Pharmacokinetics of norethindrone acetate in women after the insertion of a single subdermal implant releasing norethindrone acetate

Harbans Singh, J P Uniyal, P Jha, D Takker, K Murguesan, V Hingorani and K R Laumas

Departments of Reproductive Biology and Obstetrics and Gynaecology, All India Institute of Medical Sciences, New Delhi-110029

Abstract. The metabolic clearance rate (MCR) of norethindrone acetate\(^1\) (NETA) and norethindrone (NET) levels in plasma were studied after an iv injection of [\(^3\)H]NETA in three women before and at 1 week, 1, 2 and 6 months following the insertion of a single silastic subdermal implant releasing microquantities of NETA. No significant change in the MCR of NETA was observed at 1 week (459 ± 72 l/day), 1 month (489 ± 113 l/day) and 2 months (522 ± 144 l/day) compared with that of control (525 ± 108 l/day). However, MCR of NETA showed significant increase in women exposed to continuous presence of NETA for a period of 6 months (608 ± 121 l/day; \(P < 0.025\)). NETA was rapidly and extensively metabolized into NET. At 1 week, 1, 2 and 6 months of study, NET was observed to be present in higher amounts compared with NETA. The production rate (PR) of progesterone decreased significantly at 2 and 6 months of NETA implant insertion compared with the PR before the insertion of implant.

Norethindrone acetate has been widely used as a progestogenic component of oral pills. The successful use of this steroid through the subdermal route was first reported by Laumas and associates (Bhatnagar et al. 1975) who suggested the feasibility of the use of a single silastic capsule releasing NETA for contraception in women (Takker et al. 1978). In a previous communication (Singh et al. 1979) the pharmacokinetics of norethindrone acetate have been reported in women after a single iv administration. The fate of a progestational steroid in the body when continuously released through a subdermal implant in microquantities, is not known. This communication deals with the plasma disappearance and MCR of NETA and progesterone, and PR of progesterone in women inserted with a norethindrone acetate releasing single subdermal silastic implant.

Materials and Methods

Materials

[15,16-\(^3\)H]NETA (S.A. 57.14 Ci/m mole) Schering A G, Berlin; 6,7-\(^3\)H]progesterone (S.A. 55.7 Ci/m mole – New England Nuclear Corp. Boston); [1,2,6,7-\(^3\)H]progesterone (S.A. 110 Ci/m mole – used for radioimmunoassay was supplied by WHO, Geneva) were used. All solvents were of analytical grade and were re-distilled before use. Silica gel HF 254 was purchased from E. Merck Darmstadt. The radioactive steroid was purified on Sephadex LH-20 column (20 × 1 cm) using toluene: methanol (95:5) system for [\(^3\)H]NETA and toluene: methanol (85:15) for [\(^3\)H]progesterone.

Human subjects

Seven female volunteers with normal menstrual cycles in the reproductive age group (20–35 years with no pre-

---

\(^1\) The following trivial names of the steroids have been used: Norethindrone acetate: 17a-ethynyl-17\(\beta\)-hydroxy-4-oestren-3-one-17\(\beta\)-acetate. Norindrone: 17a-ethynyl-17\(\beta\)-hydroxy-4-oestren-3-one.
Table 1. Clinical history of the subjects.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>Parity</th>
<th>Cycle length (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.S.</td>
<td>25</td>
<td>51</td>
<td>3 + 0</td>
<td>30 ± 4</td>
</tr>
<tr>
<td>S.U.</td>
<td>27</td>
<td>59</td>
<td>4 + 0</td>
<td>25 ± 4</td>
</tr>
<tr>
<td>P.R.</td>
<td>35</td>
<td>72</td>
<td>9 + 0</td>
<td>32 ± 4</td>
</tr>
<tr>
<td>K.M.</td>
<td>30</td>
<td>55</td>
<td>3 + 0</td>
<td>28 ± 3</td>
</tr>
<tr>
<td>K.R.</td>
<td>32</td>
<td>43</td>
<td>3 + 0</td>
<td>28 ± 3</td>
</tr>
<tr>
<td>L.T.</td>
<td>30</td>
<td>60</td>
<td>3 + 0</td>
<td>30 ± 3</td>
</tr>
<tr>
<td>C.H.</td>
<td>30</td>
<td>56</td>
<td>4 + 0</td>
<td>28 ± 3</td>
</tr>
</tbody>
</table>

* Subjects for the study of MCR of norethindrone acetate.
** Subjects for the study of MCR and PR of progesterone.

Vious hormonal contraceptive history) who had completed their families and had undergone tubal ligation were selected for the study. Written consent was obtained from the volunteers before the study. The clinical history of the subjects is given in Table 1.

Design of the study

Fabrication of a silastic implant containing NETA and its insertion into the women was carried out as described (Takker et al. 1978). Plasma disappearance and MCR of NETA were studied in three women before and at 1 week, 1, 2 and 6 months on day 6–8 of the menstrual cycle after the insertion of a silastic implant releasing NETA. The plasma disappearance, MCR and PR of progesterone were studied in four women before and 2 and 6 months after insertion of the NETA implant between day 19–22 of the menstrual cycle.

Administration of [15,16-3H]NETA and [6,7-3H]progesterone

An iv injection of 20 μCi of [3H]NETA or [3H]progesterone in 10% ethanolic saline was given to each subject in the antecubital vein in the morning. Blood samples were collected in tubes containing 4% sodium citrate as an anticoagulant at 5, 10, 15, 30, 60, 120 min and 4, 6, 24, 48, and 72 h after the injection of [3H]NETA and 2, 5, 10, 15, 30, 45, 60, 90 min and 2, 4 and 6 h after the injection of [3H]progesterone.

Blood was centrifuged immediately to separate plasma. The extraction of radioactive steroids, thin layer chromatography for the separation of NETA, NET, the analysis of their disappearance from the plasma and radioactivity counting were carried out as previously reported (Singh et al. 1979). The MCR was calculated as described earlier (Laumas et al. 1971; Uniyal et al. 1976).

Estimation of serum progesterone

The serum progesterone levels were measured by a specific radioimmunoassay as described by Rahman et al. (1977). The method involved the use of anti-serum raised against progesterone-3-oxime-BSA in New Zealand white rabbits (supplied by WHO). The sensitivity of the assay was 2–5 pg. Within-assay and between-assay coefficients of variations were found to be 3.6 per cent and 17.2 per cent.

Production rate (PR) of progesterone

The PR of progesterone was determined after multiplying the levels of endogenous progesterone by MCR of progesterone (Tait & Burstein 1964).

Results

Disappearance of [3H]norethindrone acetate in plasma

[3H]NETA recovered from the free steroid fraction was expressed as per cent injected dose per litre plasma and was plotted against time on a semilogarithmic scale. The curve showed a biphasic pattern of disappearance of [3H]NETA in plasma with an initial rapid disappearance followed by a relatively slow disappearance. The disappearance curve was analysed on the basis of a two compartment model for the calculation of MCR and other pharmacokinetic parameters.

Plasma half-lives of NETA

The initial a-t1/2 before the insertion of implant (9.0 ± 0.2 min) was similar to the half-life at 1 week (7.0 ± 0.6 min), 1 month (5.5 ± 0.7 min), 2 months (7.0 ± 0.0 min) and 6 months (9.7 ± 2.1 min) after implant insertion. The β-t1/2 was found to be 51.9 ± 3.6 h before insertion of implant and 53.2 ± 4.8 h at 1 week, 53.3 ± 5.2 h at 1 month, 47.0 ± 2.9 h at 2 months and 49.8 ± 7.5 h at 6 months after the implant insertion. Thus there was no significant difference in the half-lives of NETA before and after the insertion of the implant.

Metabolic clearance rate of NETA

The MCR of NETA in women before the implant insertion was observed to be 525 ± 108 l/day. No statistically significant difference was found in the MCR of NETA at 1 week (459 ± 72 l/day), 1 month (489 ± 113 l/day) and 2 months (522 ± 144 l/day) after implant insertion from that of the MCR in women before the insertion of the implant. However, the MCR of NETA in women at 6
Disappearance of NETA and NET in the plasma following a single iv injection of [3H]NETA in three women one week after insertion of implant-D. Each point represents the mean ± SEM.

months (608 ± 121 l/day) after insertion was found to be significantly higher \( (P < 0.025) \) as compared to the MCR before insertion of the implant.

**Plasma metabolites of norethindrone acetate**

Figs. 1–4 represent the levels of [3H]NETA and [3H]NET in plasma at various time intervals after injection of [3H]NETA expressed as per cent injected dose/l plasma in three subjects after 1 week, 1, 2 and 6 months of NETA implant insertion. As seen from Fig 1 at 1 week after implant insertion there was a rapid and extensive metabolism of NETA to NET. As early as 2 h 0.28 per cent injected dose/l plasma was found to be as NET and it was found to be 0.18, 0.038 and 0.007 per cent injected dose/l plasma at 6, 24, 48 and 72 h, respectively. Whereas unconverted NETA was found to be 0.068, 0.062, 0.044, 0.037 and 0.013 per cent injected dose/l plasma at 2, 6, 24, 48 and 72 h, respectively. At 1, 2 and 6 months after insertion of implant, NET levels were always found to be higher than those of NETA at all the time intervals.
Fig. 3.
As in Fig. 1 but at 2 months after the insertion of implant.

**Ratio of NET:NETA**

Following injection of [3H]NETA after 1 week of implant insertion, a maximum conversion of NETA to NET was attained within 60 min (NET/NETA = 5.39 ± 2.44). The high level of NET was maintained till 48 h, the ratio of NET/NETA being 1.16 ± 0.03. At 72 h the level of NET was however, lower than that of NETA (NET/NETA = 0.67 ± 0.18; Fig. 5). Thus the pattern of conversion of NETA to NET was more or less similar to that found in women before implant insertion. A maximum conversion of NETA to NET was observed at

Fig. 4.
As in Fig. 1 but at 6 months after the insertion of implant.
15 min (NET/NETA = 9.69 ± 0.87), 30 min (NET/NETA = 5.72 ± 2.2) and 60 min (NET/NETA = 4.52 ± 2.09) after the iv injection of [3H]NETA to women at 1, 2 and 6 months following the implant insertion. As seen from Fig. 5, the ratio of NET/NETA increased progressively from 1 month to 6 months after the insertion of implant especially after longer intervals (48 h and 72 h).

Disappearance of progesterone in plasma

The α-t½ of progesterone was found to be 3.8 min and β-t½ being 46.0 min in women before the insertion of implant. The half-lives did not show any significant changes at 2 and 6 months after implant insertion compared with the control. The MCR of progesterone in women before implant insertion was observed to be 1810 l/day and 2168 l/day and 1655 l/day at 2 and 6 months after implant insertion, respectively. Thus, there was no significant alteration in the MCR of progesterone in women after implant insertion.

Production rate (PR) of progesterone

The PR of progesterone was calculated in women before and following insertion of the implant. Blood samples from each woman were taken before the injection of [3H]progesterone. The values of serum progesterone and PR of progesterone are given in Table 2. The PR of progesterone in women before implant insertion was found to be 14.8 mg/day while it decreased significantly at 2 months (8.8 mg/day) and at 6 months (5.5 mg/day) progressively after implant insertion.

Table 2.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Control</th>
<th>2nd cycle</th>
<th>6th cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum level (ng/ml)</td>
<td>PR (mg/day)</td>
<td>Serum level (ng/ml)</td>
</tr>
<tr>
<td>KM</td>
<td>7.9</td>
<td>20.1</td>
<td>3.1</td>
</tr>
<tr>
<td>KR</td>
<td>8.4</td>
<td>11.2</td>
<td>4.5</td>
</tr>
<tr>
<td>LT</td>
<td>8.4</td>
<td>11.1</td>
<td>3.8</td>
</tr>
<tr>
<td>CM</td>
<td>8.2</td>
<td>16.8</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Mean ± SEM 8.2 ± 0.1 14.8 ± 2.2 4.1 ± 0.2 8.8 ± 0.4 3.3 ± 0.3 5.5 ± 0.7*  

* P< 0.05.
Discussion

An interesting feature of the study was a rapid metabolism of NETA into NET in women exposed to small quantities of NETA released continuously through a subdermal silastic implant. It was observed that in women exposed to NETA for a period of 6 months, the MCR of NETA (600 l/day) increased significantly as compared to the MCR in the control women (525 l/day). Furthermore, NETA was rapidly and extensively metabolized into NET, which was always found to be in higher amounts compared with NETA. The long-term exposure of women to NETA did not affect the half-lives of NETA significantly. The MCR of NETA was found to be lower as compared to the MCR of progesterone (Little et al. 1966), but was comparable with the MCR of other potent progestogens like norethindrone (Raynaud 1970; Mills et al. 1974), and norgestrel (Uniyal et al. 1976). This suggested a prolonged retention of the steroid in the body required for its contraceptive action.

A significant increase in the MCR of NETA ($P < 0.025$) was found in women exposed to NETA for 6 months as compared with the control. However, no significant change was observed in the MCR of NETA in women at 1 week, 1 and 2 months after implant insertion. The increase in MCR of NETA in women under a continuous influence of NETA (6 months) in the body could result from an increase in the degradative metabolism of the steroid possibly via the induction of enzymes in the liver or other tissues as has been suggested in case of NET (Mills et al. 1974). The levels of NET were observed to be higher than NETA in the present study. After attaining a maximal level which was generally reached between 10–15 min NET showed a biphasic pattern of disappearance similar to that of NETA. In women exposed to NETA over prolonged periods of time, there was an increased conversion of NETA to NET as demonstrated by a progressive increase in the ratio of NET to NETA with time. This suggested the possibility of an induction of enzyme(s) responsible for the metabolism of NETA.

The continuous exposure of women to microdoses of NETA released through subdermal implant did not affect the plasma half-lives of progesterone significantly. The half-life of plasma progesterone in women was in the same range as reported by others (Little et al. 1966). Similarly, the MCR of progesterone did not change significantly after long-term exposure of women to NETA.

The PR of progesterone calculated from the values of serum progesterone and MCR, was found to decrease in the 2nd (8.8 mg/day) and 6th month (5.5 mg/day) after implant insertion as compared with that before implant insertion (14.8 mg/day). Since the MCR of progesterone was not observed to show any significant change after implant insertion, the lower serum levels of progesterone found in women after the insertion of the implant may be due to a decreased production of progesterone as observed from the values of PR of progesterone. It has further been suggested that the suppressive effect on the production of ovarian steroids may be directly related to the effect on gonadotrophin production (Rahman et al. 1977). It has been demonstrated that administration of small doses of norgestrel reduces the ability of the corpus luteum to synthesize progesterone from pregnenolone in vitro (Mukherjee et al. 1972). It may thus be possible that NETA released in small quantities form the implant has no significant effect on the clearance of progesterone but may bring about a change in the PR of progesterone either by a direct effect on the ovaries or mediated via the pituitary-ovarian axis and may bring some deterioration in the corpus luteum function with altered ovarian steroidogenesis resulting in the contraceptive action of NETA.

These studies thus show that long-term exposure of women to NETA released through subdermal silastic implant may increase its clearance with a rapid metabolism into active metabolite NET which in concert with NETA may contribute to its action.

Acknowledgments

The work was supported by grants from World Health Organization, Geneva. The authors are grateful to Schering A. G., Berlin for the generous gift of labelled norethindrone acetate and to Miss P. N. Anandlaxmi for statistical help.

References


Received on December 2nd, 1980.