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Poly(DL-Lactide-co-glycolide)/Norethisterone Microcapsules: An Injectable Biodegradable Contraceptive

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ABSTRACT

Microcapsules made from a biocompatible, biodegradable polymeric excipient, poly(DL-lactide-co-glycolide) (DL-PLGA) that contained 22 weight percent (wt %) norethisterone (NET), were prepared by a solvent-evaporation microencapsulation process. The effects of changing both the lactide-to-glycolide ratio of the DL-PLGA and the size of the microcapsules on the rate of NET release and the rate of excipient biodegradation were determined in vivo. NET release rates were determined in baboons after injecting the microcapsule formulations intramuscularly. Serum samples obtained at various times following treatment were analyzed for NET, progesterone, and estrogen by radioimmunoassay (RIA). Biodegradation kinetics were determined by injecting NET microcapsules made from radiolabeled DL-PLGA intramuscularly into the hind legs of rats. Residual radioactivity at the injection site was determined at various times after treatment by combustion analysis of the muscle tissue. Changing the ratio of the comonomers to include more glycolide (DL-lactide:glycolide–96:4, 92:8, 87:13, 74:26) increased the rate of NET release and accelerated the biodegradation of the copolymer excipient. Decreasing the size of the microcapsules increased the rate of NET release. On the basis of these studies a NET microcapsule formulation has been identified for clinical testing which releases NET for 3 months and biodegrades completely within 6 months.

INTRODUCTION

Biodegradable polymers have been effectively used to alter the kinetics, bioavailability, duration of action, and method of administration of contraceptive steroids (Benagiano and Gabelnick, 1979). There are several long-acting steroidal contraceptives under development that utilize polymeric membranes to control steroid release. These include injectable particulate systems (Beck et al., 1980b), and biodegradable (Schmitt, 1979), and nonbiodegradable (International Committee, 1978) implants. Microencapsulation of NET within poly(DL-lactide) (DL-PLA) significantly extends the duration of contraceptive action following parenteral administration.

In previous reports (Beck et al., 1979, 1980a,c) we described NET microcapsule formulations with DL-PLA excipients that provide contraceptive protection for up to 6 months following a single intramuscular injection. Other investigators have described both biodegradable and nonbiodegradable implants that provide sustained release of NET and other steroids (Pitt et al., 1979). Biodegradable microcapsule systems have the advantage that they can be administered by injection. The rate and duration of NET release from the prototype microcapsule formulation are dependent on the drug loading and the size of the microcapsules. The smaller particles have faster rates of NET release. We have shown that it takes approximately 1 year for DL-PLA microparticles to biodegrade. Accordingly, a build-up of DL-PLA

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occurs following repeated injections. Theoretically, this could be avoided, at least in part, by substituting polymeric excipients that afford microcapsules having similar NET release kinetics but that biodegrade faster. The present report describes second generation microcapsule formulations that biodegrade within 6 months. Reduction in biodegradation time, without compromising the NET release kinetics, was achieved by using a copolymer consisting of DL-lactide and glycolide in lieu of DL-lactide alone. The result is an improved formulation that has utility as either a 3- or a 6-month injectable contraceptive.

MATERIALS AND METHODS

The DL-PLGA excipient was synthesized in the following manner: Impure DL-lactide (Clinton Corn Processing Co., Clinton, IA) was recrystallized once from isopropanol and four times from benzene. During the third recrystallization, the solution was treated with charcoal and filtered while hot through a sintered-glass funnel packed with Celite and glass wool. After the fourth recrystallization from benzene, the crystals were collected by vacuum filtration in a nitrogen atmosphere and were dried under vacuum at 50°C to yield pure DL-lactide (mp 125.5 to 127.0°C). The glycolide monomer was synthesized by the method of Higgins (1954) and was purified by recrystallization three times from ethyl acetate. Its melting point was 82 to 84°C after drying under vacuum. For the copolymer synthesis, the required amount of each monomer was placed with catalyst (0.02 wt % tetraphenyltin) and lauryl alcohol (chain initiator to control the molecular weight of the copolymer) in a 250-ml, round-bottom flask equipped with a stirrer and a nitrogen inlet. The monomers were heated to 170°C under positive pressure nitrogen with stirring. After 3.5 h, the stir paddle was raised, and the polymerizate was heated for an additional 21 h. The resulting copolymer product was cooled, dissolved in methylene chloride, and precipitated into methanol to remove residual monomer, catalyst, and chain initiator. The purified copolymer was then dried under vacuum at 50°C.

To synthesize radiolabeled DL-PLGA for microcapsule-biodegradation studies, [^{14}C]-DL-lactide monomer was prepared from sodium [^{14}C]-DL-lactate by the following procedure: A solution containing 79.5 g of DL-lactic acid, 3.0 mCi of sodium DL-lactate (carbonyl-[^{14}C], New England Nuclear, Boston, MA), 3 drops of concentrated hydrochloric acid, and 74.5 g of deionized water was prepared in a 200-ml, round-bottom flask fitted with a thermometer and a vacuum-distillation head. About 54.2 g of water was removed under reduced pressure and moderate heat until distillation ceased. Zinc oxide, 1.59 g, was added as a catalyst, and an additional 10.8 g of water was removed by distillation. The water removed at this point represented 75% of the combined solution water and reaction water. After the materials left in the pot had become yellow and viscous, indicative of the formation of low molecular weight polymer, the receiver was changed, and the pot was heated to 190 to 240°C to crack this polymer thermally.

The resulting [^{14}C]-DL-lactide was distilled at 85 to 90°C at 2 torr to give a crude material that was dissolved in acetone and filtered. Subsequently, the acetone was removed by vacuum distillation. The resulting product was washed with several portions of ether, filtered, and dried under vacuum at room temperature. A yield of 36.1 g of crude product (mp 92 to 101°C) was obtained. Purification by recrystallization from benzene gave 21.0 g of [^{14}C]-DL-lactide having a melting point of 122 to 125°C. The specific activity of the [^{14}C]-DL-lactide monomer was 41.2 μCi/g as determined by liquid scintillation spectrometry. The theoretical specific activity was 47 μCi/g. Portions of the [^{14}C]-DL-lactide monomer were then combined with unlabeled DL-lactide and unlabeled glycolide to prepare a series of radiolabeled copolymers by the polymerization procedure described above.

The specific activity of the radiolabeled copolymer samples was determined in Soluene 350 (Packard Instrument Co., Inc., Downers Grove, IL) with a liquid scintillation counter (Packard Tri-Carb spectrometer, Packard Instrument Co.). The DL-lactide-to-glycolide content of the DL-PLGA was determined in deuterated chloroform by nuclear magnetic resonance (NMR) spectroscopy. The ratio of the hydrogen atoms in the copolymer was used to calculate the mole ratio of DL-lactide and glycolide in the materials. The inherent viscosity of the radiolabeled DL-PLGA was measured at 30°C in chloroform at a concentration of about 0.5 g/dl; molecular weight and molecular weight distribution were determined by gel permeation chromatography (GPC). The GPC measurements, made by Arro Laboratories (Joliet, IL), determined the number-average chain length and the weight-average chain length. From Dreiding models, the length of one repeat unit in the polymer was measured, and the Q-factor for each copolymer was calculated by dividing the relative molecular weight of each repeat unit by its chain length. The relative molecular weight of a repeat unit for the copolymers was determined from the mole fraction of each monomer unit in the copolymer multiplied by its molecular weight. The Q-factor varied from a value of 20 for the 96:4 copolymer to a value of 19 for the 76:24 copolymer. Multiplication of the chain lengths by the Q-factor gave the number-average molecular weight ($\bar{M}_n$) and the weight-average molecular weight ($\bar{M}_w$).

Batches (10-g) of NET microcapsules were prepared as follows: 7.5 g of DL-PLGA excipient was dissolved in 50 g of a mixed solvent consisting of 35 wt % acetone and 65 wt % chloroform. After this solution had been cooled to 1°C, 2.5 g of micronized NET (Ortho Pharmaceutical, Raritan, NJ, or Schering AG, Berlin, Germany) was added, and the mixture was stirred vigorously for 30 min at 1°C to obtain a homogeneous drug dispersion. (Note: 0.01 g of calco oil violet zirs was added to this mixture when microcapsules for biodegradation studies were prepared). This organic phase was then added slowly to a resin kettle containing 500 g of 5 wt % aqueous poly(vinyl alcohol) (PVA) (Vinol 205, Air Products and Chemicals, Inc., Allentown, PA). During the addition of the organic phase, the PVA solution was stirred at 800 rpm to form a stable oil-in-water emulsion. After the emulsion had been stirred for 10 min, the resin kettle

was closed, the pressure in the vessel was gradually reduced to 200 torr, and the stir rate was lowered to 600 rpm. The conditions of 200 torr, 600 rpm, and 1°C were maintained for 25 h as the volatile solvents were slowly evaporated. When the microcapsules were hard, the contents of the resin kettle were centrifuged. The resulting pellet of microcapsules was washed thoroughly with deionized water, and the microcapsules were wet sieved and dried in a vacuum chamber maintained at room temperature.

To determine the drug content (i.e., core loading) of the NET microcapsules, about 10 mg of microcapsules was dissolved in 10 ml of methylene chloride. Once the microcapsules had been dissolved, 1 ml of this solution was diluted to 10 ml with more methylene chloride. The absorbance of the sample was then measured at 240 nm. Assuming additive absorbances from copolymer, drug, and dye, the amount of NET in the solution was determined from the following equation:

$$C_1 = \frac{A_4 - E_2C_4 + C_3(E_3 - E_2) - I}{(E_1 - E_2)}$$

where C=concentration (g/dl), E=extinction coefficient (dl/g·cm), A=absorbance at 240 nm, and I=the sum of the Beer's law plot intercepts of DL-PLGA, NET, and calco oil violet zirs. The subscripts refer to (1) NET, (2) DL-PLGA, (3) calco oil violet zirs, and (4) microcapsules. This equation subtracts the small contributions of the DL-PLGA and dye to the absorbance. The NET microcapsules employed in the biodegradation studies and pharmacokinetic studies had core loadings of about 22 wt % of drug.

The specific activity of the NET microcapsules prepared with radiolabeled copolymers was determined by liquid scintillation spectrometry. Microcapsules dissolved in Soluene 350 had a specific activity of about 1.2 μCi/g; microcapsules oxidized in a Packard Model 603 oxidizer (Packard Instrument Co.) and solubilized in Permafluor (Packard Instrument Co.) had a specific activity of about 1.0 μCi/g.

Microcapsules were sterilized (for one of the biodegradation studies in rats and all of the pharmacokinetic studies in baboons) by weighing them into disposable syringes having needles closed off with corks. The syringes were then sealed in sterilizer bags and were irradiated with a 2-Mrad dose of gamma radiation.

For the biodegradation studies in rats, female Sprague-Dawley rats (Southern Animal Farms, Prattville, AL) weighing approximately 150 g were injected with 40 mg of NET microcapsules into the musculature lying along the femur of the left leg. The microcapsules were injected as a suspension in 1 ml of a viscous vehicle that consisted of 2 wt % carboxymethylcellulose (Type 7LF, Hercules Incorporated, Norcross, GA) and 1 wt % Tween 20 (ICI Americas, Wilmington, DE) dissolved in sterile U.S.P. water and autoclaved at 121°C for 20 min. The microcapsules and vehicle were vigorously mixed in 3-ml, disposable syringes having 18-gauge, 1-in. needles. After the injections, the animals were returned to their cages and were given laboratory chow and water ad libitum until the day they were killed.

The efficiency of each injection was determined by rinsing any residual microcapsules from the syringe and needle into a 20-ml vial using 70 vol % ethanol. The ethanol was evaporated in an oven (60°C), the dried residual microcapsules were dissolved in methylene chloride, and the NET concentration in the samples was determined by UV spectroscopy. From this NET concentration and the known core loading of the microcapsules, the amount of microcapsules washed from the syringe was calculated, and the injection efficiency was obtained.

For each copolymer group at each sacrifice period, five rats were killed by exsanguination. The entire leg that had been injected with microcapsules was removed, and all soft tissues were removed from the bone. The soft tissues were placed in a specimen jar and were covered with 1 N sodium hydroxide (about 30 ml). After the tissues had been allowed to digest at room temperature for 5 days, they were neutralized with 4 N hydrochloric acid. The tissues were then homogenized in a Sorvall Omni-mixer (Du Pont Instruments, Norwalk, CN), and the homogenate was placed in an oven (60°C) and brought to near dryness.

Duplicate aliquots (about 400 mg) were taken of each homogenate. These were oxidized in a Packard Model 603 oxidizer (Packard Instrument Co.), and the [^{14}C]-carbon dioxide generated was sorbed on 12 ml of Carbasorb (Packard Instrument Co.). Next, 14 ml of Permafluor (Packard Instrument Co.) was added, and the samples were counted on a Packard Tri-Carb liquid scintillation spectrometer (Packard Instrument Co.). The order of oxidation and counting of the samples was chosen by restricted randomization such that drift in the oxidizer or counter would not introduce systematic errors. A set of rats injected with microcapsules and killed immediately after injection afforded homogenate samples that served to quantify the recovery efficiency of the radioactive microcapsules from the injection sites. These samples also served as standard samples for oxidation for subsequent samples. For samples taken from rats killed at various times after treament, the radioactivity in each sample aliquot was determined, and the total radioactivity in each homogenate was calculated. The mean percentage of radioactivity remaining at the injection site for each group of rats at each time interval was normalized by the recovery efficiency.

NET microcapsule formulations prepared with copolymers selected on the basis of biodegradation kinetics were administered to normally cycling female baboons. The baboons were divided into different treatment groups (2 to 5 per group), and each group was treated with a different microcapsule formulation. Microcapsules suspended in 2.5 ml of an aqueous injection vehicle containing 2 wt % carboxymethylcellulose and 1 wt % Tween 20 were injected into the thigh muscle with a conventional syringe having an 18-gauge needle. Actual doses were ascertained by determination of residual microcapsules in the syringe and needles following injection. The difference between the actual and intended doses is a reflection of the variation in the efficiency of the injection. Baboons were observed daily for evidence of menstruation. Blood samples were obtained from all baboons daily for 1 week following treatment and twice weekly thereafter until the end of the study. Peripheral venous blood was collected (5 to 10 ml), and following

TABLE 1. Properties of radiolabeled copolymers.

DL-PLGA, lactide:glycolide (mole ratio)	Inherent viscosity (dl/g)	Specific activity (μCi/g)	$\bar{M}_n$	$\bar{M}_w$	$\bar{M}_w/\bar{M}_n$
96:4	0.76	1.68	9,930	40,320	4.1
92:8	0.75	1.73	7,400	36,700	4.9
87:13	0.83	1.73	7,500	43,000	5.7
74:26	0.75	1.61	7,950	47,000	5.9

centrifugation, serum was drawn, frozen, and stored until assayed. The total estrogens, progestins, and NET in the serum samples were quantified by RIA procedures previously described (Beck et al., 1979).

RESULTS

To determine the effect of the DL-PLGA excipient on the rate of biodegradation of microcapsule formulations, sterilized and unsterilized NET microcapsules were prepared with radiolabeled DL-PLGA excipients having lactide:glycolide mole ratios ranging from 96:4 to 74:26. Care was taken to employ DL-PLGA excipients with approximately the same molecular weight, i.e., excipients with inherent viscosities of about 0.80 dl/g. A summary of the properties of the radiolabeled copolymers used to prepare NET microcapsules for in vivo biodegradation studies is given in Table 1.

The results of NET microcapsule biodegradation studies in rats are shown in Figs. 1 and 2. Data on the biodegradation curves are expressed as the percentage of the radioactivity remaining at the injection site at various times following treatment. The curves have been standardized by correcting for recovery efficiencies at zero time post-treatment. Evaluation of the biodegradation curves obtained from unsterilized microcapsule formulations (Fig. 1) reveals that increasing the quantity of glycolide in the copolymer excipient causes a corresponding increase in the rate of biodegradation of the microcapsules. The durations of biodegradation are as follows: A, (96:4) greater than 45 weeks; B, (92:8) 35 to 45 weeks; C, (87:13) 30 to 35 weeks; D, (74:26) 20 to 25 weeks. On the basis of these biodegradation results, two candidate copolymers (i.e., Formulations B [92:8 DL-PLGA] and C [87:13 DL-PLGA]) that biodegrade in approximately 6 months were selected for further study. Figure 2 shows the biodegradation curves of NET microcapsules prepared with these two excipients following sterilization by gamma radiation. The biodegradation of the sterilized microcapsules is approximately the same, with complete resorption occurring in about 30 weeks. Although the time required for complete biodegradation of the two sterilized formulations is approximately the same, there appears to be a difference in the rate of biodegradation. Formulation C, which contains the greater amount of glycolide, begins to biode-

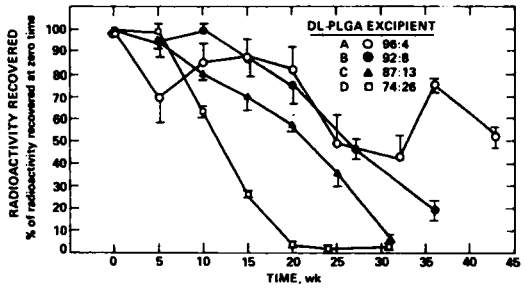

FIG. 1. In vivo biodegradation of unsterilized NET microcapsules prepared with radiolabeled DL-PLGA excipients.

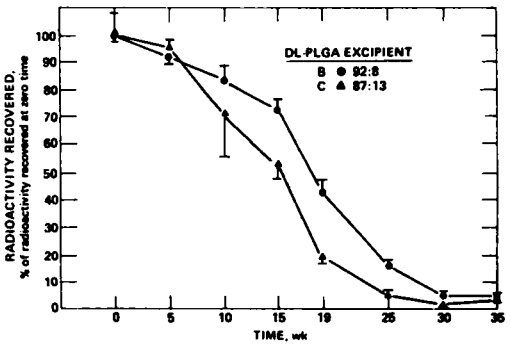

FIG. 2. In vivo biodegradation of sterilized NET microcapsules prepared with radiolabeled DL-PLGA excipients.

TABLE 2. Net microcapsule formulations used in baboon pharmacokinetics studies.

DL-PLGA, lactide:glycolide (mole ratio)	No. of baboons	NET dose (mg)		Microcapsule diameter (μm)
		intended	actual	
88:12	3	25	22.1	90–125
91:9	3	25	22.5	90–125
88:12	4	50	43.8	90–106
91:9	4	50	38.4	90–106
91:9	3	50	47.3	45–90
88:12	3	50	47.2	45–90
88:12	2	50	47.2	63–90

grade earlier, and the slope of the curve is steeper between 5 and 15 weeks. By 15 weeks following treatment, 50% of Formulation C and 70% of Formulation B remain at the injection site. We suspected, on the basis of these results, that NET release resulting from biodegradation of the DL-PLGA excipient might be greater for Formulation C, especially between 5 and 15 weeks.

Table 2 summarizes the characteristics of the NET microcapsules administered to baboons. The serum NET profiles in baboons treated with similar doses of unencapsulated NET have been previously published (Beck et al., 1979). Figures 3 and 4 show the mean serum levels of immune-reactive NET in baboons treated with a single intramuscular injection of the sterilized microcapsules—Formulation B (91:9 DL-PLGA) and Formulation C (88:12 DL-PLGA). The core loading, size distribution, and dose of the microcapsules were similar. There was a slight difference in the dose due to variation in the efficiency of the injection (Table 2). The slopes of the serum NET curves are parallel during the first 2 months following treatment; however,

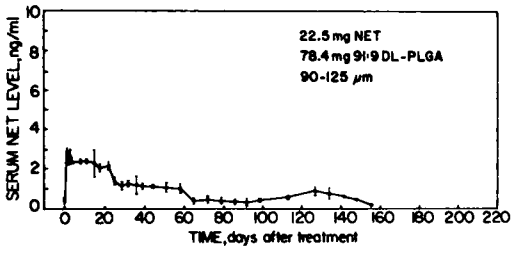

FIG. 3. Mean serum levels of immune-reactive NET in baboons (N=5) treated with a single intramuscular injection of sterilized microcapsules. The dose and composition are indicated in the figure.

after 2 months the curves are not parallel. The serum levels of NET in baboons treated with Formulation C increase between 60 and 90 days after treatment; whereas the serum NET levels in baboons treated with the slower biodegrading formulation, Formulation B, continue a gradual decline between Days 60 and 110. The secondary increase in NET release from Formulation C corresponds to the time when the 88:12 DL-PLGA excipient begins to undergo notable biodegradation (Fig. 2).

It should be emphasized that comparison between biodegradation data and NET release is only an approximation because the rate of biodegradation may not be the same in rats and baboons. Two additional groups of baboons were treated with higher doses of the identical formulations.

The size fraction of the microcapsules used for these experiments was different from that used in the first experiment: 90 to 106 μm as opposed to 90 to 125 μm. The rationale for repeating this experiment using higher doses was twofold: *1)* to demonstrate that higher doses produced proportionately higher serum levels over the entire treatment interval, thus establishing the dose-response capabilities of the system, and *2)* to confirm that the two-phase NET release profile observed in the first experiments is a reproducible characteristic of the system and not an experimental artifact.

Figures 5 and 6 show the results from this experiment. The serum NET profile obtained from Formulation C (Fig. 5) exhibits a two-phase pattern similar to that observed in the first experiment. The serum levels of NET for both Formulations B and C are proportionately higher following treatment with higher doses for both formulations. The serum NET profiles obtained in baboons from formulations having

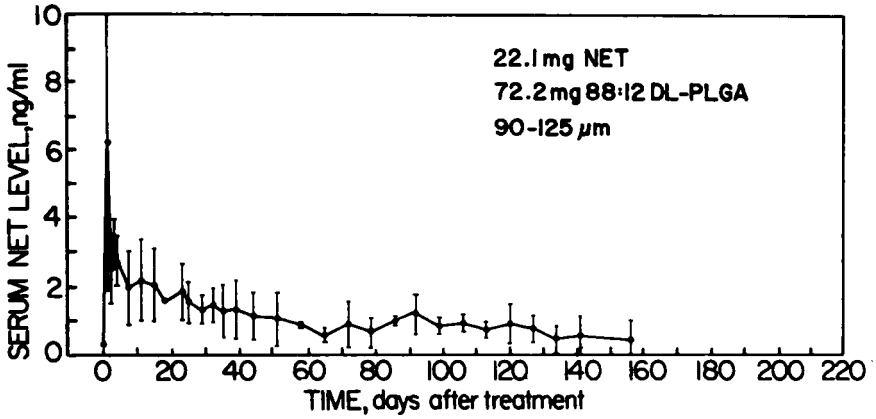

FIG. 4. Mean serum levels of immune-reactive NET in baboons treated by intramuscular injection of sterilized microcapsules. The dose and composition are indicated on the figure.

similar DL-PLGA excipients, however, are not identical. The first experiment (Fig. 3) used microcapsules that ranged in diameter from 90 to 125 μm, whereas the size fraction in the second experiment was 90 to 106 μm. We know, from past experience, that the rate and duration of NET release is dependent on the microcapsule size. Theoretically, smaller size fractions (90–106 μm) should have higher release rates and a shorter duration. NET release from both formulations occurs primarily by diffusion between Days 0 and 60. After Day 60, NET is released by both diffusion and bioerosion. The contribution from bioerosion is greater, however, for Formulation C than for Formulation B. This, in turn, accounts for the two-phase release profile. These results confirm that higher doses of the same formulation produce corresponding higher serum levels of NET over the entire treatment interval. These results

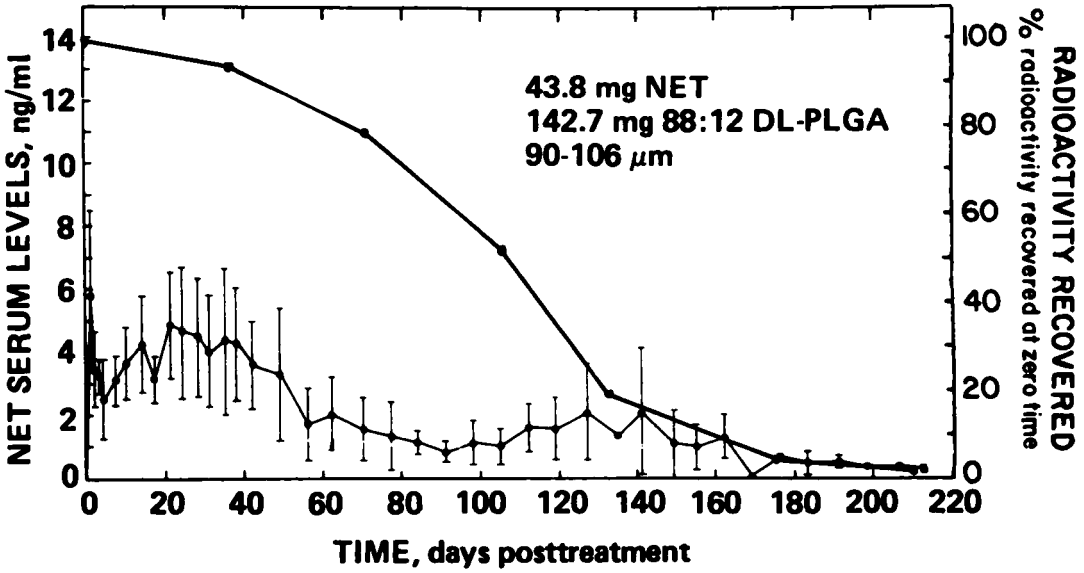

FIG. 5. Mean serum levels of immune-reactive NET in baboons (N=5) treated by intramuscular injection of sterilized microcapsules. The dose and composition are shown in the figure. The biodegradation curve *(top)* obtained in rats is included for comparison.

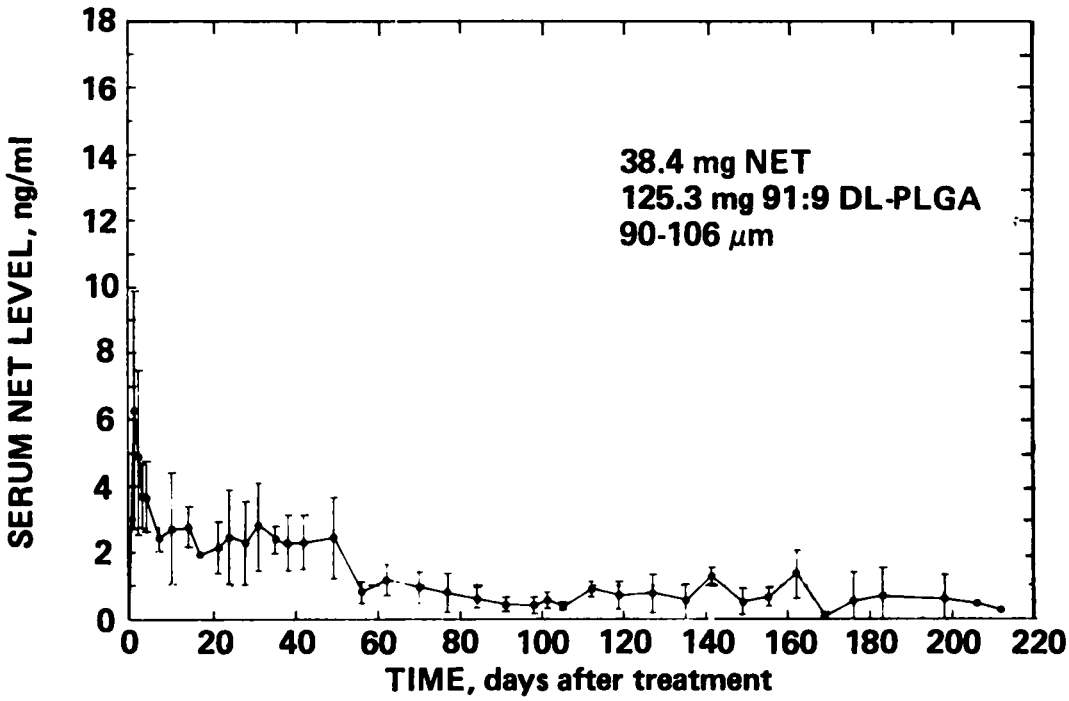

FIG. 6. Mean serum levels of immune-reactive NET in baboons (N=5) treated by intramuscular injection of sterilized microcapsules. The dose and composition are shown in the figure.

further suggest that microcapsule size influences the rate of NET release. This is evident from the observation that peak NET serum levels occur earlier in baboons treated with the smaller microcapsules.

Figure 7 compares the serum NET from two groups of baboons treated with Formulation B. The size fractions were 90 to 106 μm and 45 to 90 μm. Elimination of microcapsules greater than 90 μm reduced the duration of release from 210 days to 150 days. Comparison of the serum NET profile between Day 0 and 60 demonstrates the effect of the smaller microcapsules. Faster release rates resulting from the 45 to 90 μm microcapsules during the first 60 days produced higher serum levels of NET. The use of the slower biodegrading formulation for this comparison emphasizes diffusional release as contrasted with diffusion and bioerosion, which occur with faster biodegrading formulations.

Figure 8 illustrates the influence of microcapsule size. The curves compare the serum NET levels obtained from two groups of baboons treated with two different size fractions of Formulation C. The size fractions were 63 to 90 μm and 90 to 106 μm. Elimination of microcapsules between 90 and 106 μm reduced the tailing effect from 210 to 160 days. The addition of microcapsules between 63 and 90 μm increased the quantity of NET released during the first month. Results from this experiment demonstrate that the serum NET profile can be altered to release greater or lesser amounts of NET during specific intervals by changing the size distribution of the microcapsules. The secondary increase in the serum NET levels due to bioerosion occurs at approximately 125 days following treatment in the baboons treated with the 90 to 106 μm microcapsules and at approximately 90 days in baboons treated with the 63 to 90 μm microcapsules. Accordingly, size distribution of the microcapsules also influences bioerosion-mediated NET release.

DISCUSSION

The results of the rat biodegradation studies from this and previous studies (Beck et al., 1980b) show that the DL-PLGA microcapsules biodegrade faster than DL-PLA microcapsules.

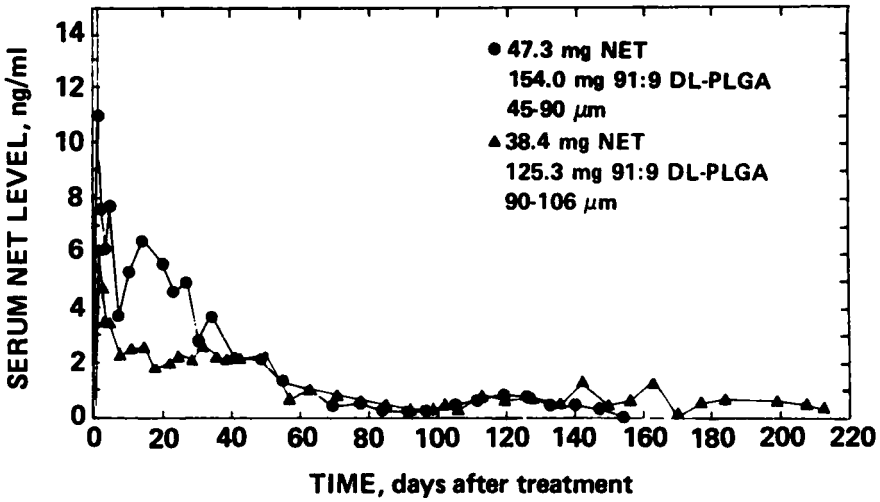

FIG. 7. Comparison of the serum levels of immune-reactive NET in two baboons treated by intramuscular injection with different size microcapsules. The dose and composition are shown in the figure.

The duration of biodegradation of the DL-PLGA excipient is dependent on the ratio of the monomers. Increasing the concentration of glycolide causes a corresponding increase in the rate of biodegradation. In previous studies we have shown in both baboons (Beck et al., 1979) and women (Beck et al., 1981) that NET release from DL-PLA microcapsules exhibits a pattern which is typical of diffusional release delivery systems. The diffusional release profile is characterized by a gradual decrease of serum levels of NET. Biodegradation of the DL-PLA microcapsules has no apparent affect on the in vivo NET release profile because the microcapsules do not biodegrade until most of the NET has been released.

The mean serum NET profiles shown in Figs. 3, 4, and 5 are atypical of a diffusional release mechanism. These curves show two phases of NET release and suggest a second mechanism of NET release. The initial phase is followed by a later phase, which causes a secondary increase

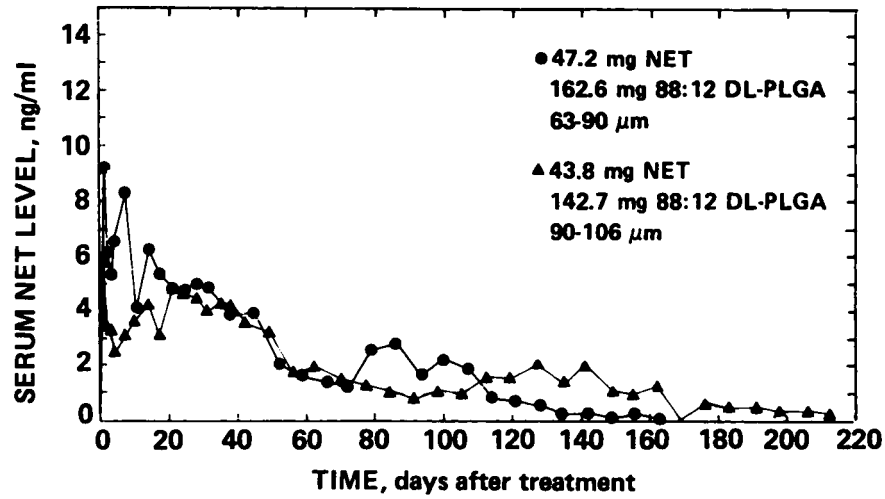

FIG. 8. Comparison of the serum levels of immune-reactive NET in two baboons treated by intramuscular injection with different size microcapsules. The dose and composition are shown in the figure.

in the serum NET levels. The second phase coincides with biodegradation of the polymer. The overall duration of NET release and the timing of the second phase varies among the different experimental groups depending on the physical/chemical properties of the microcapsules. However, all baboons treated with the copolymer formulation (88:12) had biphasic serum NET profiles.

The use of the baboon for evaluating long-acting steroidal contraceptive systems has advantages and disadvantages. The primary advantages is that the baboon is more closely related to the human than other nonprimate laboratory species. Baboons are large and blood samples can be obtained at frequent intervals over an extended period of time. The baboon has a monthly menstrual period similar to women, and the straight cervical canal permits the collection of endometrial biopsies without surgery. The histological response of the baboon endometrium to contraceptive steroids is similar to that in women, and changes in sex skin turgescence provides a noninvasive means of evaluating ovarian function. We have evaluated a variety of different long-acting steroidal contraceptive systems in baboons, and the results agree closely with the findings of human trials on the identical system.

The disadvantage is that the rarity, high purchase and maintenance costs of baboons necessitate the use of small sample sizes. Small sample size is a weakness which is less critical when testing long-acting systems because multiple samples can be obtained over an extended period of time from the same animals. In our opinion, the predictive value of the baboon model for testing long-acting steroidal contraceptive systems intended for human use outweighs the disadvantage of small sample size. We acknowledge, however, that confidence in our interpretation of these results remains open to question because of small sample sizes.

The goal of this research is to develop and perfect injectable long-acting NET microcapsule systems for contraceptive use in women. Baboon studies are an intermediate step in reaching this goal, which we find useful because of the advantages listed above. By testing variations in system design in the baboon model, we are better able to predict how the system will perform in women. After identifying a promising design, we must repeat the studies, first in Phase 1 human trials, utilizing small sample sizes, and Phase 2 and 3 trials, utilizing large sample sizes. The final proof of performance, reproducibility, contraceptive efficacy and acceptability must await the completion of Phase 2 and 3 clinical trials.

On the basis of the present study, we think we have identified a polymeric formulation that will provide at least 3 months of continuous NET release and will be biodegraded within 6 months. The biphasic serum NET profile which is characteristic of this formulation is a desirable feature because the higher serum levels of NET during the latter half of the treatment interval should help to reduce the risk of ovulation. By controlling the ratio of the monomers, drug loading, and the microcapsule size, it should be possible to produce systems in which the duration of release, as well as the shape of the release profile, can be programmed to occur within predetermined intervals.

Injection is the preferred method for taking medication in most parts of the world and the effectiveness of the long-acting injectable contraceptive is free from both user reliance and compliance on a daily basis. Accordingly, there is a great demand for a safe long-acting injectable contraceptive. The widespread use of Depo Provera in the developing world, despite the controversy that shrouds this drug, demonstrates the need for alternative injectables. Our effort to develop long-acting injectable microcapsule systems for the programmed delivery of contraceptive steroids is a response to this need.

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