

# Efficacy of Pyridoxine to Ameliorate the Cutaneous Toxicity Associated with Doxorubicin Containing Pegylated (Stealth) Liposomes: A Randomized, Double-Blind Clinical Trial using a Canine Model

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## ABSTRACT

A cutaneous reaction termed palmar-plantar erythrodysesthesia (PPES) or hand-foot syndrome can be dose limiting for Doxil, a doxorubicin containing pegylated (Stealth) liposome. The objective of this study was to determine the ability of concomitant pyridoxine therapy to prevent the development of PPES during Doxil therapy. Forty-one dogs with non-Hodgkin's lymphoma were randomized in a double-blind fashion to receive either oral pyridoxine or placebo daily during Doxil chemotherapy (1.0 mg/kg, i.v., every 3 weeks for a total of five treatments). Cutaneous toxicity was determined by clinical and histological scoring. No difference was observed in remission rates (71.4 versus 75%) achieved between groups. The likelihood of developing serious PPES and having to decrease or discontinue Doxil therapy was 4.2 times (relative risk) greater in placebo group dogs than in pyridoxine group dogs ( $P = 0.032$ ). Pyridoxine did not completely abrogate PPES; however, it occurred later and less dramatically than in placebo-treated dogs and resulted in fewer treatment delays or discontinuations, allowing a higher cumulative dose of Doxil to be received. Compared to the 5.0 mg/kg cumulative target dose, pyridoxine-treated dogs received a median cumulative dose of 4.7 mg/kg (mean, 4.1 mg/kg), and the placebo-treated dogs received a median of 2.75 mg/kg (mean, 2.9 mg/kg;  $P < 0.028$ ). A trend ( $P = 0.084$ ) toward prolongation of remission length was observed in dogs receiving pyridoxine, which was likely attributable to their ability to receive more Doxil without

delay or discontinuation. We conclude that pyridoxine is effective in delaying the onset and severity of PPES in this canine model.

## INTRODUCTION

Doxil, a doxorubicin containing pegylated (Stealth) liposome, has been shown to decrease cardiotoxicity, prolong drug circulation times, and enhance tumoricidal effects (when compared to free doxorubicin) in a variety of tumor models (1–5). Unlike free doxorubicin, a cutaneous reaction termed PPES<sup>2</sup> or hand-foot syndrome can be dose limiting for Doxil in both the canine and human species and often prevents repeated dosing (6–8). PPES is a poorly understood syndrome recognized in people undergoing long-term continuous infusions of various chemotherapeutics (1, 7, 9–15). It is theorized that continuous infusions are more likely to result in PPES due to prolonged drug circulation times and subsequent cutaneous drug accumulation. Because the stealth properties of Doxil also result in prolonged circulation times, it is likely that a similar phenomenon is occurring. An alternate possibility is that Doxil may localize in skin to a greater degree than free doxorubicin by mechanisms that are unrelated to circulation time. Clinically, PPES is a painful desquamating dermatitis characterized by skin changes, ranging from mild erythema, hyperemia, and alopecia to severe crusting, ulceration, and epidermal necrosis. Similar to humans, dogs are susceptible to PPES development following Doxil therapy, and the degree of clinical signs is associated with dose intensity and repeated treatments (6, 16). Lesions occur primarily in areas of skin contact, such as the axilla, inguinal region, and the skin surrounding the foot pads. Lameness associated with apparent paw discomfort while bearing weight has been noted in a number of dogs developing toxicity. The most significant histological features observed in dogs with PPES include hyperkeratosis, follicular atrophy, perifollicular fibrosis, and multifocal follicular necrosis (6). PPES can have devastating consequences, as it may necessitate treatment delay or discontinuation, often at the expense of antitumor therapy. The development of methods to abrogate PPES in such patients could lead to continuation of effective therapy.

Anecdotal reports have suggested that oral pyridoxine (vitamin B<sub>6</sub>) may alleviate or reverse PPES (11, 17–19). In these limited studies, institution of pyridoxine allowed continuation of chemotherapy without treatment delay in the majority of patients. The mechanism by which pyridoxine may exert a pro-

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<sup>2</sup> The abbreviations used are: PPES, palmar-plantar erythrodysesthesia; NHL, non-Hodgkin's lymphoma; t.i.d., three times a day.

Table 1 Clinical scoring criteria for cutaneous toxicity

Score	Erythema	Scale/crust	Alopecia/ulceration	Edema digit/limb	Clinical signs
0	None visible = normal lesion	None visible = normal lesion	None visible = normal lesion	None visible = normal lesion	None
1	Noticeable only upon close examination of lesion	Fine scaling, noticeable only upon close examination of lesion	Sparse thinning of hair coat at site of lesion	Noticeable only upon close examination of lesion	Licking at paws, inguinal or axillary region
2	Easily visible upon close examination	Scaling easily visible but not exfoliating in clumps; minimal crusting	Denuded area at site of lesion	Easily visible upon close examination	Pruritic, active scratching of lesion
3	Easily visible from a distance of 1 m	Easily visible scaling and crusting with exfoliation upon examination	Ulceration at site of lesion	Easily visible from a distance of 1 m	Obvious pain upon palpation of lesion

tective effect against PPES is, at present, unknown. We previously observed that Doxil is effective as a single agent in achieving remissions in dogs with spontaneously arising NHL and hypothesized that such a population could serve as a model for prospectively evaluating the efficacy of pyridoxine therapy in a controlled setting. This study was designed in a randomized, double-blind fashion to determine whether pyridoxine will affect the development of PPES when given concurrently with Doxil, using dogs with NHL as a model.

## SUBJECTS AND METHODS

**Subject Population.** Forty-one client-owned dogs from the patient population presenting to the Veterinary Medical Teaching Hospital at the University of Wisconsin (Madison, WI) and Oakland Veterinary Referral Services (Bloomfield Hill, MI) from January 1996 through February 1997 were studied. Eligible patients had histologically confirmed, previously untreated, intermediate or high-grade NHL using the Working Formulation criteria (20). Pretreatment evaluation included a complete history, physical examination, complete blood count, serum chemistry profile, urinalysis, thoracic and abdominal radiographs, and bone marrow aspirate cytology. Results of this evaluation were used to establish clinical stage for each animal according to the WHO staging system for canine lymphoma (21). Dogs were eligible for entry, provided they had adequate hematological and serum biochemical parameters to undergo chemotherapy and were free of complicating concurrent disease conditions.

**Therapy.** All dogs were assigned to receive an identical single-agent chemotherapeutic protocol using Doxil at a dosage of 1.0 mg/kg i.v. every 3 weeks for five total treatments. Concomitant with the initiation of Doxil therapy, all dogs were randomized in a double-blind fashion to receive either pyridoxine (50 mg p.o., t.i.d., for 15 weeks) or placebo (lactose), in capsules of identical size and color. The primary care clinician and the patient's caregiver were unaware of the result of randomization. Dogs that did not respond to Doxil chemotherapy and dogs that relapsed after initial response were treated with varying rescue protocols at the discretion of the clinician and caregiver involved.

**Assessment of Cutaneous Toxicity.** Prior to each Doxil treatment ( $n = 5$ ), a clinical toxicity score was determined by one of four clinicians, who was blinded to the result of random-

ization, using a previously established clinical score criteria (Table 1). Additionally, prior to each treatment, a 6-mm cutaneous punch biopsy (Keyes biopsy) was procured from the medial aspect of the calf or thigh in each dog; the site of biopsy was varied such that no biopsy was within 3 cm of a previous site. All cutaneous biopsies were scored by a single pathologist (A. J. C.), who was blinded to treatment groups, timing of biopsy, and case number. The histological score was the sum of scores (0, absent; 1, minimal; 2, mild; 3, moderate; 4, severe) from each of the following 10 categories: superficial hyperkeratosis, follicular hyperkeratosis, follicular atrophy, follicular necrosis, perifollicular fibrosis, pigmentary incontinence, apocrine gland ectasia, apocrine gland epithelial vacuolization or necrosis, sebaceous gland atrophy, and inflammation.

If clinically significant cutaneous lesions developed that were determined to be dose limiting (*i.e.*, pain, lameness, edema, and/or ulceration), the treatment code was broken, subsequent treatment was delayed for a minimum of 1 week, and the Doxil dose was decreased by 20%. In addition, if the dog in question was determined to be in the placebo group, it was crossed over to receive pyridoxine from that point forward.

**Response.** Tumor volume measurements were made immediately before each treatment and categorized on the basis of their response to chemotherapy: complete response, complete regression of all measurable lymph nodes; partial response, >50% but <100% decrease in the sum of the products of the perpendicular diameters of all measurable lymph nodes; and no response, increase in or <50% decrease in the sum of the products of the perpendicular diameters of all measurable lymph nodes or the appearance of new lesions. Once Doxil therapy was completed, dogs were reassessed monthly. Duration of first remission was defined from the time of achieving remission until disease progression. Survival time was defined as the time from initiation of therapy until death.

**Statistical Analysis.** First remission duration and overall survival times were compared between the pyridoxine and placebo groups. Survival curves were generated using the Kaplan-Meier method (22). Dogs were censored in survival analysis for the following reasons: (a) lost to follow-up ( $n = 1$ ); (b) death not caused by lymphoma ( $n = 2$ ); or (c) alive at the end of the study period ( $n = 3$ ). Dogs were censored in first remission duration analysis for the following reasons: (a) relapse had not occurred before the end of the study period ( $n = 1$ ); or (b) death

Table 2 Equality of randomization for factors known to be prognostic for dogs with NHL

Factor	Pyridoxine group	Placebo group
CD3 immunoreactivity	28%	23%
Stage V	24%	30%
Substage b	24%	25%
Sex		
Female	52%	50%
Male	48%	50%
Hypercalcemia	14%	10%
Response rate	71.4%	75%

not caused by lymphoma prior to relapse ( $n = 2$ ). Censored observations are included in all figures. Treatment groups were compared using the Logrank test to determine differences between remission or survival curves (23). Changes in clinical and histological scores of cutaneous toxicity were compared within treatment groups and between treatment groups using one-way repeated measures and multiple ANOVA, respectively. The likelihood of developing serious PPES and having to decrease or discontinue Doxil therapy was compared between treatment groups using Fisher's exact test. The difference in cumulative dose of Doxil achievable between groups was compared using the independent  $t$  test. For all analyses, a  $P$  of  $<0.05$  was considered significant.

## RESULTS

**Subject Demographics and Randomization.** Subject age and body weight ranged from 2 to 15 years (mean, 6.7 years; median, 6 years) and from 4.8 to 58.4 kg (mean, 29.1 kg; median, 28.9 kg), respectively. The randomization was successful in ensuring a nearly equal proportion of factors known to be prognostic for dogs with NHL (Table 2; Refs. 24–27).

**Cutaneous Toxicity.** In all dogs, regardless of randomized grouping, clinical scores ( $P < 0.01$ ) and histological scores ( $P < 0.01$ ) of cutaneous toxicity increased significantly during the course of Doxil therapy (Figs. 1 and 2). However, the change in clinical toxicity scores in dogs receiving pyridoxine was significantly different ( $P = 0.034$ ) than that in dogs receiving placebo. The onset and degree of cutaneous toxicity, as assessed by clinical scoring, was delayed in dogs receiving pyridoxine, allowing more Doxil therapies to take place before similar changes occurred. A similar trend was noted when cutaneous toxicity was assessed by histological score; however, the difference between groups was not significant ( $P = 0.078$ ).

The probability of developing serious PPES and having to decrease or discontinue Doxil therapy was 4.2 times (relative risk,  $P < 0.033$ ) more likely in placebo-group dogs (8 of 20) than in pyridoxine-group dogs (2 of 21). This resulted in a significant difference in cumulative dose achievable (*i.e.*, target cumulative dose of 5.0 mg/kg). Pyridoxine-treated dogs received a median cumulative dose of 4.7 mg/kg (mean, 4.1 mg/kg), compared to 2.75 mg/kg (mean, 2.9 mg/kg) in dogs receiving placebo ( $P = 0.028$ ).

**Noncutaneous Toxicities.** No incidents of myelosuppression or cardiotoxicity were encountered. Mild, self-limiting gastrointestinal toxicity consisting of diarrhea and inappetence was observed in 12 dogs (5 in the pyridoxine group and 7 in the

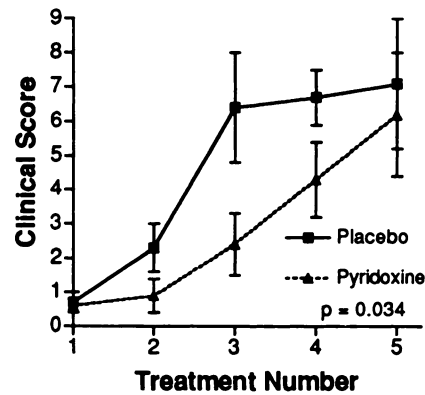


Fig. 1 Change in clinical toxicity score during Doxil therapy (1.0 mg/kg i.v., every 3 weeks for five treatments) for NHL in dogs receiving concomitant pyridoxine (50 mg p.o., t.i.d.) or placebo.

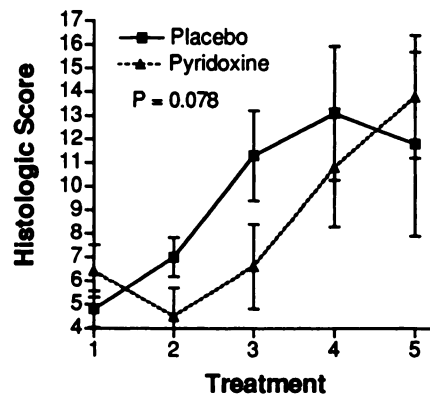


Fig. 2 Change in histological toxicity score during Doxil therapy (1.0 mg/kg i.v., every 3 weeks for five treatments) for NHL in dogs receiving concomitant pyridoxine (50 mg p.o., t.i.d.) or placebo.

placebo group). One dog in the placebo group experienced moderate gastrointestinal toxicity, including vomiting and diarrhea requiring fluid support of 1-day duration.

**Response to Therapy.** No difference was observed in remission rates between dogs receiving pyridoxine or placebo (71.4 versus 75%, respectively). All but two responders in each group were complete responders. No significant difference was observed in median first remission duration between groups (pyridoxine, 159 days; placebo, 48 days); however, a trend ( $P = 0.084$ ) toward prolongation of remission length was observed in dogs receiving pyridoxine (Fig. 3). Similarly, differences between groups for overall survival (pyridoxine, 201 days; placebo, 130 days;  $P = 0.182$ ) were not significant (Fig. 4).

## DISCUSSION

The two treatment groups compared in this study were typical of historical populations of dogs with NHL with respect to age, sex, body weight, and frequency of factors known to be associated with a more negative prognosis (Table 2; Refs. 24–27). Importantly, the frequencies of known prognostic factors

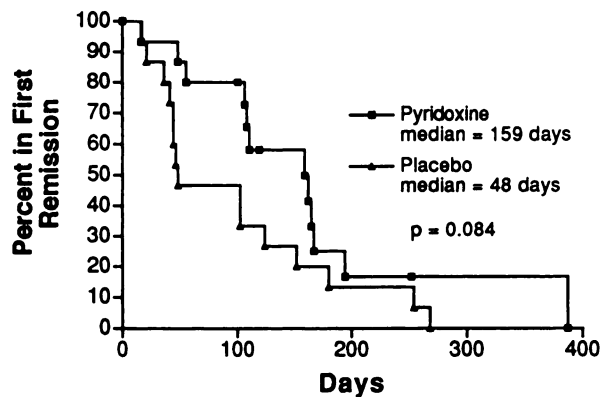


Fig. 3 Kaplan-Meier first remission duration estimates for dogs with NHL following Doxil therapy (1.0 mg/kg i.v., every 3 weeks for five treatments) receiving concomitant pyridoxine (50 mg p.o., t.i.d.) or placebo.

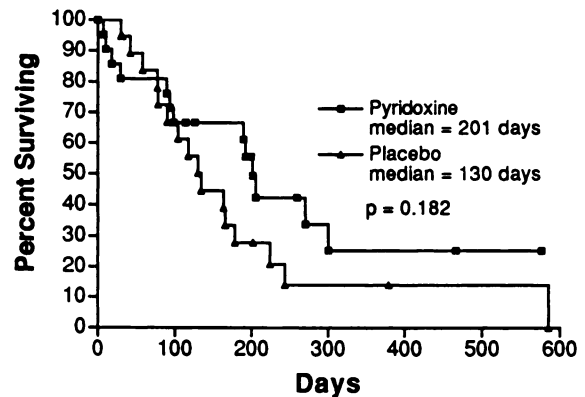


Fig. 4 Kaplan-Meier overall survival estimates for dogs with NHL following Doxil therapy (1.0 mg/kg i.v., every 3 weeks for five treatments) receiving concomitant pyridoxine (50 mg p.o., t.i.d.) or placebo.

were nearly equal between the two groups, indicating a successful randomization.

The dose of pyridoxine chosen for this trial was based on the dose described in several anecdotal reports of pyridoxine use in people with PPES (11, 17–19). The daily dose used (50 mg p.o., t.i.d.) is ~100 times greater than the published daily nutritional requirements for pyridoxine in the dog (28, 29).

The cutaneous toxicity PPES, associated with the use of Doxil, has been described previously in both humans and dogs (1, 6–16). The incidence of PPES is known to increase with repeated Doxil treatments, as was observed in the population under study. The 40% incidence of dose-limiting PPES in our placebo group was higher than that previously reported in our Phase I trial (6), likely due to the consistent use of the maximally tolerated dose established in that study and the greater number of treatments per dog used in this trial. Concomitant pyridoxine therapy did, indeed, result in a difference in cutaneous toxicity over time when clinical score was used as the criterion. Pyridoxine did not completely abrogate PPES; rather, it delayed the onset of lesions and allowed a greater number of treatment events and, subsequently, a greater cumulative dose to be received by the dogs in study. How pyridoxine, a water-soluble vitamin that plays an important role in many basic and essential cellular functions, exerts a protective effect against Doxil-induced PPES is, at present, unknown. A potential drawback of the clinical scoring system used in this study is that it is only semiobjective, requiring the observer to estimate the degree of severity. However, limiting the choices based on the proximity to the lesion where changes become obvious added some degree of objectivity. To maximize reliability, clinicians initially compared clinical scores in several cases to gain familiarity with the scheme, all were blinded to the treatment group in each case, and a nearly even distribution of the individual clinicians performing the scoring was found in each of the two treatment groups (data not shown).

Although a similar trend ( $P = 0.078$ ) toward pyridoxine protection was observed when histological score was used as the criteria, this difference was not significant. This may, in part, be due to the choice of location for biopsy procurement established

*a priori*. It was felt that repeated cutaneous biopsies of the sites more commonly associated with lesion development (*i.e.*, axilla, inguinal region, and the skin surrounding the foot pads) would have resulted in considerable morbidity in and of itself; therefore, the medial aspect of the calf or thigh was chosen. In retrospect, although histological changes were noted in the areas biopsied, more dramatic differences may have existed at other sites.

Response rates observed in dogs in this study are similar to those reported previously in dogs with NHL treated with dose-equivalent single-agent doxorubicin (30–32). The observed trend toward prolonged first remission durations in dogs in the pyridoxine group is likely attributable to their ability to receive a higher cumulative dose of Doxil without delay or discontinuation. The duration of first remission and overall survival observed in dogs in the pyridoxine group is also similar to those reported previously in dogs with NHL receiving dose-equivalent single-agent doxorubicin (30–32). Importantly, similar response durations with historical controls, taken together with the lack of an observed difference in response rates between pyridoxine and placebo groups, do not support an adverse effect on response with the addition of pyridoxine. This was an initial concern as an earlier report on the use of pyridoxine to abrogate neurotoxicity in women receiving cisplatin therapy for advanced ovarian carcinoma observed an adverse effect on remission durations (33). Other studies have failed to demonstrate a negative effect of pyridoxine on response to chemotherapy (18, 19).

Taken together, the findings in this study support the protective affects of pyridoxine for delaying the development of PPES resulting from Doxil therapy. On the basis of these results, concomitant use of pyridoxine should be explored in humans undergoing Doxil chemotherapy for a variety of tumor types. Protection from PPES would allow more individuals to continue with effective antitumor therapy without potential dose reduction or delay.

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