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COMMUNICATIONS

The Effect of Esterification on the Release of Testosterone and Estradiol from Silicone Implants

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ABSTRACT

Four experiments were conducted to determine the effect of esterification on the release of testosterone and estradiol from capsule-type silicone implants. In castrated male rats testosterone propionate and testosterone enanthate implants increased ($P < .05$) blood testosterone concentrations and seminal vesicle weights more than empty and testosterone implants. Testosterone propionate and testosterone enanthate implants released 4 times and 10 times, respectively, more equivalent testosterone per cm^2 per day than testosterone implants. The increase in blood testosterone concentrations was proportional to the increase in implant release rate for testosterone propionate, but not for testosterone enanthate. Although estradiol-17β implants increased ($P < .05$) uterine weights of ovariectomized rats, uterine weights in estradiol benzoate implanted ovariectomized rats were 1.3 times greater than in estradiol-17β implanted rats. Estradiol benzoate implants released less than 0.5 times the amount of equivalent estradiol per cm^2 per day than estradiol-17β implants.

INTRODUCTION

Silicone implants have been used by numerous researchers to increase steroid concentrations in animals since Dziuk and Cook (2) demonstrated that steroids would pass through the polymer. The amount of steroid release is related to the implant surface area, wall thickness, steroid polarity, and presence of side chains on the steroid nucleus (see reference 5 for review). Numerous researchers have altered steroid release from implants by varying implant size and implant wall thickness; however, researchers have made few attempts to alter steroid release from implants by modifying steroid polarity and steroid side chains:steroid esters.

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Numerous applications for long-term implantation of testosterone and estradiol have been developed (reference 5 for review, 9,10,12) and capsule-type implants have been extensively used in biomedical research (5). However, in all cases there is an optimal blood steroid concentration and maximal implant size may be a limiting factor. Therefore, the objective of these studies was to determine if steroid esters could be used to enhance secretion of equivalent testosterone and estradiol from silicone implants in vivo.

MATERIALS AND METHODS

All quantitative data were analyzed by analysis of variance (14).

Experiment 1

Thirty-two Lewis inbred rats were castrated at 25 days of age and each rat was implanted (subcutaneously in the neck) at 28 days of age with 3 cm implants containing either nothing, testosterone, testosterone propionate, or testosterone enanthate (8 rats per group) for 13 days. The implants were manufactured from silicone tubing (2.64 mm I.D., 4.8 mm O.D.) by sealing one end with silicone rubber adhesive, filling the tubes with one of the three testosterones or nothing, and sealing the open end of the tubes with adhesive. Rats were euthanized on the 13th day after implantation and trunk blood was collected for testosterone analysis by radioimmunoassay (3,4,12). Seminal vesicles were collected from each rat and weighed immediately after removal and 7 days after they were placed in a desiccator (16).

Experiment 2

Sixteen mature ovariectomized ewes were assigned to one of four groups (4 ewes per group). The ewes were subcutaneously implanted in the axilla with implants containing either nothing, testosterone, testosterone propionate, or testosterone enanthate. The implants were 10 cm long, 9.5 mm in diameter, and had a silicone wall thickness of 3.1 mm. The implants were manufactured as described in experiment 1. The implants were placed in a desiccator for 72 hours and then weighed before implantation. After implant removal, the implants were again placed in a desiccator for 72 hours and again weighed. Blood samples were collected daily while the implants were in situ and were assayed for testosterone concentrations via a radioimmunoassay described by Falvo et al. (3) and Falvo and Nalbandov (4) and validated by Scheffrahn et al. (12).

Experiment 3

Twenty-four female Lewis inbred rats were ovariectomized at 25 days of age and each rat was implanted (subcutaneously in the neck) at 28 days of age with a 3 cm silicone implant containing either nothing, estradiol-17β, or estradiol benzoate for 13 days. The implants were manufactured as described in experiment 1. There were 8 rats per group. Rats were euthanized on the 13th day after implantation and rat uterus were collected. Uterine weights were determined by weighing the uterus immediately after removal and 7 days after they were placed in a desiccator (16).

Experiment 4

Four ewes were subcutaneously implanted in the axilla with six implants each (two were empty, two contained estradiol-17β, and two contained estradiol benzoate). Implants were similar to those used in experiment 3 and were left in situ for 35 days. The implants were placed in a desiccator for 72 hours and then weighed before implantation. After implant removal, the implants were again placed in a desiccator for 72 hours and again weighed.

RESULTS AND DISCUSSION

Testosterone, testosterone propionate, and testosterone enanthate implants increased ($P < .05$) the wet and dry seminal vesicle weights of castrated rats (Table 1). The wet and dry seminal vesicle weights in the testosterone propionate and testosterone enanthate implanted rats were larger ($P < .05$) than in the testosterone implanted rats. When the testosterone implants were placed in ewes, testosterone concentrations were increased, again more ($P < .05$) for testosterone propionate and testosterone enanthate implanted ewes than for empty and testosterone implanted ewes (Table 2), which was the same trend observed for the rats in experiment 1 (Table 1). The equivalent testosterone released from the implants was the greatest ($P < .05$) for testosterone enanthate. The equivalent testosterone released from the testosterone propionate implants was greater ($P < .05$) than for testosterone implants but less ($P < .05$) than for testosterone enanthate implants.

Table 1

Mean[a] Seminal Vesicle Weights and Mean[a] Testosterone Concentrations of Castrated Rats Implanted with Testosterone/Silicone Implants

Group	Seminal Vesicle Weights		Testosterone Concentrations (ng/ml)
	Wet (mg)	Dry (mg)	
Control	15.3 ± 2.9[b]	9.4 ± 2.1[b]	0.1 ± 0.1[b]
Testosterone	555.7 ± 62.2[c]	128.5 ± 10.7[c]	4.8 ± 0.6[b]
Testosterone propionate	698.1 ± 48.6[d]	201.4 ± 11.1[d]	75.8 ± 6.9[e]
Testosterone enanthate	816.1 ± 26.0[d]	210.6 ± 9.4[d]	60.7 ± 7.8[c]

[a]Mean ± standard error.
[b,c,d]Values within the same column with different superscripts differ ($P < .05$).

The blood testosterone concentrations for the testosterone propionate implanted ewes were increased by 3.5-fold over the testosterone implanted ewes. Similarly, the equivalent testosterone released from the testosterone propionate implants was 4.0-fold over the testosterone implants. Therefore, blood concentration of testosterone appeared to be increased because of a greater secretion rate. For testosterone enanthate implants, blood testosterone concentrations were increased by 2.7-fold (over testosterone implants) but secretion was increased by 10.0-fold. This may suggest that although more equivalent testosterone was released as an enanthate ester, it may be metabolized more rapidly. Although testosterone enanthate has been shown to be a long-acting ester, Christensen and Kesler (1) observed a more rapid metabolism for testosterone enanthate when administered directly into the circulatory system. Previous research has been conducted with the testosterone esters administered into tissues where it may be sequestered for long-term release (see 13 for review).

Although estradiol-17β implants increased ($P < .05$) dry uterine weights (as compared to controls), estradiol benzoate increased uterine weights more ($P < .05$) than estradiol-17β implants (Table 3). Estradiol benzoate increased ($P < .05$) uterine weights even though less equivalent estradiol was released daily from the implants (Table 4). These data would suggest that estradiol benzoate implants could be 22% smaller and release only 37% of the equivalent estradiol than the estradiol-17β implants and achieve the same biological effects.

These results agree with some of the previously published data (5) and disagree with other data (15). Both

Table 2

Mean[a] Testosterone Concentrations and Equivalent Testosterone Released from Testosterone/Silicone Implants

Group	Testosterone Concentrations (ng/ml)	Equivalent Testosterone Released (μg/cm²/day)
Control	0.1[b]	—
Testosterone	0.6[b]	74.8[b]
Testosterone propionate	2.1[c]	297.5[c]
Testosterone enanthate	1.6[c]	749.4[d]

[a]Mean ± standard error.
[b,c,d]Values within the same column with different superscripts differ ($P < .05$).

Table 3

Mean[a] Uterine Weights of Ovariectomized Rats Implanted with Estradiol/Silicone Implants

Group	Uterine Weights (mg)	
	Wet	Dry
Control	176.5 ± 71.8[b]	52.1 ± 6.3[b]
Estradiol-17β	211.9 ± 16.9[b]	81.5 ± 6.7[c]
Estradiol benzoate	353.0 ± 21.4[c]	104.1 ± 4.6[d]

[a]Mean ± standard error.
[b,c,d]Values within the same column with different superscripts differ ($P < .05$).

Table 4

Mean Equivalent Estradiol Released from Estradiol/Silicone Implants

Group	Equivalent Estradiol Released (ng/cm²/day)
Control	—
Estradiol-17β	143.2[a]
Estradiol Benzoate	67.6[b]

[a,b]Values within the same column with different superscripts differ ($P < .05$).

Kesler (5) and Tojo (15) demonstrated that more testosterone propionate passed through silicone than testosterone in a given time and that testosterone cypionate passed through the silicone polymer with more difficulty than testosterone. Tojo (15) further demonstrated that as lipophilicity of the testosterone ester increased, passage through the silicone polymer increased with the addition of up to 3 carbons on the alkyl side chain. Although testosterone enanthate is more lipophilic than testosterone propionate, it has 7 carbons in the alkyl side chain. Results in this study and in the studies by Kesler (5) and Christensen and Kesler (1) suggest that more equivalent testosterone as an enanthate ester passed through silicone in a given time than testosterone propionate. The only difference between these studies and those of Tojo (15) was that these studies were conducted in vivo whereas the studies by Tojo (15) were conducted in vitro.

These results demonstrate methods of increasing blood testosterone and estradiol concentrations by altering testosterone and estradiol esters. For researchers manufacturing their own implants, use of esters may provide an additional advantage. If the ester is in the 17 position, it may not cross-react in immunoassays (i.e., in contaminated laboratories) as reported for testosterone esters by Kesler et al. (7). However, if the ester is in the 3 position, as for estradiol benzoate, cross-reactivity with immunoassays may occur (6). Another consideration is the biological activity of the ester once it is released into the circulatory system. Esters in the 17 position are not biologically active until the ester is cleaved (see 13 for review). For example, when the 17 ester of estradiol-17β, estradiol valerate, was administered to ewes, the estradiol-induced luteinizing hormone surge was delayed (11). However, for long-term release this may not be a concern and therefore the valerate ester of estradiol-17β, which has been demonstrated to be a long-acting estradiol-17β ester (reference 8 for review), should be evaluated for use in capsule-type silicone implants.

REFERENCES

1. D. A. Christensen and D. J. Kesler, The passage of testosterone, testosterone propionate and testosterone enanthate from silastic implants and the retention of testosterone once it enters the blood, Anim. Reprod. Sci., 7, 531–536 (1984).
2. P. J. Dziuk and B. Cook, Passage of steroids through silicone rubber, Endocrinology, 78, 208–211 (1966).
3. R. E. Falvo, A. Buhl, and A. V. Nalbandov, Testosterone concentrations in the peripheral plasma of androgenized female rats and in the estrous cycle of normal female rats, Endocrinology, 95, 26–29 (1974).
4. R. E. Falvo and A. V. Nalbandov, Radioimmunoassay of peripheral plasma testosterone in males from eight species using a specific antibody without chromatography, Endocrinology, 95, 1466–1468 (1974).
5. D. J. Kesler, Novel approaches and applications of steroid hormone delivery via polydimethylsiloxane. In: C. G. Gebelein, C. E. Carraher, and V. Foster, (eds.), Applied Bioactive Polymeric Materials, Plenum Publishing Co., New York, 1989.
6. D. J. Kesler, H. A. Garverick, R. S. Youngquist, R. G. Elmore, and C. J. Bierschwal, Effect of days postpartum and endogenous reproductive hormones on GnRH-induced LH release in dairy cows. J. Anim. Sci., 45, 797–803 (1977).
7. D. J. Kesler, H. Khazali, and R. J. Favero, Quantification of steroids via a polymer linked second antibody enzyme immunoassay system: Methods of linking anti-rabbit IgG to polystyrene. In: C. G. Gebelein and R. L. Dunn (eds.), Progress in Biomedical Polymers Plenum Publishing Co., New York, 1990.
8. D. J. Kesler and R. J. Favero, Estrus synchronization in beef females with Syncro-Mate B®. 1. Mechanism of action. Agri-Practice, 1996.
9. D. J. Kesler, R. J. Favero, J. C. Esarey, and L. L. Berger, Controlled delivery of testosterone propionate suppresses fertility in treated females and induces prenatal androgenization in female offspring without phenotypic masculinization. Drug Development and Industrial Pharmacy, 21, 1513–1527 (1995).
10. D. J. Kesler, T. R. Troxel, D. L. Vincent, N. S. Scheffrahn, and R. C. Noble, Detection of estrus with cows administered testosterone via injections and/or silastic implants, Theriogenology, 15, 327–334 (1981).
11. D. J. Kesler, T. R. Troxel, D. L. Vincent, and S. E. Carlin, Luteinizing hormone concentrations in anestrous ewes administered various estrogens, Theriogenology, 13, 231–235.
12. N. S. Scheffrahn, B. S. Wiseman, R. A. Nowak, and

D. J. Kesler, Induction of male sex behavior in ewes with silastic implants containing testosterone propionate, Theriogenology, 18, 1–15 (1982).
13. A. A. Sinkula, Methods to achieve sustained drug delivery. In: J. R. Robinson (ed.), Sustained and Controlled Release Drug Delivery Systems, Marcel Dekker, Inc., New York, 1978.
14. R. E. D. Steel and J. H. Torrie, Principles and Procedures of Statistics, McGraw-Hill Publishing Co., New York, 1980.
15. Kakuji Tojo, Ying Sun, and Yie W. Chien, Kinetics and thermodynamics of drug permeation through silicone elastomer. II. Effect of penetrant lipophilicity, Proceed. Int. Symp. Controlled Release of Bioactive Materials, 12, 153–154 (1985).
16. M. X. Zarrow, J. M. Yochim, J. L. McCarthy, and R. C. Sanborn, Experimental Endocrinology: A Sourcebook of Basic Techniques, Academic Press, New York, 1964.