

Long-term delivery of ivermectin by use of poly(D,L-lactic-co-glycolic)acid microparticles in dogs

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Objective—To evaluate the potential utility of poly(D,L-lactic-co-glycolic)acid (PLGA) as a long-acting biodegradable drug delivery matrix for ivermectin used in the prevention of heartworm disease in dogs.

Animals—30 adult female dogs.

Procedure—Microparticle formulations containing 25 weight percent (wt%), 35 wt%, and 50 wt% ivermectin were prepared by an oil-in-water emulsion technique with solvent extraction into excess water. A fourth formulation, consisting of a mixture of 15 wt% and 50 wt% ivermectin microparticles, was blended in a 1:1 ratio to result in a 32.5 wt% ivermectin formulation. Formulations were administered once on Day 0 to groups of 6 dogs at a dose of 0.5 mg of ivermectin/kg, SC. Half of the dogs in each treatment group and 3 untreated control dogs were infected with *Dirofilaria immitis* larvae 121 and 170 days after treatment. Six months after infection, dogs were euthanized and necropsies were performed. Pharmacokinetics and efficacy were investigated.

Results—Analysis of pharmacokinetic data revealed sustained release of ivermectin during at least 287 days in 3 distinct phases: a small initial peak, followed by release of drug through diffusion, and polymer degradation. Untreated control dogs were all infected with heartworms. Heartworms were not found in any of the dogs in the ivermectin-PLGA treated groups. Adverse clinical signs were not observed.

Conclusions and Clinical Relevance—All formulations were 100% effective in preventing development of adult heartworms. Results indicate that PLGA microparticles are a promising drug delivery matrix for use with ivermectin for the prevention of heartworm disease for at least 6 months after treatment. (*Am J Vet Res* 2004;65:752–757)

Ivermectin has been widely used for many years for the prevention of heartworm disease in dogs. Available in hard tablet and chewable formulations, this drug is administered once a month at a minimum dose of 6 µg/kg, PO.^{1,2} Since the introduction of ivermectin preventives for dogs, orally or topically administered formulations of other macrolides including selamectin, moxidectin, and milbemycin have been approved for use in the United States.^{3,5} A long-acting, nonbiodegradable injectable formulation of mox-

idectin, which provides 6 months of protection against heartworm disease, has been introduced in the United States.⁶

Poly(D,L-lactic-co-glycolic)acid (PLGA) is a safe and effective biodegradable material and has consequently been used as a drug delivery matrix for extended release applications.⁷ Pharmaceuticals have been delivered for a few weeks to several months via SC or IM injection with PLGA microparticles containing products such as leuprolide acetate,^a octreotide acetate,^b human growth hormone,^c risperidone,^d and estradiol benzoate.^e Control of drug release kinetics is obtained by varying the polymer composition (lactide-to-glycolide ratio), polymer molecular weight and end-group character, control of the drug content, and selection of process conditions.⁸ Estradiol benzoate^e is released during a period of 140 to 150 days, whereas the other products have release times from 2 weeks to as much as 4 months.

The lactide-to-glycolide ratio strongly influences the rate of PLGA degradation. For example, 50:50 (lactide-to-glycolide ratio) PLGA degrades in several weeks, whereas 100% poly(lactic acid) degrades during the course of a year.⁹ Ivermectin microparticles formulated with 65:35 PLGA have been found to control the cattle tick, *Boophilus annulatus*, on cattle during pasture conditions for a period of 4 months after SC injection.¹⁰

The purpose of the study reported here was to evaluate the potential use of PLGA biodegradable drug delivery matrix for ivermectin for the prevention of heartworm disease in dogs. We examined several 85:15 PLGA-to-ivermectin microparticle formulations that varied only in weight percent (wt%) drug content. Results of a preliminary study indicated that the initial release rate of ivermectin from PLGA microspheres in vitro varied with drug content in the microparticles. A lower drug-to-polymer ratio caused a reduced rate of drug release into a modified aqueous medium containing 40% ethanol. We postulated that varying drug content would provide a range of in vivo pharmacokinetic behavior.

Materials and Methods

United States Pharmacopoeia (USP) grade ivermectin^f (93.2% B_{1a} isomer, 3.3% B_{1b} isomer) and USP grade PLGA^g with a lactide-to-glycolide molar ratio of 85:15, mean molecular weight of 136 kd, polydispersity of 1.5, and inherent viscosity of 0.91 dL/g were used for the components of the microparticles. Polyvinyl alcohol,^h ethyl acetate,ⁱ and USP grade purified water were used during microparticle preparation. Carboxymethylcellulose,^j

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methylparaben,^k and USP grade purified water were used for vehicle preparation.

Preparation of ivermectin-loaded PLGA microparticles—Four 75 g batches of ivermectin microparticles were prepared to contain 15, 25, 35, or 50 wt% of ivermectin raw material within the PLGA matrix. The appropriate drug and PLGA quantities were dissolved in ethyl acetate and, separately, a water solution of 1% polyvinyl alcohol was prepared. The 2 phases were mixed to form a stabilized oil-in-water emulsion. The emulsion was added to a volume of excess water for a first extraction of ethyl acetate from the emulsion droplets. After 20 minutes, the hardened microparticles were collected on a 25- μ m-hand sieve and transferred to a second water volume for final solvent extraction. After 120 minutes, the contents of the second extraction were poured through a 150- μ m-hand sieve stacked on top of a 25- μ m-hand sieve. Microparticles collected on the 25- μ m-hand sieve were permitted to air dry for 2 days in a fume hood. Microparticles were transferred into a vacuum desiccator for an additional 2 days of drying. A fifth batch was made by combining a fraction of the 15 wt% batch and 50 wt% batch in equal parts by weight to yield a theoretical 32.5 wt% drug content blended sample. Microparticles were weighed into Type I borosilicate glass vials and stored at 22°C.

Preparation of injection vehicle—Injection vehicle was formulated by dissolving 2.5 wt% carboxymethylcellulose in stirring USP grade purified water heated to 80°C. When the carboxymethylcellulose was visibly dissolved, the solution was cooled to 23°C and 0.18 wt% methylparaben was added. The solution was filtered through a 0.22- μ m-syringe filter into Type I borosilicate glass vials and stored at 22°C.

Characterization of ivermectin microparticles—Drug content, calculated as the wt% of ivermectin in the final dried microparticle product, was determined by use of high-performance liquid chromatography^l (HPLC) with a photodiode array detector, a 4.6 \times 250 mm (5 μ m particle size) column,^m and computer software.ⁿ The sample was prepared by dissolving microparticles in tetrahydrofuran, which was then diluted 10-fold with mobile phase (340 mL methanol, 510 mL of acetonitrile, and 150 mL of USP grade purified water). The wt% of ivermectin was calculated from the area of the B_{1a} isomer peak measured at 245 nm relative to a separately prepared sample of ivermectin raw material, which served as the standard.

Microparticle size distribution was measured by use of a particle size analyzer.^o Mean particle size was reported as a volume weighted mean diameter.

Residual ethyl acetate was determined with a gas chromatograph.^p Ivermectin microparticle samples were dissolved in N,N-dimethylformamide with propyl acetate as an internal standard.

Scanning electron micrographs were obtained by use of a scanning electron microscope with imaging software.^q Samples were sputter coated with gold by use of a sputtering system.^r

Animals and treatment with test article—Thirty sexually intact female laboratory Beagles, ranging in age from 11 months to 4 years and weighing 9.6 to 12.3 kg were used. Dogs were housed in gang runs, with as many as 4 dogs per run, in climate-controlled rooms (16.1° to 27.2°C [61° to 81°F], 26% to 45% relative humidity, and a 12 hours on:12 hours off light cycle) and fed commercial dog food^s with ad libitum access to fresh water from an automatic watering system in each run. The institutional animal care and use committee of the animal facility approved the protocol for this study.¹

On the basis of weights obtained before treatment, 24 dogs were ranked in descending order of body weight and blocked into 6 groups of 4 dogs. Within weight groups, dogs

were randomly allocated to 1 of 4 treatment groups (T1, T2, T3, and T4, each representing a different ivermectin-PLGA formulation), each containing 6 dogs. The formulation for group T1 consisted of a 25 wt% drug content ivermectin-PLGA microparticle preparation. The formulations for groups T2 and T3 contained 35 wt% and 50 wt% drug content, respectively, whereas T4 had a 32.5 wt% drug content resulting from a 1:1 wt:wt blend of the 15 wt% and 50 wt% batches. Each of these 24 dogs was treated once on day 0 with a single dose of ivermectin (0.5 mg/kg of active ivermectin) in PLGA microparticles. Before injection, microparticles were suspended in the injection vehicle and administered SC (1 mL injection volume) into the interscapular space with a 3-mL-syringe and 22-gauge, 1-inch needle. The remaining 6 dogs served as untreated controls and were not used until dogs were infected with *Dirofilaria immitis* larvae, at which time 3 dogs were infected on day 121 and 3 dogs were infected on day 170.

Pharmacokinetic evaluation—Serum was obtained from blood samples (4 mL) taken from the jugular vein of each treated dog on days 0, 1, 2, 4, 7, 10, 14, 21, and approximately every 7 days thereafter until day 168. After day 168, samples were obtained on days 204, 224, 245, 266, and 287. On day 296, samples were obtained from only those dogs (n = 3, half of each group) euthanatized on that day. Blood was obtained from the remaining dogs (3 dogs in each treatment group) on days 315 and 345. Serum samples were stored frozen (–20°C) until analysis. Blood samples were obtained from control dogs once just prior to euthanasia. The assay for serum ivermectin concentrations was performed by use of HPLC with a fluorescence detection method.¹¹ After analytical method validation,¹² the limit of detection was determined to be 0.022 ng/mL, the limit of quantitation was 0.1 ng/mL, and the upper limit of linearity was 10 ng/mL. Correlation coefficients for all standard curves were \geq 0.99. The overall recovery was 93.8%; the recovery for samples at the low and high ends of the curve was 86.6% and 97.5%, respectively. The overall SD for the method was 7.69 with a percent relative standard deviation (%RSD) of 8.20%. The SD for the low end of the curve was 7.55 with a %RSD of 8.72%, and the SD for the high end of the curve was 5.43 with a %RSD of 5.57. Personnel performing bioassays for serum ivermectin concentrations were unaware of treatment group identification.

Efficacy evaluation—Efficacy of treatments was evaluated by comparing the number of heartworms found at necropsy in treated versus control dogs. Half (3 dogs) of the dogs in each treatment group and 3 untreated control dogs were infected with 45 infective third-stage larvae of *D immitis* on day 121. The second half of each group of treated dogs and 3 untreated control dogs were infected with 45 infective third-stage larvae on day 170. *Dirofilaria immitis* larvae were cultured and administered according to a previously published method.^{13,14} One hundred seventy five days after the induced infections (day 296 for dogs infected on day 121 and day 345 for dogs infected on day 170), dogs were euthanatized by IV injection of pentobarbital (75 mg/kg) and phenytoin^u (50 mg/kg) and heartworm counts were performed. Percentage efficacy was calculated by use of the following formula:

$$\frac{\text{Mean No. of worms recovered from control dogs} - \text{Mean No. of worms recovered from treated dogs}}{\text{Mean No. of worms recovered from control dogs}} \times 100\%$$

Personnel performing heartworm counts were unaware of treatment group identification.

Safety evaluations—Safety was evaluated by visual and tactile evaluation of the injection site of each dog for several days immediately after injection and by gross and histologic

evaluation of the injection site after euthanasia. Each dog was observed daily for food and water consumption, appearance, and evidence of clinical abnormalities. Personnel performing the clinical and injection site observations and histologic evaluations were unaware of treatment group identification.

Statistical analyses—Area under the curve (AUC), the maximum concentration (C_{max}), and the time at C_{max} (T_{max}) were calculated for each dog by use of commercial software.^v The area of a trapezoid method (equal to the sum across the entire time period of individual areas defined by the time of sampling and the concentration detected) was used to calculate AUCs. The C_{max} was determined directly from the data, and the T_{max} was determined by finding the point of C_{max} . The effect of formulation on each of these parameters was evaluated by ANOVA by use of computer software^w and the Wilcoxon signed-rank test, neither of which altered the interpretation of the results. If the effect of formulation was significant ($P < 0.05$), all pairs of means were compared by use of unpaired, 2-sided t test. Only serum ivermectin assay results through day 287 were included in the analysis because this was the last study day for which samples were available for all dogs in all treatment groups. Samples with values below the limit of quantitation (0.1 ng/mL) were considered as 0.0 ng/mL for purposes of analysis.

Results

Characterization of microparticles—Four batches of ivermectin-PLGA microparticles were formulated with target drug contents of 15, 25, 35, and 50 wt%. The actual measured drug contents ranged from 13.2 to 46.5 wt% based on the total dry weight of the microparticles (Table 1). Residual ethyl acetate varied from 4.8 to 5.7 wt%. These concentrations of ethyl acetate did not adversely affect the physical characteristics of the microparticles (ie, by fostering aggregation). If all the solvent were to leave the microparticles at the time of injection, the maximum exposure to

Table 1—In vitro characterization of ivermectin-poly(D,L-lactic-co-glycolic)acid (PLGA) microparticle formulations

Theoretical drug content (wt%)	15	25	35	50	32.5
B_{1a} drug content (wt%)	13.2	24.3	30.8	46.5	29.6
Residual ethyl acetate (wt%)	4.8	5.7	5.5	5.6	4.9
Mean particle size (μm)	92.0	91.0	90.0	89.0	90.0

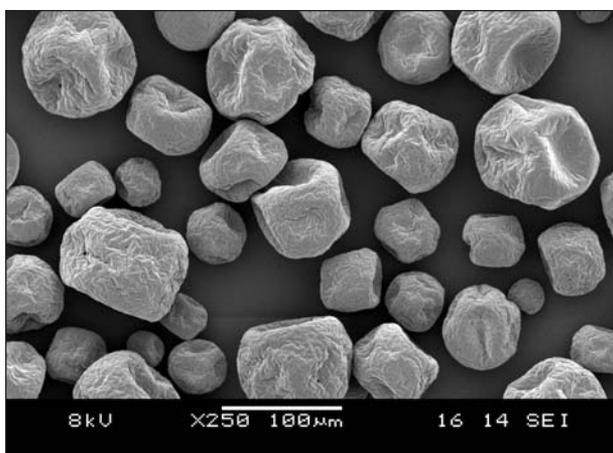


Figure 1—Scanning electron micrograph of 50 weight percent (wt%) ivermectin microparticles. Notice the microparticles are dimpled and have a rough surface texture. Bar = 100 μm .

ethyl acetate, a Class 3 solvent of low toxic potential, for the dogs in this study would have been 0.1 mg/kg, where the limit for this solvent class has been set at 50 mg/kg/day.¹⁵

The particle size distribution for each batch yielded a bell-shaped curve with the mean between 89 and 92 μm (Table 1). This consistency confirmed that process parameters were well controlled and batch-to-batch reproducibility was highly achievable with respect to particle size.

Scanning electron microscopy revealed dimpled microparticles with rough surface texture (Fig 1). There were no visible surface drug crystals. All formulations appeared similar in surface features and morphology.

Pharmacokinetics—Ivermectin was not detected in the serum of dogs before treatment or in the serum of untreated control dogs obtained just before euthanasia. Mean C_{max} of ivermectin in serum varied from 3.4 to 8.4 ng/mL, with the highest concentration observed in dogs treated with the formulation for T1 (Table 2). The T_{max} varied considerably between groups. The T_{max} for T3 was on day 1, whereas, for all other groups, T_{max} occurred later in the study. There was no difference among formulations for AUC; however, the pattern of ivermectin release varied considerably. All formulations released a small quantity of ivermectin 1 to 3 days after administration, followed by a 2- to 3-week period of minimal drug release (Fig 2 and 3). Thereafter, formulations behaved quite differently with respect to ivermectin release. The formulation for T1 released little or no detectable ivermectin until approximately day 161, after which ivermectin concentrations peaked on day 224 before decreasing again. The formulation for T2 released small quantities of ivermectin during the first 5 months of the study before it peaked on day 224, after which ivermectin concentrations decreased to < lower quantitation limit by day 314. The pharmacokinetic profile for dogs in T3 was more consistent with time than that of dogs in T1 or T2. Beginning 21 days after treatment, ivermectin concentrations remained constant until day 168, decreased somewhat on day 204, and then increased through day 266. By day 345, concentrations had decreased < limit of quantitation. Dogs in T4 had a similar pharmacokinetic profile as those in T3, although there was more

Table 2—Least squared mean \pm SD (median) values of pharmacokinetic parameters for 4 ivermectin-PLGA microparticle formulations containing 25 wt% (T1), 35 wt% (T2), 50 wt% (T3), or 32.5 wt% blend (T4) ivermectin in dogs

Group	AUC	C_{max} (ng/mL)	T_{max} (d)
T1	410 \pm 118.8 (412.6)	8.4 \pm 3.57 ^a (6.87)	228 \pm 33.2 ^a (214)
T2	400 \pm 98.4 (364.7)	7.2 \pm 4.44 ^{a,b} (5.85)	174 \pm 87.6 ^b (214)
T3	330 \pm 67.7 (325.1)	3.9 \pm 1.33 ^{b,c} (3.41)	1 \pm 0 ^b (1)
T4	375 \pm 106.4 (334.0)	3.4 \pm 1.27 ^c (2.78)	169 \pm 99.0 ^b (193)
<i>P</i> value	0.5266	0.0211	0.0001

Each dog ($n = 6$ per group) was treated once with a single dose of ivermectin (0.5 mg/kg) in PLGA microparticles.
^{a,b,c}Within a column, values with different superscript letters are significantly ($P < 0.05$) different.

between-animal variability in serum ivermectin concentrations.

Efficacy—No heartworms were recovered from any of the treated dogs (Table 3). In contrast, all control dogs had heartworms at necropsy. Mean number of heartworms found in control dogs was 27.0 (range, 21

to 33/dog) and 29.7 (range, 20 to 35/dog) for dogs infected on days 121 and 170, respectively.

Safety—There were no signs of pain during or after injection of test formulations on day 0 in any dog. All dogs were clinically normal, and no adverse events were reported during the study. Visual and tactile evaluations of the injection site revealed slight, subcutaneous edema (approx 1 cm in diameter and 1- to 2-mm-thick) in 1 dog from T1 on day 1. Palpation of the injection site did not elicit signs of pain. By day 2, the subcutaneous edema was barely detectable and, by day 4, no abnormalities were detectable. At necropsy, no gross abnormalities were observed at the injection sites in any of the ivermectin-PLGA treated dogs. Histologic evaluation of injection sites from dogs euthanatized 296 days after injection revealed no abnormalities in any of the dogs in T2, T3, and T4. Two of the 3 dogs in T1 had 1 or 2 small foci of mononuclear inflammatory cell infiltration in the subcutaneous tissue. In dogs euthanatized 345 days after injection, there were no abnormalities recorded for dogs in T3. For each of T1, T2, and T4, 2 of 3 dogs had evidence of minor inflammatory changes described as minimal focal mononuclear cell infiltration.

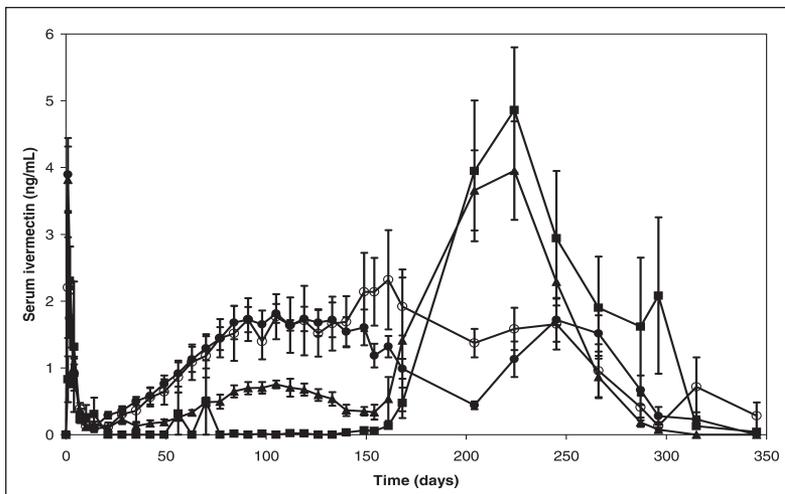


Figure 2—Mean \pm SD of serum ivermectin concentrations in dogs treated with 4 formulations of ivermectin-poly(D,L-lactic-co-glycolic)acid (PLGA) microparticles containing 25 wt% (squares), 35 wt% (triangles), 50 wt% (closed circles), or 32.5 wt% blend (open circles) ivermectin. Each dog was treated once on day 0 with a single dose of ivermectin (0.5 mg/kg) in PLGA microparticles (n = 6 dogs/treatment group for samples obtained through day 287; 3 dogs for samples obtained thereafter).

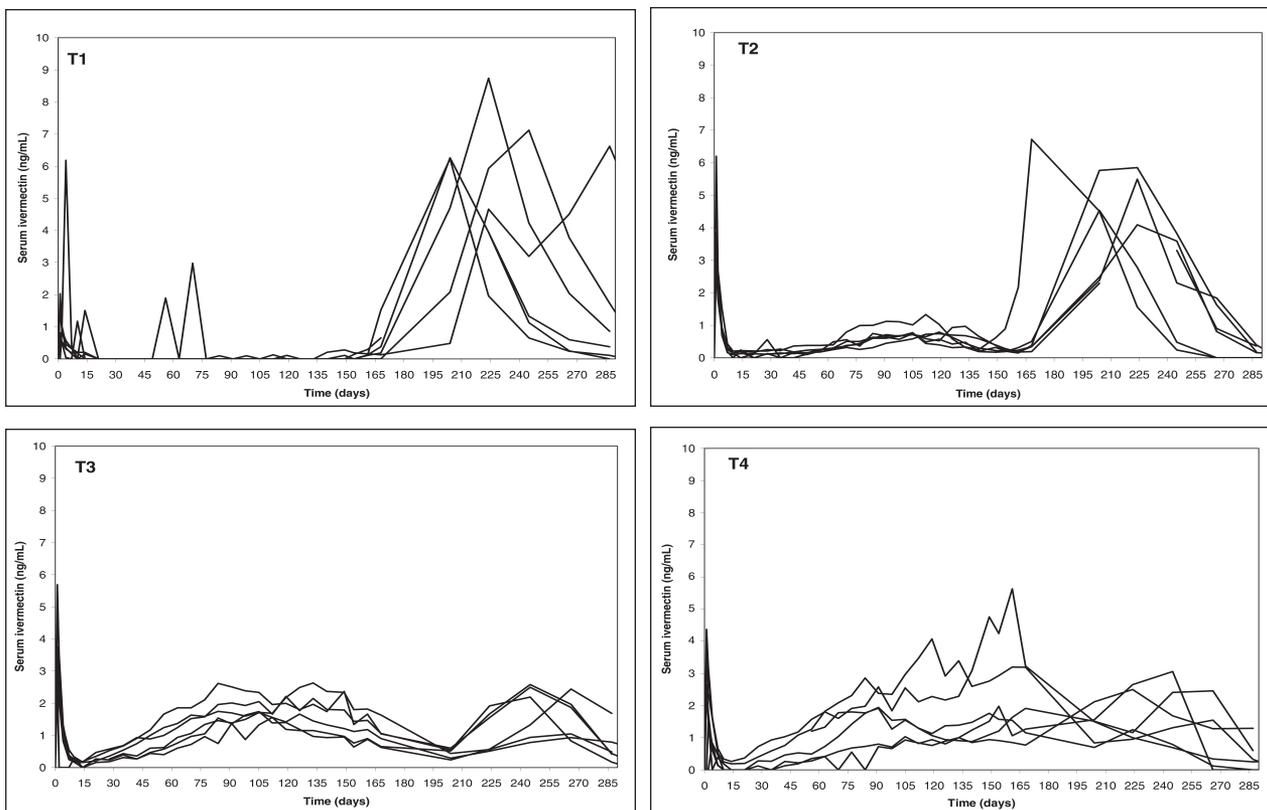


Figure 3—Individual serum ivermectin concentrations (ng/mL) in dogs (n = 6/treatment [T] group) treated with 4 formulations of ivermectin-PLGA microparticles containing 25 wt% (T1), 35 wt% (T2), 50 wt% (T3), or 32.5 wt% blend (T4) ivermectin through day 287. Each dog was treated once on day 0 with a single dose of ivermectin (0.5 mg/kg) in PLGA microparticles.

Table 3—Efficacy of 4 ivermectin-PLGA microparticle formulations containing 25 wt% (T1), 35 wt% (T2), 50 wt% (T3), or 32.5 wt% blend (T4) ivermectin against infections in dogs with *Dirofilaria immitis* induced 121 or 170 days after treatment with a single dose of ivermectin (0.5 mg/kg) in PLGA microparticles

Variable	Day of induced infection									
	121					170				
	T1	T2	T3	T4	C	T1	T2	T3	T4	C
Mean No. of heartworms	0	0	0	0	27.0	0	0	0	0	29.7
Proportion of dogs infected	0/3	0/3	0/3	0/3	3/3	0/3	0/3	0/3	0/3	3/3
% Efficacy	100	100	100	100	NA	100	100	100	100	NA

C = Control (untreated). NA = Not applicable.

Discussion

Microparticle formulations of ivermectin had excellent drug encapsulation up to 50% by weight of drug with a process capable of accurately controlling particle size and residual solvents. All formulations induced dimpled microparticles with rough surfaces. The microparticle powders were free-flowing and remained so during storage. The dried powder-like microparticles were readily suspended in the vehicle, and injections were easily made through a 22-gauge needle.

The 4 ivermectin-PLGA formulations were well tolerated in terms of injection site reactions and systemic effects. From a pharmacokinetic perspective, the AUCs were not different from 1 another, but differences in T_{max} , C_{max} , and pattern of drug release were evident. Formulations used in T1 (25 wt% ivermectin) and T2 (35 wt% ivermectin) released most of the drug in the later part of the study, whereas formulations used in T3 (50 wt% ivermectin) and T4 (15 wt% and 50 wt% blend of ivermectin) released more drug earlier and more consistently during the study. Similarities between formulations used in T3 and T4 were not unexpected in that half of the formulation for T4 was composed of 50 wt% drug content microparticles that were expected to have contributed to a pharmacokinetic profile similar to that observed in dogs in T3. Between-animal variability in serum ivermectin concentrations within treatment groups was minimal, suggesting that the formulations behaved similarly in all dogs. Importantly, all formulations induced detectable serum ivermectin concentrations for at least 287 days.

The difference in pharmacokinetic profiles between the 25 wt% and 50 wt% ivermectin content microparticles was striking. At 25 wt% ivermectin content, the PLGA polymer network was believed to be sufficiently dense that little drug escaped until the process of polymer degradation provided escape routes beginning on approximately day 150. Similarly, Beck et al⁹ reported a 50% molecular weight reduction of 87:13 PLGA polymer 150 days after injection of norethisterone microparticle formulation. Beginning around 150 days after injection, the influence of polymer degradation became apparent for the 25 wt% ivermectin microspheres, and the drug was released during the subsequent 100 to 150 days.

With the 50 wt% ivermectin content microparticles, the polymer network made up only half the mass of the particles. After the initial release of a small percentage of

the surface-bound ivermectin during the first week, serum ivermectin concentrations increased steadily up to approximately 100 days and then diminished moderately at approximately 200 days. Drug release during this early phase occurred largely by diffusion of ivermectin out of the microparticles.⁷ The balance of the encapsulated ivermectin was released during the polymer degradation phase from 200 to approximately 300 days. The 35 wt% ivermectin content microparticles had a pharmacokinetic profile intermediate to the 50 wt% and 25 wt% microparticles. Finally, the blend of 50 wt% and 15 wt% ivermectin microparticles had a profile similar to that of the 50 wt% microparticles (which contributed 77% of the drug in the blend) but with a less pronounced trough between the diffusion and degradation phases probably caused by contributions from the 15 wt% ivermectin microparticles.

All 4 formulations were 100% effective in preventing development of adult heartworms following infections induced 121 or 170 days after treatment. Further research is required to evaluate the efficacy of these formulations in preventing heartworm infections beyond 170 days because serum concentrations of ivermectin were detectable until at least day 287. To the authors' knowledge, the minimum serum ivermectin concentrations for efficacy against heartworms have not been published, although peak concentrations of 2.9 and 3.4 ng/mL have been reported for single radiolabeled doses of ivermectin (6 µg/kg, PO) from tablets and chewable formulations, respectively.¹⁶ By 3 days after administration, serum ivermectin concentrations decreased to < 0.5 ng/mL. Ivermectin administered at a dosage of 6 µg/kg, PO, once a month is 100% effective in preventing heartworm disease in dogs despite the short (1.8 days) intrinsic half-life of ivermectin.^{2,17} In our study, serum ivermectin concentrations peaked from 1 to 228 days and varied from 3.4 to 8.4 ng/mL depending on the formulation and duration after treatment. Because of the exquisite sensitivity of *D immitis* to ivermectin, the short spike of ivermectin administered at a dosage of 6 µg/kg, PO, once a month provides efficacy against heartworm larvae as much as 30 days old. A continuous low dose of ivermectin, as provided by a sustained-release formulation, may also provide similar efficacy. In the study reported here, the limit of detection of the assay may not have permitted for full characterization of the pharmacokinetics. Therefore, although dogs in T1 did not have detectable concentrations of ivermectin in serum at the time of infection on day 121, efficacy was still 100%. Thus, it

is possible that doses lower than that used in our study would be effective. In addition, the safety of this delivery system needs to be evaluated at multiples of the ultimate effective dose. On the basis of the pharmacokinetic profiles, formulations used in T3 and T4 would be preferred because they provided the most uniform kinetic profiles during the drug release period. Results of the study reported here indicate that 85:15 PLGA microparticles are a promising drug delivery matrix for use with ivermectin for the prevention of heartworm disease for at least 6 months after treatment.

- ^aLupron Depot, TAP Pharmaceuticals Inc, Lake Front, Ill.
^bSandostatin LAR, Novartis Pharmaceuticals Corp, East Hanover, NJ.
^cNutropin Depot, Genentech Inc, South San Francisco, Calif.
^dRisperdal Consta, Janssen Pharmaceutica Products LP, Titusville, NJ.
^eCelerin, PR Pharmaceuticals Inc, Fort Collins, Colo.
^fEcomectin, ECO Animal Health Inc, London, UK.
^gVetisorb 8515 DL EB1, Alkermes, Wilmington, Ohio.
^hAirvol 205 PV alcohol, Air Products, Allentown, Pa.
ⁱEthyl acetate ACS grade, Fisher Scientific, Houston, Tex.
^jCMC 7LF PH, Aqualon, Hopewell, Va.
^kMethylparaben, Sigma Chemical Co, St Louis, Mo.
^lWaters 2690 HPLC, Waters Corp, Milford, Mass.
^mHewlett Packard Zorbax ODS, Hewlett-Packard Co, San Fernando, Calif.
ⁿMillennium 32, version 4.0, Waters Corp, Milford, Mass.
^oCoulter LS 100Q particle size analyzer with small volume module, Beckman Coulter, Miami, Fla.
^pVarian 3800 GC with 1079 Universal Capillary Injector, FID, and 8200/SPME Autosampler, Varian, Walnut Creek, Calif.
^qJEOL JSM-5600 scanning electron microscope with imaging software, version 4.08, JEOL, Tokyo, Japan.
^rAnatech Hummer 6.2 Sputtering System, Anatech LTD, Denver, NC.
^sWayne Dog Chow, Royal Canin, St Peters, Mo.
^tInhausen Research Institute, Fort Collins, Colo.
^uBeuthanasia D, Schering-Plough Corp, Kenilworth, NJ.
^vMicrosoft Excel 97, Microsoft Corp, Redmond, Wash.
^wGLM, SAS Institute Inc, Cary, NC.

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