SUSTAINED RELEASE HORMONAL PREPARATIONS

5. Absorption of 6-methyl-17α-acetoxyprogna-4,6-diene-3,20-dione from polydimethylsiloxane implants in vivo

By

G. Benagiano, M. Ermini, C. C. Chang, K. Sundaram and Fred A. Kind

ABSTRACT

Determination of radioactivity in urine and faeces was used to monitor absorption of labelled 6-methyl-17α-acetoxyprogna-4,6-diene-3,20-dione from polydimethylsiloxane implants. Based on previously determined in vitro diffusion rates, mean absorption was 60% in hamsters, 80% in rabbits, and 85% in rats during the first month after implantation. Thereafter, excretion of radioactive material has slowly decreased. After six months, the rate declined to 40% of the original value.

Polydimethylsiloxane (PDS) membranes are permeable to organic compounds, including hormones. In vitro diffusion rates are proportionate to membrane area, inversely proportionate to membrane thickness and Fick’s law is fully applicable (Garrett & Chemburkar 1968; Kind et al. 1968; Kratochvil et al., in prep.). This assures constancy of hormone supply from PDS implants; capsules made by filling a PDS tubing with dry, crystalline material have shown a remarkable diffusion constancy when incubated in distilled water (Kind et al. 1968). No data, however, were available describing absorption of steroids from PDS implants in vivo. The present communication describes measurements of absorption of 6-methyl-17α-acetoxyprogna-4,6-diene-3,20-dione (megestrol acetate) released from PDS implants in several species of laboratory animals.
MATERIALS AND METHODS

General conditions: Virgin female rats (weight 180–220 g), virgin female golden hamsters (weight 80–100 g), and virgin female albino rabbits (weight 2200 g) kept in metabolic cages with food and water ad libitum were used. The implants were made by sealing dry crystalline powder into PDS tubing no. 602–265 (wall thickness 0.42 mm) with Medical Adhesive Type A cement (obtained from Dow-Corning Corp., Midland, Michigan). In one experiment (see text) the steroid was suspended in an aqueous carboxymethylcellulose (CMC) solution and a larger tubing was used. The average length of the implants was 16 mm; the average length of each seal was 2–3 mm. [6-14C]Megestrol acetate (diluted to a specific activity of 0.2 μCi/mg) was obtained from British Drug Houses, Ltd., London; 3H-megestrol acetate (specific activity 913 μCi/mg) was a gift from Mead Johnson Co., Evansville, Indiana. Prior to, and following the in vitro studies, the amounts of the steroid diffusing in vitro through each implant were determined by incubation in distilled water as described (Kincl et al. 1968). The implants were inserted subcutaneously, under slight ether anaesthesia, in the dorsal region. In rabbits, urine and faeces were collected separately every 1–2 days; in rats and hamsters every 2–4 days. At autopsy plasma was separated from heparinized blood; selected organs were removed and weighed.

Preparation of the samples for the determination of radioactivity: Two different methods were used. Initially, urine samples were diluted to a constant volume with methanol to make a 70%/ aqueous methanol solution. The solution was cooled to −5°C, centrifuged, and 1 ml aliquots of the supernatant were counted. To count faeces, an aliquot was homogenized with 70%/ aqueous methanol in a Waring blender; the supernatant was separated by centrifugation, the precipitated fraction was extracted three times with 3 volumes of 70%/ aqueous methanol, all supernatants were combined, evaporated to dryness, dissolved in 50 to 100 ml of 70%/ aqueous methanol and kept at −5°C for 48 h. The samples were then centrifuged, the clear supernatant was evaporated to dryness, redissolved in 10 ml of methanol, and 1 ml aliquot were counted. In later experiments we used Bio-solv solubilizer formula BBS-3 (Beckman Instruments, Inc., Fullerton, California): urine was diluted to a constant volume (25 ml to 100 ml depending on the amounts) and 1 ml aliquots were counted using BBS-3 scintillation media (vide infra) without further processing. Faeces were homogenized with 70%/ aqueous methanol (the usual volume was 50 ml), the mixture was centrifuged, the solids were resuspended in 70%/ aqueous methanol (20–25 ml) and centrifuged again. The supernatants were combined, diluted with 70%/ aqueous methanol to 100 ml and 1 ml aliquots were counted in BBS-3 scintillation liquid. The above procedure was also suitable for processing faeces and urine together.

Heparinized plasma was diluted with absolute ethanol to 70%/ aqueous solution, precipitated plasma proteins were removed by centrifugation and the solvent was removed by distillation under reduced pressure. The residue was redissolved in 5 ml of methanol, and 2 ml aliquots were counted in scintillation media (vide infra). The following organs were processed separately in each animal: liver, kidneys, adrenals, uterus, ovaries, and vagina; all other organs were extracted together. All samples were extracted in a routine manner (e.g. Mikhail et al. 1963).

Measurement of radioactivity: BBS-3 scintillation medium was prepared by adding 250 ml of solution A (naphthalene, 60 g; butyl-PBD [2-(4-tert-butylphenyl)-5-(4-biphenyl)-1,3,4-oxidazole], 10 g; ethylene glycol, 20 ml; and methanol, 100 ml dissolved in 1000 ml of dioxane) to a solution prepared by dissolving 5 g of butyl-PBD and 75 ml of BBS-3 in 500 ml of toluene. The final solution solubilized well up to 2 ml
of urine, or urine-faeces mixtures with a counting efficiency for tritium of about 20 %.
All samples were counted in duplicate or triplicate in a Packard Tri-Carb Scintillation
Spectrometer Model 3375 using either external, or internal standardization (14C or 3H
toluene) to calculate counting efficiency.

Calculation of steroid absorption from the implant: The absorption of the steroid
from the implant was calculated on the basis of the total urinary and faecal excretion
of radioactive metabolites and is expressed as µg of megestrol acetate per 24 h.

Statistical analysis: The following abbreviations were used: M. V., median values;
SD, standard deviation; se, standard error; ss, variation; r, Bravais coefficient.

RESULTS

1. Short term absorption studies: Large PDS capsules (wall thickness, 0.66
mm; area, 250–350 mm²), filled with megestrol acetate suspended in aqueous
carboxymethyl cellulose were used in the initial experiments. The capsules
were implanted first in hamsters; after an observation period of 40 days the
implants were removed and incubated in distilled water at 37°C for 14 days.
They were then implanted into 4 rabbits (1 animal had 2 implants) for a 40
day period. The data are listed in Table 1. In vitro diffusion of the individual
implants ranged from 30 µg/24 h to 45 µg/24 h. The variation was due to
different implant lengths. The amounts of megestrol acetate that diffused prior
to, and following implantation into hamsters was the same for individual im¬
plants. This indicates that during 40 days there has been no accumulation of
foreign material into implant’s membrane which would interfere with the dif¬
fusion process. During the observation period there was a total of 18 hamster
urine and faeces collections. Only 7 rabbit collections were considered. After

<table>
<thead>
<tr>
<th>Implant number</th>
<th>Average excretion µg ± S. E. /24 h</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hamsters</td>
<td>Rabbits</td>
</tr>
<tr>
<td>1</td>
<td>25.9 ± 2.2</td>
<td>32.8 ± 4.1</td>
</tr>
<tr>
<td>2</td>
<td>20.5 ± 1.2</td>
<td>30.4 ± 1.8</td>
</tr>
<tr>
<td>3</td>
<td>29.2 ± 2.2</td>
<td>55.9 ± 5.0a</td>
</tr>
<tr>
<td>4</td>
<td>23.1 ± 1.8</td>
<td>27.4 ± 2.2</td>
</tr>
<tr>
<td>5</td>
<td>17.1 ± 1.1</td>
<td></td>
</tr>
</tbody>
</table>

Ratios: H = Hamster in vitro; R = Rabbit in vitro.
a Implants 3 and 5 combined.
this time (16 days) the values for excreted megestrol acetate declined sharply and it became apparent that the implants became exhausted. This was confirmed at autopsy. The mean values for this period (individual rabbits 6 µg, 14 µg, 24 µg, and 13 µg per 24 h, respectively) were excluded from calculations.

Variations in detected amounts of excreted megestrol acetate in individual animals were greatest during the first two weeks after implantation. In hamsters two animals excreted high amounts (about twice the mean value) during the first four days, and one animal excreted only about one half of the mean value found later. Thereafter the excretion patterns were more uniform but variations of ± 30% from the mean were encountered. In rabbits one animal excreted high amounts and one animal low amounts during the first 4 days of observations. Thereafter, the values became more stable. Excretion patterns in the remaining two animals were more uniform.

For the stated periods of observation mean absorption from individual implants in both species (in vivo/in vitro) was 61% in hamsters and 80% in rabbits; average ratio between urinary and faecal extraction was 1.91 and 1.85, respectively.

Absorption from implants filled with crystalline material was measured only in hamsters. Prior to implantation the capsules (thickness 0.42 mm) were incubated in vitro to measure the release rates. Five hamsters had two implants each (average diffusion 34.5 µg/24 h); one animal had two smaller implants releasing a total of 29.3 µg per 24 h. Average excretion values for this group (40 days of observation, a total of 12 collections) are given in Table 2.

During the first two weeks the amounts of excreted radioactivity were increasing in all the five animals; thereafter excretion patterns appeared more stable. The variations during the whole period of observation were large.

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Table 2.
Absorption of [14C]megestrol acetate from PDS implants in hamsters during 60 days.

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Average excretion µg ± S. E. 24 h⁻¹</th>
<th>Ratioª</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.1 ± 2.3</td>
<td>0.50</td>
</tr>
<tr>
<td>2</td>
<td>13.2 ± 2.4</td>
<td>0.38*</td>
</tr>
<tr>
<td>3</td>
<td>18.6 ± 3.4</td>
<td>0.54</td>
</tr>
<tr>
<td>4</td>
<td>19.6 ± 2.8</td>
<td>0.57</td>
</tr>
<tr>
<td>5</td>
<td>17.8 ± 2.8</td>
<td>0.52</td>
</tr>
<tr>
<td>6</td>
<td>15.9 ± 3.6</td>
<td>0.54</td>
</tr>
</tbody>
</table>

ª Infection at implantation site.

ª In vivo/in vitro.
similar to that seen in rats (vide infra). This is reflected in the relatively large standard errors of the mean.

Mean excretion for this group was 53% of the in vitro values, excepting animal No. 2 where this was only 38%. Infection at the implantation site was found at autopsy which may explain lower values.

2. Long term absorption studies: Constancy of megestrol acetate release was measured in 10 female rats. Each animal had one implant (wall thickness 0.42 mm) providing in vitro 19.8 µg/24 h. The observation period was six months. The following values were excluded from calculations: animal No. 7 lost its implant and was withdrawn from the study on day 29; on day 21 and on day 29 faecal material accumulated in collecting funnels and was added to that day collection; this contributed to higher than average values; on day 68 one animal (collection No. 24) had an abnormally high urinary value of 12.8 µg; on day 81 (collection No. 28) the mean excretion value (5.0 µg/24 h) was significantly lower than any other determination. The main metabolic elimination was via faecal excretion; only about 10% of total radioactivity was present in the urine. This ratio remained stable throughout the period of observation.

The mean initial absorption rate during the first 3 weeks after implantation was 16 µg per 24 h (84% of the in vitro value). This had declined to a mean of 5.5 µg per 24 h (29% of the initial in vitro diffusion) at the end of 180 days (Benagiano & Ermini 1969). Assuming that this reflects a diminished absorption from the implant the general slope of the regression line calculated by the least square method was =0.266 for the whole period. It appeared to fall into two distinct phases. During the first period (from day 1 to 44) the mean value was 14 µg/24 h and the slope appeared to be negligible but calculations were precluded because of the wide variation ($s^2 = 4.002$) of individual measurements (Fig. 1). Absorption was uniform ($s^2 = 0.530$), the slope was =0.254 and the coefficient $r = -0.806$ days for 47 to 112. The mean value for this period was about 10 µg/24 h (Fig. 2). The decline persisted towards the end of the experiment (mean 4 µg/24 h, slope =0.226) but variance between individual measurements has increased ($s^2 = -2.076$; Fig. 3).

3 Megestrol acetate plasma levels: Rabbits were implanted in succession with two implants filled with tritiated megestrol acetate (specific activity 913 µCi/mg). In vitro diffusion for the two implants was 30.2 µg/24 h. Total radioactivity was determined in plasma collected 30 min, 1 h, 2 h, 4 h, and 24 h, on the first day and then on days 2, 5, 6, and 7 of the experiment. Radioactivity was measured in urine and faeces collected every 24 h. The results are shown in Table 3.

The high specific activity of the steroid used permitted detection of radioactive material already 30 min after implantation. The initial value for the first rabbit was 15 ng/100 ml of plasma. This rose to 40 ng/100 ml at the end
Fig. 1.
Excretion of megestrol acetate and metabolites in rats absorbed from PDS implants during the first 44 days.

Fig. 2.
Excretion of megestrol acetate and metabolites in rats absorbed from PDS implants during days 44 to 112.

of 4 h. For the second animal the values with the same implant were 6 ng and 15 ng per 100 ml plasma, respectively. The amounts circulating in plasma were still rising during the first 48 h post-implantation but were relatively constant between days 5 and 7. Similarly, less material was excreted during the first two days; thereafter the values were uniform. Mean excretion (days 3 to 7) was 26 μg/24 h in the first animal and only 15 μg/24 h in the second rabbit.
Fig. 3.
Excretion of megestrol acetate and metabolites in rats absorbed from PDS implants during days 119 to 178.

Table 3.
Absorption of megestrol acetate from DPS implants in two rabbits.

<table>
<thead>
<tr>
<th>Sample collection, days</th>
<th>Plasma levels, µg/100 ml</th>
<th>Total excretion, µg/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.44; 0.37</td>
<td>6.7; 1.4</td>
</tr>
<tr>
<td>2</td>
<td>0.81; 0.77</td>
<td>18.6; 8.9</td>
</tr>
<tr>
<td>3</td>
<td>c</td>
<td>24.7; 15.7</td>
</tr>
<tr>
<td>4</td>
<td>c</td>
<td>28.8; 15.9</td>
</tr>
<tr>
<td>5</td>
<td>1.70; 1.39</td>
<td>27.2; 13.3</td>
</tr>
<tr>
<td>6</td>
<td>1.66; 1.43</td>
<td>24.4; 14.1</td>
</tr>
<tr>
<td>7</td>
<td>1.99; 1.63</td>
<td>23.5; 15.1</td>
</tr>
</tbody>
</table>

a, b Values for animal No. 1 and No. 2, respectively.
c No measurement.

4. Accumulation of radioactivity into various organs: Accumulation of radioactivity into various organs of hamsters and rabbits was expressed in terms of megestrol acetate even though it is recognized that in most instances the parent steroid has been metabolized*. Table 4 shows total accumulation as ng/g of tissue into various organs.

In hamsters highest accumulation was in the carcass (including blood). The average amount per animal was 22 µg which corresponds roughly to the mean daily release. This probably represents material circulating in blood and

* The distribution and identification of metabolites in the various organs will be reported at a later date.
Of interest is the high accumulation of the radioactive material in the vagina; this represents real accumulation, not urine contamination since most of the radioactive material was unconjugated; in urine, 99% of the metabolites were present in a conjugated form. Since we have not analyzed this fraction as yet, no conclusions can be drawn to possible biological significance of this finding. In rabbits, high amounts accumulated in the liver and kidneys but the relative concentration was much lower than in hamsters. This was due to weight differences. Total accumulation per organ was 0.41 µg and 1.9 µg in the liver, 0.14 and 0.8 µg in kidneys for hamsters and for rabbits, respectively. This was about 50 times less than was found in animals which were given the same steroid by gavage (Kincl et al., in prep.). Accumulation of radioactivity at implantation site was not excessive. In rabbits it was only 0.05 µg/g of tissue. This compares to 0.003 µg/g found in a muscle distal to implant site, and 0.02 µg/g found in intestinal fat. In the digestive tract the accumulation was 0.03 µg/g in the duodenum and 0.02 µg/g in the ileum. These amounts represent most likely the enterohepatic recirculation of the metabolites. The high specific activity of [3H]megestrol acetate used made it possible to determine also incorporation of radioactivity into the brain and the pituitary gland. Total accumulation in the pituitary gland was only 0.25 ng. Based on tissue weights, this represents about 0.01 µg/g. We have not seen differential uptake of radioactivity in the various brain centers that were studied. The mean value for the hypothalamus was 0.002 µg/g of tissue, for the cerebellum 0.002 µg/g, and 0.001 µg/g for the forebrain.

**DISCUSSION**

Diffusion of megestrol acetate from PDS implants *in vitro* was constant; the rate for one set of implants was 16.0 ± 0.04 (SE) µg/24 h for days 10–20 and
16.2 ± 0.03 µg/24 h for days 21–40 of incubation (Kincl et al. 1968). Compared to the previous studies, values obtained in the present in vivo experiments were less satisfactory. A six months study in rats revealed that absorption from implants prepared by filling polydimethylsiloxane tubing with dry crystalline steroid was not constant. The rate decreased to less than one-half of the mean initial value at the end of 180 days and large variations were noted between individual collections in the same animal during the first month after implantation.

Several factors may be responsible for the initial irregular release. After insertion the injured tissue must heal, a proper vascularization be established, the diffusing compound removed into circulation and come to an equilibrium with various tissue compartments. Possibly, the site of implantation may play a role. That this may be so was indicated by different absorption values found with the same implant in different animals. Additional errors could be due to difficulty in radioactivity measurement with materials of low specific activity.

The decline of diffusion rates is more difficult to explain. The geometry of the implant remains constant which should insure a sustained release for long periods of time. Several possibilities could explain altered permeability: body lipids could have diffused »into« the membrane; the presence of silica filler could be a contributory factor the membrane acting as a miniature chromatographic column; the method of using dry, crystalline material to make the implants could be the main factor.

The rate of diffusion of steroids through polydimethylsiloxane membranes depends besides the influence of membrane area and thickness mainly on the concentration gradient across the membrane. To use steroids without a suspending medium may create irregular concentration gradients across the membrane due to non-uniform distribution of the solid particles on the inner surface. We have noted that results obtained with implants containing carboxymethyl cellulose suspension were more uniform but additional experiments would be needed to establish this with certainty.

For biological experiments lasting few weeks implants filled with crystalline material are probably satisfactory. Based on values obtained in in vitro studies it can be expected that for most steroids average in vivo absorption from DPS implants during the first few weeks after implantation would be about 60% in hamsters, 80% in rabbits and 85% in rats, assuming that losses by excretion through organs other than the kidneys and the digestive tract are negligible. Fairly constant plasma levels can be expected about 3 days following implantation.

Despite the seemingly disappointing results, PDS implants appear to offer more promise as a drug delivery system than the conventional, once-a-day administration since slowly diffusing steroids are utilized more efficiently resulting in increased biological effectiveness (Chang & Kincl 1968 and in prep.).
ACKNOWLEDGMENTS

We wish to thank Mr. Enzo Polsivelli (University of Rome), Miss I. Angee and Miss Y. Froix (The Population Council) for able technical assistance.

The expense of this research has been partly covered by Population Council grant No. M. 68. 105 to G. Benagiano.

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Received on July 3rd, 1969.