Controlled Release of Contraceptive Steroids from Biodegradable and Injectable Gel Formulations: *In Vivo* Evaluation

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Purpose. The purpose of this study was to investigate *in vivo* biocompatibility, biodegradability and biological effects of contraceptive steroids, such as levonorgestrel and ethinyl estradiol, released from gels prepared with a combination of derivatized vegetable oil (Labrafil 1944 CS) and glyceryl ester of fatty acids (Precirol ATO 5). ***Methods.*** Biocompatibility, biodegradability, and *in vivo* effects of levonorgestrel and ethinyl estradiol were studied by histologic evaluation of rat tissue, visual estimate of changes in gel size, and assessment of drug effects on reproductive cyclicity of female rats, respectively, following subcutaneous injection of gel formulations. ***Results.*** Histological evaluation of the tissue samples following an injection of the gel revealed an inflammatory reaction for about 7 days, after which the tissues did not show any inflammatory response. Complete degradation of the gels containing 10% wax was observed between 5 and 6 weeks. Normal rat estrous cycles were completely blocked by the contraceptive steroids released from the gels. Gel formulations containing 0.25% w/w levonorgestrel were more effective in blocking the estrous cycle of female rats compared to the oil formulations containing an identical drug loading. The duration of the biological effect induced by levonorgestrel appears to be dose-related. The gel formulation containing 2.00% ethinyl estradiol was superior to oil formulation containing an identical drug loading in terms of controlling drug release and toxicity. ***Conclusions.*** These observations suggest that Labrafil-Precirol gels are biocompatible and biodegradable. Moreover, controlled release of steroids is possible *in vivo* for a prolonged period of time.

KEY WORDS: levonorgestrel; ethinyl estradiol; precirol; labrafil; controlled release; injectable gels; biodegradability; biocompatibility.

INTRODUCTION

Hormonal contraception involves two classes of sex hormones: the estrogens and the progestogens. The most important natural sex hormones are estradiol and progesterone. The synthetic estrogen, ethinyl estradiol, and the synthetic progestogen, levonorgestrel, are more potent than their natural counterparts (1). The major biological effects of levonorgestrel and ethinyl estradiol are to suppress the secretion of gonadotropins, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) from the anterior pituitary gland, thereby inhibiting follicular development, ovarian steroid secretion, and ovulation (2).

These synthetic steroids have been widely used as contraceptive agents in conventional delivery systems, such as pills. Extensive reviews on controlled release systems for contraception have also been published (3–6) and several investigators have described slow-release devices for the delivery of levonorgestrel, including intrauterine devices or IUDs (7), intravaginal rings (8), intracervical devices (9) and Silastic implants (10). However, major drawbacks of the IUDs, vaginal rings and intracervical devices include discomfort, increased frequency of vaginal infection, increased menstrual bleeding, frequent expulsion of the devices after insertion and unwanted pregnancies that are more likely to be ectopic (11,12).

More recently, biodegradable polymers have been used in the development of injectable and implantable controlled release contraceptive delivery systems, such as microspheres, rods, cylinders and films (13–16). The advantages of these systems include complete degradation of the polymers to non-toxic products after all the drug is released from the system, thus obviating the need for surgical removal of the system following implantation or injection. However, major drawbacks of the systems include: remains of residual toxic solvents such as methylene chloride and chloroform which are used during the preparation of the microspheres; syringeability and injectability problems associated with microspheres; requirement of minor surgery for the implantation of rods, cylinders or films; and the possibility of an infection and formation of a permanent scar-tissue at the implant site.

Injectable gels prepared from natural and synthetic polymers, waxes and metallic stearates are another class of injectable drug delivery systems (17–20). Potential advantages of the gels, particularly those prepared with natural substances such as blends of glyceryl esters of fatty acids and vegetable oils, include ease of preparation without the use of toxic organic solvents, ease of administration and complete biodegradation. In the companion report by the present authors (21), the gel formulations containing combinations of polyglycolyzed apricot kernel oil (Labrafil 1944 CS) and glyceryl palmitostearate (Precirol ATO 5) were prepared, and formulation factors affecting drug release from these gel formulations were investigated using levonorgestrel as a model drug. The objective of this study was to evaluate how drug-loaded gel formulations behave *in vivo* after subcutaneous administration in female rats. Therefore, in this report, the authors investigated *in vivo* degradation of the gel, the foreign-body reaction of tissue surrounding the injected gel, and the biological effects of the controlled release gel formulation of levonorgestrel and ethinyl estradiol in female rats.

EXPERIMENTAL

In Vivo Biocompatibility and Biodegradability Tests

Sprague-Dawley (200–225 g) albino female rats were

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supplied by Sasco Laboratories and kept in standard animal facilities. NIH guidelines for the care and use of laboratory animals were observed. The rats were randomly assigned to control and experimental groups. The animals used for these studies were first anesthetized with ether. The hair on the dorsal lower body surface was removed by shaving. The exposed skin was cleaned and sanitized with 70% alcohol.

Preparation of the oil formulation and gel formulation containing 10% Precirol has been described in the companion paper (21). In the experimental group, 1 mL of a blank gel formulation (10% Precirol gel containing no drug) was injected subcutaneously on the dorsal upper body surface using a 22 gauge needle. In control animals, a 22 gauge needle was inserted subcutaneously into the rats and withdrawn without injection. Three rats from each group were sacrificed with an overdose of methoxyflurane at 1, 7, 14, 21, 28, and 45 days following the injections. An incision was made in the vicinity of the original injection site, and the skin was pulled back to expose the gel. The size of the gel at the injected site was visually examined in each of the three rats and photographs of the gel *in situ* were taken in one of the animals at each time point. In each animal, the adjacent tissue containing the gel was removed and fixed in 10% saline-buffered formalin for histologic evaluation. The tissue samples were embedded in paraffin, sectioned with a microtome, and stained with hematoxylin and eosin for microscopic examination. Tissue samples following the blank needle punctures were also examined by the same procedures.

Biological Effects of Contraceptive Steroids Released from the Gel Formulations

The biological effects of two different contraceptive steroids were evaluated by injecting 1 mL of the following formulations subcutaneously on the dorsal lower body surface of gonadally intact, cycling female rats: (a) gel formulations (10% w/w Precirol) loaded with 3 different concentrations of levonorgestrel (0.25%, 0.50% and 2.00% w/w); (b) oil formulation containing 0.25% w/w levonorgestrel and (c) a gel formulation (10% Precirol) and an oil formulation containing 2.00% w/w ethinyl estradiol. A daily vaginal lavage was taken using standard procedures and then transferred to a slide for microscopic examination of vaginal cell morphological changes in estrous cycles (22).

Unmated laboratory female rats exhibit cyclic changes in vaginal cytology over the course of the estrous cycle, and these changes are biological indicators of fluctuations in release of ovarian hormones (22). In most non-pregnant laboratory rats, a complete cycle of such changes occurs every 4 or 5 days. Arbitrarily, the first day of the cycle was considered the day of estrus, which is characterized by the predominance of large cornified cells (CC). This stage lasts about 36 hours. The second stage is diestrus, in which the cornified cells become less numerous and small round cells (leucocytes, LC) are present for about 48 hours. The next stage (proestrus) is characterized by the presence of many nucleated epithelial cells (NC), and the duration of this stage is about 12 hours. Levonorgestrel, a synthetic progestogen, will block the estrous cycle of female rats and retain them in diestrus, whereas ethinyl estradiol, a synthetic estrogen, will stop the cycle in a constantly estrous condition.

After three consecutive normal 4-day estrous cycles had occurred, animals in each group (n = 4) received subcutaneous injections of either the drug-loaded gel or oil suspension, containing either levonorgestrel or ethinyl estradiol. Additional control groups of an equal number of animals received blank (drug-free) gel or oil formulation. Daily observation of the vaginal lavage was continued until the experimental group resumed at least one normal cycle. The smears were scored as to the predominance of LC (indicative of diestrus), NC (indicative of proestrus), and CC (indicative of estrus). Only animals showing 4–5 day cycles with progression from CC to LC to PC in the pre-injection period were used.

Statistical Analysis

An estrogen such as ethinyl estradiol interrupts the estrous cycle by producing a state characterized by the predominance of CC, while progestins such as levonorgestrel produce a persistent LC state. In normal cycles, it is expected that female rats spend two consecutive days with LC and 1–2 days with CC. To quantify the ability of the steroid-gel treatments to block estrous cyclicity, the mean number of consecutive days in LC or CC for each animal was scored for the first 40 days after gel implant. Differences in this measure were assessed with unpaired t-test.

RESULTS AND DISCUSSION

In Vivo Biocompatibility and Biodegradability Tests

Representative hematoxylin- and eoxin-stained sections of tissues seven days after subcutaneous injection of 1 mL blank gel are shown in Figure 1. Numerous white blood cells with round nuclei (shown by arrows in Figure 1a), which are indicative of an inflammatory reaction, were visible in the region surrounding the gel at this time. However, no evidence of inflammation was observed at the injection site in

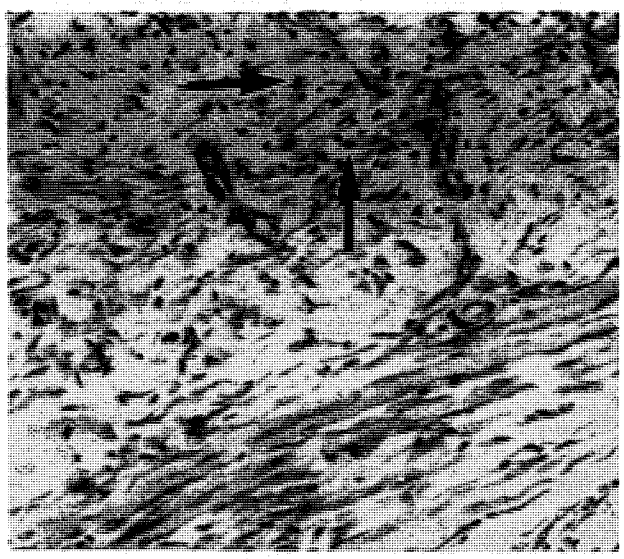

Fig. 1a. Hematoxylin- and eosin-stained sections of tissue 7 days after subcutaneous injection of 1 mL gel. Arrows show round nuclei of white blood cells. (Magnification - 2000×).

control animals who received only a needle puncture seven days previously. In this condition, cells with elongated thin nuclei (shown by arrows in Figure 1b), which are indicative of a normal connective tissue, were observed. Fourteen days following the injection of the gel formulation, no white blood cells were evident in the hypodermis. These results are representatives of all animals studied.

To study the biodegradation of the gel, photographs of subcutaneously injected blank gel formulation were taken in individual animals at 0, 4, 7, 10, 14, 21, 28 and 35 days after injection. The size of the gel progressively decreased, and the color of the gel changed from white to yellow with time (Figure 2). The gel was still visible at the injection site after 28 days (shown by arrows in Figure 2b), but no gel was observed after 35 to 45 days.

Effects of Levonorgestrel and Ethinyl Estradiol Released from Various Formulations on Reproductive Cyclicity of Female Rats

In vivo effects of levonorgestrel released from the gel formulation were measured in terms of changes in estrous cyclicity of female rats. After injection of 1 mL of a gel formulation containing 2.00% levonorgestrel, the normal estrous cycles of rats were suppressed and remained in the diestrous condition for 38.7 ± 0.6 consecutive days (mean ± SEM, n = 4). The control animals that received 1 mL of blank gel injection (without drug) maintained normal cyclic changes in vaginal cytology and remained in diestrus for the normal period of 2.0 ± 0.1 consecutive days (mean ± SEM, n = 4).

The effects of the varying levonorgestrel loading on estrous cyclicity of the animals were compared. Normal estrous cycles were suppressed for 20, 27, and 41 consecutive days in animals receiving the gel formulations containing 0.25% w/w, 0.50% w/w and 2.00% w/w levonorgestrel loading, respectively, and the number of consecutive days in diestrus was significantly prolonged ($p < 0.05$) by the 2.00% w/w formulation compared to the 0.25% w/w formulation. When the number of consecutive days that female rats stayed in diestrus after receiving levonorgestrel gel formulations was plotted against the logarithm of percent drug loading, an apparent linear relationship was obtained (Figure 3).

A significant difference ($p < 0.05$) in the duration of estrous cycle suppression was observed between animals

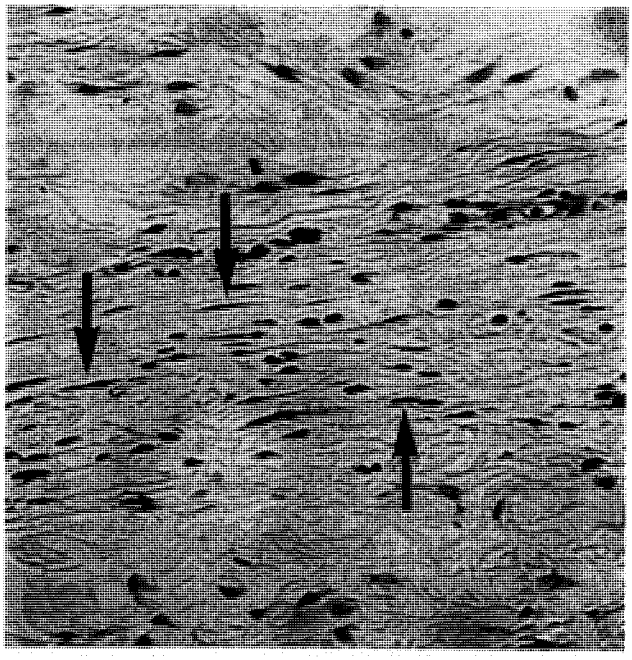

Fig. 1b. Hematoxylin- and eosin-stained sections of tissue 14 days after subcutaneous injection of 1 mL gel. Arrows show elongated thin nuclei of normal connective tissue. Magnification - 200×).

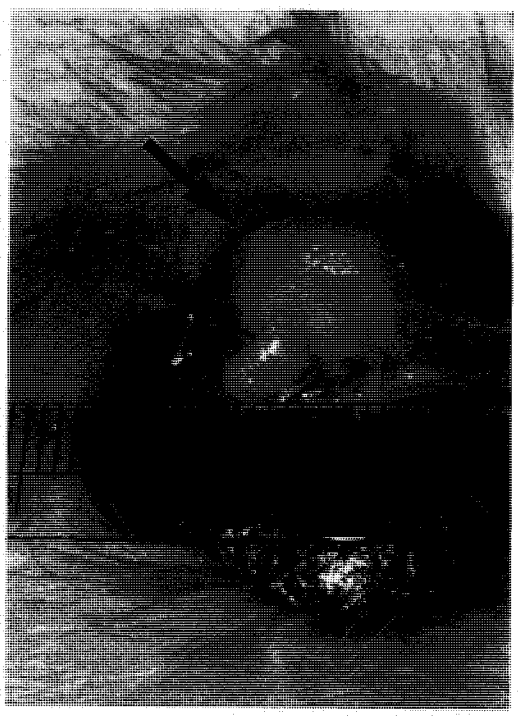

Fig. 2a. The size of the gel (shown by arrow) 0 day after subcutaneous injection of blank gel.

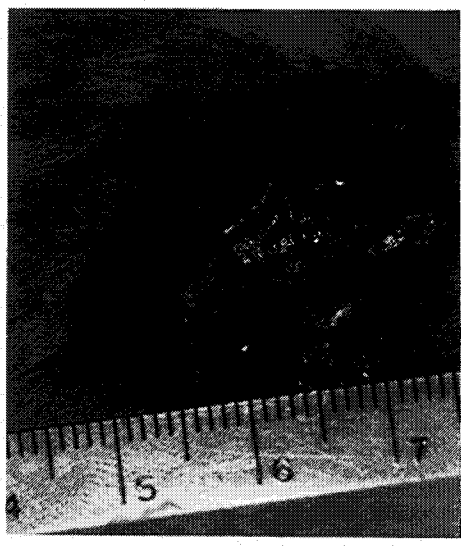

Fig. 2b. The size of the gel (shown by arrows) 28 days after subcutaneous injection of blank gel.

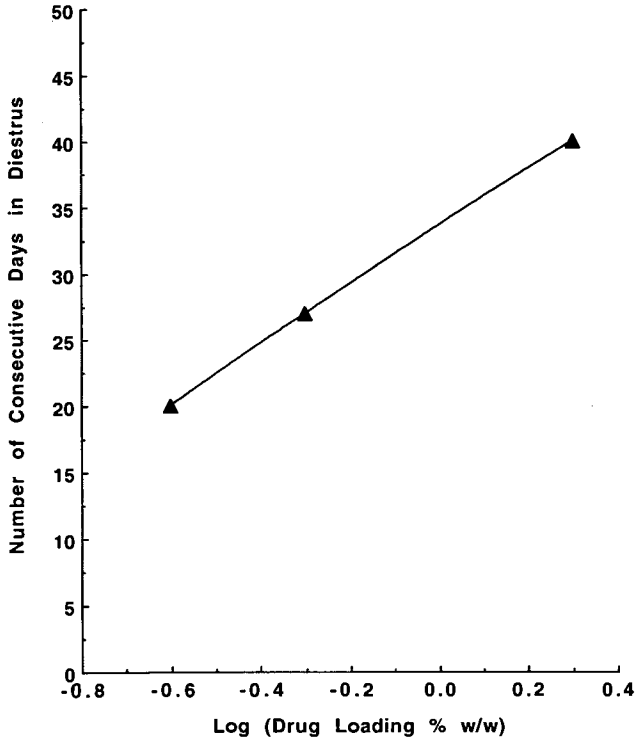

Fig. 3. Effect of drug loading on biological effect of levonorgestrel released from the gel formulations.

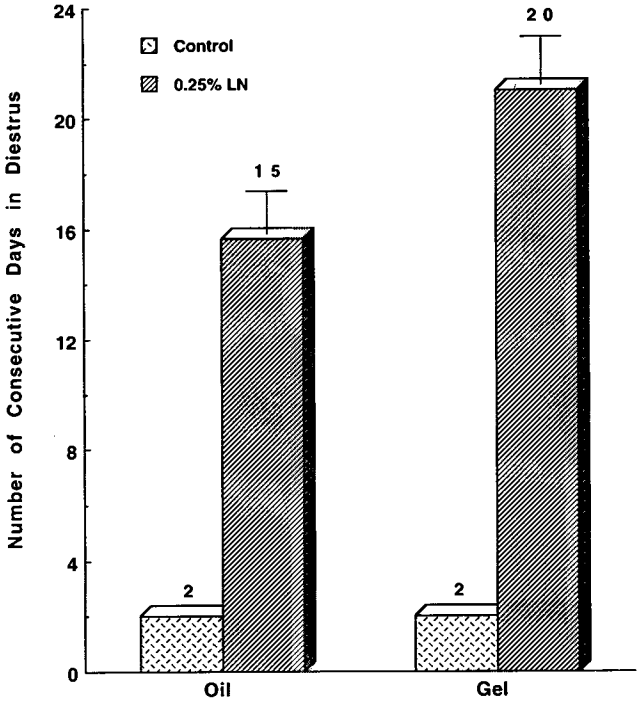

Fig. 4. Comparison of the number of consecutive days of suppression of estrous cycles by levonorgestrel (LN) released from oil and gel formulations with 0.25% w/w drug loading.

receiving 0.25% w/w levonorgestrel-loaded gels and those receiving the same amount of drug-loaded oil. The estrous cycles were blocked for 20.0 ± 0.9 consecutive days (n = 4) in animals receiving the gels, whereas, the cycles were blocked for 15.0 ± 0.7 consecutive days (n = 4) in those animals that received the oil formulation (Figure 4).

The effects of ethinyl estradiol released from oil and gel formulations, consisting of 2.00% w/w drug loadings, on female rats were also investigated. A significant difference ($p < 0.05$) in the duration of estrous cycle suppression was observed between animals receiving 2.00% w/w ethinyl estradiol gel and those receiving the same amount of drug-loaded oil. During the first 40 days post injection, the estrous cycles were blocked for 35 ± 0.7 consecutive days (n = 4) in animals receiving the gel formulation, whereas the average number of consecutive days in estrus was only 4.0 ± 0.5 consecutive days (n = 4) in those animals which received the oil formulation (Figure 5). These latter animals showed irregular cycles, interrupted by periods of constant CC, and symptoms of systemic toxicity of ethinyl estradiol, such as alopecia at or near the injection site, were observed in all animals (n = 4) receiving the oil formulation. On the other hand, the animals treated with the gel formulation containing 2% w/w ethinyl estradiol did not show any signs of systemic drug toxicity such as alopecia.

Slightly longer duration of *in vivo* activity of both steroids was observed in the animals after complete degradation of the gels. This phenomenon could occur if the steroids released from the gel formulations were absorbed in the subcutaneous fatty tissue. The tissue could have then acted as a drug reservoir for a few more days, following complete biodegradation of the gels.

CONCLUSIONS

The results of this study show that a gel formulation prepared with a combination of Labrafil and Precirol is biocompatible and biodegradable. Moreover, the sustained re-

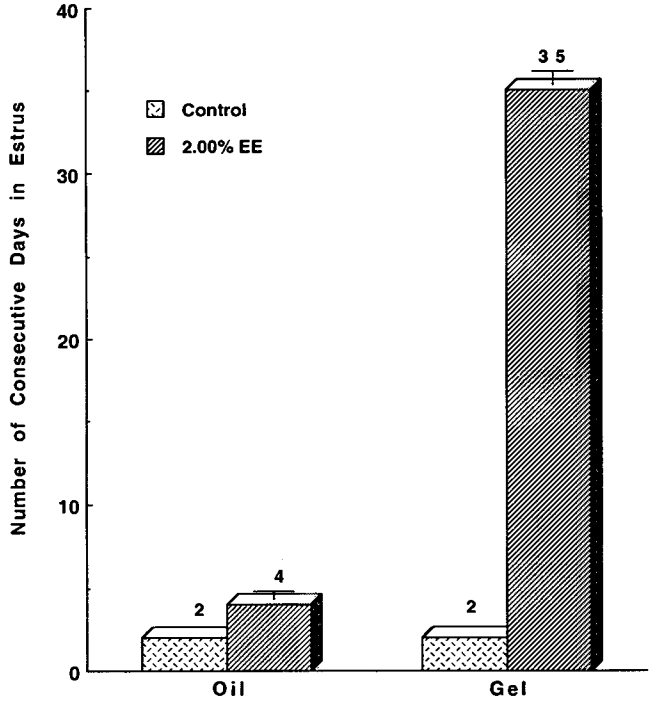

Fig. 5. Comparison of the number of consecutive days of suppression of estrous cycles by ethinyl estradiol (EE) released from oil and gel formulations with 2.00% w/w drug loading.

lease of the contraceptive steroids can be achieved from the gel formulation when administered subcutaneously to female rats. The duration of the biological effect of levonorgestrel is directly proportional to the logarithm of the dose. These observations suggested that Labrafil-Precirol gels may be an effective means of administering biologically active agents with minimal tissue toxicity.

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