

PHARMACODYNAMIC EVALUATION OF BIODEGRADABLE DL-LACTIDE-GLYCOLIDE MICROPARTICLES FOR A 30 DAY CONTROLLED RELEASE THYROXINE FORMULATION USING A NEW SOLVENT FREE EXTRUSION PROCESS

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Introduction

An accurate figure for the incidence of equine hypothyroidism is difficult to estimate but has been reported to be high, especially in young horses and broodmares (Irvine and Evans 1975, Chen and Riley 1981). Lowe et al., (1974) failed to observe reproductive problems in thyroidectomized mares however, many practitioners continue to report clinical improvements in fertility after thyroid hormone supplementation. Because many nonthyroid-related illnesses and drugs can depress thyroid hormone levels one should take care with diagnosis of hypothyroidism. Drugs affecting thyroid hormone levels include phenylbutazone and glucocorticoids, management practices such as stall confinement, shipping or a sudden change in routine can also suppress T4 levels. Also, dietary factors such as endophyte infected tall fescue pastures or pastures containing goitrogenic factors such as thiocyanates and perchlorates can cause problems, diets high or low in iodine can alter thyroid levels and selenium deficiency alters conversion of T4 to T3. Lastly, chronic conditions such as Cushings disease, laminitis, gastric ulcers can lead to lowered T4 levels.

Nachreiner and Hyland (1993) advocate using baseline Total T3 and T4 as the most economical and practical diagnostic approach and suggest if thyroid replacement is initiated, 5 to 10 mg L-thyroxine per 500 KG body weight per day is a good starting dose.

Recently, Burns (2003) reported on using biodegradable microparticles to deliver T4 for a period of 30 days. Preliminary results suggested that a 30 day controlled release T4 formulation was possible. The present study was conducted to examine the use of new solvent free extrusion process on in-vivo release profiles .

Experimental Methods

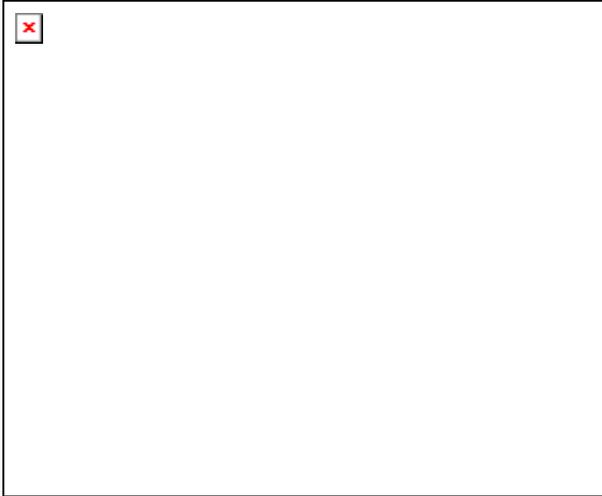
In the first study, 9 healthy mares weighing about 1000-1100 lbs (450-500 kg) were used. Pharmacodynamic endpoints evaluated included Injection site swelling scores and serum thyroxine concentrations. Blood samples were collected by veni-puncture immediately before treatment and on days 1, 4, 9, 14, 18, 23, 30, 37 and 42 after treatment. Experimental microparticle formulations were prepared by solvent free extrusion based process whereby a mixture of poly (DL-lactide-co-glycolide) and thyroxine were mixed and extruded. Resulting polymer thyroxine complex were ground into microparticles suitable for intra-muscular injection (< 150 microns) and packaged in 5 mL single dose vials. Prior to injection the microparticles containing 100 mg T4 were suspended in a CMC based vehicle and shaken well for 30 seconds, drawn into a standard syringe and injected intramuscularly using an 18-gauge needle.

A second study designed to evaluate the effect of sterilization using Gama radiation. Experimental T4 microparticle formulations prepared as described above and then subjected to a sterilizing dose (2.5 Mega-rads) of gama radiation.

Pharmacodynamic endpoints evaluated included injection site swelling scores and serum thyroxine concentrations. Blood samples were collected by veni-puncture immediately before treatment and on days 1, 4, 6, 8, 11, 13, 15, 18, 20, 22, 28, and 30 after treatment using 11 healthy mares similar in type as those used in the first study. Mares were randomly divided into 2 groups: Controls (n=5) which received 50 mg of non-sterilized T4 microparticles or treated (n=6) which received 50 mg of T4 microparticles subjected to a sterilizing dose 2.5 Mega-rads.

Results

Results from study 1 are illustrated in the figure below.



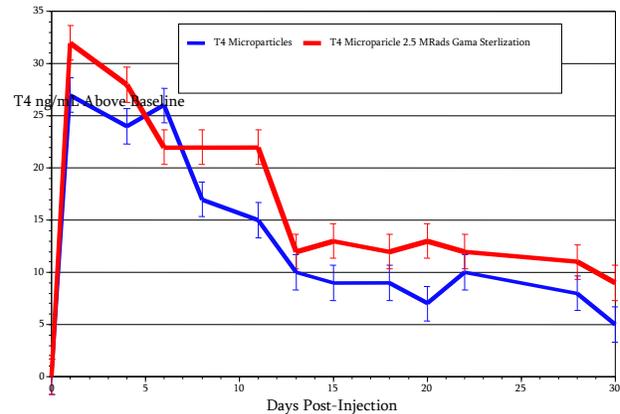
Statistical examination of the data indicated a significant treatment by time interaction ($p < 0.01$) for T4 concentrations in study one.

Mean T4 concentrations averaged 20.8 ng/ml on day 0 prior to treatment for all mares ($n=9$). Control mares averaged 22 ng/ml over the next 42 days ranging between 14 and 28 ng/mL ($n=5$). All treated mares ($n=4$) receiving the 100 mg - T4 MicroParticles (Solvent Free Extrusion Formulation) showed significant T4 increases following treatment peaking 1 day post injection and averaged 19.1 ng/mL above their pretreatment baseline over the next 6 weeks ranging between 24.7 and 58.6 ng/mL ($n=4$).

Injection site swelling scores were all zeros in both studies so the data were not analyzed. In addition there were no reported adverse effect with either of the experimental treatments

In experiment 2, mean T4 concentrations averaged 16 ng/ml on day 0 prior to treatment in control mares while mares receiving the sterilized microparticles averaged 18.2 ng/ml. Statistical examination of the data indicated there were no significant treatment or treatment by time interactions for T4 concentrations. All mares showed significant T4 increases following treatment peaking 1 day post injection and averaged 15.5 ng/mL above their

pre-treatment baseline over the next 30 days ranging between 27.7 and 48.4 ng/mL ($n=11$). Results from study 2 are illustrated in the figure below.



Conclusions

The present studies indicates that a 50 mg dose the new solvent free extrusion formulation subjected to sterilization by gamma radiation produced biocompatible T4 microparticles capable of delivering T4 for at least 30 days .

References

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