

BIOLOGICAL EFFICACY AND PLASMA NORETHISTERONE LEVELS OF ORALLY ADMINISTERED NORETHISTERONE ENANTHATE IN RAT AND HAMSTER

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Abstract : Norethisterone enanthate (NET-En) is a well known intramuscular contraceptive drug. The long acting nature of this preparation when administered orally was evaluated in female rats and hamsters using fertility inhibition test and from the plasma levels of norethisterone (NET). An oral dose of 20-60 mg NET-En was administered to random groups of six female rats and hamsters and were mated after five and ten days with males of proven fertility. The fertility inhibition rate was determined from vaginal delivery. A dose-dependent reduction in fertility was seen in rats 5 days after oral administration of NET-En. This effect was found to be less pronounced and not significant 10 days after administration of similar doses of NET-En. In hamsters, a similar but less pronounced effect was noted. The decrease in fertility was significant only at the 60 mg dose. The plasma levels of NET estimated by RIA over a period of 15 days, in a different set of treated rats, suggested rapid absorption of NET-En within a day, and drug concentration decreased slowly, the levels on the 4th day ranged from 0.9-2.3 with the 10 mg and 1.0-4.0 ng/ml with the 20 mg dose. Detection of adequate levels of NET in plasma during the estrous cycle in rats, and the fertility inhibition observed in female rats and at higher doses in hamsters, suggest that NET-En is orally active.

Key words : biological efficacy
oral administration

norethisterone enanthate
plasma levels

INTRODUCTION

Fatty acid esters of steroids are known to have longer half life when compared to the parent compound (1). These esters act as prodrugs and on biotransformation are cleaved to the active compound.

Norethisterone acetate (NET-Ac) and NET-En are the fatty acid esters of NET and are converted to the more active norethisterone. NET-Ac is used as progestogen in many combination oral pills while NET-En is used as an intramuscular injectable preparation. A 200 mg dose of NET-En was earlier tested

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for its effectiveness in a multicentric phase III-clinical trial (2). Preparations containing 20–200 mg have been investigated in India and the results showed a pregnancy rate of 0.18 per 100 women years and a continuation rate of 63.7% at the end of one year (3, 4). Though the preparation was found to be effective, the reasons for discontinuation were irregular bleeding and amenorrhea. The mode of administration i.e. intramuscular injection poses yet another major operational problem in the National context. Considering the long-acting nature of intramuscularly administered NET-En, the antifertility effects of single oral dose of NET-En was studied in two species with short estrous cycle and gestation like rats and hamsters. In addition, the plasma levels of the active component NET, were monitored in rats to confirm the availability of the drug for prolonged period:

METHODS

Injectable NET-En (200 mg/ml) and [³H]-15, 16 NET were gifts from Schering A.G., West Germany. Norethisterone was from Sigma Chemicals Co. (St. Louis, USA), activated charcoal from Sarabhai Chemicals, (Baroda, India) and Dextran T-70 from Pharmacia Fine Chemicals (Uppsala, Sweden).

Female adult virgin hamsters (Golden, Syrian) and albino rats (Wistar/NIN) both weighing around 160-180 g were obtained from the National Centre for Laboratory Animal Sciences (NCLAS, NIN, Hyderabad). Animals with proven cyclicity as assessed by vaginal smear cytology for a period of 2 cycles and males of proven fertility were

used in the study. Animals were housed individually and maintained on a 12-hr light and dark cycles. Free access to water and pelleted stock diet were ensured.

Assessment of fertility in rat and hamsters :

As our primary objective was to assess the biological efficacy of the drug on overall fertility of the females, vaginal delivery was monitored. Initially, the methodology was standardised in control animals. The procedure consisted of keeping the animals for mating for a period of one estrous cycle (i.e. 4-5 days), monitoring the weekly body weights, palpation for pregnancy on the 10th day after the separation of the males and allowing for vaginal delivery. Care was taken to ensure recording all deliveries by frequent monitoring. There was a good agreement amongst the three indicators of pregnancy and confirmed the validity of the methodology and adequacy of sample size of 6. The procedure adopted here resulted in greater than 80% deliveries and is being routinely followed in the breeding colonies of NCLAS (NIN, Hyderabad).

In the initial experiments with rats, litter size was monitored both in control and NET-En treated rats. The mean litter size was 9.5 ± 3.7 (n=6) in controls and 6.5 ± 3.7 , 6.7 ± 4.7 and 8.5 ± 1.5 (n=>3) in rats receiving 30, 40, and 50 mg NET-En orally and continued their pregnancy till delivery. The differences among the groups were not significant. The pooled mean values of experimental group 7.1 ± 3.28 (n=12) was also not statistically different from the controls. Therefore, litter size was not considered in the later experiments.

Experimental protocol:

The animals were allowed to acclimatize for 4 to 5 days before administering different oral doses of NET-En at 9.00 a.m. using a polyvinyl tube (1 mm X 10 cm length) attached to a tuberculin syringe. A single oral dose of 20, 30, 40, 50, and 60 mg NET-En diluted in peanut oil in a total volume of 0.5 ml was administered to rats and hamsters. Animals receiving only groundnut oil vehicle orally, served as controls. Animals administered intramuscularly with a 20 mg dose in 0.1 ml peanut oil served as positive controls. For each dose a minimum of 6 animals were used for fertility studies. On the 5th day after the administration of NET-En or vehicle alone, males were introduced and left with their female partners for a period for one estrous cycle to ensure successful mating. The biological efficacy of NET-En was evaluated by computing the fertility rates based on the deliveries that took place. Similarly the biological efficacy of a single dose of 50 and 60 mg NET-En was tested 10 days after the oral administration in rats. The males were introduced on the 10th day and left for a period of 5 days.

Plasma levels of NET:

For the monitoring of plasma NET levels, a set of 3 rats each were given 10 and 20 mg of NET-En orally. Blood samples were collected on days 1, 4, 8, 11, and 15 after administration of the dose. Plasma was separated and subjected to NET analysis using a RIA procedure as described earlier using the same antiserum (5). The minimum detectable level was 10 pg and inter assay and intra assay variations were less than 10%.

RESULTS

The results on fertility with different doses of NET-En in hamsters and rats are presented in Figs. 1 and 2. The mean fertility seen in control rats and hamsters receiving vehicle alone was around 75%. An intramuscular injection of 20 mg NET-En to rats and hamsters resulted in zero conceptions in these two species (0/6 and 0/6 respectively). A similar dose administered by oral route brought about 33 and 43% fertility respectively in hamster and rat. A significant reduction in fertility was seen with doses 30 to 60 mg in rats. A 60 mg dose administered orally showed a

TABLE I : Plasma levels of NET-En (ng/ml) after 10 or 20 mg oral dose of NET-En in rats.

Dose	Rat No.	1st day	4th day	8th day	11th day	15th day
10 mg	15	12.5	2.3	0.50	0.24	0.20
	23	3.3	0.9	0.61	0.32	0.59
	24	8.4	2.1	0.14	0.12	0.21
Mean±SD		8.07 ± 4.61	1.76 ± 0.76	0.42 ± 0.24	0.23 ± 0.1	0.33 ± 0.22
20 mg	4	15.8	4.0	1.5	0.29	0.28
	6	13.9	1.0	1.1	1.08	0.20
	13	8.8	1.4	0.7	0.77	0.29
Mean ± SD		12.8 ± 3.6	2.1 ± 1.6	1.0 ± 0.4	0.7 ± 0.4	0.2 ± 0.05

fertility of only 12%. A small reduction in fertility was seen in rats mated after 10 days after 50 and 60 mg oral dose which was not significant. A significant reduction in fertility was seen in hamsters receiving 50 mg oral dose. However, at lower dosage levels (20 and 30 mg NET-En), the fertility reduction seen was not significant. Though the fertility assay was not sensitive to increments of 10 mg dose, there was a dose dependent drop in fertility with increasing dose in rats. In repeated experiments a minimum of 20 mg dose difference was necessary to find a change in the fertility rate (data not shown).

The plasma levels of NET reached a peak within a day after oral administration of 10 and 20 mg NET-En (Table I). The peak NET levels ranged between 3.3-12.5 ng/ml and 8.8-15.8 ng/ml respectively with these doses of NET-En. Thereafter, the mean values declined rapidly and reached close to 0.3 ng/ml between 8 and 15 days with 10 mg

dose. A similar decline in plasma NET levels was also seen with 20 mg dose, but the plasma levels of NET were higher until day 11.

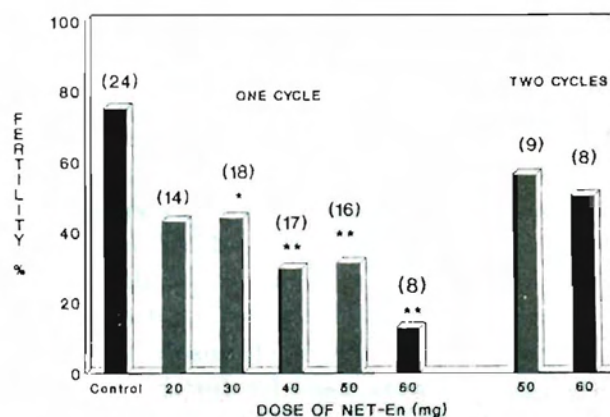


Fig. 2: Percentage fertility in female rats given oral NET-En after one or two cycles after the dose. *P<0.05 as compared to control by proportionality test. **P<0.01 as compared to control by proportionality test. Parenthesis indicates 'n'.

DISCUSSION

The WHO Special Programme on Research on Human Reproduction prepared and tested a number of esters of levonorgestrel and norethisterone (6) to identify promising intramuscular contraceptive drugs.

NET-En is a well known depot injectable contraceptive extensively used in developing countries like Indonesia. However, this method has not found favour in India due to many operational problems, especially its route of administration and side effects like breakthrough bleeding. Therefore, the feasibility of NET-En as a long acting oral preparation was tested in different species of animals.

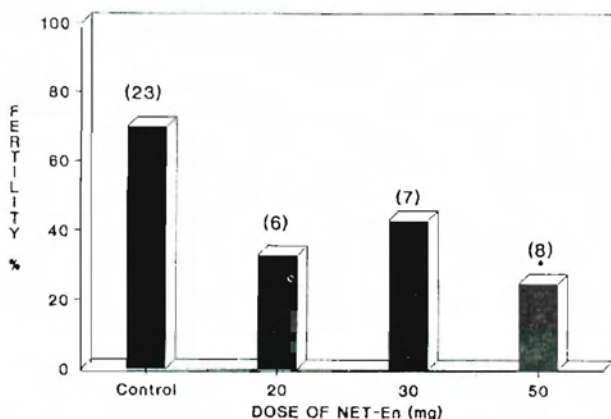


Fig. 1: Percentage fertility in female hamsters given NET-En. *P<0.05 as compared to control by proportionality test. Parenthesis indicates 'n'.

Oral administration of NET-En has decreased fertility in both the species. The inhibition of fertility was also found to be directly related to the amount of the dose administered. Fertility inhibition was greater with a dose of 60 mg as compared with 20 mg dose of NET-En in rats. In view of this, the dose was not increased further to demonstrate the maximum effect. There was a strong contraceptive effect at 50 mg and not at a lower oral dose in female hamsters. As expected, there was a time dependent reduction in the inhibition of fertility 10 days after oral dose of NET-En compared to that after 5 days in rats. With a 16 mg IM dose of NET-En, Bialy et al (6) reported estrous suppression for a period of 13 days in rats, whereas in the present study, a single oral dose of 50 or 60 mg of NET-En showed only a marginal reduction in fertility of about 40% after 10 days in rats. These differences between oral and IM dose may be due to the lower intestinal absorption of NET-En or due to rapid hydrolysis of NET-En to NET by the esterases present in the intestinal tract, causing lowered bioavailability of NET-En (7, 8).

The plasma NET profile revealed that the NET-En is absorbed rapidly, biotransformed to NET and is finally eliminated from circulation. Detectable levels of NET were seen in plasma even after 15 days with 20 and 10 mg NET-En given orally in rats. This suggests that NET-

En is stored in a deep compartment like adipose tissue and biotransformed to NET subsequently. A decrease in biological efficacy was noted after 10 days and this is in tune with the decrease in plasma NET concentration.

In a separate study we compared pharmacokinetics of orally administered NET-En with that after intramuscular dose in three species viz., rabbit, monkey and women. Effective levels of NET were shown to be present 15-20 days after an oral dose in monkeys and women (9). Even the plasma levels of progesterone were suppressed during the luteal phase in women supporting the fertility control by oral NET-En. The results of the present study confirm the presence of adequate plasma level of NET along with direct evidence for suppression of fertility in rats and hamsters. This is in consonance with the findings earlier obtained in other species including women.

Thus, it can be concluded from this study that the orally administered NET-En is biologically active and effective in inhibiting fertility in the two species tested here.

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