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Polyethylene Glycol-L-asparaginase versus Native L-asparaginase in Canine Non-Hodgkin's Lymphoma

Erik Teske, Gerard R. Rutteman, Peter van Heerde and Wim Misdorp

42 dogs with non-Hodgkin's lymphoma (NHL) were randomized for treatment with either PEG-L-asparaginase 10 IU/kg intramuscularly (n = 22) or L-asparaginase 400 IU/kg intraperitoneally (n = 20). Another 20 dogs were treated with either PEG-L-asparaginase 30 IU/kg (n = 10) or L-asparaginase 400 IU/kg (n = 10). Each treatment protocol consisted of two asparaginase treatments followed by a 10-week period of induction chemotherapy and then maintenance on asparaginase until progression occurred. No significant differences were found between treatments in the response rates after 2 weeks of asparaginase therapy or in the time to relapse, the time to treatment failure or the remission period. The reaction to asparaginase after the initial 2 weeks was a prognostic factor for the total duration of remission under asparaginase maintenance therapy. No side-effects were noted in the dogs treated with PEG-L-asparaginase, whereas 14 (48%) of the L-asparaginase treated dogs had side-effects related to this drug, including anaphylactic shock (9), anorexia or vomiting (4), hypersensitivity-related oedema (3), seizures (1) and acute pancreatitis (1). No abnormalities in clotting times, fibrinogen levels or antithrombin-III levels were found in any of the 62 dogs. PEG-L-asparaginase has the same anti-tumour activity as native L-asparaginase in dogs with NHL, but lacks side-effects.
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INTRODUCTION

ALTHOUGH L-asparaginase has been used extensively for more than two decades in man to treat acute lymphoblastic leukaemia of childhood and occasionally some other haematopoietic malignancies [1, 2] the drug has some serious disadvantages. Apart from coagulation disorders [3–5] and pancreatitis [6], major limitations are its strong immunogenicity [1, 2, 6, 7] and short plasma half-life (approximately 18 h) [8]. L-asparaginase from *Escherichia coli* has been modified by covalent attachment to monomethoxypolyethylene glycol (PEG). The polymer-enzyme conjugate (PEG-L-asparaginase) has a prolonged plasma half-life of 16–25 days and minimal immunogenicity [9]. In preliminary studies PEG-L-asparaginase was effective against murine tumours and canine malignant lymphomas [10]. Malignant lymphomas in dogs are a good experimental–therapeutic model for non-Hodgkin's lymphoma (NHL) in man [11]. The spontaneous lymphomas predominantly affect middle-aged dogs and spontaneous regression is rare. Average survival without treatment is 6 weeks. Many categories of human NHL recognized by histological and immunological methods also occur in dogs [12]. L-asparaginase alone or in combination chemotherapy has been used successfully in the treatment of malignant lymphomas in the dog [13, 14]. Our objective was to compare PEG-L-asparaginase with L-asparaginase in dogs with spontaneous malignant lymphomas.

MATERIALS AND METHODS

Animals

From 1986 to 1988, 62 dogs with malignant lymphoma, admitted to the Clinic for Companion Animals of the University of Utrecht, were studied. Eligibility was based upon cytological diagnosis by fine-needle aspiration biopsy with histological confirmation on a surgically excised lymph node. A histological classification was applied according to the Working Formulation [15]. For staging a haematological profile, a bone-marrow aspirate and thoracic and abdominal radiographs were evaluated. The modified World Health Organization staging system for canine lymphosarcoma was used [16]. Exclusion criteria were previous cytostatic treatment (including hormonal therapy), the absence of measurable tumour disease, the presence of another life-threatening disease and score less than 3 on an adapted performance scale [17].

Randomization and treatment

Dogs in groups A1 and A2 received native L-asparaginase 400 IU/kg intraperitoneally, those in group B received PEG-L-asparaginase 10 IU/kg intramuscularly and those in group C received PEG-L-asparaginase 30 IU/kg intramuscularly. The first 42 dogs were randomized without stratification to group A1 (n = 20) or group B (n = 22). The subsequent 20 dogs were randomized to group A2 (n = 10) or group C (n = 10) (Table 1). There were no significant imbalances between the groups for age, weight, stage, performance scale or histological classification. According to the protocol (Table 2) the dogs received asparaginase for the first 2 weeks, combination chemotherapy for the following 10 weeks and then asparaginase every other week. When the disease began to progress, asparaginase was discontinued and a second-line combination (usually cyclophosphamide, vincristine, doxorubicin and prednisone) was instituted.

Table 1. Dogs' characteristics

	Group			
	A1	A2	B	C
Number	20	10	22	10
Median age (range)	6 (2–11)	6 (2–10)	7 (3–14)	5 (4–13)
M/F	9/11	6/4	10/12	5/5
Clinical stage				
III	8	4	6	6
IV	4	2	6	3
V	8	4	10	1
Performance scale				
0	4	2	6	2
1	6	4	6	3
2	4	2	8	3
3	5	2	2	2
4	1	0	0	0
Working formulation				
Low grade	3	2	2	0
Intermediate grade	16	8	19	9
High grade	1	0	0	1
Miscellaneous	0	0	1	0

Response criteria

In all dogs a complete physical examination, including three-dimensional measurement of lymph nodes, and a haematological evaluation were done at the start and were repeated after the first two doses of asparaginase and thereafter at each treatment.

Disappearance of all signs of disease was considered to be a complete response (CR). Decrease in total tumour size by greater than 50% was defined as a partial response (PR), provided that no new lesions or progression of any lesion developed. No change (NC) was defined as less than 50% decrease in tumour size and no increase of more than 25% in any of the measurable

Table 2. Induction chemotherapy

	Week												
Drug	1	2	3	4	5	6	7	8	9	10	11	12	14
Asparaginase*	+	+		+		+			+		+	+	+
Vincristine†			+		+			+		+			
Cyclophosphamide‡				+									
Doxorubicin§						+					+		
Chlorambucil‖									+				
Prednisone¶			+	+	+	+	+	+	+				

*See text. †0.6 mg/m² intravenously. ‡200 mg/m² orally. §30 mg/m² intravenously. ‖30 mg/m² orally. ¶2 mg/kg descending to 0.5 mg/kg daily orally.

Correspondence to E. Teske.
E. Teske, G.R. Rutteman and W. Misdorp are at the Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, State University of Utrecht, Postbox 80.154, 3508 TD Utrecht; and P. van Heerde is at, and W. Misdorp is also at, the Department of Pathology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

lesions. A 25% or more increase in the size of one or more measurable lesions, or the appearance of new lesions, was considered as progressive disease (PD). Response rate was defined as the percentage of CR and PR.

The following endpoints were used [18]. Survival was the interval between random assignment for treatment and death or the date on which the dog was last known to be alive, including all dogs, and counting all deaths as events. Time to treatment failure (TTF) was the interval between randomization date and relapse (for those dogs achieving CR or PR), or disease progression (for dogs known to progress), or treatment-related death or the date when the dog was last known to be alive, including all dogs and counting relapses, progressions and treatment-related deaths (not deaths resulting from unrelated causes) as events. Time to relapse (TTR) was the interval between randomization date and relapse or the date on which the dog was last known to be free of disease, including only dogs with CR, and counting only relapses as events. Remission time was the interval between the first day of CR or PR and first relapse.

Coagulation studies

Before every treatment blood samples were collected in 3.8% sodium citrate. Plasma was obtained by centrifugation at 2000 *g* for 10 min at 4°C. Prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen levels were measured by clotting assays. Antithrombin III (AT-III) was assayed by a chromozym substrate method (Boehringer Mannheim Diagnostics) in which absorbance was measured at 405 nm and AT-III levels were computed from the mean change in absorbance.

Statistics

Differences among treatment groups were evaluated by the χ^2 test for sex, by the Kruskal–Wallis test for ordinal or ratio data (stage, performance scale, histological grading and treatment responses) and by one-way parametric analysis of variance for interval data (age, weight). Survival curves were drawn with the Kaplan–Meier method. Tests for comparison of groups of survival data were made with the log rank test. The 95% CI for the proportions (P) were calculated as $P \pm (1.96 \times$ S.E.). CI for medians were calculated with tables [19]. Differences were considered to be significant at $P < 0.05$.

RESULTS

Response

The response rates observed in each treatment group are shown in Table 3. After the first two treatments with asparaginase CR rates were higher in the two groups receiving PEG-L-asparaginase, but this difference was not significant. The differences in response rates after induction chemotherapy were also not significant. The median durations of TTR, TTF and total survival tended to be higher in the groups receiving PEG-L-asparaginase than in those receiving native L-asparaginase, but the differences were not significant (Table 4). The values for group C may be an underestimation of the real values, as most of these dogs are still in remission.

Table 3. Reponse to first two treatments with asparaginase (A) and subsequent induction chemotherapy (CT)

	CR		PR		NC		PD	
Group	A	CT	A	CT	A	CT	A	CT
A1	3	15	9	3	8	1	0	1
A2	2	8	8	2	0	0	0	0
B	6	18	10	1	6	2	0	1
C	4	8	6	2	0	0	0	0

Table 4. Median duration of responses (95% CI)

Response times (days)	Group A1	A2	B	C
TTR	205 (84–294)	245 (70–259)	217 (147–420)	309 (105–476)
TTF	150 (70–245)	199 (70–259)	171 (105–305)	280 (105–476)
Remission	126 (63–238)	196 (56–256)	168 (119–294)	259 (91–462)
Survival	220 (98–483)	234 (70–406)	325 (119–588)	386 (112–476)

Since no significant differences were found in the response rates and durations of responses between the paired treatment groups, the time data of groups A1 and A2 were combined and those of groups B and C were combined. The median TTR was 266 days (95% CI 161–420) in the combined PEG-L-asparaginase group and 245 days (95% CI 112–259) in the native L-asparaginase group. The median TTF was 210 days (95% CI 140–308) and 154 days (95% CI 84–245), respectively, and the median remission time was 206 days (95% CI 133–294) and 133 days (95% CI 84–224), respectively. Survival curves for these groups are shown in Fig. 1. The 1 year estimated survival rate was 52% for the PEG-L-asparaginase group and 38% for the native L-asparaginase group. The difference between the two survival curves was not significant.

The duration of remission on maintenance therapy with asparaginase was related to the response to the first two treatments with asparaginase. The remission time in dogs with CR after the first 2 weeks of therapy (median 286 days) was significantly longer than that in those with NC (median 77

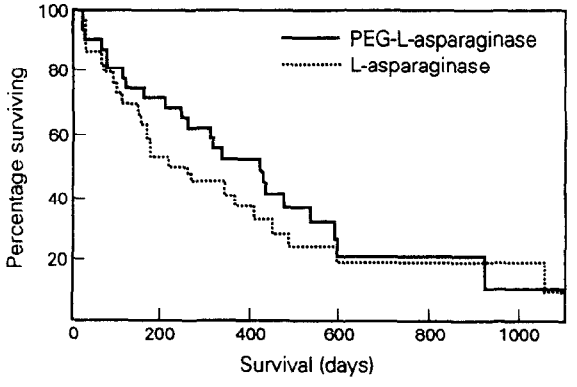

Fig. 1. Survival of dogs treated with PEG-L-asparaginase (groups B and C, $n = 32$) and dogs treated with native L-asparaginase (groups A1 and A2, $n = 30$).

days). Even dogs with PR tended ($P = 0.06$) to have a shorter remission time (median 140 days) than dogs with CR.

Toxicity

No side-effects of PEG-L-asparaginase were noted with doses of either 10 or 30 IU/kg and the intramuscular injections were well tolerated. In contrast, 14 (47%) of the dogs treated with native L-asparaginase had some side-effects, beginning between the 2nd and the 36th week: anaphylactic shock (9 dogs), oedema (3), anorexia (2), vomiting (2), acute pancreatitis (1) and seizures (1). PT, APTT and fibrinogen levels were measured in all dogs at every treatment. Apart from an incidental small prolongation of APTT, there were no abnormalities in clotting times and fibrinogen levels in any treatment group. AT-III was measured in 7 dogs in both the native L-asparaginase and the PEG-L-asparaginase groups: no decrease was found.

DISCUSSION

Despite the longer plasma half-life of PEG-L-asparaginase we did not find its anti-tumour effects to be better than those of native L-asparaginase. Neither the low nor the high dose of PEG-L-asparaginase was significantly better than native L-asparaginase as determined by the response rate after two injections or the duration of TTR, TTF and remission times. Median remission times we saw after PEG-L-asparaginase were similar to that (126 days) reported by MacEwen *et al.* [10]. The small differences in TTR, TTF and remission time between the high and the low doses of PEG-L-asparaginase were not significant and might have been due to a time-dependent effect, since the results were also slightly better in the second group of dogs treated with native L-asparaginase. Since the effect of PEG-L-asparaginase was not dose-dependent, it is unlikely that the lack of a difference in its efficacy from that of native L-asparaginase was the result of too low a dosage.

The initial response to two doses of asparaginase alone appeared to be prognostic for the eventual duration of remission on asparaginase maintenance therapy. The short median remission time (77 days) in dogs having NC after starting asparaginase therapy suggests that this drug can be omitted in further treatment of such dogs, especially in view of the contribution of the induction combination chemotherapy (63 days) to the duration of the remission.

In man, the side-effects of native L-asparaginase are well known and can be divided into those related to the immunogenicity of the enzyme and those resulting from inhibition of protein synthesis due to depletion of asparagine in the plasma. Clinical signs due to hypersensitivity may vary from urticaria to hypotension, bronchospasm and cardiac arrest [2, 6]. Nausea and vomiting also occur. Inhibition of protein synthesis may lead to hypoalbuminaemia, and a decrease in clotting factors and AT-III, such that haemorrhagic complications or hypercoagulability occur [3–5, 20, 21]. Hypersensitivity reactions, thrombosis and haemorrhages have also been reported in dogs as complications in L-asparaginase therapy [14, 22, 23]. In our study, 47% of the dogs treated with L-asparaginase had side-effects. Anaphylactic shock was seen most frequently, but urticaria, anorexia and vomiting also occurred and 1 dog had acute pancreatitis, which has been reported earlier in both man and dog [6, 22]. Diminished plasma asparagine or glutamine levels have been related to cerebral dysfunction during asparaginase therapy and may be the reason for the seizures seen in 1 dog [6]. The side-effects of asparaginase in our dogs appeared during the second week or later, as they do in man [24]. In contrast, there were no side-effects in the dogs treated with PEG-L-asparaginase. Others have reported that PEG-L-asparaginase caused no side-effects in dogs, even at doses of 200-1200 IU/kg [10].

No abnormalities in clotting times, fibrinogen levels or AT-III levels were observed in our study. Since the plasma half-life of native L-asparaginase is only about 18 h, weekly or bi-weekly injections would not be expected to deplete coagulation factors, but PEG-L-asparaginase apparently also had no adverse effects on blood coagulation, despite its much longer plasma half-life. Because the antitumour activity of PEG-L-asparaginase was at least as effective as that of native L-asparaginase and the drug lacked toxic side-effects, and combined with the long plasma half-life of PEG-L-asparaginase, study of this drug in the treatment of human lymphoid malignancies may be worthwhile.

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An Oestradiol-linked Nitrosourea and Site-directed Chemotherapy in Mammary Carcinoma

B. Betsch, M.R. Berger, B. Spiegelhalder, D. Schmähl and G. Eisenbrand

The half-life, peak concentration, peak accumulation and tissue availability of the DNA-crosslinking nitrosourea 1-(2-chloroethyl)-1-nitrosocarbamoyl-L-alanine (CNC-alanine) and its oestradiol-linked derivate (CNC-alanine-oestradiol-17-ester) were studied in liver, lung, spleen, uterus and mammary carcinomas in female Sprague-Dawley rats with chemically induced mammary carcinomas. Compared with CNC-alanine, the ester had a longer half-life, higher peak concentration, increased peak accumulation and enhanced tissue availability in all tissues. In oestradiol receptor positive mammary carcinomas, the oestradiol-linked drug showed a 2 times higher peak concentration, a 5 times longer half-life, a 10 times increased peak accumulation and a 20 times greater tissue availability compared with CNC-alanine. Oestradiol-linked nitrosoureas may offer new perspectives for site-directed chemotherapy of oestradiol receptor positive breast cancer.
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INTRODUCTION

OESTRADIOL-LINKED nitrosoureas have modified pharmacokinetic [1–3] and antitumour properties compared with the corresponding single agents. Increased half-life ($T_{1/2}$) and volume of distribution of the conjugate cannot fully explain the pharmacodynamic superiority of 1-(2-chloroethyl)-1-nitrosocarbamoyl-L-alanine-oestradiol-17-ester (CNC-alanine-oestradiol-17-ester). For the improved antineoplastic activity and decreased toxicity that have been observed in preclinical studies [4–6], a specific pattern of drug distribution can be supposed [3, 7]. Although preferential drug distribution into oestradiol receptor positive tumours is the rationale for the use of oestradiol-linked anticancer agents, there is hardly any knowledge about the disposition of such drugs [8].

For site-directed chemotherapy of receptor positive breast cancer the hormone should function as a carrier [9] that leads to receptor-mediated drug accumulation in receptor positive mammary carcinoma cells [10, 11]. In this way, at least some sparing of non-target tissues should be achievable, allowing more selective, less toxic chemotherapy.

We have studied the disposition of CNC-alanine and its oestradiol conjugate in receptor positive tissues (uterus and mammary carcinomas), the liver and in receptor negative tissues (lung and spleen) of rats with chemically induced mammary carcinoma.

Correspondence to B. Betsch.
B. Betsch, M.R. Berger, B. Spiegelhalder and D. Schmähl are at the Institute for Toxicology and Chemotherapy, German Cancer Research Center, Heidelberg, and G. Eisenbrand is at the Department of Food Chemistry and Environmental Toxicology, University of Kaiserslautern, F.R.G.

MATERIALS AND METHODS

Drugs

CNC-alanine and CNC-alanine-oestradiol-17-ester were synthesized [7] and checked for purity by thin-layer chromatography and nuclear magnetic resonance. CNC-alanine and CNC-alanine-oestradiol-17-ester were dissolved in physiological saline and dimethylsulphoxide, respectively. Equimolar doses (137 μmol/kg) were administered via the tail vein, corresponding to 30 mg/kg CNC-alanine and 66 mg/kg CNC-alanine-oestradiol-17-ester.

The relative binding affinity (RBA) to an oestradiol receptor preparation from calf uterus cytosol is known for both compounds [7]. For CNC-alanine no oestradiol receptor affinity was detectable (RBA = 0%), while for CNC-alanine-oestradiol-17-ester an RBA of 1% was measured compared with that for oestradiol (100%).