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Prednisolone inclusion in a first-line multidrug cytostatic protocol for the treatment of canine lymphoma does not affect therapy results

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ABSTRACT

Chemotherapy protocols for canine lymphoma include the routine use of glucocorticoids for their lympholytic effect. However, glucocorticoids are associated with side effects (e.g. polyphagia, polyuria, and weight gain), limit the use of non-steroidal anti-inflammatory drugs, and can induce drug transporter expression that could lead to drug resistance. Despite these negative effects, there are no data to support the use of glucocorticoids as part of a multidrug chemotherapy protocol for the treatment of canine lymphoma.

A prospective, randomized clinical trial was conducted in 81 dogs with multicentric lymphoma and no history of recent glucocorticoid use. All dogs were staged and treated with the same chemotherapy protocol (L-asparaginase, cyclophosphamide, doxorubicin, vincristine, and prednisolone) with half of the dogs receiving prednisolone. Both treatment groups were similar with respect to demographics, immunophenotype, and clinical stage, except for a higher number of substage b patients in the prednisolone group (5 vs. 14; $P = 0.015$). Treatment results obtained with the initial treatment (complete response rate 75%, disease-free period 176 days) and rescue treatment (complete response rate 45%, disease-free period 133 days), overall survival (283 days) and adverse events (number and grade) were similar for both groups. In conclusion, prednisolone, as part of a multidrug chemotherapy protocol, has no additional effect on treatment results and can be omitted from first-line multidrug protocols used for the treatment of canine lymphoma.

Introduction

Canine lymphoma (cL) is the most common haematopoietic neoplasia in the dog (Teske, 1994; Dobson et al., 2002) and typically presents as generalized lymphadenopathy (Vezzali et al., 2010). Treatment for cL has evolved from a monotherapy with glucocorticoids (GCs) (Squire et al., 1973) to a multidrug therapy with the GC prednisolone (P) and cytostatic agents; those included initially cyclophosphamide (C) and vincristine (O) (COP protocol) (Cotter and Goldstein, 1983), and later also doxorubicin (H) (CHOP protocol) (Teske et al., 1994a; Myers et al., 1997; Moore et al., 2001; Garrett et al., 2002).

CHOP-based protocols are the current standard of care for treating cL like in human oncology prior to the introduction of rituximab (Fisher et al., 1993). They resulted in a 70–90% complete response (CR) rate and a disease-free period (DFP) of 9–11 months (Vail et al., 2013). Since only a small proportion of dogs is cured, most animals will experience tumour recurrence after which the disease is more difficult to control (Flory et al., 2011).

The routine use of GCs in the treatment of lymphoid neoplasia, including cL, is based on the fact that GCs are capable of inducing apoptosis of lymphoid cells (Ammersbach et al., 2006; Kfir-Erenfeld et al., 2010). However, GCs are associated with adverse effects (e.g. polydipsia, polyuria, polyphagia and weight gain), predispose to subclinical (urinary tract) infections (Torres et al., 2005) and diabetes mellitus (Jeffers et al., 1991), muscle atrophy, and have negative effects on the quality of tendons and ligamentous structures (Rewerts et al., 1997). Furthermore GCs limit the concurrent use of non-steroidal anti-inflammatory drugs (NSAIDs) and GC administration prior to starting chemotherapy has been associated with a poorer prognosis (Price et al., 1991; Piek et al., 1999; Gavazza et al., 2008; Marconato et al., 2011).

Failure of a tumour to respond to chemotherapy can result from tumour cell resistance to the cytotoxic agents used. Although drug resistance can arise through various mechanisms (Gillet and Gottesman, 2010), overexpression of drug transporters of the ATP-binding cassette superfamily, and P-glycoprotein (Pgp) in particular, appears to be the most important mechanism in human oncology (Gottesman et al., 2002). In dogs, Pgp expression was demonstrated in therapy-resistant and relapsed cL cases and correlated with a poorer

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response to chemotherapy and shorter survival (Bergman et al., 1996; Lee et al., 1996; Steingold et al., 1998).

GCs can increase Pgp expression in human lymphocytes, both in vitro (Manceau et al., 2012) and in vivo (Wasilewska et al., 2006), as well as in the dog (Allenspach et al., 2006). It can therefore be speculated that GCs can induce Pgp expression in neoplastic lymphoblasts, leading to the development of drug resistance, which would explain poorer therapy results in relapsed cL cases. On the other hand, removing the lympholytic effect of GCs from a chemotherapy protocol could lead to inferior therapy results.

To establish the effect of prednisolone within a multidrug chemotherapy protocol, a prospective, randomized study was conducted. Dogs, which had just been diagnosed with multicentric lymphoma and had no recent treatment with GCs, received the same doxorubicin-based multidrug chemotherapy protocol, but were randomly assigned to receive GCs or not. Our hypothesis was that prednisolone use would not affect treatment results in the first treatment round, but would represent a negative factor on treatment results following relapse.

Material and methods

Patient population

All dogs were privately owned and presented to the Small Animal University Clinic at the Faculty of Veterinary Medicine, Utrecht University between September 2005 and January 2010 for the treatment of multicentric lymphoma. Dogs that had received steroids <3 months prior to diagnosis or required NSAIDs were excluded from the study. Breed, sex, age and weight were recorded. Stage and substage were determined using the World Health Organization (WHO)-staging system for cL (Owen, 1980). The minimum diagnostic dataset obtained for each dog included a haematological and clinical chemistry profile, bone marrow aspiration biopsy, and cytological examination of a lymph node aspirate to confirm the diagnosis of cL, establish grade (high vs. low according to Updated Kiel after Ponce et al. (2010) and Teske et al. (1994b)) and immunophenotype using CD79a (pan–B marker) and CD3 (pan–T marker) (Dako) (Aulbach et al., 2010). Thoracic radiographs, abdominal ultrasound or other diagnostic tests were performed when indicated by clinical signs.

The study was evaluated and approved by the Research Scientific and Ethical Committee at Utrecht University. Following an informed consent from the owners, dogs were randomized to one of the treatment groups, chemotherapy 'with' or 'without' prednisolone. A randomization scheme was generated for 120 dogs (60 per group) and the randomization for each individual was enclosed in consecutively numbered, sealed envelopes. Dogs were numbered based on order of entry into the study after which randomization was performed by opening the corresponding envelope. Because the signs associated with GC use (polydipsia, polyphagia) might prohibit a blinded study, no placebo was used for the dogs in the 'without prednisolone' group.

Treatment

All dogs received the same doxorubicin-based multidrug protocol (Table 1) and some received prednisolone (Table 2). A complete haematological analysis was performed immediately before each treatment with doxorubicin. Prior to doxorubicin treatment, the dogs in the 'prednisolone' group received an intravenous (IV) injection of dexamethasone, and those in the 'without prednisolone' group were given the antihistamine clemastine (2 mg/m^2 IV). In case of a clinically relevant chemotherapy-related adverse event, the scheduled treatment was either delayed or a 20% dose reduction was applied. Following relapse, further treatment was dependent on owner's wishes and individual clinician's preferences.

Laboratory results

Anaemia was defined as a haematocrit <0.42 L/L (mild anaemia 0.35–0.42 L/L), leukopenia as a total white blood cell count <4 × 10^9/L, thrombocytopenia as a thrombocyte count <144 × 10^9/L (mild thrombocytopenia 100–144 × 10^9/L), hypoalbuminaemia as a plasma albumin concentration <26 g/L and hypercalcaemia as a plasma calcium level >3.0 mmol/L.

Response and toxicity

Response was assessed by palpation of peripheral lymph nodes. Objective response (CR; PR, partial response; SD, stable disease; PD, progressive disease), DFP, response duration (RD), time to progression (TTP), and overall survival (OS) were recorded using the definitions proposed in the Veterinary Co-Operative Oncology Group (VCOG) consensus document (Vail et al., 2010).

In the first and second treatment round DFP, RD and TTP were calculated for those dogs that received treatment with cytostatic drugs. For dogs that received a monotherapy with GCs only TTP was calculated. All deaths during the study were considered disease- or treatment-related, except for those cases where a clear cause of death was established that was not connected with lymphoma or chemotherapy. All dogs alive at the end of the study were censored at the time of analysis. Toxicity was graded based on the VCOG criteria for adverse events (VCOG, 2004).

Statistical analysis

Results were described per group as frequencies (categorical variables) or mean/median (continuous variables). Differences between groups were tested for significance with the Chi Square or Fisher Exact test for categorical variables and either the Kruskal–Wallis test (non-parametric) or the unpaired *t* test (parametric) for continuous variables after testing for normality. The Kaplan–Meier product limit method was used for estimating DFP, RD, TTP and OS and tested for significance (Log-rank or Breslow method). Prognostic variables for outcome (DFP first and second treatment round, OS) were individually assessed in the univariate Cox Proportional Hazard analysis and variables with $P < 0.20$ were subsequently included in a multivariate analysis, with a backward elimination procedure. The level of significance was set at $P < 0.05$. Significant differences are indicated in the graphs and mentioned in the text, unless otherwise stated. All statistical analyses were performed using the software package IBM SPSS Statistics v.9.

Results

Patient population

Eighty-eight dogs were entered into the study, but seven cases were excluded from the final analysis due to failure to return for further therapy after the first treatment (three based on owner's decision, one dog died from gastric dilatation volvulus), breach of therapy protocol (two cases) and improper inclusion (one case).

The remaining 81 dogs were equally distributed over both treatment groups (41 with no prednisolone vs. 40 with prednisolone) and included eight mixed-breed dogs and 73 purebred dogs representing 35 different breeds. The most commonly represented dog breeds were the German shepherd, Rottweiler (six of each), Bernese mountain dog, Boxer, Golden retriever (five each), English Cocker Spaniel, Flat-coated retriever (four each), Dogue de Bordeaux, Bullmastiff (three each), Border collie, Bouvier des Flandres, Great Dane, Rhodesian Ridgeback, Tibetan terrier, West Highland White terrier (two each) and the other 20 breeds were represented by one dog each. There were 50 male (24 intact, 26 neutered) and 31 female (6 intact, 25 spayed) dogs with a mean age of 7.5 years

Table 1
Chemotherapy protocol.

Day	1	8	22	33	43	57	60	71	80	92	113	127–148
L-asparaginase (10.000 IU/m^2 IM)	×											
Doxorubicin (30 mg/m^2 IV)		×	×		×			×		×		
Chlorambucil (25 mg/m^2 PO over 2 days)				×					×			
Vincristine (0.5–0.7 mg/m^2 IV)						×						
Cyclophosphamide (200–250 mg/m^2 PO)							×				×	
Chlorambucil (2 mg/m^2 once daily PO)												×

IM, intramuscular; IV, intravenous; PO, per os.