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Pharmacokinetics of total thyroxine in dogs after administration of an oral solution of levothyroxine sodium

G. LE TRAON*
S. BURGAUD* &
L. J. I. HORSPOOL†

*Intervet Pharma R&D, a part of Schering-Plough Corporation, Beaucouzé, France; †Intervet International bv, a part of Schering-Plough Corporation, Boxmeer, The Netherlands

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Oral L-thyroxine (L-T4) supplementation is used to replace thyroid hormone concentrations in dogs with hypothyroidism. The pharmacokinetics of L-T4 following administration of a solution (Leventa®) was investigated in healthy dogs. L-T4 was absorbed fairly rapidly (t_{max} 3 h). A mean bioavailability of 22% was calculated following a single oral administration of 40 μg L-T4/kg body weight. Repeated oral administration at the same dose for 14 consecutive days did not lead to any accumulation of T4 in serum. After intravenous administration of L-T4, a serum half-life of 11.6 h was calculated. Food intake concomitant with L-T4 oral administration delayed L-T4 absorption and decreased its rate and extent by about 45%. The relative bioavailability of L-T4 following administration of a tablet formulation was about 50% of that of the L-T4 solution. The pharmacokinetic properties of liquid L-T4 after oral administration support the use of a dose rate of 20 μg/kg once daily, as a starting dose for replacement therapy in dogs with hypothyroidism.

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L. J. I. Horspool, Intervet International bv, International Marketing, PO Box 31, 5820 AA Boxmeer, The Netherlands. E-mail: Linda.Franken-Horspool@intervet.com

INTRODUCTION

Hypothyroidism, a metabolic disease resulting from deficiency of thyroid hormones, thyroxine (T4) and triiodothyronine (T3), is one of the most common endocrine diseases in dogs (Feldman & Nelson, 2004), with a prevalence of 0.3% (Milne & Hayes, 1981).

As in humans, daily and lifelong levothyroxine (L-T4) replacement therapy has to be initiated to normalize thyroid hormone concentrations [total thyroxine (tT4), free fraction of thyroxine (fT4) and thyroid stimulating hormone (TSH)] in dogs with hypothyroidism and consequently to improve and finally resolve associated clinical signs. The posology of L-T4 replacement therapy has to be tailored individually to each dog and the general dosage recommendation for L-T4 tablets, corresponding to adequate therapy for the majority of hypothyroid dogs, is twice daily administration at a dose rate of 20 μg/kg body weight. These dosage recommendations are primarily empirical but are supported by pharmacokinetic data generated following administration of L-T4 tablets to thyroidectomized dogs (Nachreiner *et al.*, 1993).

Long-term compliance with treatment is essential in ensuring optimal clinical and hormonal response and this is often a challenge for lifelong treatment such as L-T4 supplementation. Thus, any improvement leading to simplification of the dosing schedule, *e.g.* from twice-a-day to once-a-day supplementation, may improve long-term control of hypothyroidism. Until very recently, only tablet formulations containing L-T4 were available for use in veterinary medicine. A novel oral solution of L-T4 sodium (Leventa®, 1 mg/mL; Intervet Inc., Millsbaro, DE, USA) was recently introduced in the USA with a recommendation for once daily administration to hypothyroid dogs.

The general pharmacokinetic properties of the novel oral solution of L-T4 are described here. Pharmacokinetic profiles following single and repeated oral administration (including evaluation of bioavailability Inc., Millsboro, DE, USA), influence of food intake and a comparison of the pharmacokinetic profiles of the liquid and a commercially available tablet formulation of L-T4 using their respective dosage schedule were studied.

MATERIALS AND METHODS

Four studies were performed in clinically healthy male and female Beagle dogs. The dogs were fed a commercial dry diet once daily in the morning and had free access to water. In studies A and C, food was supplied 10 h after treatment on the days of dosing, except for the 'fed' dogs in study C. In study B on

the first and last treatment days, the dogs were fed 10 h after the administration of treatment. On the other days, the dogs were fed 2 h after treatment. In study D, food was withheld on the treatment days.

For each study, the treatment schedule is described in detail in the sections that follow.

Study A: bioavailability of T4 after oral administration of the oral solution of L-T4

The pharmacokinetics of total T4 (tT4) in serum, including the bioavailability, were determined in dogs ($n = 6$) following a single intravenous (T4 for injection, 0.1 mg/mL, Bedford Laboratories, Bedford, OH, USA) and oral administration (Leventa®, 1 mg/mL; Intervet Inc.) at a dose rate of 40 μg L-T4 sodium/kg body weight, using a cross-over (2 × 3) study design with a washout period of 1 week.

Study B: pharmacokinetics of T4 after repeated oral administration of the oral solution of L-T4

Dogs ($n = 8$) were administered L-T4 sodium orally (Leventa®) at a dose rate of 40 μg/kg once daily for 14 consecutive days. The pharmacokinetic profiles of tT4 were determined on the first and last treatment days, and before (trough) and 2 h after (peak) once daily treatment every 2 days until the last treatment day.

Study C: effect of food intake on the pharmacokinetics of T4 after oral administration of the oral solution of L-T4

The pharmacokinetics of tT4 following oral administration of L-T4 sodium at 40 μg/kg (Leventa®) were compared in fasted and fed dogs ($n = 8$), using a cross-over (2 × 4) study design with a washout period of 1 week. All dogs were fasted overnight and food was either supplied immediately after treatment (fed) or 10 h after treatment (fasted).

Study D: comparative pharmacokinetic study of liquid and tablet formulations of L-T4

The pharmacokinetics of tT4 were compared in dogs ($n = 8$) administered a single dose (200 μg) of an oral solution of L-T4 sodium (Leventa®) and two doses at an interval of 12 h (200 μg per dose) of a tablet formulation of L-T4 sodium (Soloxine®; Virbac AH Inc., Fort Worth, TX, USA). For practical reasons, this study was conducted using a paired design with an 8-day washout period between treatment periods.

Blood samples

For all the studies, 2 mL of blood samples were taken prior to L-T4 sodium administration (−1, −0.5 and 0 h). Blood samples were taken following treatment according to the schedule in Table 1.

Blood was collected via a jugular catheter or by jugular venipuncture (study B on days 4, 6, 8, 10 and 12 only) into dry tubes. Tubes were placed at ambient temperature for 30 min and centrifuged at +4 °C and 2000 ***g*** for 10 min. Serum was separated and frozen at −20 °C until analysis.

Table 1. Blood sampling schedule following administration of L-T4 sodium

Study	Period	Sampling time points
A	Oral route	0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 24, 34 and 48 h
	Intravenous route	5, 15, 30 min and 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 24, 34 and 48 h
B	Treatment no. 1 (day 1)	0.5, 1, 1.5, 2, 3, 4, 5, 6, 8 and 10 h
	Treatments no. 2, 4, 6, 8, 10, 12	Prior to treatment (trough) and 2 h after (peak).
	Treatment no. 14 (day 14)	0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 24, 34, 48, 58, 72 and 96 h
C	Fasted/fed	0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 24, 36 and 48 h
D	Leventa®	0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h
	Soloxine®	0.5, 1, 1.5, 2, 3, 4, 6, 8 and 12 h after each administration

Determination of tT4 concentration in canine serum samples

One radioimmunoassay (RIA) kit (IM342301; Beckman Coulter, Roissy, France) was used for studies A, B and C and a second (TKC41; Diagnostic Products Corporation, La Garenne Colombes, France) for study D. The kits were validated for the determination of tT4 concentrations in canine serum by the respective manufacturers: intra-assay coefficients of variation (four and three T4 concentrations, respectively, 10 tests per concentration) ranged from 3.4 to 6.2% and from 3.1 to 9.3%, respectively. Sensitivity was 6 and 2.8 nmol/L, respectively. Linearity and recovery characteristics were satisfactory for both tests. Neither assay had cross reactivity with major analogs of T4. Determination of tT4 was performed twice for each sample. Samples with a coefficient of variation of >20% were not included in the pharmacokinetic analysis. The limits of quantification (LOQ) of assays were 15 and 6.4 nmol/L, respectively. LOQ was arbitrarily assigned to all measurements below LOQ.

Pharmacokinetic and statistical analysis

Pharmacokinetic parameters were calculated using noncompartmental analysis (WinNonlin; Pharsight, Mountain View, CA, USA). For studies A, C and D prior to analysis, tT4 concentrations were corrected for endogenous (basal) concentration (mean of concentrations measured at −1, −0.5 and 0 h before treatment, calculated individually for each dog). For study B, tT4 concentrations were not corrected for initial endogenous (basal) concentrations. The maximum serum concentrations (C_{max}) and the time (t_{max}) to reach C_{max} were the observed values from the serum concentration–time profiles. The terminal half-life (intravenous route) or apparent elimination half-life

(oral route) ($t_{1/2}$) were calculated from the terminal phase of the serum concentration–time curve. The area under the serum concentration vs. time curve (*AUC*) was calculated using the linear trapezoidal rule, up to the last measurable concentration. Mean residence time (*MRT*), clearance (*Cl*) and apparent volume of distribution (V_d) were determined using WinNonlin standard algorithms. Finally, absolute and relative bioavailabilities (*F*) of L-T4 sodium were calculated using the generic formula:

$$F = (AUC \times \text{dose}_{\text{ref}})/(AUC_{\text{ref}} \times \text{dose})$$

where the reference (ref) was either intravenous route (study A vs. oral), fasted state (study C vs. fed) or tablet formulation (study D vs. oral solution).

Statistical analysis was performed using SAS (SAS Institute Inc., Cary, NC, USA) with the level of significance (α) set at 0.05. C_{max} and *AUC* were log-transformed prior to analysis. For study B, *AUC* was calculated from 0 to 24 h post-treatment on both day 1 and day 14. C_{max} and *AUC* on days 1 and 14 were compared using ANOVA with day as factor and subject as random effect. t_{max} and $t_{1/2}$ values were compared using a Wilcoxon signed rank test and paired *t*-test, respectively. C_{trough} and C_{peak} were compared using a Dunnett adjusted *t*-test. For study C, comparisons were made for C_{max} and *AUC* using ANOVA with feeding state, sequence, dog within sequence and period as factors. t_{max} was compared using a Wilcoxon signed rank test. For study D, C_{max} and *AUC* over 24 h after first treatment were compared using Wilcoxon test (only C_{max} values reported after the first treatment with the tablet formulation were considered for the comparison).

Table 2. Pharmacokinetic parameters* calculated following intravenous and oral administration of L-T4 sodium to fasted dogs ($n = 6$)

Parameters	Intravenous	Oral
Actual L-T4 dose rate (μg/kg)	39.7 ± 0.8	41.1 ± 0.3
C_{max} (nmol/L)†	–	78 ± 19.2
t_{max} (h)	–	3.0 (1.5–4.0)
AUC (nmol·h/L)†	4379 ± 635	976 ± 253
$t_{1/2}$ (h)	11.6 ± 0.4	14.8 ± 2.5
MRT (h)	9.1 ± 0.8	10.5 ± 1.1
Cl_B (L/h/kg)	0.01 ± 0.001	–
Cl_{tot} (L/h/kg)	–	0.06 ± 0.02
V_d (L/kg)	0.12 ± 0.02	
V_z (L/kg)	–	0.63 ± 0.32
Bioavailability (*F*)	–	0.22 ± 0.05

*Data expressed as mean ± SD if distributed normally or as median (range), except for $t_{1/2}$ expressed as harmonic mean with pseudo SD.
†Corrected for endogenous tT4 concentration.

RESULTS

Study A: bioavailability study

The pharmacokinetic parameters calculated are summarized in Table 2. The serum concentrations of tT4 following intravenous and oral administration are depicted in Fig. 1.

Following a single oral dose, a C_{max} of approximately 86 nmol/L (median corrected for endogenous concentration, interquartile range (IQR) 71–91 nmol/L) was reached around 3 h (median t_{max}, IQR 3–4 h) post-treatment. Comparable terminal $t_{1/2}$ was calculated after intravenous and oral administration. The bioavailability of T4 following oral administration of the oral solution of L-T4 sodium to fasted dogs was 22 ± 5%.

Study B: repeated oral administration

The actual dose rate of L-T4 administered daily was 41.3 ± 0.23 μg/kg. The pharmacokinetic parameters calculated for the first and last days of treatment are summarized in Table 3. The tT4 concentration–time profile over the 14-day treatment period is shown in Fig. 2.

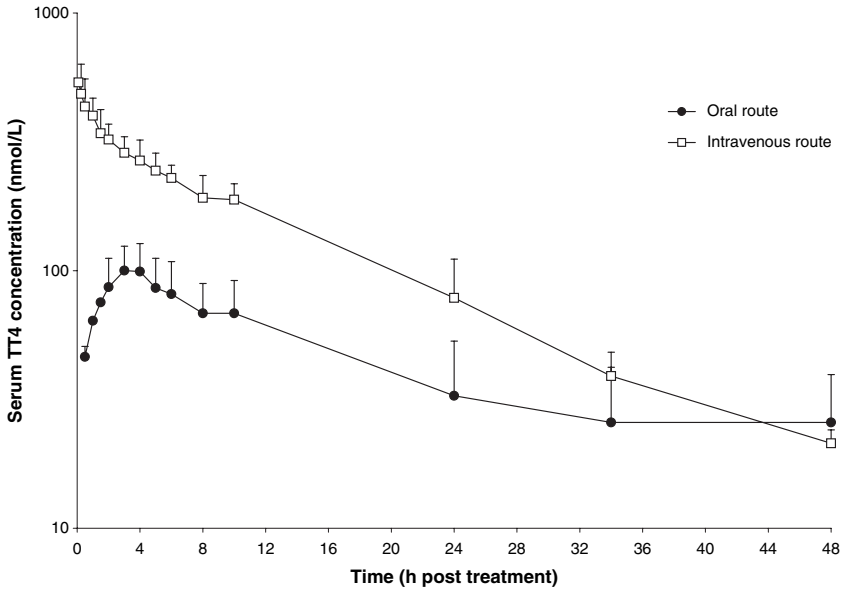

Fig. 1. Serum tT4 concentrations* (mean ± SD) following intravenous and oral (Leventa®) administration of L-T4 sodium to dogs ($n = 6$). *Observed values.

Table 3. Pharmacokinetic parameters* calculated on first and last days of once daily treatment with L-T4 sodium in fasted dogs (n = 8)

Parameters	Day 1 (0–24 h)	Day 14 (0–96 h)
C_{max} (nmol/L)	103 ± 31.8	103 ± 41.8
t_{max} (h)	2.5 (1.5–5.0)	2.0 (2.0–5.0)
C_{peak} (nmol/L)	92 ± 23.7	101 ± 44.8
C_{trough} (nmol/L)	24 ± 4.9	23 ± 7.7
$t_{1/2}$ (h)	14.3 ± 1.0	14.0 ± 2.9
MRT (h)	9.5 ± 0.2	13.0 ± 1.4
AUC (nmol.h/L)†	1486 ± 439	1492 ± 532
Ratio of accumulation‡	1.1 ± 0.47	

*Data expressed as mean ± SD if distributed normally, or as median (range), except for $t_{1/2}$ expressed as harmonic mean with pseudo SD. †*AUC* calculated from t_0 to t_{24h}. ‡Ratio of accumulation of L-T4 after repeated administration from day 1 to day 14 defined as $(AUC_{day14} \times dose_{day1})/(AUC_{day1} \times dose_{day14})$.

Table 4. Pharmacokinetic parameters* calculated following oral administration of L-T4 sodium to fasted and fed dogs (n = 8)

Parameters	Fasted	Fed	*P*-value‡
Actual L-T4 dose rate (μg/kg)	40.4 ± 0.2	40.7 ± 0.3	–
C_{max} (nmol/L)†	76 ± 21.1	42 ± 17.4	<0.05
t_{max} (h)	2.5 (1.5–5.0)	5.0 (4.0–8.0)	<0.05
AUC (nmol·h/L)†	922 ± 402.6	509 ± 323.2	<0.05
$t_{1/2}$ (h)	11.4 ± 1.4	14.1 ± 2.8	–
MRT (h)	8.8 ± 2.9	9.5 ± 4.1	–
Cl_{tot} (L/h/kg)	0.068 ± 0.051	0.151 ± 0.149	–
V_z (L/kg)	0.66 ± 0.37	1.10 ± 0.75	–
Relative bioavailability (*F*)	–	0.55 ± 0.29	

*Data expressed as mean ± SD if distributed normally or as median (range), except for $t_{1/2}$ expressed as harmonic mean with pseudo SD. †Corrected for endogenous tT4 concentration. ‡See "Pharmacokinetic and statistical analysis" section for further details.

Basal concentrations were 24 nmol/L (median, IQR 21–28 nmol/L). T4 was absorbed rapidly after oral administration of the oral solution of L-T4 sodium. Peak concentrations did not increase during the 14 days of treatment. Trough concentrations were slightly but significantly increased after the first day of treatment and stabilized at approximately 30 nmol/L during the first week of treatment. C_{max}, t_{max} and *AUC* values calculated on day 14 were not significantly different from those observed on day 1.

A ratio of accumulation of 1.1 (median, IQR 0.7–1.2) was calculated. tT4 concentrations had returned to basal level within 34 h after the last treatment in all the dogs. At 96 h after the last treatment, tT4 concentrations were lower than basal concentration on day 1 in four of the eight dogs.

Study C: effect of food intake

The pharmacokinetic parameters calculated are summarized in Table 4. The serum concentrations of tT4 after oral administration to fasted and fed dogs are shown in Fig. 3.

L-T4 absorption was significantly delayed and its rate and extent reduced when the oral solution of L-T4 sodium was administered with food, as shown by a significant increase in t_{max} and significant decreases in C_{max} and *AUC*. C_{max} in fasted dogs was 83 nmol/L (median corrected for endogenous concentration, IQR 71–90 nmol/L) and in fed dogs was 39 nmol/L (IQR 32–57 nmol/L). t_{max} was 3 h in fasted dogs (median, IQR 2–4 h) and 5 h in fed dogs (IQR 4–7 h). The relative bioavailability of L-T4 following oral administration to fed dogs was 55 ± 29% of the value in fasted dogs.

Study D: comparative pharmacokinetic study

The pharmacokinetic parameters calculated following intravenous and oral administration are summarized in Table 5.

Following the first administration of 200 μg L-T4 sodium, C_{max} after the oral solution of L-T4 sodium was 41 nmol/L (median corrected for endogenous concentration, IQR 36–48 nmol/L), significantly higher than C_{max} after the tablet formulation of L-T4 sodium (median 30 nmol/L, IQR 24–36 nmol/L). Similar t_{max} values were observed for both dosage forms. Although dogs received 400 μg of L-T4 as tablets vs. only 200 μg of L-T4 of the oral solution, similar *AUC* values ($P > 0.05$) were observed over the 24-h post-treatment period.

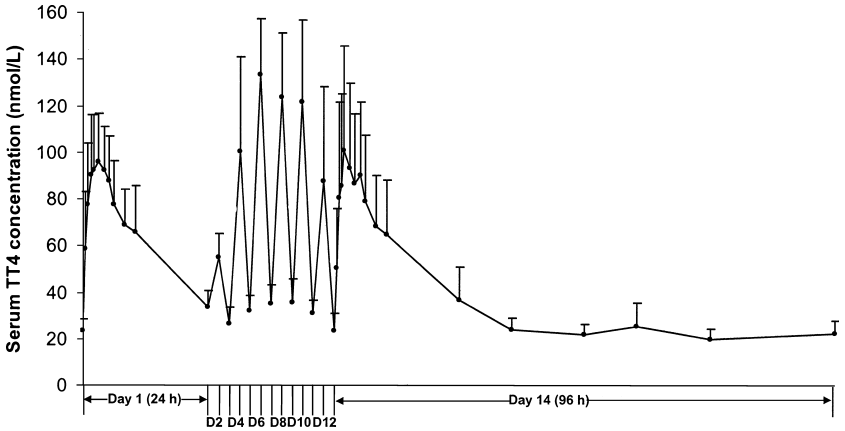

Fig. 2. Serum tT4 concentrations* (mean ± SD) in dogs (n = 8) administered L-T4 sodium as Leventa® once daily for 14 days. *Observed values.

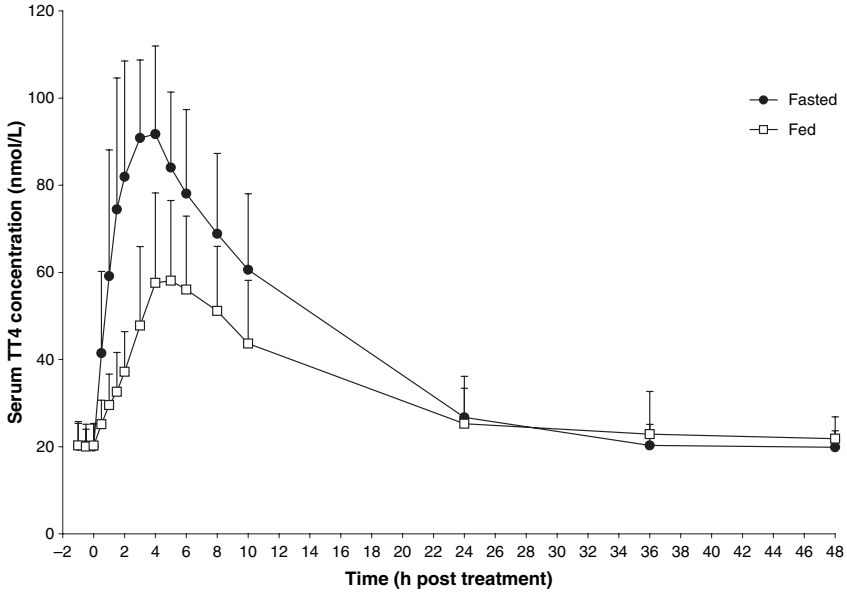

Fig. 3. Serum tT4 concentrations* (mean ± SD) in dogs (n = 8) following administration of Leventa® with and without food. *Observed values.

Table 5. Pharmacokinetic parameters* calculated following oral administration of 200 μg L-T4 sodium to dogs (n = 8) as either a tablet twice daily (q12h) or an oral solution once daily

	Tablet		Liquid
Parameters	1st administration	2nd administration	Single administration
C_{max} (nmol/L)†	30 ± 8.7	28 ± 11.5	40 ± 10.4
t_{max} (h)	3.0 (2.0–4.0)	3.0 (1.5–3.0)	3.0 (1.0–4.0)
$AUC_{0\text{-}12h}$ (nmol·h/l)†,‡	218 ± 70.2	191 ± 87.5	293 ± 66.8
$AUC_{0\text{-}24h}$ (nmol·h/l)†	409 ± 144.3		370 ± 110.5

*Data expressed as mean ± SD if distributed normally or as median (range). †Corrected for endogenous tT4 concentration. ‡Corresponds to *AUC* calculated over the first 12 h after each oral administration of 200 μg L-T4 sodium to dogs.

Consequently, the relative bioavailability of a single dose of the oral solution was 206% (median, IQR 137–242%) that of the same dose of the tablet formulation.

DISCUSSION

L-T4 oral replacement therapy has been used extensively in human and veterinary medicine for several decades. The pharmacokinetic properties of L-T4 *per os*, mainly in the form of tablets, have been studied in a number of species, including dogs. The results of the present studies show that the pharmacokinetics of L-T4 as an oral solution displays some specificities that have potential clinical implications.

The pharmacokinetic profiles of tT4 and not free T4 (by equilibrium dialysis) were evaluated during the four studies for two reasons. Firstly, changes in serum tT4 concentrations directly reflect the absorption of the exogenous moiety following its oral administration to dogs. Secondly, although free t4 (by equilibrium dialysis) is considered essential in establishing a diagnosis of hypothyroidism in dogs (Dixon & Mooney, 1999), tT4 concentrations are valuable for monitoring the degree of control of hypothyroidism (Dixon & Reid, 2002).

A dose rate of 40 μg L-T4/kg once daily of the oral solution was selected because it corresponds with the highest daily dose of this formulation required to control clinical hypothyroidism in dogs (Brennan *et al.*, 2006). The administration of a high dosage of L-T4 may result in high peak serum tT4 concentrations and inhibition of endogenous tT4 secretion. Therefore, the effects of this high dosage on the pharmacokinetics of tT4 were assessed in comparison with the results from study D where 50% of this dose rate was administered. In fasted dogs, the mean C_{max} value reported following a 20 μg/kg oral dosage (around 40 mol/L) was half of that after 40 μg/kg oral administration (around 78 nmol/L) and t_{max} was comparable. This suggests that the inhibition of tT4 by oral administration of L-T4 solution at 40 μg/kg was either negligible or would only be observed following long-term repeated administrations.

After intravenous administration of L-T4, a mean serum half-life of 11.6 h was calculated. This is longer than that has been described in previous studies (Nachreiner *et al.*, 1993). Following a single oral administration of L-T4 of the oral solution, pharmacokinetic parameters, such as t_{max}, $t_{1/2}$ and bioavailability, were within the range of values reported previously in dogs (Nachreiner *et al.*, 1993; Kaptein *et al.*, 1994). Dogs have a relatively low bioavailability and relatively short serum terminal $t_{1/2}$ of T4 compared with humans (bioavailability ~ 60%, $t_{1/2}$ ~ 1 week) (Kaptein *et al.*, 1994) which may explain that under clinical conditions, the dose rates of L-T4 needed to achieve adequate tT4 concentrations in hypothyroid dogs are about ten times those reported for humans.

The profile, rate and extent of absorption of L-T4 remained unchanged after oral administration of the oral solution of L-T4

(40 μg/kg) to dogs once daily for 14 consecutive days. Steady state was reached from the first day of treatment, as indicated by the stabilization of trough and peak concentrations after the first treatment and by the absence of significant difference between pharmacokinetic parameters C_{max}, t_{max}, and *AUC* on day 1 and on day 14, and there was no accumulation of T4 in serum during treatment. Only a slight increase in trough tT4 concentrations was observed during the treatment period. During repeated oral administration of L-T4 tablets (Soloxine®) at dose rates up to 44 μg/kg once or twice daily, it was concluded that steady state was reached from the 7th day of treatment (Refsal & Nachreiner, 1995). However, in that study, peak and trough blood samples were performed only once a week between the first and the last treatment. The results of the present study allowed to state more precisely the time needed to reach steady state during repeated treatment with oral L-T4 solution, by a closer monitoring of trough and peak concentrations throughout the entire study period.

The findings that the apparent serum terminal half-life of T4 was not increased by repeated dosing and that tT4 serum concentrations regained basal concentrations at about 34 h after the last oral administration to normal dogs are in line with published data from thyroidectomized dogs (Nachreiner *et al.*, 1993) reporting a relatively short half-life of T4 in the circulation at a dose rate of 44 μg/kg once daily (mean $t_{1/2}$ value of 11.5 h). The apparent elimination half-life evaluated following oral administration of L-T4 was longer than the terminal half-life observed after intravenous administration (about 14 and 11 h, respectively), suggesting the existence of a 'flip-flop' kinetics for oral L-T4 in dogs, something that has not been reported previously.

In humans, L-T4 absorption following oral treatment is reduced when administered concomitantly with food, compared with administration on an empty stomach (Wenzel & Kirschsieper, 1977). In humans, it is recommended that L-T4 should be taken on an empty stomach. The present study showed for the first time, that a food interaction is present in dogs, the bioavailability of L-T4 being almost double when the oral solution was administered on an empty stomach compared with concomitantly with food. The absorption of L-T4 was also delayed in fed dogs. The therapeutic correlate of this observation in hypothyroid dogs is that L-T4 absorption will be maximized if it is administered on an empty stomach in dogs. In addition, standardization of the temporal relationship between L-T4 administration and feeding is advised so as to minimize inter-day variation in serum tT4 concentration and thus, a consistent response to treatment in dogs with hypothyroidism. As in humans, where potential for interactions with food constituents, such as walnuts, liver, albumin and soybean have been identified (Choe & Hays, 1995; Liel *et al.*, 1996), an effect of specific dietary constituents might exist in dogs. This warrants further investigation.

In the pharmacokinetic studies presented here, there was a high inter-individual variation in the extent of absorption of L-T4 following oral administration to healthy dogs. This observation was previously reported in thyroidectomized dogs (Nachreiner *et al.*, 1993). Such high variability, together with interindividual physiological variability in T4 requirements, provides evidence for the empirical practice that the L-T4 dose should be adjusted individually to achieve therapeutic concentrations of tT4.

In study D, treatment recommendations for each L-T4 product were followed and the subsequent tT4 concentrations achieved compared. It was demonstrated that about 50% less T4 was absorbed following administration of the tablet formulation compared with the oral solution, showing that a single dose of the oral solution of L-T4 sodium can be considered as equivalent to two doses of the tablet formulation administered at 12-h intervals. Following oral administration of the oral solution of L-T4 at dose rate of 20 μg/kg once daily, tT4 concentrations were also within the euthyroid reference range over the 24-h period post-treatment. A similar observation was made for the tablet formulation at a dose rate of 20 μg/kg twice daily, which is fully in line with the results of Nachreiner *et al.* (1993). Following treatment with the oral L-T4 solution, tT4 concentrations reached a peak of 40 ± 10.4 nmol/L once during the 24-h treatment interval. Following twice daily treatment with L-T4 tablets, peak concentrations of around 30 nmol/L were reached once during each 12-h dosing interval. This difference is not expected to affect the clinical efficacy of the L-T4 solution as efficacy is more likely related to total exposure of tissues to thyroxine, which is reflected by the total amount of thyroxine in the body (as determined by the *AUC*) rather than by peak tT4. These results are fully in agreement with recent clinical observations where the oral solution of L-T4 at a dose rate of L-T4 of 20 μg/kg once daily was effective in and sufficient for controlling clinical and hormonal signs of hypothyroidism in almost all dogs (Brennan *et al.*, 2006).

In the present study, healthy normal (with intact functioning thyroid glands) dogs were used. Methodological considerations and calculation methods comparable to what is proposed by the Food and Drug Administration since 2003 for evaluation of relative bioavailability of L-T4 in humans have been applied, *i.e.* correction of post-treatment tT4 concentrations by endogenous tT4 concentrations, calculated as the mean of three pretreatment time points. This was considered as a valid approach as (i) the contribution of endogenous tT4 to the *AUC* cannot be considered as negligible when sampling is for a short period (*e.g.* 24 h), especially in case of administration of low L-T4 doses (20 to 40 μg/kg) and (ii) it is assumed that the effect of single exogenous administration of L-T4 on endogenous T4 secretion is negligible, related to the short duration of each single dose pharmacokinetic study (studies A, C and D) compared with the time required to evidence inhibition of T4 secretion. This was confirmed by the last determinations of tT4 concentrations in each study: tT4 concentrations regained pretreatment basal (and not lower) levels in all the dogs. The correction method using three predose baseline values was considered as reasonable and more appropriate approach than more complex methods of correction (Bolton, 2005). In a previous study (Nachreiner *et al.*, 1993), dogs were thyroidectomized prior to pharmacokinetic evaluations. However, nonnegligible tT4 concentrations were still observed following surgical removal of thyroid gland (mean:

11 ± 3.4 nmol/L, range: 6–18 nmol/L), possibly related to synthesis of thyroid hormones from nonthyroidal tissue, according to authors. In contrast, no correction of tT4 concentrations from the endogenous concentration was performed in the long-term repeated dose study (study B). Indeed, it was considered that (i) the inhibition of endogenous secretion by repeated oral supply of exogenous T4 cannot be neglected. This was confirmed by the lower serum thyroxine concentrations reported at 96 h after the last treatment than on day 1 in four of eight dogs). (ii) Such a correction would attenuate the potential accumulation of T4 assessed following repeated daily oral administration. Future studies will focus on confirming that the pharmacokinetics of this L-T4 solution in healthy dogs accurately reflects the pharmacokinetics in dogs with naturally-occurring hypothyroidism. Such studies will require careful selection of clinical cases with little or no residual thyroxine secretion.

Regarding bioanalytical methods used to determine serum tT4 concentrations, the RIA kit used for the three studies A, B and C had a rather high limit of quantification (LOQ, 15 nmol/L). This value is at the lower limit of the reference range for serum tT4 concentration in healthy dogs. The use of such a method for samples from hypothyroid dogs would have resulted in a poor description of basal and post-treatment tT4 concentrations. However, as the present studies were performed in healthy, euthyroid dogs, the LOQ was not a limiting factor in the interpretation of the study results. The LOQ value was assigned to all tT4 concentrations below LOQ. Prior to treatment, basal tT4 values below the LOQ were rarely seen and three tT4 values were averaged to calculate the basal tT4 concentration for each dog, thus limiting any impact approximation to LOQ would have, if any. Some values below LOQ were reported at the end of the blood sampling period. However, when reported, these values were in the middle of values for other sampling points just above the LOQ. Thus, the values below LOQ were also likely close to LOQ and approximation to the LOQ value could be justified.

In conclusion, in dogs, the pharmacokinetic profile of tT4 following administration of L-T4 as oral solution is similar to that observed for L-T4 tablet formulations in terms of t_{max} and $t_{1/2}$; however, the bioavailability of T4 from the oral solution is approximately double that of the tablet formulation. tT4 concentrations reported after oral administration of the L-T4 solution support the general recommendation to use the oral solution of L-T4 at a dose rate of 20 μg/kg once daily for the treatment of hypothyroidism in dogs. It is important to standardize of the timing of L-T4 administration in relation to feeding to minimize inter-day variation in serum tT4 concentrations and a thus a consistent response to treatment.

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