ELSEVIER

International Journal of Pharmaceutics 194 (2000) 181–191

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Parameters affecting the efficacy of a sustained release polymeric implant of leuprolide

Harish B. Ravivarapu *, Katie L. Moyer, Richard L. Dunn

Atrix Laboratories, Incorporated, 2579 Midpoint Drive, Fort Collins, CO 80525, USA

Received 8 June 1999; received in revised form 6 October 1999; accepted 15 October 1999

Abstract

The objective of this study was to evaluate the formulation parameters critical to the efficacy of an injectable polymeric implant of leuprolide acetate, formed in situ, in suppressing and maintaining serum testosterone levels of animals in the range 0.5 ng/ml for over 90 days. The formulation evaluated contained 45% (w/w) 75/25 poly (DL-lactide-co-glycolide) polymer having an intrinsic viscosity of 0.20 dl/g, dissolved in 55% (w/w) *N*-methyl-2-pyrrolidone with 3% (w/w) leuprolide acetate added either as a homogeneous solution or a two-part suspension (A/B) system, in which the drug was dispersed within the polymer solution immediately prior to use. The formulation parameters evaluated in this study included polymer molecular weight, polymer concentration, and drug loading. Both rat and dog models were used to evaluate efficacy. Serum testosterone was assayed by radioimmunoassay to determine efficacy, and retrieved implants from the rats at the termination of the study were analyzed by HPLC for residual drug content to determine the extent of drug release. With the candidate formulation, testosterone levels in dogs diminished to the targeted levels of 0.5 ng/ml by day 14 and remained suppressed up to day 91, reproducing the results seen in rats. Variations in polymer concentration (40–50%), and drug load (3–6% (w/w)) did not have a significant effect on the apparent level and duration of efficacy. However, employing lower molecular weight polymer decreased the duration of efficacy of the formulation.

Keywords: Leurpolide acetate; In situ implant; Testosterone suppression; Rats; Dogs; Formulation parameters

1. Introduction

Recently developed polymer-based sustained release products of leuprolide (leuprorelin), a LHRH superagonist analog, have circumvented the discomfiture associated with the daily subcutaneous (s.c.) injections of the analog solution (Sharifi and Soloway 1990; Okada et al., 1991a), useful in the palliative treatment of hormone dependent prostate carcinoma, endometriosis and precocious puberty (Redding and Schally 1981; Lemay and Quesnel 1982; Okada et al., 1983; Garnick et al., 1984; Plosker and Brodgen 1994). Injectable microspheres that are capable of sustaining required drug levels over a period of 1, 3, and 4 months after a single administration are

* Corresponding author. Tel.: +1-970-482-5868; fax: +1-970-482-9735.

E-mail address: hraviv@atrixlab.com (H.B. Ravivarapu)

0378-5173/00/$ - see front matter
PII: S0378-5173(99)00371-3

currently available on the market and are being widely used. These systems, in addition to augmenting the convenience and patient's compliance, reduced the needed dose to 1/4–1/8 of the aqueous drug solution by virtue of sustaining therapeutic drug levels at the target receptor sites (Okada and Toguchi, 1995). However, manufacturing of these systems is complex and expensive. In addition, removal of the intramuscularly injected microspheres in case of drug adverse reactions is not easy.

A recent series of patents by Dunn et al. (Dunn et al., 1990; Dunn and Tipton, 1997) describes a novel polymer drug delivery system (Atrigel®) that is injectable and forms an implant in situ combining the ease of injection of microspheres and the simplicity of implants for drug delivery. In this system, a water-insoluble and biodegradable polymer is dissolved in a biocompatible organic solvent. When the polymer solution is injected into the body, the organic solvent dissipates into the surrounding tissue as the water permeates into the implant. This process leads to phase separation and subsequent coagulation of the polymer to form an implant in situ. Active drugs are added to the polymer solution to produce a ready-to-use homogeneous solution or dispersion depending upon the solubility of the drug ('uniphase'). However, if product stability is an issue due to interaction between the drug, polymer, and solvent, the product can be prepared as a two-part system ('A/B'). In this system, one syringe contains an appropriate amount of the polymer solution with the other syringe containing the drug as a dry powder. The final product is made sterile by aseptic manufacturing or terminal sterilization with γ-irradiation, and the effect of irradiation on the performance of the product is taken into account. Before administration to the patient, the two syringes are coupled and the contents mixed with back and forth cycles between the two syringes. Once the drug containing implant is formed in vivo, drug release is controlled by the properties of the polymer, solvent, and drug employed.

Using the Atrigel® technology, an uniphase solution system of 75/25 poly (DL-lactide-co-glycolide) polymer and *N*-methyl-2-pyrrolidone in a 45:55 ratio with 3% (w/w) leuprolide acetate has been developed ('candidate formulation') and shown to effectively suppress serum testosterone levels in rats for 3 months or more (Ravivarapu et al., 1999). However, subsequent in vitro stability studies with this formulation showed a significant degradation of polymer (loss of molecular weight) in the presence of leuprolide acetate, even though the drug remained stable (unpublished results). Hence an A/B suspension system of the same composition was considered as an alternative delivery system. The A/B suspension system avoids polymer instability by avoiding the long-term contact between polymer solution and drug. However, it was not known if the efficacy seen with the uniphase solution formulation would be reproduced with the A/B suspension system, due to possible variation in the drug release in vivo.

This study compares the efficacy of the A/B suspension and uniphase solution systems of the candidate formulation in suppressing serum testosterone levels in rats. The efficacy with the A/B suspension formulation was also evaluated in a different animal model, the dog. In addition, the effect of various polymer concentrations, molecular weights, and drug loads on the efficacy of the A/B suspension formulation was evaluated in rats.

2. Materials and methods

2.1. Materials

Leuprolide acetate was purchased from Bachem California (Torrance, CA). *N*-methyl-2-pyrrolidone (NMP, Pharmasolve™) was obtained from International Speciality Products (Wayne, NJ). 75/25 poly (DL-lactide-co-glycolide) (PLG) with an intrinsic viscosity (IV) of 0.20 dl/g was purchased from Birmingham Polymers (Birmingham, AL). All other reagents used were of HPLC grade.

2.2. Methods

2.2.1. Preparation of uniphase solution formulation

Appropriate amounts of 75/25 PLG (IV 0.20)

polymer and NMP were weighed into glass vials. After initial mixing of the contents the vials were placed on a continuous shaker (Labline® orbit shaker, Melrose Park, IL) overnight at room temperature to completely dissolve the polymer. The polymer solution in glass scintillation vials was sent to a contract irradiation facility (Isomedix, Morton Grove, IL) for γ-irradiation at a dose of 20–25 kGy. Thirty to 45 min before animal injections the appropriate amount of non-irradiated leuprolide acetate was added to the irradiated polymer solution and kept on a continuous shaker with occasional mixing. Final formulation was filled into 1-cm^3 polypropylene syringes (Becton Dickinson, B.D., Franklin Lines, NJ) and the required weight of formulation was injected into the animals.

2.2.2. Preparation of A/B suspension formulations

A solution of the 75/25 PLG (IV 0.2) polymer and NMP was prepared as described above. The proper amount of the polymer solution was filled into either 1.25-cm^3 male B-Braun (B-Braun Medical, Bethlehem, PA) or 3-cm^3 male B.D. (Becton Dickinson, Franklin Lines, NJ) syringes. These syringes were double pouched in aluminum foils with a desiccant bag, heat-sealed under nitrogen and sent out for γ-irradiation at a dose of 20–25 kGy. For the drug part of the system, 1.25-cm^3 female B-Braun or 3-cm^3 male B.D. syringes were filled with the calculated volumes of leuprolide acetate aqueous solution. These syringes were lyophilized overnight using a Labconco® freeze dry system (Kansas City, MO) to form a leuprolide acetate cake. Just before dosage administration, the syringes containing the polymer solution and leuprolide acetate were joined; with a polypropylene syringe coupler in case of the 3-cm^3 male–male syringes, and the contents mixed with 40 back and forth mixing cycles. The resulting homogeneous dispersion was drawn into the male syringe and injected into the animals. The 1.25-cm^3 syringes were used for the rat studies and the 3-cm^3 syringes for the dog studies.

To determine the formulation parameters critical to the efficacy of the product in animals, a range of polymer molecular weights, concentrations, or drug loads was employed. The compositions of these formulations are shown in Table 1.

2.2.3. Polymer molecular weight

Irradiated polymer solution was quantitatively dissolved in tetrahydrofuran to yield a polymer concentration of approximately 0.5% (w/v). Filtered samples were analyzed by gel permeation

Table 1
Evaluated Atrigel®–leuprolide acetate formulations

Formulation	Polymer/solvent[a]	Drug load (% w/w)	Animal ($n = 5$)	Average dose (μg/kg per day)	Polymer Mol. wt. (wt. avg.)
A	75/25 PLG (IV 0.2):NMP 45:55	3	Rat	125.2	15705
B	75/25 PLG (IV 0.2):NMP 45:55	3	Rat	115.6	15678
C	75/25 PLG (IV 0.2):NMP 50:50	3	Rat	112.0	15629
D	75/25 PLG (IV 0.2):NMP 40:60	3	Rat	116.0	15429
E	75/25 PLG (IV 0.31):NMP 45:55	3	Rat	119.3	26762
F	75/25 PLG (IV 0.11):NMP 45:55	3	Rat	138.4	6518
G	75/25 PLG (IV 0.2):NMP 45:55	4.5	Rat	128.0	15606
H	75/25 PLG (IV 0.2):NMP 45:55	6	Rat	126.9	15560
I	75/25 PLG (IV 0.2):NMP 45:55	3	Dog	24.5	15094

[a] Polymer solution was irradiated at 21 kGy. All formulations except for A are A/B systems and composition ratios are w/w.

chromatography (GPC) to determine the weight average polymer molecular weight. Narrow molecular weight polystyrenes in the range of 580–370 000 Da (Polymer Laboratories, Amherst, MA) were used as standards. The chromatography conditions were: Polymer Laboratories MIXED-D (5 μm, 30 cm × 7.5 mm) column maintained at 40°C, Hewlett Packard (Santa Clara, CA) 1050 series isocratic pump, autosampler, 1047A refractive index detector, and 50 μl injection volume. Tetrahydrofuran at a flow rate of 1 ml/min was used as the mobile phase. Polymer Laboratories CALIBER software was employed for GPC calculations.

2.2.4. In vivo evaluation

2.2.4.1. Rat studies. Adult male Sprague–Dawley (Harlan Sprague Dawley, Chicago, IL) rats with a baseline weight range of 242.6–283 g were used in this study. The animals were housed in polycarbonate cages with 12 h on/off lighting cycle. Laboratory rodent chow and tap water were provided ad libitum. The animals were maintained according to AAALAC requirements and were in accordance with the Guide for the Care and Use of Laboratory Animals (DHEW Pub. No. (NIH) 78-23, revised). Randomized rats were identified by ear notch and cage cards, and were acclimated for at least a week before the studies. Each treatment group had five rats.

On the study date the rats were weighed, anesthetized with isoflurane, and each given a single s.c. injection of the specific formulation in the dorsothoracic region using 20–21 gauge needles. Based on the literature reports, the targeted dose for rats was set at 100 μg/kg per day (Okada et al., 1991b). The syringes were weighed before and after dosage administration to determine the formulation amount received by the animals. Dose information for each group of rats is provided in Table 1. On days 0 (pre-dose), 3, 9, 14, 21, 35, 49, 63, 70, 80, and 91, approximately 0.7 ml of blood was collected into a clot tube from the lateral tail vein by serial bleeding from each animal; the rats were periodically observed for overt toxicity and weight changes. On day 105 the rats were anesthetized, bled by cardiac puncture, and euthanized with carbon dioxide. Any remaining polymer/drug implants at the injection site were retrieved for the residual drug content analysis. At termination the injection site was evaluated macroscopically for any tissue reactions.

2.2.4.2. Dog studies. Five adult male beagle dogs (Ridglan Farms, Mt. Horeb, WI) with a baseline weight range of 9.1–16.4 kg were used as one treatment group. The dogs were acclimated for at least a month and identified by ear tattoos. Conditions to maintain the dogs were similar to those employed for the rats.

On the start day of the study (day 0) the dogs were weighed and given a single bolus s.c. injection of the test formulation between the shoulder blades via a 20 gauge needle. Based on the literature reports the dose was set at 25.6 μg/kg per day (Okada et al., 1991b). On days 0 (pre-dose), 1, 2, 3, 4, 7, 14, 21, 28, 35, 49, 63, 71, 81, 91, 105, and 121, approximately 8 ml of blood was collected from the anterior jugular vein. The dogs were observed for overt toxicity and any adverse conditions at the injection site through out the study period. One dog died on day 77 of the study due to intestinal strangulation. Complete necropsy determined the death was not formulation related. In contrast to the rat studies the dogs were not sacrificed at the termination of the study and accordingly, no implants were retrieved.

2.2.5. Serum testosterone assay

Serum was separated from the blood by centrifugation at 3500 rpm for 10 min and frozen at −20°C for later analysis by solid-phase radioimmunoassay (RIA). Standard commercial RIA kits were used and the mean ± S.D. of the testosterone levels (ng/ml) were reported. Samples, standards, and controls were analyzed in duplicate. The average values that were reported as nil or that fell below 0.1 ng/ml were considered to be equal to 0.1 ng/ml, the lower detection limit for the assay. The average of pre-dose serum testosterone values from all the study animals was used as the baseline value.

2.2.6. Retrieved implant analysis by HPLC

The excised implants from the rats were dissolved in the sample diluent solution which was a 1:1 mixture of dimethyl sulfoxide and methanol with 1% (w/v) polyethyleneimine, by placing the contents on a continuous shaker for 24 h at room temperature. The samples were then vortex-mixed for 5 s and filtered through 0.45 μm PTFE syringe filters into HPLC vials. Leuprolide acetate was separated on a Vydac (Hesperia, CA) protein and peptide C-18 column (4.6 × 250 mm, 5 μm particle size) with a modified literature method (Sutherland and Menon, 1987). The chromatographic equipment employed was a Shimadzu LC-10A (Columbia, MD) that consisted of an autosampler, pump, variable UV–Vis detector, computer, and data acquisition/analysis software. The mobile phase was 23:77 acetonitrile and 87 mM ammonium acetate buffer solution that was pH adjusted to 6.5 with 6 M HCl. The flow rate was set at 1 ml/min and the detection wavelength was 280 nm. Sample run time was 30 min with leuprolide eluting at 15 min. The limit of detection was 1 μg/ml and the leuprolide standard curve was linear over the range of 1–1200 μg/ml.

2.2.7. Statistical analysis

Results from the multivariate repeated measures analysis of the data indicated a significant day effect ($P < 0.001$) and also suggested day-by-treatment group effect ($P < 0.25$), indicating that the treatment groups should be compared at each day. Comparisons were performed for each active group against the control group (formulation B) at each time point using Dunnett's t-test at $P < 0.05$ and the significant differences were indicated (*) on the figures.

3. Results and discussion

The first objective of this study was to compare the efficacy of the A/B suspension and uniphase solution systems of the candidate formulation; 75/25 PLG (IV 0.20):NMP 45:55 with 3% (w/w) leuprolide acetate, in suppressing serum testosterone levels to approximately 0.5 ng/ml ('castration') for at least 3 months. Additionally, changes in the formulation of the A/B suspension system were evaluated for their effect upon the efficacy of the product as determined by testosterone suppression. The compositions of the formulations evaluated in rats and dogs are presented in Table 1. The 75/25 PLG polymer and NMP were employed in preparing all these formulations. Formulation A was earlier shown to be efficacious in suppressing and maintaining serum testosterone levels of rats and thus was used to compare the A/B suspension system (formulation B) of the same composition (Ravivarapu et al., 1999). Formulation I is identical to formulation B, but was prepared as a different batch and was evaluated in dogs for its efficacy in a larger animal. Other formulations evaluated in rats (formulations C–H) had varied polymer concentration, molecular weight, or drug load to investigate the effect of these variables on efficacy. Polymer solutions were irradiated at approximately 21 kGy and all the formulations except for formulation A were A/B mixing systems. Leuprolide acetate was not sterilized in these studies. If this formulation were to be developed into a clinical product, leuprolide acetate would be sterilized either by filtration or irradiation. In case of irradiation, it would also be warranted to characterize the possible degradants.

Table 1 also shows the weight average polymer molecular weight as determined by GPC. As expected, polymer concentration did not affect the polymer molecular weight (formulations B–D). Polymers employed in formulations B, E and F have different starting IVs. and thus these formulations after irradiation provided a wide range of polymer molecular weights (6518–26 762) for evaluation. The polymer solution of formulations A, B, G, H, and I had the same composition, and accordingly no significant difference in the polymer molecular weight was noticed. With all the A/B suspension formulations, polymer solution and drug could be mixed easily and the final dispersion was visually homogeneous. All the formulations were easily injected using 20–21 gauge needles with no needle clogging.

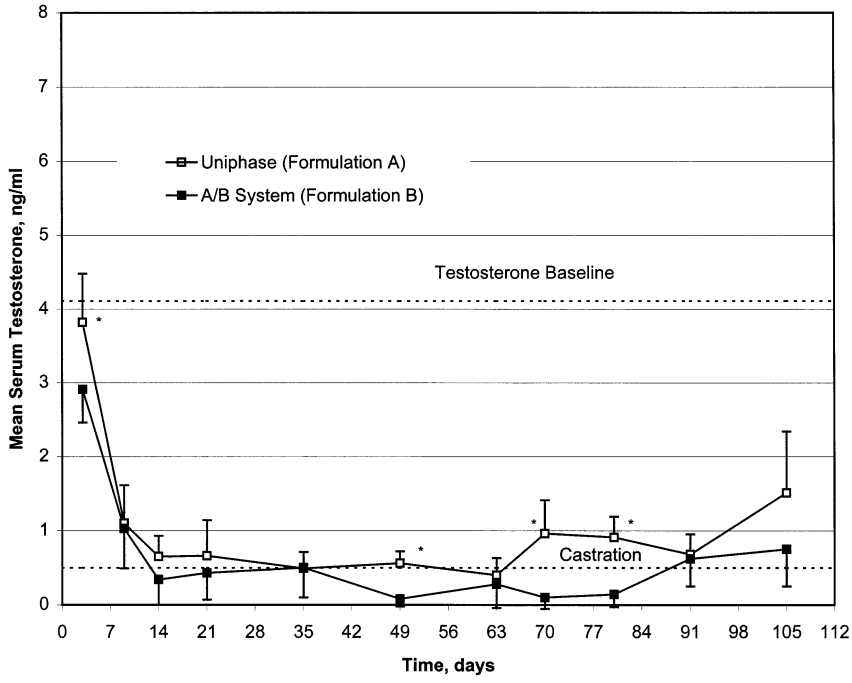

Fig. 1. Testosterone suppression in rats with formulations A and B: uniphase suspension vs. A/B solution system. * Indicates a significant difference ($P < 0.05$).

Based on the literature reports, the target doses for rats and dogs were set at 100 and 25.6 μg/kg per day, respectively (Okada et al., 1991b). However, the actual doses given were slightly higher than intended. Due to the high number of rats involved, the required dose was calculated based on an approximate average rat weight of 285 g. However, as all the study rats weighed below this estimated average value, higher than intended weight-based drug doses resulted. The average dog dose was closer to the intended dose as the dosage was individually prepared based on the weight of each dog. No overt toxicity or external appearance of tissue reaction was seen throughout these studies. All the rats and dogs gained weight steadily. There was no to minimal vasodilation, erythema, or edema noted on macroscopic observation of the injection sites in the rats at the termination. All the retrievable implants were located subcutaneously and were clearly fragmented.

Fig. 1 compares the efficacy profiles obtained after administering 45% 75/25 PLG (IV 0.20) + 55% NMP with 3% (w/w) leuprolide acetate as an uniphase solution or A/B suspension system to rats. As an A/B suspension, this formulation appeared to be even more efficacious than the uniphase solution. At days 3, 49, 70, and 80 testosterone levels with the uniphase solution were significantly higher than those obtained with the A/B suspension system ($P < 0.05$). Starting from day 14 to day 91, the serum testosterone levels after administering the A/B suspension formulation were below 0.5 ng/ml at each sampling time point. Even after that, up to 105 days the levels were very close to castrate levels and much below the baseline values. Based on these results and the stability concerns with an uniphase solution system, the A/B suspension system of the formulation was considered the best choice for further development.

In the next experiment, the same A/B suspension (formulation I) was evaluated in dogs. As is shown in Fig. 2, testosterone levels reached castration by day 14 and were maintained close to castration for the entire duration of the study

(day 105). Later evaluation at day 120 showed elevated levels of testosterone, possibly indicating exhaustion of the drug or a reduction in the rate of release. One of the study dogs whose testosterone values were significantly different from those of other dogs at many time points was deemed to be an outlier based on Dixon's test ($P < 0.05$) (Caulcutt, 1983). When the data from this particular dog was excluded ($n = 4$ in Fig. 2), the mean of the other values was consistently very low, with a low S.D. At several time points, the values were reported to be 'nil' and for the purpose of data analysis they were considered to be equal to 0.1 ng/ml, the lower detection limit for the assay. Even at day 105 of the study, the testosterone levels remained suppressed, adding a 15-day (day 90–day 105) margin of safety for the repeat administration. It was also perceived that due to this continued suppression at 90 days, testosterone flare up that leads to hot flushes and other side effects would not be seen on subsequent chronic dosage administrations. These studies thus confirmed the A/B suspension formulation to be efficacious in suppressing the pituitary–gonodal axis and testosterone levels for 3 months.

The A/B suspension formulation was further evaluated to determine the effects of formulation variables on its efficacy. Figs. 3–5 show the serum testosterone profiles obtained from rats after administering formulations B–H. The relevant data are presented together to directly compare the effects of polymer concentration (Fig. 3), polymer molecular weight (Fig. 4) and drug load (Fig. 5). The profile obtained with the standard formulation (formulation B) was included in all the figures for comparison. Groups that have at least a single rat from which an implant could be retrieved at the termination (day 105) are reported in Table 2. Except for group E (formulation E with 0.31 IV polymer), the residual leuprolide acetate in implants as a percentage of initially administered dose was insignificant. The higher residual amount in case of the higher molecular weight polymer (formulation E) indicates that polymer degradation played a key role in the drug release. Formulations with lower IV (B and F)

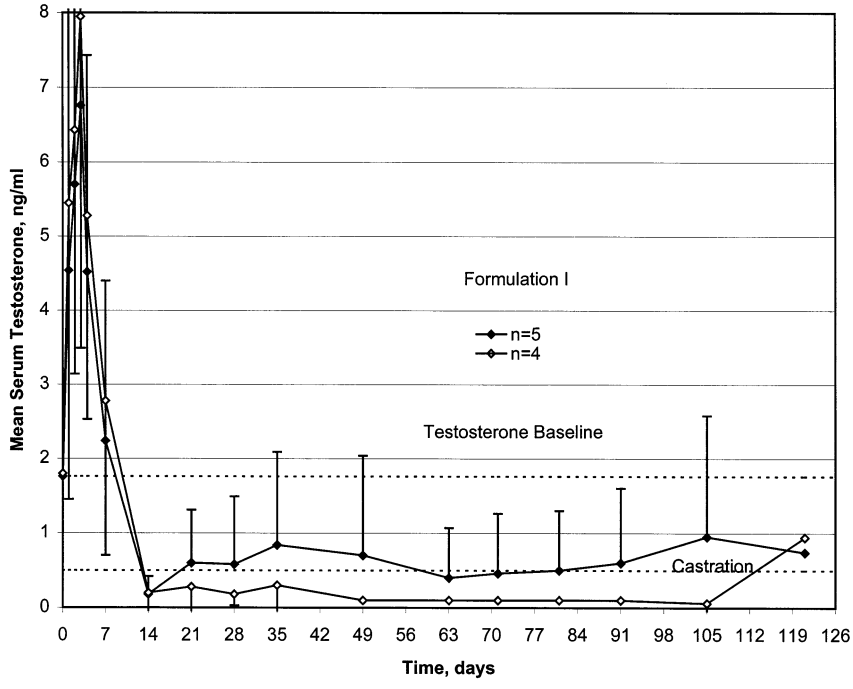

Fig. 2. Serum testosterone profile of dogs with formulation I.

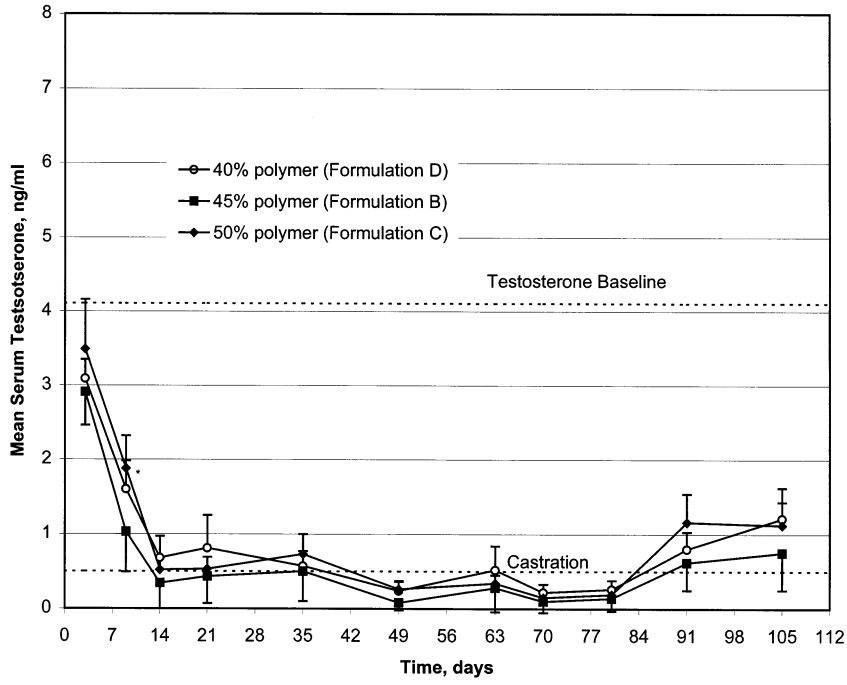

Fig. 3. Testosterone suppression in rats with formulations B, C, and D: effect of polymer concentration. * Indicates a significant difference ($P < 0.05$).

had minimal or no residual implants and drug at the termination, as they are expected to lose polymer mass comparatively faster.

No significant effect of the polymer concentration on the formulation efficacy could be noted from Fig. 3 except for day 9 when the mean testosterone level with formulation C (50% polymer) was statistically ($P < 0.05$) higher than that with the standard formulation B. The polymer concentration ranged from 40 to 50% (w/w) with the control formulation having 45% (w/w) polymer. Close to castration levels were obtained with all the three formulations by day 14 and were maintained at those levels up to day 91. At day 91, formulations C and D showed elevated levels of testosterone, which, however, were still very much lower than the baseline. A relatively wide range of polymer concentration, 40–50% (w/w), thus appears to be suitable in producing efficacious formulations.

Fig. 4 compares the effect of polymer molecular weight in suppressing rat testosterone levels. Formulation F from a low IV polymer (molecular weight 6518), as compared to the other formulations, was not completely efficacious with elevated testosterone levels being observed from day 63 onwards. Due to its low molecular weight this polymer was expected to lose mass faster as it reached the critical molecular weight when it became soluble in the surrounding aqueous environment. Thus, it was not surprising that it did not release the drug for a longer time. From Fig. 4, it is suspected that complete loss of polymeric implant occurred at about 63 days after dosage administration for this lower molecular weight polymer formulation. These results indicate that polymer molecular weight is a critical parameter in the efficacy of the formulation.

Polymers with IVs 0.20 and 0.31 (formulations B and E) were not significantly different in their efficacy profiles except for a single time point at day 49. Slightly elevated levels of testosterone were observed from day 91 onwards. Rats from these groups had retrievable implants and residual drug (Table 2). Residual drug with formulation B was insignificant explaining the lack of leuprolide

release and corresponding efficacy beyond that point. Even though a higher amount of drug was found with formulation E rats, apparently the rate of release was not sufficient to maintain the suppression. From these studies it appeared that formulations that have a 75/25 PLG polymer with a molecular weight in the range 15 600–27 000 could provide efficacious formulations. It was also apparent that polymers with a molecular weight of approximately 6000 Da or lower would not be suitable for 90-day release of the drug. However, the present studies could not determine a lower specification on the molecular weight of polymer that still yields an efficacious formulation.

Fig. 5 shows the efficacy of candidate formulation with different drug loads (3–6%, formulations B, G, and H). The objective of this study was to determine the maximum drug load, without compromising the injectability and efficacy of the formulation. An efficacious formulation with a higher drug load would mean lesser amount of formulation to be administered to

Table 2
Residual drug content in retrieved implants on day 105

Formulation[a]	Remaining drug (μg)	Injected amount (%)
B	16.84	0.56
	5.93	0.22
C	4.76	0.21
	2.91	0.13
	2.45	0.08
	7.93	0.28
D	15.57	0.57
	14.97	0.49
E	138.07	4.76
	151.75	4.94
	89.95	3.01
	57.53	2.09
	92.04	3.36
G	41.33	1.32
	23.24	0.86
H	44.84	1.23
	70.60	2.92

[a] No retrievable implants could be found in the groups not mentioned.

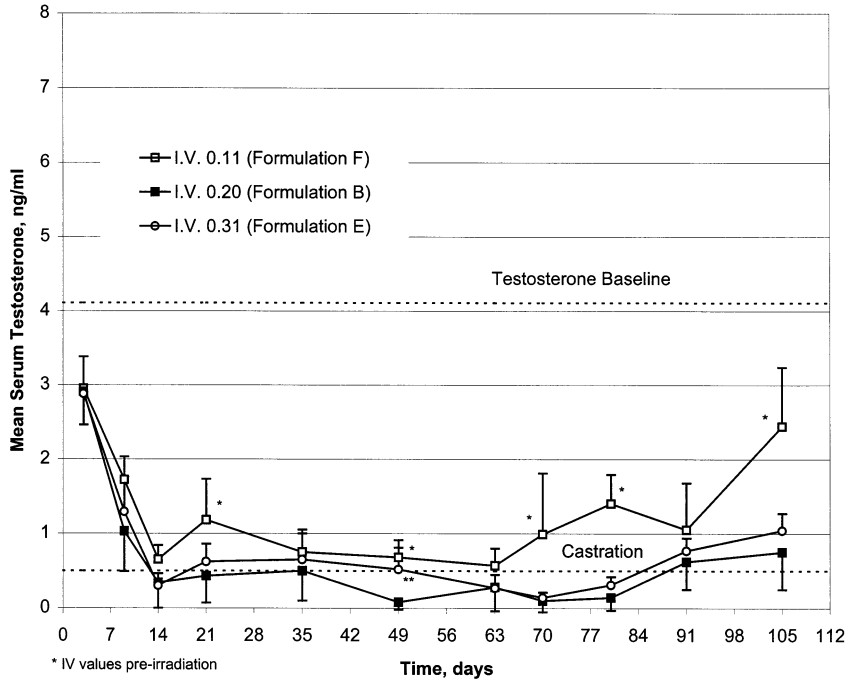

Fig. 4. Testosterone suppression in rats with formulations B, E, and F: effect of polymer molecular weight. *, ** Indicate a significant difference ($P < 0.05$).

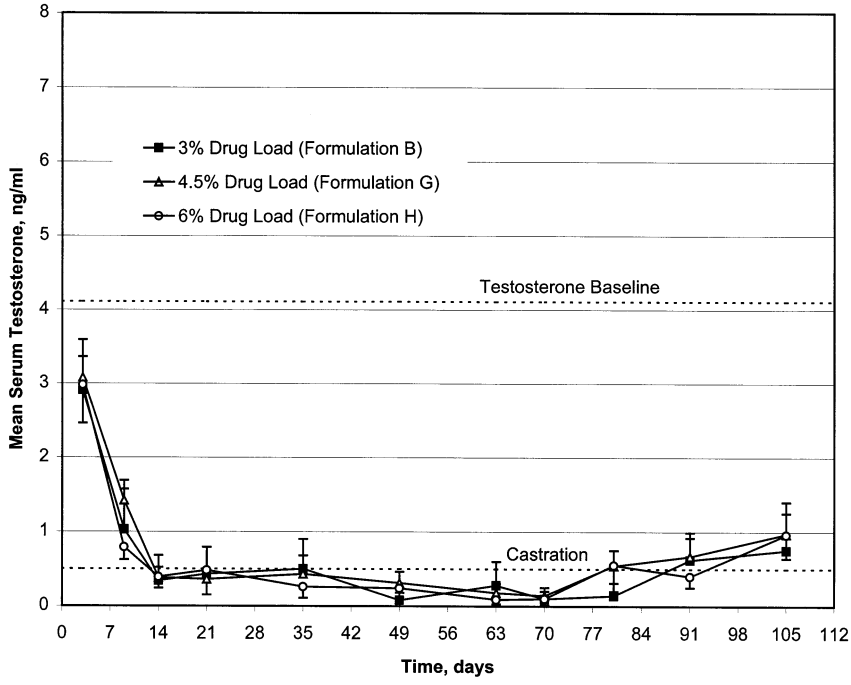

Fig. 5. Testosterone suppression in rats with formulations B, G, and H: effect of drug load. * Indicates a significant difference ($P < 0.05$).

deliver the needed drug amount. From the in vitro studies it was apparent that a 6% load may be the maximum limit with regards to injectability. Thus, formulations with 3, 4.5 and 6% (w/w) drug load were included in this evaluation and the drug dose was kept similar by adjusting the formulation amount administered to the animals.

The efficacy obtained with 4.5 or 6% drug load formulations in rats was very similar to that of the control formulation with 3% drug load (Fig. 5) and statistically ($P < 0.05$) there was no significant difference at any sampling time points. The testosterone levels were below castration levels by day 14 and were maintained through out the study. In comparison to control formulation with 3% drug load, the amounts of polymer and NMP injected with 6% drug load formulation are halved, but still the formulation remained efficacious.

4. Conclusions

In conclusion, an A/B suspension system of 75/25 PLG (IV 0.2):NMP 45:55 with 3% leuprolide acetate formulation suppressed serum testosterone levels in rats to castration (0.5 ng/ml) over a period of 3 months, and produced a similar efficacy in dogs. Polymer concentration (40–50%) and drug load (3–6%) did not affect the efficacy of the product, however, the use of a low molecular weight polymer gave a shorter duration of efficacy. Based on these studies this formulation appears promising for development into a clinical product. With additional benefits of a less painful subcutaneous injection, simple manufacturing process and reduced cost compared to marketed microsphere and implant products, this product should offer a more acceptable alternative to the patient.

Acknowledgements

The authors would like to sincerely thank the Atrix Laboratories, Incorporated, personnel Cathy Balliu, Steve Volker, Michele Gehring, and Dennis Wilson for their assistance with the animal studies

and the analysis of the test formulations. We also want to thank Graham Carron for his statistical analysis of the data.

References

Caulcutt, R., 1983. Statistics in Research and Development, first ed. Chapman and Hall, New York.

Dunn, R.L., Tipton, A.J., 1997. Polymeric compositions useful as controlled release implants. US Patent 5,702,716.

Dunn, R.L., English, J.P., Cowsar, D.R., Vanderbilt, D.P., 1990. Biodegradable in situ forming implants and methods of producing the same. US Patent 4,938,763.

Leuprolide Study Group, Garnick, M.B., 1984. Leuprolide versus diethylstilbestrol for metastatic cancer. New Engl. J. Med. 311, 1281–1286.

Lemay, A., Quesnel, G., 1982. Potential new treatment of endometriosis: reversible inhibition of pituitary–ovarian function by chronic intranasal administration of a leutinizing hormone-releasing hormone (LHRH) agonist. Fertil. Steril. 38, 376–379.

Okada, H., Toguchi, H., 1995. Biodegradable microspheres in drug delivery. Clin. Rev. Ther. Drug Sys. 12 (1), 1–99.

Okada, H., Sakura, Y., Kawaji, T., Yashiki, T., Mima, H., 1983. Regression of rat mammary tumors by a potent leutinizing hormone-releasing hormone (leuprolide) administered vaginally. Cancer Res. 43, 1869–1874.

Okada, H., Heya, T., Ogawa, Y., Toguchi, H., Shimamoto, T., 1991a. Sustained pharmacological activities in rats following single and repeated administration of once-a month injectable microspheres of leuprolide acetate. Pharm. Res. 8 (5), 584–587.

Okada, H., Inoue, Y., Heya, T., Ueno, H., Ogawa, Y., Toguchi, H., 1991b. Pharmacokinetics of once-a-month injectable microspheres of leuprolide acetate. Pharm. Res. 8 (6), 787–791.

Plosker, G.L., Brodgen, R.N., 1994. Leuprorelin. A review of its pharmacology and therapeutic use in prostate cancer, endometriosis and other sex hormone-related disorders. Drugs 48 (6), 930–967.

Redding, T.W., Schally, A.V., 1981. Inhibition of prostate tumor growth in two rat models by chronic administration of D-Trp^6 analogue of leutinizing hormone-releasing hormone. Proc. Natl. Acad. Sci. USA 78, 6509–6512.

Ravivarapu, H.B., Moyer, K.L., Dunn, R.L., 1999. Sustained suppression of pituitary-gonodal axis with an injectable, in situ forming implant of leuprolide acetate. J. Pharm. Sci. (submitted).

Sharifi, R., Soloway, M., 1990. Clinical study of leuprolide depot formulation in the treatment of advanced prostate cancer. J. Urol. 143, 68–71.

Sutherland, J.W., Menon, G.N., 1987. HPLC of leuprolide acetate in injectable solutions. J. Liq. Chromat. 10 (10), 2281–2289.