an erosive dermatopathy with a multifocal distribution. Early skin lesions of erythema and papules rapidly progress to scaling and crusted erosions. Primary bullous and vesicular lesions occasionally are seen. Typically, the footpads are affected with crusting and hyperkeratosis and the appearance of fissures. Painful footpad lesions result in inactivity, lethargy, and reluctance to walk. Other affected sites include the mucocutaneous junctions of the mouth, eyes, and genitalia, as well as hocks, elbows, pinnae, axillae and groin, and interdigital areas.[1,3,5,6,10,11]

Concurrent hepatic abnormalities and low plasma amino acid concentrations have been linked to the dermatologic lesion. Affected dogs typically have mild to moderate hepatopathy and pronounced reductions in plasma amino acid concentrations, especially proline, glutamine, threonine, alanine, citrulline, and arginine.[1,4,11,12] Studies suggest that dogs with SND might be in a hypermetabolic state with exaggerated amino acid catabolism[11] that could explain the hypoaminoacidemia found in affected dogs.

SND has been associated with concurrent disease conditions but its relation to these is uncertain. An association between diabetes mellitus and SND originally was described by Walton et al.[13] In a recent retrospective study of SND, preexisting endocrine disorders, including diabetes mellitus, hyperadrenocorticism, and hypothyroidism were present in approximately 40% of affected dogs.[11] A minority of dogs and a majority of humans with SND have hyperglucagonemia secondary to a glucagon-secreting pancreatic tumor.[4,5,14–20] Glucagon stimulates gluconeogenesis and increases amino acid catabolism and can contribute to low plasma amino acid concentrations and glucose intolerance reported in dogs with glucagonomas.[4,5,14,16,17] However, the majority of dogs with SND have no identifiable glucagonoma or defect in hepatic glucagon degradation. Plasma glucagon concentrations measured in SND dogs with no evidence of glucagonoma have either been normal or mildly increased.[4,16,17] Sporadic cases of infectious, inflammatory, and neoplastic conditions associated with SND in dogs also have been described.[8,9,21,22]

Case reports of 2 dogs with SND that have a history of either primidone or phenobarbital (PB) administration have been published.[23,24] In addition, 3 of 36 dogs in a recent retrospective study of SND also had a history of chronic PB administration.[11] Chronic PB use is associated with hepatotoxicity and eventual cirrhosis.[25,26] Dogs with progressive PB-induced hepatotoxicity typically have poor body condition, ascites, low serum albumin and blood urea nitrogen (BUN) concentrations, normal to moderately increased serum bilirubin concentrations, and abnormally high fasting and postprandial serum bile acid concentrations. Grossly, the liver usually is reduced in size with extensive fibrosis interspersed with areas of nodular hyperplasia.[25–27] In contrast, dogs with SND usually do not have concurrent clinical and clinicopathologic findings that would support a diagnosis of cirrhosis and hepatic insufficiency.[1–3,11,12] Furthermore, dogs with SND and a history of

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Table 1. Age of disease onset, duration of PB administration, and dosages and serum concentrations of PB in each dog group.[a]

Patient Group (n)	Median Age of Disease Onset (years)	Median Duration of PB Administration (years)	Mean Maximal PB Dosage ± SD (mg/kg q24h)	Mean Maximal Trough Serum PB Concentration ± SD (μg/mL)
SND/PB (11)	10[b] (6.15–14.5)	6[c] (1.7–11)	12.4 ± 5.7 (3.8–19.8)	43.5 ± 15.1 (22.8–66)
SND/No PB (9)	11[b] (7–12)	Not applicable	Not applicable	Not applicable
PB/hepatotoxicity-A (18)	6.5 (NA)	4 (0.4–6.8)	17.2 ± 6.9 (5.1–24.7)	NA
PB/hepatotoxicity-B (6)	5.25 (4.2–5.5)	3.1[c] (2–4)	13.0 ± 2.1 (11.6–16)	50.5 ± 10.0 (40–62.5)

SND, superficial necrolytic dermatitis; PB, history of phenobarbital administration; PB/hepatotoxicity-A and -B, dogs with hepatotoxicity and a history of PB administration reported in the literature[25] and from Ohio State University Veterinary Teaching Hospital records, respectively; NA, information not available.

[a] Numbers in parentheses are ranges of values for the parameter.

[b] Significantly different from the PB/hepatotoxicity-B group ($P < .05$).

[c] Significantly different from the PB/hepatotoxicity-A group ($P < .05$).

PB administration (SND/PB) have not been described as having clinical signs of liver failure,[11,23] and pathologic findings in the few dogs examined have not been compatible with cirrhosis.[11]

The objective of this retrospective study was to assess the frequency of concurrent PB administration in dogs that were diagnosed with SND between 1995 and 2002 and to evaluate clinical, clinicopathologic, ultrasonographic, and pathologic abnormalities in affected dogs. Diagnostic findings are compared with those in dogs with SND without prior PB exposure (SND/No PB) and with those in dogs with PB-associated hepatotoxicity without skin lesions (PB/hepatotoxicity).

Materials and Methods

Data for the retrospective study on SND/PB dogs was obtained from records of patients admitted to the Ohio State University Veterinary Teaching Hospital (OSU-VTH; 7 patients), the University of Illinois College of Veterinary Medicine Veterinary Teaching Hospital (1 patient), and a private veterinary referral hospital (3 patients) between April 1995 and February 2002. Inclusion criteria were a history of seizures and PB administration, typical cutaneous lesions associated with SND, and typical histopathologic findings of SND on skin biopsy samples. Other diagnostic tests reviewed included CBC and serum biochemistry at the time of SND diagnosis, fasting and 2-hour postprandial serum bile acid concentrations, urinalysis, ultrasonography, liver biopsy, and postmortem examination findings. Sections of paraffin-embedded hepatic tissue were stained with hematoxylin and eosin, periodic acid–Schiff (PAS) with and without diastase digestion, Masson's trichrome, and reticulin stains and evaluated by a board-certified pathologist (SEW). Historical information on seizure etiology, duration of seizures, and PB use and use of other antiepileptic drugs (AEDs) was evaluated in all dogs. Serial results of biochemical tests, AED concentrations, AED dosages, and body weights available from 2 weeks to 2.5 years before the onset of skin lesions were assessed in those dogs with detailed medical histories.

Results of clinicopathologic data were compared with those of patients with either SND without PB exposure (SND/No PB) or PB-associated hepatotoxicity without SND (PB/hepatotoxicity) in order to identify any differences in these results among groups. Information for the PB/hepatotoxicity patient group was obtained from a previously reported study[25] and from records of patients seen at the OSU-VTH. Inclusion criteria for the OSU PB/hepatotoxicity group were a history of PB administration of at least 12 months, clinical signs of hepatic encephalopathy, and serial clinicopathologic records for dogs demonstrating hepatic disease over at least 2 months. Information for the SND/No PB patient group was obtained from medical records of all dogs seen at the OSU-VTH between 1995 and 2002 diagnosed with SND on the basis of typical histopathology on skin biopsy. Clinicopathologic test results are expressed as mean ± standard deviation (SD), and age and time variables are expressed as median and range. Mean and median values for each group were compared by a Kruskal-Wallis test, and Dunn's multiple comparison tests were performed if significant group differences were found.

Results

Retrospective Findings in Dogs with SND and a History of PB Administration

Signalment and Clinical History. Eleven dogs met inclusion criteria for the SND/PB group. Breeds affected were Samoyed (n = 1), Weimaraner (1), miniature Schnauzer (1), Collie (1), Shetland Sheepdog (1), Australian terrier (1), and mixed breed (5). The male to female ratio was 0.83 : 1. Four of the 5 male dogs were neutered, and 5 of the 6 female dogs were spayed. Ten dogs had idiopathic epilepsy, and the remaining dog was diagnosed at 9 years of age with a meningioma as the cause of seizures. The median age at onset of SND, the median duration of PB administration before the diagnosis of SND, the mean maximal PB dosage, and the mean maximal trough serum PB concentration are listed in Table 1. The median duration of PB administration in dogs with idiopathic epilepsy before the onset of skin lesions was 7 years (range 4–11 years), and 82% of affected dogs had been on PB for more than 4 years. A majority of dogs had maximal PB dosages >10 mg/kg q24h and maximal trough serum PB concentrations >35 μg/mL (64 and 73%, respectively). In 6 of 11 dogs, PB had been discontinued a median of 4 weeks (range 2–72 weeks) before onset of skin lesions. Phenobarbital was tapered and discontinued in these dogs because of persistent increases in serum alanine aminotransferase (ALT) activity. Of the remaining 5 dogs, 4 were being treated with maximal dosages of PB, and 1 was being weaned off PB at the time of diagnosis of SND. Six of 11 dogs were receiving bromide therapy for a median duration of 15 months (range 1.5–48 months). Three dogs were receiving thyroid hormone supplementation for a previously diagnosed hypothyroid condition.

Skin lesions developed in all dogs within the 4 weeks

Table 2. Hepatic enzyme activities in dogs with SND/PB, SND/No PB, and PB/hepatotoxicity.[a]

Patient Group (n)	Parameter	ALT (U/L)[b]	AST (U/L)[c]	ALP (U/L)[d]
SND/PB (11)	Mean ± SD (range)	239 ± 139 (104–520)	72 ± 18 (53–101)	1,047 ± 1,299 (136–4,215)
	Relative change	2.5 ± 1.3	1.6 ± 0.4	7.6 ± 10.0
SND/No PB (9)	Mean ± SD (range)	298 ± 215 (62–677)	93 ± 61 (47–233)	1,109 ± 913 (122–2,425)
	Relative change	2.7 ± 1.9	2.1 ± 1.4	8.5 ± 7.0
PB/hepatotoxicity-A (18)	Mean ± SD (range)	387 ± 446 (53–1,830)	NA	1,293 ± 1,201 (226–5,240)
	Relative change	6.8 ± 7.8	NA	7.6 ± 7.1
PB/hepatotoxicity-B (6)	Mean ± SD (range)	302 ± 191 (80–556)	138 ± 107 (21–308)	912 ± 528 (336–1,594)
	Relative change	2.8 ± 1.7	3.1 ± 2.4	7.0 ± 4.1

SND, superficial necrolytic dermatitis; PB, history of phenobarbital administration; PB/hepatotoxicity-A and -B, dogs with hepatotoxicity and a history of PB adminstration reported in the literature[25] and from Ohio State University (OSU) Veterinary Teaching Hospital records, respectively; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; NA, information not available.

[a] Relative change is the multiple of each parameter relative to the reference range, calculated as the actual value divided by the upper limit of the reference range.

[b] OSU reference range 15–110 U/L; reference range for PB hepatotoxicity-A 13–57 U/L.

[c] OSU reference range 7–44 U/L.

[d] OSU reference range 20–130 U/L; reference range for PB hepatotoxicity-A 35–169 U/L.

before presentation. Owners observed skin lesions affecting the footpads 1st in 10 of 11 dogs. One dog initially developed lesions in the nasal region. Ten of 11 dogs had a history of difficulty walking. Licking and chewing the feet was reported in 3 dogs. Other historical problems included ptyalism (2 dogs), diarrhea (1 dog), ataxia (1 dog), ocular discharge (1 dog), sneezing (1 dog), coughing (1 dog), and difficulty prehending food (1 dog). Dog 2 had a history of sudden collapse 2.5 months previously from hemorrhagic pericardial effusion secondary to a heart base hemangiosarcoma. The mass was successfully resected and metastatic disease was not evident at the time of surgery. Decreased appetite was reported in 6 of 11 dogs over a time period ranging from 1 to 84 days (median 27 days). For those dogs that had serial measurements of body weight (n = 6), only 2 dogs experienced >10% reduction in body weight over a 3–4 month period, pre-SND diagnosis.

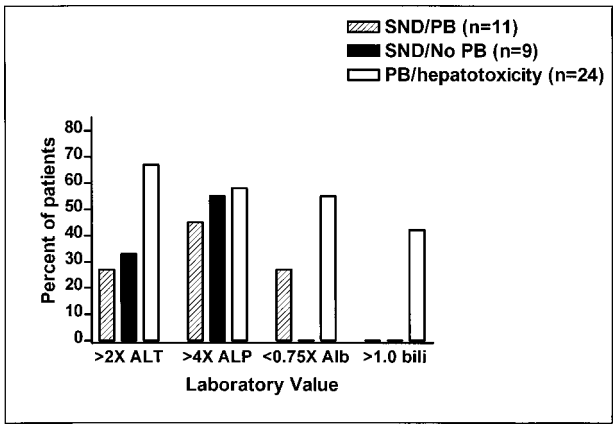

Fig 1. Comparison of serum alanine aminotransferase (ALT) activities, serum alkaline phosphatase (ALP) activities, serum albumin concentrations, and total bilirubin concentrations in dogs with superficial necrolytic dermatitis (SND) and a history of phenobarbital (PB) administration (SND/PB), dogs with SND and no history of PB administration (SND/No PB), and dogs with PB-associated hepatotoxicity (PB/hepatotoxicity). The PB/hepatotoxicity group consisted of pooled affected dogs from the Ohio State University Veterinary Teaching Hospital and the literature.[25] A greater number of dogs in the PB/hepatotoxicity group had serum ALT activities >2 times the upper limit of the reference range (>2× ALT), serum albumin concentrations <0.75 times the lower limit of the reference range (<0.75× Alb), and serum total bilirubin concentrations >1.0 mg/dL compared with the SND/PB and SND/No PB groups. The percentage of patients with serum ALP activity >4 times the upper limit of the reference range (>4× ALP) was >40% in all groups.

Physical Examination Findings. At presentation, all dogs were lethargic, but only 2 dogs had decreased responsiveness and mentation. One dog (dog 2) had moderate abdominal distension that later was found to be from abdominal effusion. Ten of 11 dogs had noticeable lameness upon examination. Affected skin areas at the time of presentation included the footpads (11 dogs), oral mucocutaneous junctions (6 dogs), nose or nasal planum (3 dogs), periocular areas (2 dogs), ear pinnae (2 dogs), perianal (2 dogs) and perivulvar area (2 dogs), ventral abdominal or inguinal region (4 dogs), ventral neck or thorax (3 dogs), elbows (4 dogs), and hocks (1 dog). Skin lesions in the majority of dogs were characterized by footpad hyperkeratosis (10 dogs), erythema (10 dogs), crusting dermatitis (11 dogs), alopecia (7 dogs), and erosions/ulcers (8 dogs). Other lesions included footpad fissuring (4 dogs), scales (3 dogs), pustules (3 dogs), and papules (2 dogs).

Median survival time for 9 dogs was 12 weeks (range 2.5–32 weeks). Dog 2 was lost to follow-up, and dog 7 was alive 12 months after the diagnosis of SND.

Clinicopathologic Data. Biochemical abnormalities at the time of SND diagnosis included high ALT, alkaline phosphatase (ALP), and aspartate aminotransferase (AST) activities; high glucose concentrations; and mild decreases in serum albumin, BUN, and creatinine concentrations. Mean ALT, AST, and ALP activities were above the normal reference range. Mean ALT activity was 2.5-fold greater than the upper limit of the reference range (Table 2). Twenty-seven percent of affected dogs had a 2-fold or higher increase in ALT activity (Fig 1). AST activities were higher than the reference range in 9 of 11 dogs. Mean ALP activity was increased 7.5-fold above reference values. Mean BUN concentration was 9.0 ± 4.4 mg/dL (OSU reference range 7–31 mg/dL), and mean serum creatinine concentration was

Table 3. Serum albumin and bile acid concentrations in dogs with SND/PB, SND/No PB, and PB/hepatotoxicity.[a]

Patient Group (n)	Parameter	Albumin (g/L)[b]	Fasting SBA (μmol/L)[c]	Postprandial SBA (μmol/L)[d]
SND/PB (11)	Mean ± SD (range)	2.6 ± 0.5 (1.8–3.5)[e]	24 ± 18 (9–57)	75 ± 37 (44–140)[ef]
	Relative change	0.94 ± 0.16	2.4 ± 1.8	5.0 ± 2.5
SND/No PB (9)	Mean ± SD (range)	3.1 ± 0.6 (2.4–4.2)[e]	26 ± 32 (3–97)	30 ± 14 (9–49)[e]
	Relative change	1.0 ± 0.2	5.2 ± 3.2	2.0 ± 0.9
PB/hepatotoxicity-A (18)	Mean ± SD (range)	2.1 ± 0.7 (1.1–3.6)	NA	NA
	Relative change	0.8 ± 0.3	NA	NA
PB/hepatotoxicity-B (6)	Mean ± SD (range)	1.5 ± 0.3 (1.1–1.8)[f]	206 ± 60 (164–249)	368 ± 39 (341–396)[f]
	Relative change	0.5 ± 0.1	20.6 ± 6.0	24.5 ± 2.6

SND, superficial necrolytic dermatitis; PB, history of phenobarbital administration; PB/hepatotoxicity-A and -B, dogs with hepatotoxicity and a history of PB adminstration reported in the literature[25] and from Ohio State University (OSU) Veterinary Teaching Hospital records, respectively; SBA, serum bile acid; NA, information not available.

[a] Relative change is the multiple of each parameter relative to the reference range, calculated as the actual value divided by the upper or lower limit of the reference range.

[b] OSU reference range 3.1–4.4 g/dL; reference range for PB/hepatotoxicity-A 2.7–3.6 g/dL.

[c] OSU reference range 0–10 μmol/L.

[d] OSU reference range 0–15 μmol/L.

[e] Significantly different from the PB/hepatotoxicity-B group ($P < .05$).

[f] Significantly different from the SND/No PB group ($P < .05$).

0.6 ± 0.2 mg/dL (OSU reference range 0.7–1.4 mg/dL). Individual serum albumin concentrations were either below the normal range (6 dogs) or in the low normal range (5 dogs) for the respective testing laboratories (Table 3) and was <0.75 times the lower limit of the reference range in 27% of dogs (Fig 1). Serum glucose concentrations were in the normal range for the majority of dogs tested. Two dogs with concurrent diabetes mellitus had resting serum glucose concentrations of 405 and 241 mg/dL. The mean serum glucose concentration for all dogs was 160 ± 96 mg/dL (range 93–405 mg/dL, OSU reference range 63–120 mg/dL).

Serial biochemistry results up to 2.5 years before the onset of SND were available in 7 dogs (dogs 2–8), and serial PB dosages and trough PB concentrations were available in 4 of these dogs (dogs 3, 4, 6, 7). Serum ALT activity was increased above the reference range a median of 6.25 months (range 1–30 months) before the development of skin lesions. Serum ALT activity was monitored over a minimum of 12 months before the diagnosis of SND in 4 dogs (dogs 3–6). In these 4 dogs, increased enzyme activity was modest but persistent and peaked a median of 3.75 months before signs of cutaneous disease. Five of these 7 dogs eventually were weaned off PB, and the drug had been discontinued before the onset of SND. Abnormally high serum ALT activity was observed over time despite gradual tapering of the PB dosage, and ALT activity remained above the reference range after discontinuation of PB. Mean trough serum PB concentrations were in the high therapeutic range (mean concentration 41.6 ± 13.6 μg/mL, range 28.6–65.3 μg/mL) before a tapering dosage schedule was initiated. PB serum concentrations eventually declined over time in parallel with decreasing PB dosages but remained high (mean 40.7 ± 18.2 μg/mL) in those dogs (n = 4) in which the PB dosage had not been decreased.

Serial albumin concentrations over time were available in dogs 2–8. In all 7 dogs, serum albumin concentrations were below the lower limit of the reference range for a median duration of 3.75 months (range 1–26 months) before the development of skin lesions. Decreased serum albumin concentrations persisted in 6 of these dogs but gradually increased into the normal reference range in 1 dog (dog 6) immediately after the diagnosis of SND. BUN and serum creatinine concentrations decreased before the onset of and during the course of SND in these 7 dogs.

Complete blood counts were obtained in 9 dogs. Mean packed cell volume (PCV) was 32.8 ± 6.9% (range 22–48%). The PCV was below the lower limit of the reference range in 8 of the 9 dogs tested. Mean corpuscular volume (MCV) values ranged from 59 to 68 fL (reference range 64–74 fL), and mean MCV was 62.6 ± 3.3 fL. Hemostasis profiles were performed in 5 dogs, and all values were within normal reference ranges.

Fasting and 2-hour postprandial serum bile acid assays were performed in dogs 1–4, 7, and 8. The mean fasting and the mean 2-hour postprandial serum bile acid concentrations were normal to moderately high compared with reference range values (Table 3). Serum bile acid assays were performed at the time of SND diagnosis in 4 of these 6 dogs. Serum bile acid assays were performed at least 1 month from the onset of SND in the 2 dogs with the highest 2-hour postprandial serum bile acid concentration (dogs 2 and 8). In these 2 dogs, PB had not been tapered and was still being administered at high dosages (dog 2: 13.3 mg/kg q24h; dog 8: 15 mg/kg q24h). At the time serum bile acid concentrations were measured, trough serum PB concentrations in these 2 dogs were 57 and 38 μg/mL, respectively, and ALT activities were at or near their peak (563 and 520 U/L in dogs 2 and 8, respectively). After this time, PB was weaned and liver enzyme activities decreased, but serum bile acid assays were not repeated.

Plasma amino acid concentrations were measured in 1 dog (dog 8). Alanine, methionine, lysine, threonine, glutamine, proline, taurine, serine, glycine, tyrosine, histidine, arginine, and isoleucine concentrations were reduced when compared with normal controls. At the time of SND diagnosis, plasma glutamine, proline, threonine, and arginine concentrations were <20% of normal, and alanine, lysine,

histidine, and methionine were <40% of normal. On day 76 after diagnosis of SND, glutamine, arginine, glycine, lysine, and methionine concentrations were further reduced, some by as much as 90% of control concentrations. Plasma concentrations of tryptophan, phenylalanine, and glutamate were minimally affected at both time points compared with other amino acids.

Hepatic Ultrasound. Ultrasonographic findings were characterized by hypoechoic nodules with a hyperechoic trabecular network surrounding the nodules (''honeycomb'' pattern) in 6 of the 7 dogs in which ultrasonography was performed (dogs 1, 3, 4, 6–8). The remaining dog (dog 5) had a less well defined nodular pattern with mixed echogenicity. Hypoechoic nodular foci were diffuse and numerous and measured between 1.0 and 1.5 cm in diameter. Livers were subjectively assessed to be small in all dogs, and a nodular, irregular margin was noted in 5 of 7 dogs. Ascites was absent in 4 dogs and minimal in 3.

Histopathologic Findings. Dermatohistopathology was characterized by parakeratotic hyperkeratosis, epidermal spongiosus, and epidermal hyperplasia in all dogs. Basal cell hyperplasia and presence of bacteria (cocci) in surface crust, epidermal pustules, or within hair follicles were observed in 10 dogs. Skin lesions were also characterized by follicular hyperkeratosis with occasional follicular casts and cysts in 5 dogs, epidermal necrosis or apoptosis in 3 dogs, and hydropic degeneration of the basal layer in 2 dogs. Superficial perivascular dermal inflammation was present in all dogs with concurrent interface dermatitis present (4 dogs). The inflammatory cell infiltrate was overwhelmingly mononuclear, with lymphocytes and plasma cells observed most commonly.

Liver biopsies were obtained in 5 of 11 dogs (dogs 1, 4–6, 8), and postmortem examinations were performed in 2 dogs (dogs 1 and 4). In the 2 dogs that were examined postmortem, the livers were grossly nodular in appearance and had irregular margins. Histologic findings in dogs 1 and 4–6 were characterized by regions of severe parenchymal collapse containing hepatocytes with marked vacuolization. Vacuolization was predominantly a result of glycogen deposition (confirmed by PAS with diastase digestion) in all dogs, but microvacuolar lipid was also found in dogs 2 and 4. Regions of collapse surrounded sharply demarcated nodules of normal hepatic parenchyma with preserved lobular architecture in dogs 1, 5, and 6. Dog 4 had mild to moderate disorganization of lobular architecture from multifocal areas of neutrophilic inflammation and fibrosis. Fibrosis in collapsed regions was mild to moderate in all dogs. Very few reticulin-positive fibers were demonstrated in regions of collapse, and hepatocellular necrosis was not evident in any dog. Hepatic tissues on dog 8 were evaluated by a commercial diagnostic laboratory and were unavailable for review by the authors. Reported histologic characteristics included hepatocellular vacuolization, increased connective tissue elements, mild periportal fibrosis, and mild multifocal neutrophilic hepatitis.

Retrospective Findings in Dogs with SND without a History of PB Administration

Medical records of 9 dogs with SND (confirmed by typical skin histopathology) but without a history of PB exposure (SND/No PB) were reviewed. Signalment, history, physical, clinicopathologic, and ultrasonographic findings were available in all dogs. Pathologic findings on hepatic biopsy or postmortem examination were available in 4 of these 9 dogs. Median age of onset of SND was 11 years (range 7–12 years). Mean ALT, ALP, and AST activities at the time of diagnosis were above the upper limits of the reference ranges (Table 2). Mean fasting and postprandial serum bile acid concentrations were normal to mildly high. Mean serum albumin, BUN, and creatinine concentrations were at the lower limits of their respective reference ranges (Table 3). Figure 1 compares the abnormal biochemical findings in this group of dogs with those dogs in the SND/PB and PB/hepatotoxicity groups. Serum ALT activity was increased at least 2-fold over the upper limit of the reference range in 33% of dogs in the SND/PB group. Individual serum albumin concentrations were never <0.75 times the lower limit of the reference range (Fig 1).

Mean serum glucose concentration was mildly increased (155 ± 100 mg/dL; range 83–373 mg/dL), but only 3 of 9 dogs had serum glucose concentrations above the upper limit of the reference range. Mean hematocrit was 36 ± 7% (reference range 37–52%), and mean MCV was 57 ± 5 fL (reference range 64–74 fL).

Ultrasonographic examination identified multiple small (~1 cm diameter) hypoechoic nodules surrounded by hyperechoic borders (honeycomb appearance) in all affected dogs. Histopathologic findings in hepatic tissues from 4 dogs were characterized by regions of marked parenchymal collapse containing hepatocytes with moderate to marked vacuolization. Variably thick collagen fibers with interspersed bile ducts and Kupffer cells also were present in the collapsed areas. Hepatocellular micro- to macrovacuolar lipidosis predominated in 2 dogs, but a less extensive lacelike clearing of the cytoplasm as a result of glycogen deposition was found in both dogs. One of these dogs also had scattered foci of hepatocellular necrosis, lymphohistiocytic lobular hepatitis, and portal fibrosis. In the other 2 dogs, vacuolization was predominantly a result of glycogen deposition. Collapsed regions surrounded well-demarcated, nodular areas of normal hepatic parenchyma in 3 dogs. Lobular architecture was mildly disrupted in the dog with multifocal lymphohistiocytic hepatitis and portal fibrosis. Regenerative nodules were absent to rare.

Retrospective Findings in Dogs with PB-Associated Hepatotoxicity

Complete historical, physical, clinicopathologic, and pathologic information was available in 6 dogs that had a history of PB use and later developed hepatotoxicity. Four of these 6 dogs were euthanized because of the severity of their clinical signs. Three of these 4 dogs had complete postmortem examinations, and the 4th dog had a liver biopsy performed antemortem. The other 2 dogs had clinicopathologic findings compatible with hepatotoxicity, including abnormally high hepatic enzymes, high serum bile acid concentrations, and persistent decreases in serum albumin concentration. Clinical signs of hepatic insufficiency were less severe in these 2 dogs than in the other 4 dogs. Liver biopsies were obtained in these 2 dogs, but owners

did not elect euthanasia. Median age of disease onset, median duration of PB administration, mean maximal PB dosage, and mean maximal trough PB concentration are listed in Table 1. Mean ALT, AST, and ALP activities were above the upper limits of the reference ranges (Table 2). Mean serum albumin concentration was reduced by 52% compared with the lower limit of the reference range. Mean BUN concentration was 4.7 ± 1.0 mg/dL (32% reduction), and mean serum creatinine concentration was 0.8 ± 0.2 mg/dL (reference range 0.7–1.4 mg/dL). Mean serum total bilirubin was 1.45 ± 1.6 mg/dL (reference range 0.1–0.4 mg/dL). Mean fasting and postprandial serum bile acid concentrations were markedly high (Table 3). Ultrasonographic findings included hepatic echogenicity that was normal to diffusely increased in all dogs. Liver size was reduced and margins were irregular in only 1 dog. Hypoechoic nodules in a hyperechoic network were not observed in any of the dogs. A single hypoechoic nodule was found in 1 dog, and a single hyperechoic mass was found in another dog. Four of 6 dogs had moderate to severe ascites. Liver histopathology in 3 of the 4 dogs that were euthanized was characterized by cirrhosis, with mild to marked fibrosis. Collagen fibers replaced parenchymal tissue in regions of fibrosis. One of these 3 dogs also had evidence of chronic hepatitis characterized by multifocal areas of mild neutrophilic and lymphoplasmacytic hepatitis located within regions of hepatocellular collapse. The 4th dog had chronic active hepatitis characterized by moderate to marked neutrophilic portal, periportal, and lobular hepatitis with moderate to marked bridging fibrosis. Liver biopsies from the additional 2 dogs with biochemical signs of hepatotoxicity but without severe signs of hepatic insufficiency revealed widespread mild to marked hepatocellular vacuolization secondary to glycogen deposition. One of these dogs also had multifocal minimal lymphoplasmacytic and neutrophilic perivascular and lobular hepatitis. Neither dog had evidence of parenchymal collapse.

Clinical and clinicopathologic data from a report of 18 dogs with hepatotoxicity secondary to PB were reviewed.[25] Twelve of these 18 dogs were being treated with PB alone. Six of the 18 dogs were being treated concurrently with primidone, phenytoin, corticosteroids, or some combination of these drugs. Median age of disease onset, median duration of PB use, and mean maximal PB dosage before onset of hepatotoxicity are listed in Table 1. Mean ALT activity was increased (Table 2) and mean serum albumin concentration was decreased (Table 3) relative to reference range values. Mean serum bilirubin concentration was 1.3 ± 1.2 mg/dL. When PB/hepatoxicity data was pooled from all groups, 45% of affected dogs had been treated with PB for a period of >4 years, 86% were receiving a PB dosage >10 mg/kg q24h, 67% had serum ALT activities >2 times the upper limit of the reference range, and 42% had bilirubin concentrations >1.0 mg/dL (Fig 1).

Discussion

Prolonged PB administration was a consistent finding in the dogs of this study. A history of administration of PB, primidone, phenytoin, or some combination of these drugs has been reported in 5 dogs with SND,[11,23,24] but duration of AED therapy before the onset of SND was not defined in 4 of the 5 dogs. The median duration of PB therapy in the dogs of this study was 6 years before the onset of SND, and no previous exposure to either primidone or phenytoin was identified. A review of all histologically confirmed cases of SND at the OSU-VTH between 1995 and 2002 disclosed that, of 16 dogs diagnosed with SND, 9 (56%) had no history of PB exposure and 7 (44%) had received chronic PB therapy. These 7 dogs represent 7 of the 11 dogs evaluated here. The history of PB administration in nearly half of the confirmed cases of SND at a single institution suggests that prolonged PB exposure might be a risk factor for the development of SND in dogs. Previous retrospective studies of SND have not identified a high frequency of PB use in affected dogs, but this might reflect differences in the scope of the retrospective studies and in the types of patients typically referred to the authors of these studies.[1–3,6,11,12]

Clinical signs and other indices of overt hepatic failure were not present in 10 of the 11 dogs in this study. Affected dogs generally were in good health until the development of skin lesions; had not experienced clinically relevant weight loss; and did not have a history of polyuria, polydipsia, or gastrointestinal problems. Increases in ALT activity were mild to moderate, serum albumin concentrations were nearly normal, hyperbilirubinemia was absent in all dogs, and only 1 dog (dog 2) had moderate abdominal effusion. With the exception of dog 2, fasting serum bile acid concentrations did not exceed 30 μmol/L, and postprandial serum bile acid concentrations did not exceed 100 μmol/L. In the majority of dogs in this study, ultrasonographic and histopathologic abnormalities were compatible with sharply demarcated nodular regions of normal hepatic parenchyma surrounded by areas of collapsed parenchyma containing vacuolated hepatocytes, variable degrees of collagen deposition, and numerous surviving biliary ducts. Previously reported histologic features of hepatic changes in dogs with SND/No PB are similar.[1–3,10–12] In the SND/No PB and SND/PB dogs that presented to the OSU-VTH, the characteristic honeycomb liver observed on ultrasonographic examination in most dogs was consistent with the histopathologic findings. Hypoechoic nodular foci in both conditions corresponded to the well-demarcated areas of normal ''spared'' hepatic parenchyma. The hyperechoic network consisted of areas of moderate to severe hepatocellular vacuolization contained within regions of parenchymal collapse. The major difference between the vacuolated hepatocytes in the 2 disorders was the predominance of lipid vacuoles in SND/No PB and the predominance of glycogen vacuoles in SND/PB, although considerable overlap in vacuole type was present in both conditions. Interestingly, the only feature that SND/PB dogs shared with dogs experiencing early hepatotoxicity secondary to PB was a predominance of glycogen within vacuolated hepatocytes. Regenerative nodules, dense fibrosis, and other features of hepatic cirrhosis were rare to absent in the SND/PB and the SND/No PB groups.

Chronic PB administration has been linked to hepatotoxicity and hepatic failure in dogs.[26,28,29] Mean fasting serum bile acid concentration was 187 μmol/L (reference range 0–10 μmol/L) and mean serum albumin concentration was 1.5 g/dL (reference range 2.0–3.3 g/dL) in 3 dogs with

hepatic cirrhosis secondary to chronic PB therapy.[26] Fifty percent of dogs with PB-associated hepatic disease had total bilirubin concentrations >1.0 mg/dL in another study.[25] Three dogs in the OSU-VTH data base with histologically confirmed cirrhosis and a history of PB administration had postprandial serum bile acid concentrations >150 μmol/L and had an average reduction in serum albumin concentration that exceeded 50% of normal values. On ultrasonographic examination, all 3 of these dogs had ascites, hepatic echogenicity was either normal or diffusely increased, and small hypoechoic nodules were not observed. The ultrasonographic appearance of diffuse, ill-defined areas of increased echogenicity is typical of hepatic cirrhosis and bears no resemblance to ultrasonographic findings in dogs with SND.[2,12] Histologic findings in the PB/hepatotoxicity dogs were similar to those reported in other dogs with PB-associated cirrhosis[25,26] and included chronic fibrosis with nodular regeneration, biliary hyperplasia, and mild inflammatory cell infiltrates and necrosis. Although the magnitude of the increase in serum bile acid concentrations might not correlate with the severity of hepatic dysfunction, the marked increase in serum bile acid concentrations, as well as the clinical and pathologic abnormalities in dogs with PB-associated hepatotoxicity and cirrhosis, signifies a severe state of hepatic insufficiency that typically is not found in dogs with SND. It is uncertain whether or not the hepatic changes in SND/PB represent a precirrhotic state, but evidence for this conclusion is lacking in the dogs of this report and in other studies.[11,23]

Increases in ALT activity above the normal reference range preceded the onset of SND by a median of 6 months in the 7 dogs in which it was serially measured, and median age at onset of skin lesions was 10 years in all dogs. Furthermore, increases in ALT activity persisted in those dogs in which PB was tapered or discontinued. Although a history of high ALT activity can be found in dogs with PB-induced hepatic cirrhosis or chronic active hepatitis (CAH), progressive signs of hepatic dysfunction, including polyuria, polydipsia, anorexia, weight loss, and icterus, also are evident in most affected dogs.[25,29–32] Age of onset in both conditions typically is <7 years, and liver disease frequently is advanced by the time clinical signs are noticed.[25,29–32] Our review of dogs with PB-associated hepatotoxicity also identified a shorter duration of PB administration (median 3.1 years for OSU cases and 4.0 years for cases in the literature[25]) compared with dogs with SND/PB (median 6 years). The high frequency of rapidly progressive signs of hepatic failure after 3–4 years of PB therapy in dogs with PB-associated hepatotoxicity contrasts with the subclinical, slowly progressive changes in hepatic biochemical parameters that precede the development of skin lesions in older dogs with SND/PB or SND/No PB. Consequently, persistently high ALT values in an older dog without accompanying signs of progressive hepatic failure could be an early warning sign of SND.

The marked reduction in serum amino acid concentrations in 1 dog of the present study and in other dogs with SND[1,4,11,12] likely is a result of excessive utilization of amino acids in hepatocytes.[11] Plasma amino acid profiles in SND are very different from those that characterize CAH or hepatic disease of other cause[11,33,34] and could, in part, be explained by excessive hepatic uptake and catabolism of circulating amino acids. The liver utilizes amino acids for gluconeogenesis and for the urea cycle.[35–39] Gluconeogenic amino acids include glutamine, alanine, threonine, and serine, but the liver also is efficient at extracting glycine and proline from portal venous blood.[35,36,40] Glucagon and catecholamines (eg, epinephrine, norepinephrine) stimulate amino acid uptake.[20,35,39,41–43] Specific carrier transport systems for different classes of amino acids are present in hepatocellular membranes.[36,44,45] Transport of gluconeogenic amino acids is competitive and limited by the saturability of the carrier transport system. Transport efficacy of these systems is determined partially by hormonal influences[44] but also is sensitive to extracellular amino acid concentrations.[45] Excess glucagon or increased adrenergic stimulation could produce a state of increased amino acid uptake by these transport systems and also stimulate increased amino acid catabolism in gluconeogenic and urea cycle pathways.[40,44–47]

The plasma amino acid profiles of dogs with SND are very similar to those observed in association with glucagonoma in dogs[4,14,15] and humans.[40] Seven dogs with SND associated with a pancreatic glucagonoma have been described.[4,5,14–19] All dogs had tumors identified at postmortem examination or during celiotomy. Hepatic metastases were present in some cases, but nonneoplastic hepatocytes were histologically normal or only mildly vacuolated. Serum glucagon concentrations were high in 5 dogs in which it was measured.[4,5,14–18] In dogs with SND but without glucagonoma in which serum glucagon concentrations have been measured, concentrations have been normal to moderately increased but not to the magnitude seen in dogs with glucagonomas.[4,6,12] Glucagon concentrations were not measured in the dogs of the present study, but neither ultrasonographic examination in 7 dogs nor complete postmortem examinations in 2 dogs identified any pancreatic mass.

Normal changes in hepatic function associated with aging could play a role in SND. In previous retrospective studies of SND in dogs, mean age of disease onset ranged from 9.1 to 10.9 years. Median age of SND onset in the present study was 10 years. Age-associated changes in hepatocellular membrane stability increase hepatocyte responsiveness to adrenergic stimulation,[41,47,48] resulting in enhanced amino acid utilization for gluconeogenesis. Membrane instability also alters the sensitivity of the cell to extracellular amino acid concentrations so that hepatocellular carrier system transport becomes poorly regulated.[49] The latter defect allows amino acid uptake to occur more readily and subjects the cell to amino acid imbalances that might be present in the circulation. Aging also has been associated with alterations in hepatic sensitivity to glucagon.[42,50,51]

Increased hepatic uptake of amino acids can be stimulated by agents that promote hepatic protein synthesis. Induction of microsomal enzymes by PB places a great demand on the hepatocyte to generate a majority of the cytochrome P450 enzymes for phase I reactions of hepatic metabolism.[28,29,52–57] PB also induces microsomal UDP glucuronyl transferase[55] and cytoplasmic ALP and ALT and stimulates a 4-fold increase in α-1 acid glycoprotein (AGP) synthesis.[27,56] AGP is an important binding protein for basic drugs in plasma.[56] PB stimulates a generalized increase in

hepatic recruitment and utilization of circulating amino acids for microsomal enzyme synthesis,[56,58–61] decreases amino acid utilization in other metabolic pathways such as the urea cycle and gluconeogenesis,[62] and decreases protein catabolism in the hepatocyte.[63] Excessive utilization of amino acids secondary to chronic microsomal enzyme induction by PB potentially could lead to depletion of amino acids that are important for normal physiologic functions in many tissues. Microsomal enzyme induction by glucocorticoids (endogenous or exogenous), thyroid supplementation, or administration of other pharmaceutical compounds likewise might increase amino acid utilization.[36,38,41,55,64,65] In a recent report of SND in dogs, at least 6 of 36 dogs had a history of recent corticosteroid administration, 3 dogs had spontaneous hyperadrenocorticism, and 2 dogs were receiving thyroid supplementation.[11] A more thorough examination of hepatic enzyme induction and synthesis in a larger population of dogs with SND is needed to determine the relative importance of these events in disease progression.

Glutamine, proline, alanine, arginine, threonine, and glycine were proportionally the most severely affected amino acids in dogs described in this and other studies of SND.[1,4,11,12] Normally, glutamine and alanine are found in the highest concentrations in plasma, and muscle and skin are the most important sources for these circulating amino acids.[35,37,66,67] Severe depletion of amino acids can affect normal dermatologic, gastrointestinal, central nervous system, and hepatic functions. Glutamine, proline, arginine, and glycine have important dermatologic functions that include differentiation of keratinocytes, synthesis of matrix metalloproteinases, and formation of elastin and collagen.[68,69] Persistent depletion of these amino acids may contribute to the hyperkeratotic skin lesions seen in SND. Glutamine is an important energy source in enterocytes and is involved in glutathione synthesis. These 2 functions promote enterocyte health and prevent necrosis or apoptosis secondary to inadequate energy stores or oxidant damage.[37,67,70] Marked glutamine deficiency could explain the gastrointestinal signs of vomiting, diarrhea, and anorexia reported in late stages of SND.[8,9,13] Glutamine also is coupled to glucose utilization in the cerebral cortex,[71] is important in removal of nitrogenous wastes and glutamate from the interstitium of the brain, and is critical for normal amino acid exchange at the blood-brain barrier.[72] Clinical signs of lethargy and depression in affected dogs probably are multifactorial, but reduced brain glucose consumption and neurotransmitter imbalances because of a glutamine deficiency could have an effect on normal brain function.[71,73]

Within hepatocytes, glutamine participates directly in ammonia detoxification, pH regulation, gluconeogenesis, and protein and nucleotide synthesis. It also stimulates bile acid secretion, inhibits proteolysis, and can be utilized as an oxidative energy source.[37,67,74] Both glutamine and glycine are utilized in phase II reactions to conjugate toxic xenobiotic intermediates.[35,55] Other amino acids have functions that involve normal microsomal enzyme activity; methylation reactions; and albumin, glutathione, and nucleic acid synthesis.[35,36] Consequently, although hepatic metabolism might be enhanced during early and moderately advanced stages of SND in dogs, hepatic function in advanced disease potentially could be compromised if amino acid concentrations in tissues are severely decreased.

The long-term prognosis for SND generally is poor. Dogs in this study were euthanized a median of 12 weeks after diagnosis. PB had been discontinued in 6 dogs, but discontinuation occurred just before the onset of skin lesions in the majority of these dogs. SND outcome in this study was similar to that reported in other studies. Twenty-four of 30 dogs with SND had a mean survival time of approximately 3 months in a recent study.[11] Three of these 30 patients had a history of PB administration, and none survived more than 8 months despite drug discontinuation.[11] Reasons for euthanasia could not be ascertained from the medical records of the dogs in this study, but lethargy, reluctance to walk, and diminished appetite were noted in many dogs at the time of euthanasia. Recurrence of seizure activity during PB withdrawal was a concern and might have been a factor in owners' decisions to euthanize their dogs. In 1 dog, signs of SND were recognized at an early stage, PB was discontinued, and oral amino acid supplementation and treatment of secondary pyoderma was initiated. This dog was still alive 1 year after the diagnosis of SND, had normal pre- and postprandial serum bile acid concentrations, and had no recurrence of skin lesions. Although this dog might be the exception, prompt recognition of SND, management of systemic disorders, withdrawal of drugs that could adversely affect hepatic metabolism, and aggressive therapy of the amino acid deficiency have improved long-term survival in dogs in other studies.[1,5,11,23,24]

The sequence of pathophysiologic events that lead to SND are not well understood, but amino acid utilization by hepatocytes appears to be up-regulated. A hypercatabolic state secondary to hormonal imbalances and changes in hepatocellular amino acid uptake with age could be an important predisposing factor. Persistent increases in ALP and ALT activity before development of skin lesions appear to be a common theme in SND even if there is no history of PB exposure. More studies of older dogs with early signs of hepatopathy but without hepatic insufficiency are needed to understand the early metabolic derangements in SND.

A history of chronic PB therapy was a common finding in affected dogs in this study. Prolonged exposure to high concentrations of PB would be expected to produce chronic microsomal enzyme induction and increased amino acid utilization. This was a retrospective study and so cannot be used to identify cause and effect. Prospective studies of serial amino acid profiles and alterations in hepatic metabolism in dogs on chronic PB therapy are warranted to assess the possible contribution of PB to the metabolic defect in SND. Comparisons of early clinicopathologic and amino acid abnormalities in both SND/PB and SND/No PB dogs might identify different pathogenic mechanisms in each condition that independently lead to the same clinical outcome. Finally, identification and selective elimination of risk factors for the development of SND in dogs will aid in assessing the relative importance of each risk factor in SND pathogenesis.

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