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Evaluation of PEG-L-asparaginase in asparagine suppression and anti-drug antibody development in healthy Beagle dogs: A multi-phase preclinical study

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ABSTRACT

L-asparaginase is a frequently used drug in the treatment of canine malignant lymphoma. Since production and availability of native *E. coli*-derived L-asparaginase are limited, PEG-L-asparaginase (PEG-ASP) is an alternative. However, recommended doses and dosing intervals are mainly empirically determined. A multi-phase clinical dose-finding study with seven healthy Beagle dogs was conducted to find the minimum effective dose and, potentially, a dosing interval for PEG-ASP in dogs. Plasma concentrations of amino acids and PEG-ASP activity were measured at various time points after administration of different doses of PEG-ASP. Anti-PEG and anti-asparaginase antibody titres were measured.

Administration of 10 IU/kg PEG-ASP resulted in asparagine depletion in all dogs, albeit for various durations: for 9 days in all dogs, 15 days in five dogs, 21 days in three dogs and 29 days in one dog. Asparagine suppression occurred at PEG-ASP plasma concentrations < 25 IU/L. Subsequent administrations of a second and third dose of 20 IU/kg and 40 IU/kg PEG-ASP resulted in asparagine suppression at < 9 days in five dogs, accompanied by the development of antibodies against PEG and L-asparaginase. Two dogs with prolonged asparagine suppression after the second and third administration did not develop antibodies. Marked individual variation in the mechanism and duration of response to PEG-ASP was noted. Antibody formation against PEG-ASP was frequently observed and sometimes occurred after one injection. This study suggests that PEG-ASP doses as high as the currently used dose of 40 IU/kg might not be needed in treatment of canine malignant lymphoma.

Introduction

L-asparaginase (ASP) is a cornerstone drug in the treatment of human acute lymphoblastic leukaemia and lymphoma and has therapeutic efficacy in treating canine malignant lymphoma (mL; Hill et al., 1967; Valerius et al., 1997; Jeffreys et al., 2005; Kamen, 2005; MacDonald et al., 2005; Rizzari et al., 2013; Cawley et al., 2020). ASP can be part of a first-line L-CHOP treatment, as a monotherapy or as part of a rescue treatment (MacEwen et al., 1987; Piek et al., 1999; Jeffreys et al., 2005; MacDonald et al., 2005; Cawley et al., 2020; Nakagawa et al., 2022). ASP is an enzyme that catalyses the conversion of asparagine to aspartic acid and ammonia. Aspartic acid can be converted back into asparagine in normal cells with the help of the enzyme asparagine synthetase. This enzyme is not expressed in leukaemic cells, making them dependent on extracellular sources of asparagine (Ho et al., 1970; Story et al., 1993). Treatment with ASP causes a reduction in serum

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asparagine, which results in reduced asparagine-dependent protein synthesis, leading to cell cycle arrest in the G1 phase and, finally, to apoptosis of the leukaemic cells (Capizzi et al., 1971). Currently used asparaginases also harbour co-glutaminase activity, which causes the breakdown of glutamine, an essential building block in protein, RNA and DNA synthesis (Capizzi et al., 1971). This co-glutaminase activity is thought to contribute to the clinical toxicity of asparaginase therapy (Chan et al., 2014). Additionally, as a foreign protein, ASP is known to cause adverse immune responses, including drug resistance by antibody formation (Goldberg et al., 1973), thereby facilitating the removal of the ASP enzyme from circulation (Goldberg et al., 1973; Viau et al., 1986). Advantages of ASP are low toxicity and the fact that multi-drug resistance efflux pumps do not affect ASP, making it an interesting treatment option in dogs with MDR expression to regular cytostatic drugs.

The availability of native *E. coli*-derived ASP varies, since it is no longer marketed in many countries. Therefore, pegaspargase (PEG-ASP), ASP linked covalently with polyethyleneglycol, has been introduced for the treatment of canine mL and is as effective as native ASP and less toxic (Teske et al., 1990; MacEwen et al., 1992). Adverse events such as anaphylaxis, coagulopathies, pancreatitis, and hepatotoxicity are reported with native ASP but appear to be uncommon with PEG-ASP (Rogers, 1989; Teske et al., 1990; Rogers et al., 1992; Schleis et al., 2011; Blake et al., 2016). As in humans, PEG-ASP is thought to have a longer plasma half-life in dogs than does native ASP: respectively, 4–6 days vs. 12–40 h, (MacEwen et al., 1987; Rogers, 1989; Borghorst et al., 2014) and the pegylation renders the asparaginase less immunogenic (Kidd et al., 2015). A disadvantage of PEG-ASP is the higher cost compared to native ASP. Furthermore, the PEG moiety not only shields the protein from anti-ASP antibody binding, but also has immunogenic potential due to anti-PEG antibodies (Mima et al., 2015; Elsadek et al., 2020; Freire Haddad et al., 2022). Basic knowledge about pharmacokinetics and mechanism of action is sparse in dogs and is mostly extrapolated from human and laboratory animal data (Viau et al., 1986; Rogers, 1989; Borghorst et al., 2014). Consequentially, various doses and dose intervals of ASP and PEG-ASP are used in veterinary practice on empirical basis, with 40 IU/kg typically used (MacEwen et al., 1987; Teske et al., 1990; Valerius et al., 1997; Jeffreys et al., 2005). Additionally, the number of publications on the use of PEG-ASP as monotherapy in dogs is limited (MacEwen et al., 1987; Teske et al., 1990; MacEwen et al., 1992).

In this study, we aimed to identify the minimum effective dose and dose interval of PEG-ASP in dogs and evaluate possible antibody formation. Minimal effective dose was defined as a completely suppressed asparagine concentration during ASP activity. To this end, plasma concentrations of amino acids and PEG-ASP activity were measured at various time points after administration of different doses of PEG-ASP. In addition, to evaluate potential drug resistance to PEG-ASP, anti-PEG and anti-ASP antibodies were determined.

Materials and methods

PEG-asparaginase

PEG-ASP (Oncaspar, Servier Nederland Farma B.V.) was, in accordance with the summary of product characteristics, dissolved with 5.2 mL water for injection to yield a solution of pegaspargase 750 IU/mL and was administered subcutaneously at doses of 10 IU/kg, 20 IU/kg and 40 IU/kg.

Animal studies and sampling

Seven healthy adult 5–8 year old female Beagle dogs from the Utrecht University animal colony were selected for this study, and experiments were approved by the Dutch Animal Research Committee (DEC, IvD; Approval number 4847–1–8; Approval date, 24 April, 2019). Descriptive veterinary pharmacokinetic studies are mostly conducted with a sample size of six or more animals, which is the sample size needed to obtain estimates of the pharmacokinetic parameters within 5 % of the actual value for the population with 95 % confidence, assuming a relative variability in the parameters of no more than 20 % (Chittenden, 2011). Here, we did not expect variability to be higher than 20 %, as the study population was relatively homogenous, consisting of healthy Beagle dogs of the same sex, and of similar age and weight (see Appendix A: Supplementary Table S1).

Each animal received a single PEG-ASP administration of 10 IU/kg, 20 IU/kg and 40 IU/kg SC (referred to as round one, two and three, respectively), with a follow-up of 29 days after each administration and a wash-out period of 1 month between the different doses (Fig. 1). Dogs were sampled by jugular venepuncture at day 0 (before PEG-ASP administration) and at days 1, 9, 11, 13, 15, 19, 21, 29 after administration. Heparin coated tubes were used. After blood sampling, tubes were immediately placed on ice (within 1 min of sampling) and centrifuged within 10 min after collection (5 min at 4000 RPM at 4 °C). As no sulfosalicylic acid was added to the samples before freezing, the supernatant was frozen (stored at −80 °C) within 15 min of collection to diminish continued asparaginase activity after blood draw (Valerius et al., 1999; Borghorst et al., 2014). Samples were frozen and measured in batches.

Amino acid analysis

Analysis of plasma amino acids (AA) was performed as previously described (Prinsen et al., 2016). The lower levels of detection and quantification (LOD/LOQ-values) of the amino acids were 0.0 and 0.0 μM, respectively, for asparagine, 0.0 and 0.0 μM for aspartic acid, 0.1 and 0.2 μM for glutamine, and 0.0 and 0.1 μM for glutamic acid (Prinsen et al., 2016). See Supplementary materials and methods for a detailed description.

L-Asparaginase activity in serum

ASP activity in serum was measured as described previously (Mondelaers et al., 2020). Activity was quantified by incubating the samples with an excess amount of L-aspartic acid β-hydroxamate (AHA; Sigma-Aldrich A6508) at 37.0 °C. L-asparaginase hydrolyses AHA to L-aspartic acid and hydroxylamine, which was detected at 690 nm with a SpectraMax M3 (Molecular Devices) spectrophotometer, after condensation with 8-hydroxyquinoline (Merck 8.20261) and oxidation to indooxine. See Supplementary materials and methods for a detailed description.

Antibody measurement

Anti-ASP and anti-PEG antibodies were measured using indirect enzyme-linked immunosorbent assay (ELISA) protocols by the Diagnostics Development department of Medac GmbH. See Supplementary materials and methods for a detailed description.

Results

Adverse events

Dogs were closely monitored daily, and no adverse events were observed after administration of PEG-ASP.

ASP activity and amino acid concentrations

Concentration of amino acids, asparagine and aspartic acid, as well as plasma ASP activity at various time points after PEG-ASP administration, are shown in Fig. 2. Twenty-four hours after each PEG-ASP injection, plasma asparagine concentrations dropped to below the limit of quantification (LOQ). Since the LOQ of PEG-ASP was 5 IU/L, values < 5

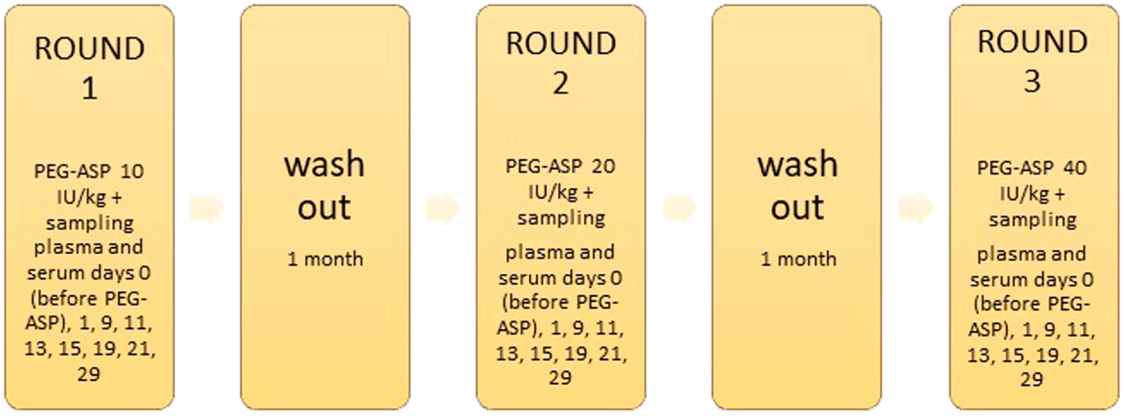

Fig. 1. Study design setup. PEG-ASP, polyethyleneglycol L-asparaginase.

IU/L were extrapolated.

After the first injection (10 IU/kg), plasma asparaginase activity was present in all dogs for at least 9 days (Fig. 2A). Five of seven treated dogs had measurable plasma asparaginase activity at day 15 and one dog on day 29. A dose of 10 IU/kg PEG-ASP was sufficient in all dogs for asparagine suppression until day 9 after injection, with asparaginase activities as low as 12 IU/L on day 9. Similar to the activity concentration, variation existed between dogs in the maximal duration of asparagine suppression. Asparagine was suppressed for at least 15 days in five of seven dogs, for 21 days in three dogs, and for 29 days in one dog (1102).

After the second injection (20 IU/kg), two dogs (1102 and 1702) showed prolonged measurable asparaginase activity and asparagine suppression (Fig. 2B). This inhibition lasted until day 13 (dog 1702) and for more than 29 days (dog 1102). As observed for the 20 IU/kg dosing, after injection with 40 IU/kg only dogs 1702 and 1102 showed prolonged asparaginase activity and asparagine suppression for more than 29 days (Fig. 2C).

After the first injection (10 IU/kg), aspartic acid concentrations increased in all dogs, which was opposite to the course of asparagine concentrations (Fig. 2A). This was similar after the second (20 IU/kg; Fig. 2B) and third (40 IU/kg) injections (Fig. 2C), where there was an initial increase in aspartic acid after 24 h.

The effect of PEG-ASP on glutamine and glutamic acid concentrations was also investigated (see Appendix A: Supplementary Fig. S1-S6). Concentrations of glutamine remained in the same range before and after all three injections and no decline in concentration was observed after PEG-ASP administration (Supplementary Fig. S1, S3 and S5). In most dogs, an increase in glutamic acid could be seen after all three injections (Supplementary Fig. S2, S4 and S6).

Low plasma L-asparaginase activity is sufficient for asparagine depletion

A linear correlation between plasma asparagine concentration and ASP activity could not be assessed in this cohort, although a similar pattern was observed in the two dogs with prolonged asparagine suppression (dog 1102 and 1702). Table 1 depicts asparagine concentrations and corresponding PEG-ASP activity for the three dosage rounds. Plasma asparagine suppression was seen at PEG-ASP activity of 22 IU/L in dog 1702 and at 10 IU/L in dog 1102. Asparagine suppression occurred even with very low PEG-ASP activity. This was seen in all dogs on day 1 of round two, when asparagine concentrations were <LOQ, while PEG-ASP activity was below 5 IU/L.

Anti-PEG and anti-asparaginase antibodies

Antibodies against both asparaginase and PEG were measured on day 0 of round one (asparaginase-naïve situation), on day 0 of round two, on day 0 of round three and on day 29 of round three. Results are presented in Fig. 3. For PEG, both anti-PEG IgG and IgM were measured. For ASP, anti-ASP IgG was measured. Antibody results are presented as optical density (OD) signal to cut-off ratios (S/C values; positive: ≥ 1, and negative: < 1). Titres were determined for anti-ASP IgG and for anti-PEG IgG and IgM. While two dogs (1102, 1702) remained negative for all antibodies (except for one temporarily low anti-PEG-IgG-positive result on day 0 of round 2), the five other dogs developed anti-ASP IgG as well as anti-PEG IgG. S/C values appeared to increase over time from round to round. There was a decrease in anti-ASP IgG in dog 1703 and in anti-PEG IgG in dog 1301 between day 0 and day 29 of round three; however, all values remained positive. Dog 85 had pre-existing anti-PEG IgG antibodies.

Table 2 shows anti-PEG and anti-ASP IgG titres. The weakly anti-PEG-IgG positive dog 85 showed an antibody increase after each PEG-ASP application, but the observed booster effect was moderate.

Discussion

L-asparaginase is a frequently used drug in the treatment of canine malignant lymphoma. Here, we aimed to identify a minimum effective dose and dosing interval for PEG-ASP in dogs by measuring the effect of ASP on asparagine depletion. To evaluate potential drug resistance to PEG-ASP, antibody formation was assessed. We noted marked individual variation in response to PEG-ASP. Nevertheless, an initial dose of 10 IU/kg PEG-ASP was sufficient in all dogs for minimal asparagine suppression of 9 days, although wide variation existed in the maximal duration of suppression.

Asparagine concentrations decreased after the three injections. Although a wider individual range was present, all dogs showed an increase in aspartic acid after all three injections. The duration of this aspartic acid increase was of comparable duration as its corresponding asparagine concentration decline. This was expected since ASP promotes the formation of aspartic acid from asparagine.

It is known that ASP also has some glutaminase effect, although no uniform decline in glutamine concentration after PEG-ASP administration was observed in this study. The increased formation of glutamic acid was observed in most dogs after all three PEG-ASP injections.

Experimental variation in response to PEG-ASP, and the degree of asparagine suppression described in literature is likely multifactorial. In a clinical setting, different doses and intervals of both ASP and PEG-ASP have been used (MacEwen et al., 1992; Piek et al., 1999; Jeffreys et al., 2005; Cawley et al., 2020). MacEwen et al., and Teske et al., reported a significantly higher percentage of complete remissions after one injection of PEG-ASP in dogs receiving 30 IU/kg versus those receiving 10 IU/kg (Teske et al., 1990; MacEwen et al., 1992). Nevertheless, the total response rate in dogs treated with two injections of 10 IU/kg PEG-ASP was 72.7%. No significant difference was noted between the two doses

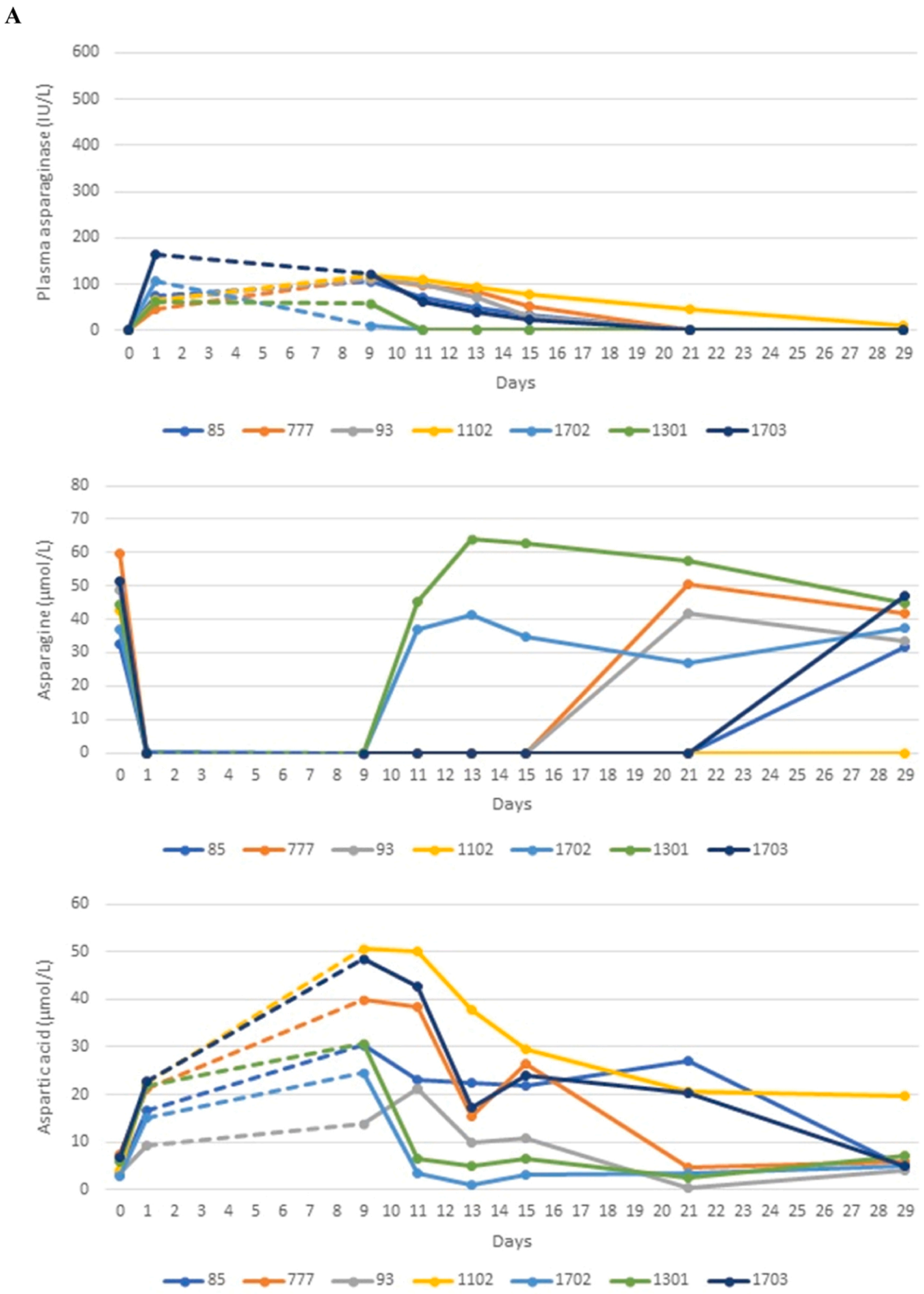

Fig. 2. Plasma asparagine and aspartic acid concentrations and plasma asparaginase activity at different time points after each of three consecutive polyethyleneglycol L-asparaginase (PEG-ASP) dosing rounds: (A) 10 IU/kg (B) 20 IU/kg and (C) 40 IU/kg. Different colours and numbers present the individual seven dogs. Day 0 was set as immediately before administration. Since no samples were taken between day 1 and day 9, concentrations between these timepoints are depicted as a dotted line.

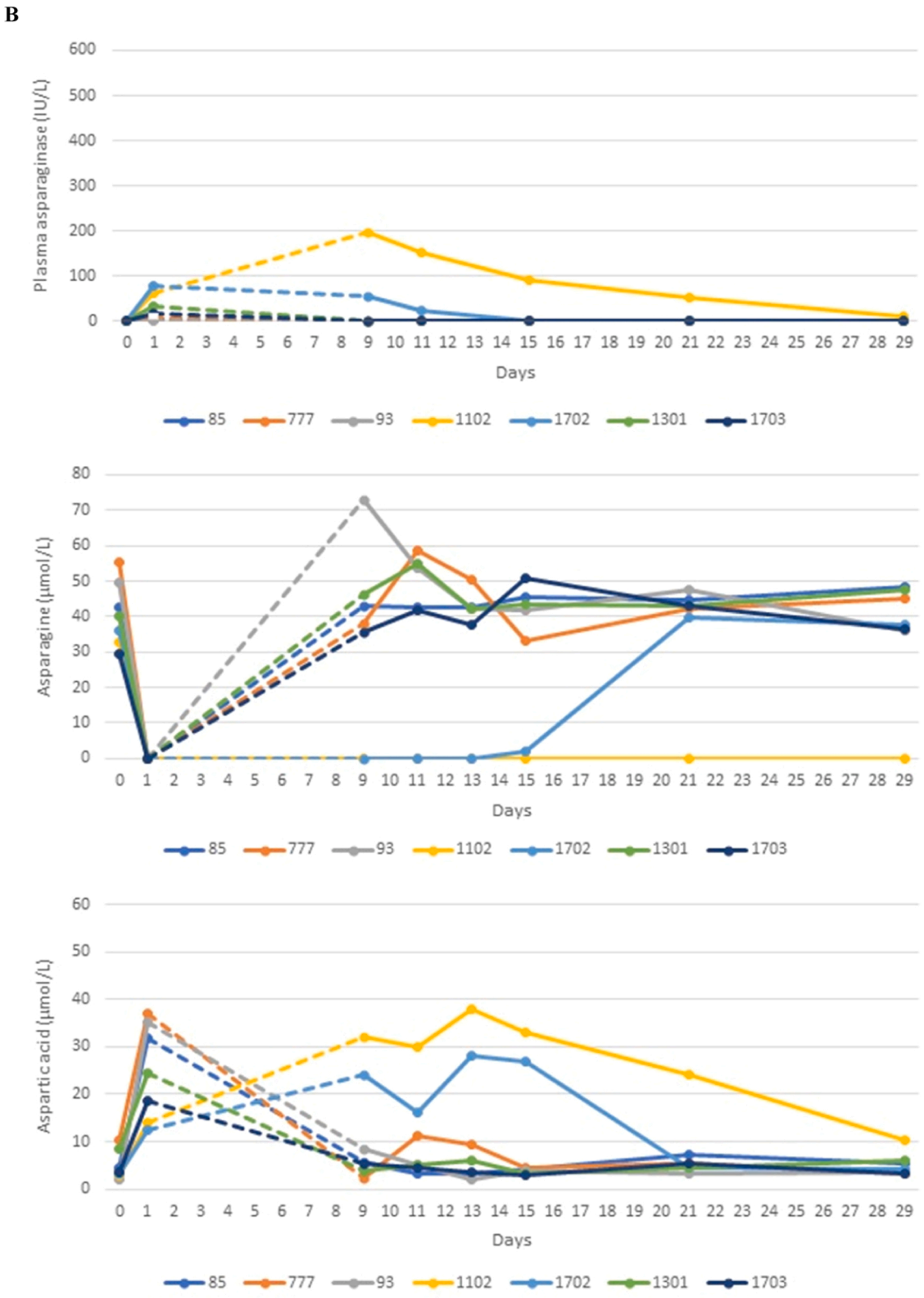

Fig. 2. (*continued*).

in time to relapse, to treatment failure, or to remission (Teske et al., 1990).

Currently, there is no established optimal dosage protocol for ASP and PEG-ASP in veterinary medicine. Publications where ASP or PEG-ASP were used as monotherapy are sparse (MacEwen et al., 1987; Teske et al., 1990; Nakagawa et al., 2022). Due to the variation in responses to ASP administration seen in this study, both in duration of asparagine depletion and in antibody development, it is not yet possible to propose an evidence-based standardised treatment schedule.

In humans, ASP plasma activity of >100 IU/L are generally considered to be therapeutic (Riccardi et al., 1981; Boos et al., 1996; Vieira Pinheiro et al., 2001; van der Sluis et al., 2016). Some studies

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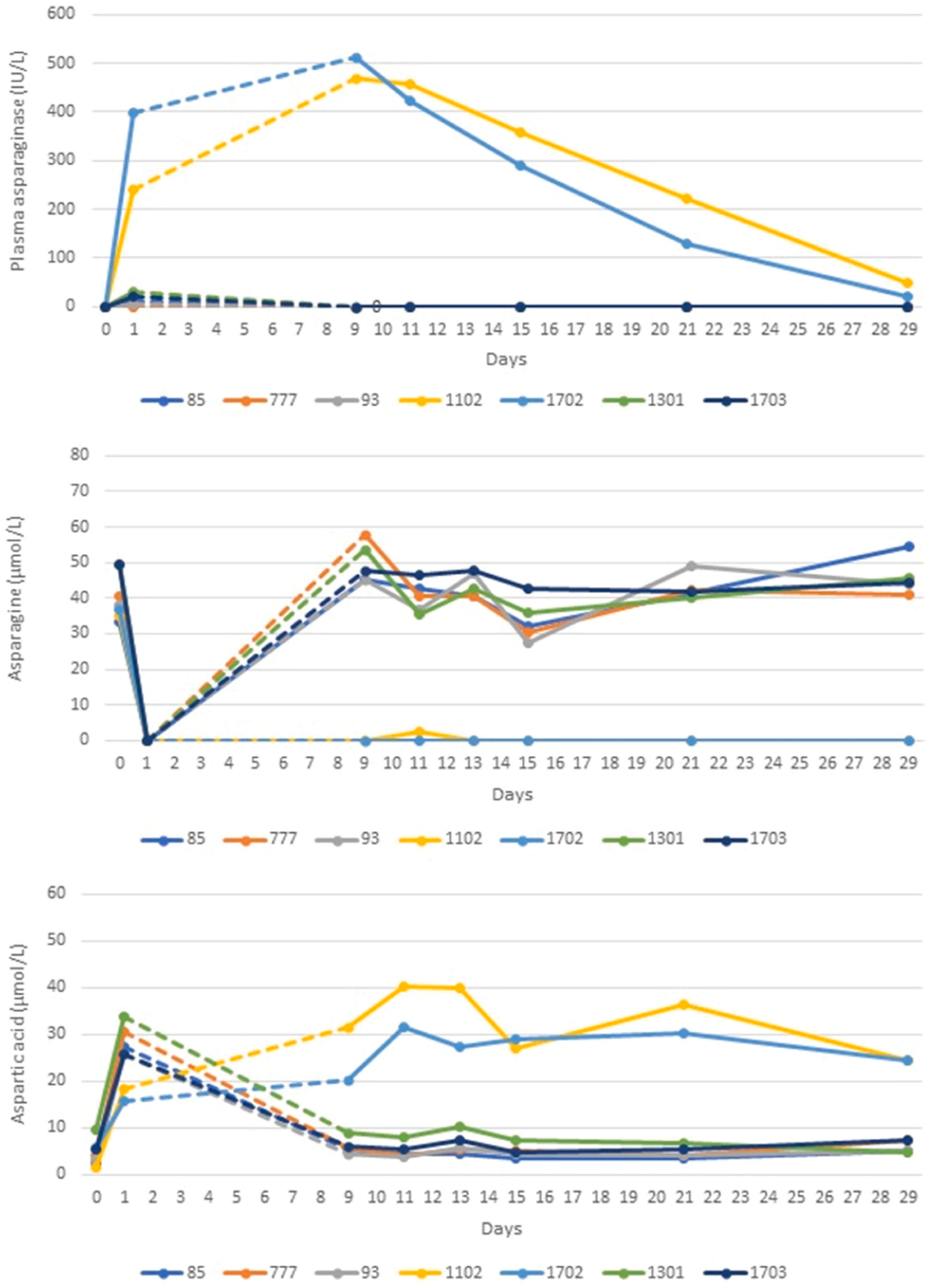

Fig. 2. (*continued*).

have suggested that sustainable asparagine depletion also occurs at ASP concentrations < 50 IU/L (Rizzari et al., 2000), while others suggested that activity of > 400 IU/L are needed (Avramis and Panosyan, 2005; Angiolillo et al., 2014). Minimum effective ASP plasma activity concentrations in dogs are not yet defined. In this study we demonstrated that asparagine suppression can already occur at PEG-ASP plasma concentrations < 5 U/L, although this is not proof of sustainable asparagine depletion in canine patients.

The plasma half-life of ASP in dogs appears to be shorter than that of PEG-ASP and is between 12 and 40 h (Viau et al., 1986; Rogers, 1989). However, Valerius et al. described suppressed plasma asparagine concentrations for at least 1 week after ASP administration (Valerius et al., 1999). ASP activity was not evaluated in that study; but combining this information with that of the current study, it appears that asparagine

Table 1
Asparagine concentrations (μmol/L) and its corresponding polyethyleneglycol L-asparaginase (PEG-ASP) activity (IU/L) for all dogs at various time points during rounds (R) one (10 IU/kg), two (20 IU/kg) and three (40 IU/kg).

Dog		85		777		93		1102		1702		1301		1703	
R	Day	Asparagine	PEG-ASP	Asparagine	PEG-ASP	Asparagine	PEG-ASP	Asparagine	PEG-ASP	Asparagine	PEG-ASP	Asparagine	PEG-ASP	Asparagine	PEG-ASP
1	0	32.4	< 5	59.9	< 5	48.7	< 5	42.8	< 5	36.8	< 5	44.6	< 5	51.3	< 5
	1	0.2	76	0	45	0	66	0	64	0	106	0	63	0	164
	9	0	106	0	115	0	114	0	119	0	12	0	59	0	122
	11	0	73	0	99	0	100	0	109	36.8	< 5	45.3	< 5	0	63
	15	0	34	0	54	0	30	0	78	34.9	< 5	62.9	< 5	0	24
	21	0	< 5	50.5	< 5	41.9	< 5	0	46	27	< 5	57.6	< 5	0	< 5
	29	31.5	< 5	41.8	< 5	33.6	< 5	0	< 5	37.6	< 5	44.7	< 5	46.8	< 5
2	0	42.7	< 5	55.5	< 5	49.4	< 5	32.7	< 5	36	< 5	40.1	< 5	29.6	< 5
	1	0	5	0	9	0	< 5	0	62	0	79	0	32	0	16
	9	43.2	< 5	37.9	< 5	72.8	< 5	0	196	0	57	46.2	< 5	35.8	< 5
	11	42.4	< 5	58.6	< 5	53.5	< 5	0	152	0	22	55	< 5	41.6	< 5
	15	45.3	< 5	33.3	< 5	41.8	< 5	0	90	2.1	< 5	43.3	< 5	50.7	< 5
	21	44.5	< 5	42.3	< 5	47.5	< 5	0	52	39.6	< 5	43.1	< 5	43.2	< 5
	29	48.3	< 5	45.1	< 5	36	< 5	0	10	37.6	< 5	47.5	< 5	36.6	< 5
3	0	33.4	< 5	40.4	< 5	38	< 5	34.9	< 5	36.7	< 5	49.5	< 5	49.6	< 5
	1	0,1	12	0	< 5	0.1	6	0	241	0	399	0	30	0	20
	9	45	< 5	57.7	< 5	45	< 5	0	470	0	511	53.8	< 5	47.6	< 5
	11	42.8	< 5	40.5	< 5	36.6	< 5	2.3	458	0	422	35.3	< 5	46.4	< 5
	15	31.9	< 5	30.3	< 5	27.4	< 5	0	359	0	289	35.7	< 5	42.7	< 5
	21	41.5	< 5	42.4	< 5	49	< 5	0.1	222	0	129	39.9	< 5	41.6	< 5
	29	54.3	< 5	40.9	< 5	43.9	< 5	0	47	0	21	45.6	< 5	44.5	< 5

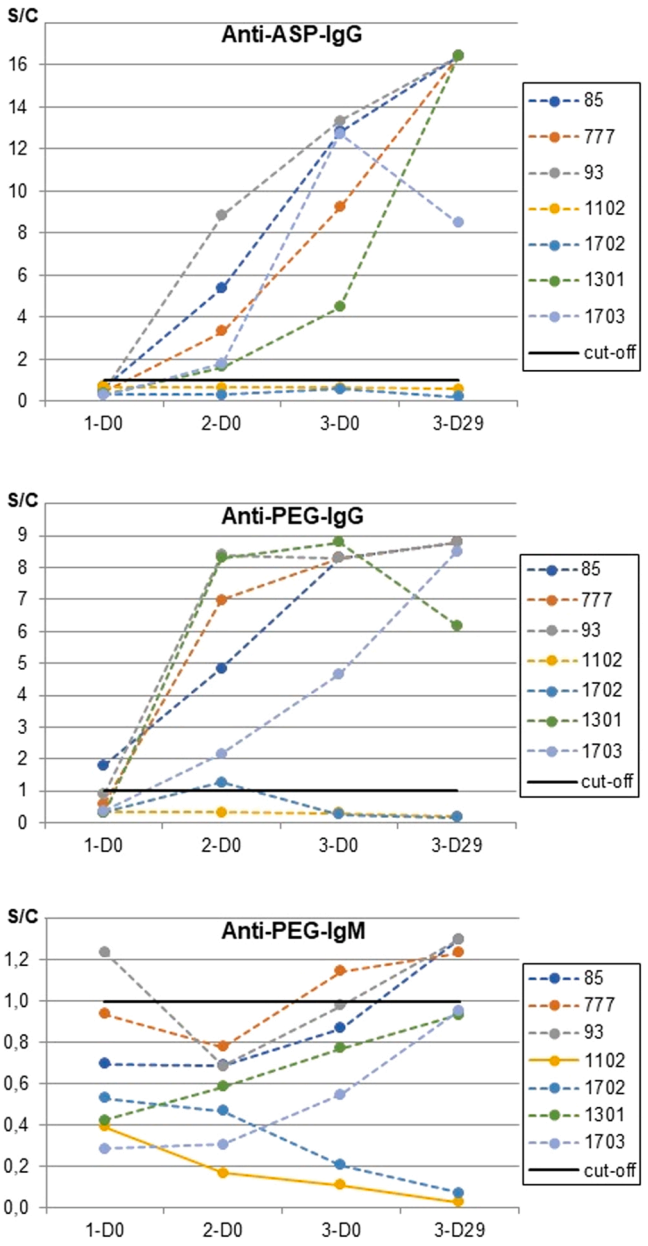

Fig. 3. Signal to cut-off ratios (S/C values) of anti-asparaginase (anti-ASP) IgG, anti-polyethyleneglycol (anti-PEG) IgG and anti-PEG IgM on day (D) 0(D0) of round one, day 0 of round two, day 0 of round three and day 29 of round three. S/C ≥ 1 was positive. Different colours and numbers represent each of the seven dogs.

Table 2
Anti-asparaginase (anti-ASP) and anti polyethyleneglycol (anti-PEG) IgG titres at day 0 of three rounds (R) and day 29 of round three. (0) not detectable, (*N*) detectable with corresponding titre.

Dog	Anti-ASP IgG				Anti-PEG IgG			
	R1 day 0	R2 day 0	R3 day 0	R3 day 29	R1 day 0	R2 day 0	R3 day 0	R3 day 29
85	0	8	32	256	1	2	16	32
777	0	4	16	64	0	2	4	16
93	0	16	32	256	0	2	8	16
1102	0	0	0	0	0	0	0	0
1702	0	0	0	0	0	0	0	0
1301	0	0	4	128	0	2	4	2
1703	0	2	32	8	0	0	2	4

suppression could occur even at low ASP and PEG-ASP activity.

The sparse sampling times are a limitation of this study. Exact peak ASP plasma activity could not be assessed due to the low number of measurements between day 0 and day 9. In this study PEG-ASP was administered SC while, in several studies, IM administration was performed. To our knowledge, there are no publications comparing ASP activity after IM versus SC PEG-ASP administration in dogs. One study compared differences in asparagine plasma concentrations following SC and IM ASP administration and, in this study, no difference in asparagine concentrations was found (Valerius et al., 1999).

An overall dose effect could not be evaluated because successful asparagine suppression was only seen in two dogs after the second and third injection of PEG-ASP (20 and 40 IU/kg). There appears to be a relationship between the dose and the duration, but not with the magnitude of effect. In dog 1102, asparaginase was suppressed for more than 29 days after all three injections. Dog 1702 showed a dose effect with 9 days of asparagine suppression after the 10 IU/kg injection, 15 days suppression following the 20 IU/kg dose and more than 29 days after 40 IU/kg. Borghorst et al. reported asparagine suppression varying from 11 to 36 days after 20 IU/kg PEG-ASP injection, with corresponding PEG-ASP activity of more than 500 IU/L (Borghorst et al., 2014). An older toxicology study demonstrated measurable PEG-ASP activity up to 3–4 weeks after administration (Viau et al., 1986), although doses administered in that study were higher than in this study.

Antibody formation seems to terminate asparaginase activity quickly and completely, while dogs that do not produce antibodies show prolonged activity. In the current study, anti-ASP and anti-PEG antibody development was already found in five out of seven dogs after one injection with PEG-ASP. Previous studies suggested PEG-ASP is less immunogenic than native ASP (Kidd et al., 2015). Kidd et al. found that anti-ASP antibody formation occurred in 3/10 dogs with lymphoma after the first injection of native ASP and in 4/7 dogs after the second dose, although the time between the first and second dose varied greatly (54 – 575 days; Kidd et al., 2015). Correlation between ASP activity and ASP antibody titres is also observed in children with acute leukaemia and is associated with a worse clinical outcome (Panosyan et al., 2004). The antibody analysis of anti-PEG IgG and anti-ASP IgG in this study shows consistent and explainable values. The results of the anti-PEG IgM ELISA are less consistent, and the cut-off definition of that assay proved to be challenging because of background reactivity. We found higher baseline concentrations of anti-PEG antibodies in study subjects (before first PEG-ASP administration) as compared to the 20 other healthy control dogs. This might be due to (repeated) previous exposure to other PEGylated products (vaccine, deworming, cleaning product, etc.). There is a potential rise in anti-PEG sensitisation in recent years due to broader use of PEG, hampering future immunogenicity studies on PEGylated therapeutics (Zhou et al., 2021).

In the current study, random assignment to different doses was not used. As such, a possible phase effect, versus a dose-effect, could not be assessed. There is evidence to suggest that anti-drug antibody development is dose-dependent and frequently occurs after administration of higher doses (Borghorst et al., 2014). It can be argued that the wash-out phases might have been in part responsible for the antibody results, since longer intervals between doses could trigger a more profound immune response (Panosyan et al., 2004).

This study was performed in healthy Beagle dogs and further studies in canine lymphoma patients are needed to correlate plasma asparagine depletion and ASP activity with clinical response. In dogs, it is not known what duration of asparagine suppression is needed to achieve a sufficient clinical response. Antibody formation might be different in veterinary patients compared to healthy immunocompetent Beagle dogs and should also be evaluated in dogs with lymphoma. In the future, measuring plasma ASP activity and antibodies in canine lymphoma patients would facilitate optimal, individualised treatment plans and could help prevent administration of expensive and potentially toxic drugs to non-responsive animals.

Conclusions

In this study, substantial individual variation was observed in the level and duration of asparaginase activity and of corresponding asparagine concentration in healthy Beagle dogs. Asparagine suppression occurred with PEG-ASP plasma concentrations < 5 IU/L. Importantly, antibody formation against PEG-ASP is often encountered and can occur after one injection. This study suggests that doses < 40 IU/kg of PEG-ASP could be effective in some dogs and that monitoring of ASP activity enabled individualisation of treatment plans.

Declaration of Competing Interest

T. König is an employee of Medac. None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tvjl.2022.105854.

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