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Particle size and release of norethindrone acetate through silastic tubing: an in vitro and in vivo study

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Summary

The diffusion of size graded crystalline norethindrone acetate (NETA) from a carefully prepared silastic tubing was studied in vitro in physiological saline solution and in vivo as a subdermal steroid releasing implant device in the mouse. Four types of implants of varying particle size (a) micronised < 8 μm, (b) 75–150 μm, (c) 150–250 μm and (d) 250–550 μm were used. The studies revealed that (i) the in vitro release rate of NETA is always less than the in vivo release rate, (2) the size of the crystal and its solubility within the silastic membrane are inversely correlated and (3) the release rate from micronised NETA filled implants was more uniform than the devices filled with crystalline steroid. Therefore, use of micronised, more uniform particle sized NETA in implants should provide better, sustained release device for long and effective therapeutic application.

Introduction

The single silastic implant, filled with 40 mg of norethindrone acetate, was developed for long-term contraception in women (Bhatnagar et al., 1975). No study is available to show the release characteristics of crystalline and amorphous NETA from silastic devices under both in vitro and in vivo conditions. Therefore, the present study was carried out to determine the optimum microcrystalline size of norethindrone acetate which could be used for sustained release of norethindrone acetate from a single silastic implant.

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Materials and Methods

Implants

Silastic implants were prepared by filling 40 mg norethindrone acetate (17α-ethynyl-17β-acetoxy-4-estren-3-one) in a silastic tubing (Dow Corning, Midland, MI) of 22 mm length, 0.60 mm wall thickness and 3.19 mm outside diameter. These implants were prepared strictly according to the following guidelines. The silastic tubing was cut into small pieces of one foot length. The uniformity of the tubing was tested. The pieces which showed any bulge were discarded. The tube lengths

were washed in a light detergent solution for 20–30 minutes and rinsed in running distilled water. The tubing was cut into smaller pieces of 22 mm length and washed in a mixture of isopropyl alcohol/water (87.2 : 12.8) for 30 min. The smaller pieces were dried in a desiccator over anhydrous calcium chloride. One end of the dried pieces was sealed with medical grade adhesive type A (Dow Corning) and kept for 24 h in an atmosphere saturated with water vapour for proper cross-linking and hardening. From the open end of each piece 40 mg of NETA was filled and this was also sealed with medical grade adhesive. These implants were kept in moist chambers for 24–48 h and were rinsed in 50% methanol and dried. After ensuring proper sealing of both ends, these implants were individually packaged and gas sterilised with ethylene oxide (Anprolene, H.W. Anderson Products, NY). NETA (Schering AG, Berlin) was re-crystallized. Its purity was checked by gas liquid chromatography. The crystallized steroid was ground in a mortar and pestle and was passed through graded sieves (U.S. standard sieve series, Tyler) to obtain crystals of size ranging from 250–550 μm (30–60 mesh), 150–250 μm (60–100 mesh) and 75–150 μm (100–200 mesh). Three types of implants were prepared using these crystals, the fourth being prepared from amorphous micronised NETA (98% less than 8 μm) obtained from Balpharm, Basel.

In vitro release rate

Six implants each, from the four batches of implants, containing crystals of different sizes were incubated in 25 ml isotonic saline in a shaker cum incubator at 37 °C. The incubation medium was replaced every day at a particular time and aliquots collected at specified intervals. The released steroid in the incubation medium was extracted twice with an equal volume of double-distilled ethyl acetate. The pooled extract was dried and redissolved in methanol. The quantitation of steroid was done spectrophotometrically by measuring the absorbance at 240 nm against a methanol blank.

In vivo release rate

Implants were inserted subdermally with implants in the scapular region into Swiss mature female mice of uniform weight (obtained from the Experimental Animal Facility of the All India Institute of Medical Sciences) with the help of a thin-walled 11-gauge trocar and cannula. These implants were removed at monthly intervals up to 8 months and then after 10 and 12 months of use in batches of 5 to 10. The residual steroid in each implant was estimated spectrophotometrically at 240 nm. The release rate of norethindrone acetate from implants was calculated as follows:

$$\text{Amount of NETA released} = \frac{\text{Amount of NETA filled} - \text{Amount of NETA remaining}}{\text{Number of days in situ}}$$

Radioimmunoassay of NET (17α-ethynyl-17β-hydroxy-4-estren-3-one)

Blood was collected from mice before killing them for implant removal and allowed to clot. The serum was separated by centrifugation and stored at −20 °C till assayed. Serum norethindrone concentration was estimated using specifically labelled [15,16-^{3}H]NET (spec. act. 57.14 Ci/mmol) and antiserum raised against NET-3-BSA in rabbits. Each sample was extracted once with diethyl ether and taken further without purification for assay in duplicate (Hillier et al., 1977; Laumas et al., 1978). The intra- and inter-assay co-efficient of variation was 9 and 18%, respectively.

Results and Discussion

The release rate of crystalline and micronised NETA from silastic tubing has been studied in vitro for a period up to 193 days (Fig. 1). The results showed that NETA diffusion from micronised implant was higher than that from the crystalline implants (Table 1, $p < 0.05$–0.001). The in vivo release from micronised and crystalline NETA implants is shown in Fig. 2. Again, it is clear that the in vivo release rate of micronised implants was significantly higher (Table 2, $p < 0.05$–0.001) as compared to crystalline NETA implants. A comparison of in vitro and in vivo release rates from

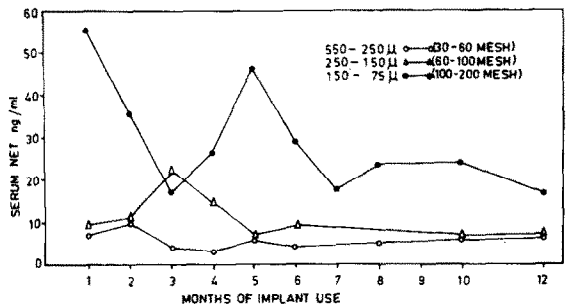

Fig. 1. In vitro release rate pattern of NETA from silastic implant containing different crystal sizes of NETA.

the four types of implants showed that the in vivo release rate was about 2-fold greater than the in vitro release rate up to 3 months, after which it was about 1.5-fold greater than the in vitro release rate. Further, it was observed from the release rate data that an inverse relationship exists between the particle size and the release rate from the silastic implant. It was observed that the larger the crystal size the lower the release rate from the implant and vice versa.

The bioavailability of serum NET, one of the bioactive metabolites of NETA, was measured

TABLE 1

Effect of crystal size of NETA on in vitro release from silastic implant (mean ± S.D., mg / day)

Days	Micronised (98% < 8 μm) (1)	75–150 μm (2)	150–250 μm (3)	250–550 μm (4)	Inter-group comparison, p value (unpaired Student's t-test)	
1	0.107 ± 0.037	0.102 ± 0.015	0.116 ± 0.008	0.110 ± 0.009	–	
3	0.082 ± 0.024	0.064 ± 0.008	0.083 ± 0.006	0.071 ± 0.013	–	
5	0.073 ± 0.015	0.060 ± 0.009	0.056 ± 0.009	0.062 ± 0.011	–	
7	0.080 ± 0.018	0.055 ± 0.005	0.055 ± 0.004	0.056 ± 0.006	1 vs 2; 1 vs 3; 1 vs 4	< 0.025; < 0.025; < 0.025
9	0.078 ± 0.022	0.052 ± 0.009	0.070 ± 0.019	0.063 ± 0.017	1 vs 2	< 0.025
11	0.081 ± 0.031	0.048 ± 0.014	0.056 ± 0.005	0.050 ± 0.014	1 vs 2; 1 vs 4	< 0.05; < 0.05
13	0.081 ± 0.006	0.051 ± 0.001	0.054 ± 0.012	0.058 ± 0.015	1 vs 2; 1 vs 3; 1 vs 4	< 0.001; < 0.001; < 0.001
17	0.070 ± 0.012	0.052 ± 0.004	0.051 ± 0.013	0.057 ± 0.008	1 vs 2; 1 vs 3	< 0.01; < 0.05
36	0.078 ± 0.016	0.059 ± 0.009	0.054 ± 0.007	0.058 ± 0.012	1 vs 2; 1 vs 3; 1 vs 4	< 0.05; < 0.01; < 0.05
43	0.067 ± 0.010	0.042 ± 0.008	0.049 ± 0.009	0.039 ± 0.003	1 vs 2; 1 vs 3; 1 vs 4	< 0.005; < 0.025; < 0.001
58	0.075 ± 0.025	0.053 ± 0.005	0.053 ± 0.004	–	–	
103	0.065 ± 0.016	0.053 ± 0.006	0.053 ± 0.007	0.042 ± 0.005	–	
133	0.074 ± 0.013	0.045 ± 0.014	0.047 ± 0.012	0.045 ± 0.008	1 vs 2; 1 vs 3; 1 vs 4	< 0.005; < 0.005; < 0.01
163	0.068 ± 0.018	0.060 ± 0.014	0.053 ± 0.007	0.051 ± 0.015	–	
193	0.067 ± 0.021	0.049 ± 0.009	0.040 ± 0.014	0.047 ± 0.015	1 vs 2	< 0.025

TABLE 2

Effect of crystal size of NETA on in vivo release from silastic implant in mice (mean ± S.D., mg / day)

Month of use	Micronised (98% < 8 μm) (1)	75–150 μm (2)	150–250 μm (3)	250–550 μm (4)	Inter-group comparison, p value (unpaired Student's t-test)	
1	0.278 ± 0.030	0.234 ± 0.400	0.196 ± 0.018	0.202 ± 0.014	1 vs 2; 1 vs 3; 1 vs 4	< 0.025; < 0.001; < 0.001
2	0.165 ± 0.007	0.115 ± 0.020	0.115 ± 0.020	0.124 ± 0.007	1 vs 4	< 0.05
3	0.128 ± 0.007	0.120 ± 0.010	0.130 ± 0.021	0.120 ± 0.008	1 vs 4	< 0.05
4	0.108 ± 0.007	0.102 ± 0.013	0.104 ± 0.003	0.093 ± 0.011	1 vs 4	< 0.05
5	0.110 ± 0.008	0.081 ± 0.003	0.081 ± 0.004	0.082 ± 0.005	1 vs 2; 1 vs 3; 1 vs 4	< 0.001; < 0.001; < 0.001
6	0.093 ± 0.009	0.085 ± 0.014	0.078 ± 0.005	0.079 ± 0.009	1 vs 3; 1 vs 4	< 0.001; < 0.01
7	–	0.071 ± 0.008	0.086 ± 0.005	0.069 ± 0.016	–	
8	0.090 ± 0.010	0.072 ± 0.008	0.076 ± 0.003	0.073 ± 0.004	1 vs 2; 1 vs 3; 1 vs 4	< 0.005; < 0.005; < 0.001
10	0.085 ± 0.004	0.076 ± 0.006	0.066 ± 0.001	0.064 ± 0.002	1 vs 2; 1 vs 3; 1 vs 4	< 0.025; < 0.025; < 0.001
12	0.080 ± 0.004	0.080 ± 0.003	0.065 ± 0.001	0.064 ± 0.002	1 vs 3; 1 vs 4	< 0.001; < 0.001

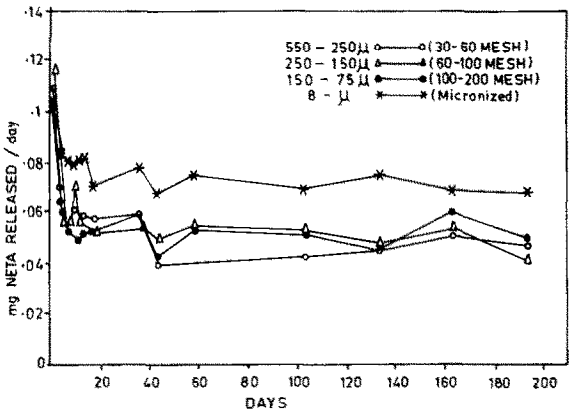

Fig. 2. In vivo release rate pattern of NETA from silastic implant containing different crystal sizes of NETA, removed from mice after different intervals of use.

after the insertion of crystalline implants in mice. Fig. 3 shows distinctly that the three different crystalline NETA implants produced different serum levels of NET. Further, it was observed that implants filled with smaller crystalline NETA resulted in higher in vivo serum NET levels.

The present study has demonstrated that silastic tubing of uniform thickness filled with varying

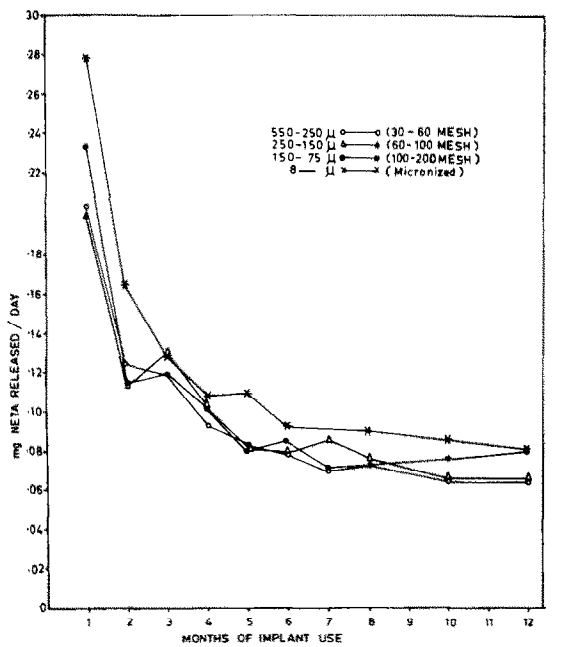

Fig. 3. Serum levels of NET in mice inserted with silastic implant containing 3 crystal sizes of NETA.

size graded crystalline NETA shows significant differences in steroid release over the period of use. The in vitro release rate pattern of NETA from the three types of silastic implants showed that there was an initial high release of NETA for a short duration followed by a more or less constant release with a gradual decline in all three grades of crystalline steroid releasing implants whereas the micronised NETA implants showed much higher in vitro release rate over the other three crystalline NETA implants.

A steady decline in the in vivo NETA release rate pattern from 30–120 days was observed. However, after 120–150 days, the release rate was observed to be reasonably constant. A similar in vivo release rate pattern demonstrating an initial high release followed by a relatively constant release has been reported by Coutinho et al. (1970), Benagiano et al. (1973), Wiese et al. (1976) and Nash et al. (1978) with silastic implants containing different steroids.

The in vivo and in vitro release rate from implants containing different crystal sizes of NETA showed quantitative differences. The rate of diffusion of a particular steroid from silastic capsule is being influenced by factors such as membrane thickness, molecular structure and polarity of the substances. The significantly higher in vivo release rate of NETA over the in vitro release clearly suggests that not only physical and chemical factors but biological factors as well influence the diffusibility of a steroid through silastic tubing. Similar phenomenon has been observed with other steroids as well as NETA in other studies (Kincl et al., 1968; Sundaram and Kincl, 1969; Kratochvil et al., 1970).

Both in vitro and in vivo NETA release rate studies indicated that the crystal size of NETA is a determining factor, again indicating an inverse relation between crystal size and diffusion rate. Between the micronised (8 μm) and 250–550 μm sized crystalline NETA exists a graded variation in diffusion rate and utilising this physical property a desired release can be established. However, the in vivo levels of NET, one of the biologically active metabolites of NETA, has shown distinct differences in the three types of implants. Further, the serum NET levels showed a rise after an initial

stabilization. The rise in serum NET could be due to its binding to sex hormone binding globulin (SHBG) and its pharmacokinetic characteristics in the body. The cumulative accumulation and higher serum levels of NETA and NET have been shown in oral as well as subdermal NETA implant users (Singh et al., 1982). It is likely that the concentration of NET/NETA could have induced varying levels of SHBG and a consequent difference in NET levels attained with the three different crystalline implants. Our present study on the diffusion characteristics of the four types of crystalline NETA showed that finer crystals diffuse in greater quantity than larger crystals both under in vitro and in vivo conditions. It may be suggested from this study that silastic implants containing uniform crystalline NETA could be used for achieving a sustained in vivo release for long-acting contraception.

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