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Preparation and pharmacokinetic evaluation of a modified long-acting injectable norethisterone microsphere

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Abstract

Prototype long-acting formulations of norethisterone in the form of injectable microspheres made of biodegradable co-polymer of polylactic acid and glycolic acid have been successfully used as injectable contraceptives in women. Second-generation formulations with improved *in vitro* and *in vivo* norethisterone-release profiles have been developed and tested in baboons. A cyclic pattern of norethisterone release has been achieved by modification of the surface properties of the prototype formulation. One such modification, and the resulting effects on the *in vitro* and *in vivo* norethisterone release profiles will be presented and discussed.

Introduction

In the ongoing search for improved methods of delivering steroids for fertility control, injectable biodegradable microcapsules have been considered, in concept at least, to have certain advantages over other delivery systems, including effectiveness, simplicity of use and independence from coitus [1,2].

A major problem associated with most long-acting injectable steroid formulations is the irregularity of the menstrual bleeding cycle, varying from breakthrough bleeding and spotting to amenorrhea [3,4]. Bioavailability of norethisterone (NET) released from an injectable microcapsule formulation is not synchronous with the normal cyclic profile of progesterone release from the ovaries, and it is possible that this contributes to irregular menstrual cycles. According to traditional concept, the ideal release

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profile of long-acting steroid delivery systems should have zero-order release kinetics [5,6]. Zero-order release may not be an option for all steroid hormones. The ovarian steroid hormones occur in regular cyclic patterns in the blood, and the contraceptive steroids, such as NET, prevent pregnancy by inhibiting ovulation and natural cyclic secretion of both estrogen and progesterone. When NET in the blood is kept constant at a dose high enough to suppress ovarian function, this usually results in irregular bleeding or amenorrhea, because cyclic secretion of ovarian hormones is necessary to maintain the menstrual bleeding cycle. The long-acting steady-state release of NET, although effective for contraception, causes irregularity in the menstrual cycle. One possible approach to achieving better control of breakthrough bleeding is to deliver continuous NET in a cyclic pattern. Recently, we prepared modified microsphere formulations containing NET by changing the solvent-evaporation procedure used in the manufacturing process. Our objective was to develop a long-acting injectable contraceptive delivery system that releases NET in a cyclic pattern. The purpose of this communication is to report the outcome of this research.

Preparation of microspheres

A prototype NET microsphere formulation was prepared by the method described by Beck *et al.* [7]. The microspheres, made from a co-polymer of polylactic acid and polyglycolic acid (85:15), ranging in diameter from 25–45 μm and containing 43–45% NET weight, were used as the starting material for preparation of a modified microsphere formulation.

The prototype microspheres were suspended in a reaction flask containing 5% by weight aqueous alcohol. A polymer/chloroform solution was slowly added to the mixture with constant stirring at 54°C. A sample of the reaction solution was taken at frequent intervals, and examined using an optical microscope. When the average particle size reached 100 microns in diameter, a vacuum was introduced to the reaction system to evaporate the chloroform. After 30–40 minutes, the reaction solution was transferred to a cold water bath with stirring to harden the microspheres. After thorough washing in water, the suspension was sieved to remove the microspheres less than 25 μm and greater than 250 μm in diameter. Microspheres ranging between 150 and 212 μm in diameter obtained by a second sieving, and were dried under vacuum to remove the residual solvent.

Prototype microspheres of equivalent size and drug loading were prepared as previously described.

In vitro *release studies*

Control and modified microspheres (25 mg of each) were placed individually in diffusion chambers made from nylon mesh (5–25 μm pore size). The nylon chambers were immersed in 100 ml of 27.5% by weight of aqueous ethanol in individual glass vials placed in a shaker bath at 37°C. The fluid in the flask was changed daily, and a

3 ml sample was analyzed for NET concentration by measuring the absorbance at 247 nm using a Perkin–Elmer Model 575 Spectrophotometer.

In vivo *studies*

Six adult female baboons with normal menstrual cycles were used for the *in vivo* evaluation of the prototype and modified microsphere formulations. The animals were randomly divided into two groups of three baboons each. Group A was treated by intramuscular injection of the modified microsphere formulation containing 75 mg NET; Group B was injected with an equal dose of the prototype formulation. Blood samples were obtained twice weekly, and the changes in sex skin turgescence and menstrual bleeding were observed daily. NET concentration in the serum was measured by radioimmunoassay.

Results and discussion

There are some obvious differences in the structure of modified and prototype microspheres, as determined by examination using scanning electron microscopy (Figure 1). The surface of the modified microsphere formulation is smoother than the surface of the prototype, and the amount of crystalline NET on the surface of the modified microsphere is less than that on the prototype. The surface of the modified microsphere is similar in appearance to the surface of the placebo microsphere, prepared with polymer only (see Figure 1).

Comparison of the *in vitro* NET release profiles (Figure 2) showed a different pattern of release. The burst from the modified microsphere is less pronounced than the prototype. The *in vitro* release profile suggest that there are more NET crystals close to the surface of the prototype microspheres.

The results from the *in vivo* studies (Figure 3) revealed different NET release profiles. There is a prominent second peak in NET blood curve in baboons treated with the modified microsphere. There is no corresponding second peak in the blood NET curve in baboons treated with the prototype formulation. The duration of NET release from the modified microsphere is 40–50 days longer than the prototype. Both the *in vitro* and the *in vivo* results suggest that the structure of the modified microsphere formulation is different from the prototype.

The modified microsphere formulation has a layer of drug-free polymer on the surface of the original microsphere. This layer of drug-free polymer alters the release kinetics of NET both *in vitro* and *in vivo*. According to the Fick first law, the release rate of a sphere can represented by the following relationship:

$$\frac{dMt}{dt} = 4\pi DK\Delta C \qquad \frac{dMt}{dt} = 4\pi DK\Delta C \frac{r_o r_i}{r_o - r_i}$$

where d Mt/dt is the steady-state release rate at time t; D is the diffusion coefficient of the drug in the membrane in cm/sec; K is the distribution coefficient; C is the

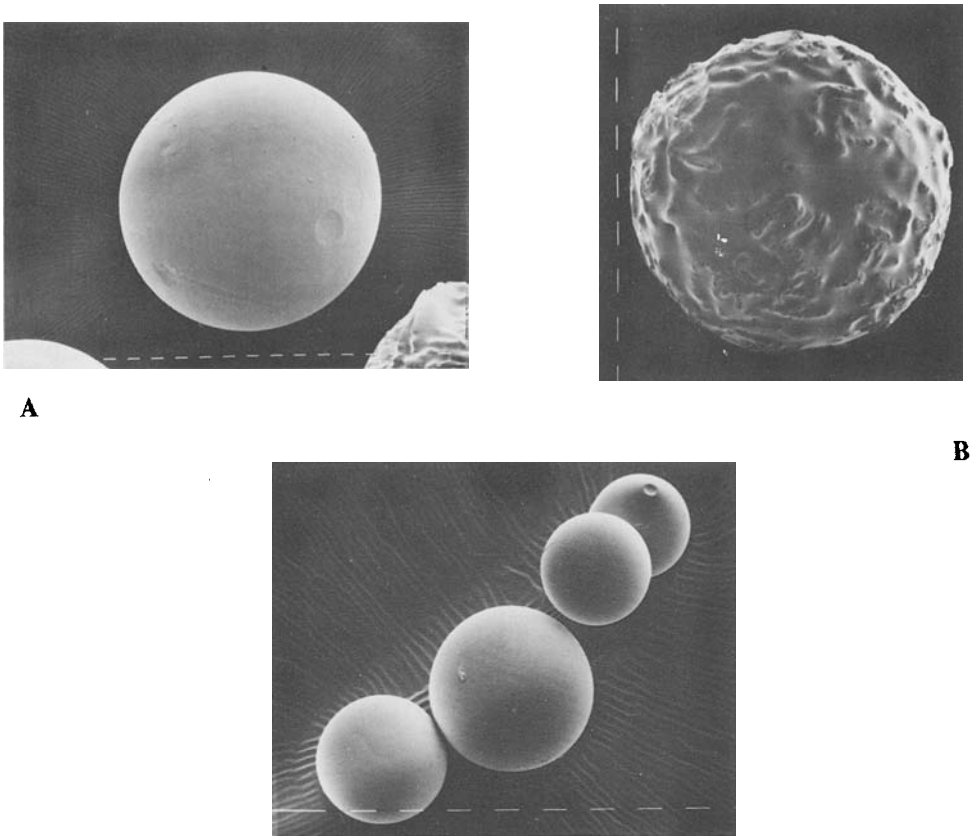

Figure 1 SEMs from the three microspheres, respectively
A: modified microsphere; B: prototype microsphere; C: placebo microsphere

difference in the drug concentration between the internal and the external surface of the membrane; and r_i r_o are the outer and inner radii of the capsule wall, respectively.

The diffusion rate of a drug depends on the thickness of the rate-limiting membrane which encapsulates the drug. Coating the surface of the prototype microsphere with a drug-free layer of polymer increases the thickness of the rate-limiting layer. This reduces the initial burst of NET release and extends the duration of release. The results from both the *in vitro* and the *in vivo* experiments support the predicted result. In the presence of water, the biodegradation of polymer contributes to the second peak seen in the *in vivo* release profile. The timing of the second peak can be controlled by changing the thickness of the drug-free polymer coating and/or the type of polymer (different polymers have various permeability and biodegradation rates). Using this approach, it is possible to produce a series of microsphere formulations having the second peak programmed to appear at preselected times, post-treatment. By blending different microsphere formulations, repeating cycles of NET release can be included in a single injection. We anticipate

that it should be possible to achieve better control of menstrual bleeding by delivering NET in repeating cycles, rather than by steady-state rate of delivery.

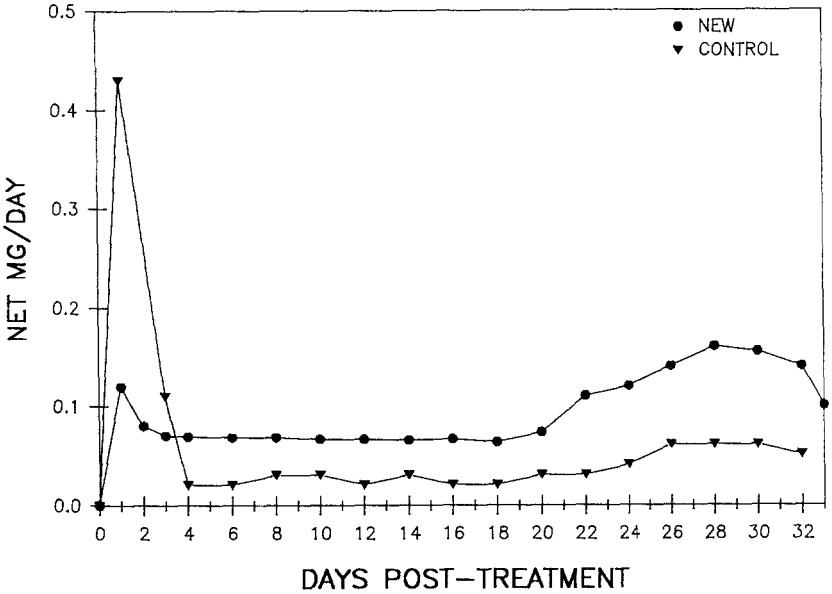

Figure 2 *In vitro* release of NET from the modified microsphere (150–212 μm, 12–13% by weight of NET) and the prototype (150–212 μm, 12–13% by weight of NET) into 27.5% by weight of aqueous ethanol at 37°C

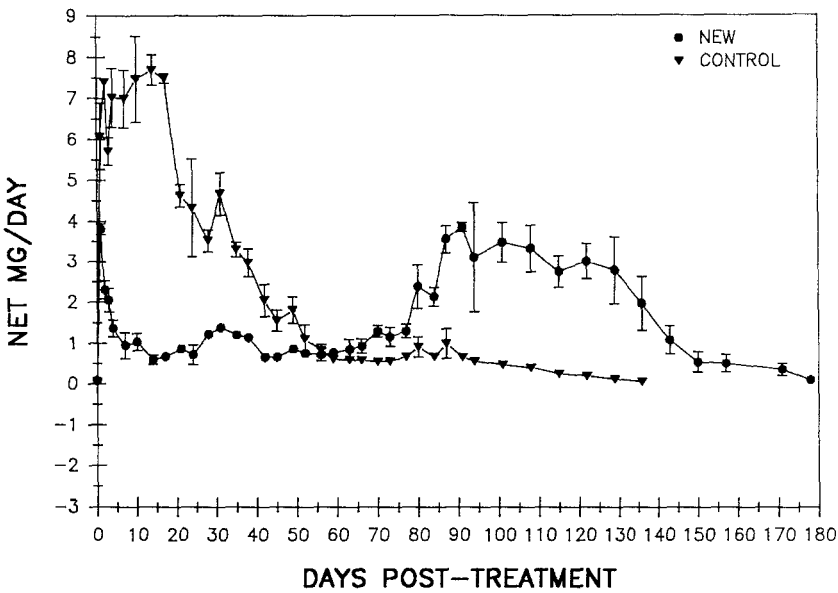

Figure 3 Comparison of NET profiles in the serum of baboons receiving the modified microsphere formulation (150–212 μm, 75 mg of NET) and the prototype (150–212 μm, 75 mg of NET)

Acknowledgements

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References

1. Fraser, I.S. and Weisberg, E. (1981). A comprehensive review of injectable contraception with special emphasis on depot medroxyprogesterone acetate. *Med. J. Aust.*, **1** (special suppl.), 1–19
2. Injectable and implants. (1983). *Pop. Rep. Series K*, **2**
3. Beck, L.R., Flowers, C.E., Pope, V.Z., Tice, T.R., Dunn, R.L. and Gilley, R.M. (1983). Poly(dl-lactide-co-glycolide)/norethisterone microcapsules: clinical study. In: Zatuchni, G.I., Goldsmith, A., Shelton, J.D. and Sciarra, J.J. (eds.) *Long-acting Contraceptive Delivery Systems*. Harper and Row, Philadelphia, pp. 407–417
4. Vecchio, T.J. (1976). Long-acting injectable contraception. In: Briggs, M.H. and Christie, G.A. (eds.) *Advances in Steroid Biochemistry and Pharmacology*. London, pp. 1–46
5. Longer, R.S. (1984). Polymers and drug delivery systems. In: Zatuchni, G.I., Goldsmith, A., Shelton, J.D. and Sciarra, J.J. (eds.) *Long-acting Contraceptive Delivery Systems*. Harper and Row, Philadelphia, pp. 23–32
6. Nuways, E.S., Williams, D.L., Kerrigan, L.H., Nucefora, W.A. and Armstrong, L.C. (1984). Microcapsulation of contraceptive steroids. In: Zatuchni, G.L., Goldsmith, A., Shelton, J.D. and Sciarra, J.J. (eds.) *Long-acting Contraceptive Delivery Systems*. Harper and Row, Philadelphia, pp. 64–76
7. Beck, L.R., Pope, V.Z., Flowers, C.E., Cowsar, D.R., Tice, T.R. and Lewis, D.H. (1983). Poly(dl-lactide-co-glycolide/norethisterone microcapsules: a injectable biodegradable contraceptive. *Biol. Reprod.*, **28**, 186–195
8. Beck, L.R., Cowsar, D.R., Lewis, D.H. and Tice, T.R. (1980). Evaluation of a new three months injectable contraceptive microsphere system in primates (baboon). *J. Contracept. Deliv. Syst.*, **1**, 79–86

Resumé

Les préparations d'un prototype ayant une action prolongée, qui contient de la noréthistérone sous forme de microsphères injectables composées d'un copolymère biodégradable d'acide polyactique et d'acide glycolique, ont été utilisées avec succès chez les femmes en tant que contraceptif injectable tous les 90 jours. Une deuxième génération de ces produits, dont les séquences de libération *in vivo* et *in vitro* de noréthistérone ont été améliorées, a été mise au point et testée chez les babouins. Un schéma de libération cyclique de noréthistérone a été réalisé en modifiant les propriétés superficielles de la formule prototype. Cet article présente et examine cette modification et les effets qui en résultent sur la courbe de libération *in vivo* et *in vitro* de la noréthistérone.

Resumen

Las preparaciones de un prototipo de acción prolongada, que contiene noretisterona en forma de microesferas inyectables compuestas de un copolímero biodegradable de ácido poliáctico y de ácido glicólico, fueron utilizadas con éxito en mujeres como anticonceptivo inyectable cada 90 días. La segunda generación de estos productos, con secuencias mejoradas de liberación de noretisterona *in vivo* e *in vitro*, fue desarrollada y sometida a prueba en babuinos. Se logró un esquema de liberación cíclica de noretisterona modificando las propiedades superficiales de la fórmula prototipo. Esta monografía presenta y examina esta modificación y los efectos resultantes sobre la curva de liberación de noretisterona *in vivo* e *in vitro*.