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PHARMACOKINETIC STUDY OF NORETHINDRONE COPRECIPITATE

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ABSTRACT

Pharmacokinetic parameters of norethindrone (NE) were studied in 12 adult female guinea pigs following either oral or rectal administration of a single dose of 2.5 mg. The animals were divided into three groups (4 animals each) and each group was given the single dose either in capsule,tablet or suppository form. NE in blood plasma was determined by HPLC at various time intervals (0.5, 1, 2, 4, 6, 8, 12 hour). The results indicate that NE was readily absorbed by both routes but the maximum plasma concentration (Cmax) and the area under the plasma concentration time curve AUC of NE were significantly high by the rectal route. No significant differences in the plasma half-life (t1/2), absorption (Ka) and elimination (Ke) rate constants and the time to maximum plasma concentration (tmax) were observed between the oral and rectal administrations. These findings suggest that the rectal route offers the possibility of reducing the effective dose of NE, which is widely used for contraception and hormone replacement therapy.

INTRODUCTION

Progestogens are amongst the most widely used steroidal contraceptives and used for the treatment of premenstrual syndrome and hormone replacement therapy[1]. They are conventionally administered orally or parenterally. Most of the steroids administered orally are metabolized in the gut wall and the liver, as a consequence of which their bioavailability is markedly reduced[2]. The synthetic progestogen norethindrone (NE) also undergoes considerable first-pass metabolism in the human and its bioavailability has been reported to be between 47% to 73%[3].

Administration of drugs as rectal suppository is one of the methods which considerably enhances the bioavailability and prevents drug loss by first-pass metabolism[4]. It may offer a possibility of reducing the effective dose for administering progestogens. Therefore, the pharmacokinetics of NE was studied following its oral or rectal administration using HPLC to analyze NE in blood plasma.

MATERIALS AND METHODS

Drug formulation:

Coprecipitate system of 2.5 mg NE with polyvinylpyrrolidone (PVP) 44,000 was prepared according to Sumnu, 1986[5] (using different ratios of NE to PVP 44,000, 1:1, 1:3, 1:4, 1:8 and 1:12). The dried mass was powdered and the sieve fraction of 90-100 μM was collected. In vitro release test was conducted[6,7] on the different ratios of the coprecipitate systems. The results indicated that the release rate of NE was enhanced as the ratio of NE to PVP 44,000 was increased up to 1:8 (Fig. 1). Therefore, this ratio was formulated in each of polyethyleneglycol (PEG) suppositories and hard gelatin capsules and compared with marketed tablets (Micronor tablets, ORTHO Pharmaceutical Co., Raritan, NJ 08869. opc 1990, Dialpak tablet dispenser 28's, NDC 0062-1411-07, Exp. Dec. 1995, made in USA).

Animals: Twelve adult female guinea pigs, each weighing 700-900 g, were used. They were divided into three groups, 4 animals each. Each animal in group one was orally given a 2.5 mg tablet of NE, purchased from the market, while in group 2 each animal was orally given a formulated 2.5 mg gelatin capsule of NE and in group 3 a formulated 2.5 mg rectal suppository of NE was inserted to each animal.

Sample collection: Blood samples were collected, from each animal in each group, on EDTA (8 mg/ml) from the orbital sinus at intervals of 0, 1/2, 1, 2, 4, 6, 8,and 12 hr after drug administration. Plasma was separated, extracted with hexane-ethyl acetate (2:1) for 10 minute, the extract was evaporated to dryness and the residue was kept frozen at -20°C until analyzed[8,9].

HPLC determination of NE: HPLC grade methanol and double distilled water were used in the mobile phase. The mobile phase was filtered through a 0.45 μm cellulose triacetate membrane (GA-6 Metricel; Gelman Science, Ann Arbor, MI, U.S.A.) and then degassed with ultrasonic bath and vacuumed prior to use. All chemicals used were of analytical grade.

Sample preparation: The dried residue was dissolved in 0.2 ml of 70% methanol and filtered through a 0.45 μm cellulose triacetate membrane. A 100 μl aliquot of the filtrate was injected into the HPLC system. If necessary, the sample was diluted further with 70% methanol.

Apparatus and operating conditions: The analysis was performed using a SP8810 liquid chromatograph (Spectra-Physics, San Jose, CA, U.S.A), with a 240 nm ultraviolet detector and a Rheodyne injection valve (Cotati, CA, U.S.A.) with a 100 μl loop. Samples were separated on a Zorbax ODS column (4.6 mm x 250 mm) with particles diameter 5 μm (Du Pont, Wilmington, DE, U.S.A) using isocratic elution with 70% methanol as a mobile phase at flow rate of 1.0 ml/min. A SP4100 integrator was used to identify and quantitate the peaks.

Standard curve: Calculations were carried out using standard curve constructed by analyzing aliquots containing 1 - 30 ng of NE in 70% methanol. The injection was repeated 4 times for each concentration and the mean of the peak areas was plotted versus the corresponding concentration. The standard curve data were subjected to least-squares linear regression analysis, and the resulting ($y=981.6 + 121467.7X$; $r= 0.9994$) equation was used for the calculation of the drug concentration in the unknown samples. The described procedure for the extraction of NE from the plasma is simple and efficient. There was no back-extraction involved. The percent recovery of NE from plasma samples spiked with known amount of standard NE was 97.87 ± 5.12 ($n = 12$) with excellent precision.

Pharmacokinetics of NE: A two-compartment model was used to analyze the data. Plasma half-life for disposition curve (t 1/2) was calculated by log-linear regression analysis. The area under the plasma concentration time curve (AUC) was calculated by the trapezoidal rule. The absorption (Ka) and elimination (Ke) rate constants, maximum plasma concentration (Cmax) and time to maximum plasma concentration (Tmax) were calculated using a computer programme. The difference between the tablet from the market, and either the formulated capsule or rectal suppository was subjected to student's "t" test.

RESULTS

To achieve high pressure chromatographic analysis the selection of the appropriate stationary and mobile phases, column temperature, detector were investigated. Based on reported studies of norethindrone chromatographic separation[8,9], isocratic elution with 70% methanol as a mobile phase and UV detection at 240 nm were chosen. The standard graph showed excellent linearity between the peak area and NE concentrations ($r = 0.9994$, $p < 0.0001$) throughout the range of 2 - 20 ng. The limit for detection was 1.2 ng. Fig. 2. shows a typical chromatogram of NE. The specificity of this method is clearly demonstrated by the absence of interfering peaks.

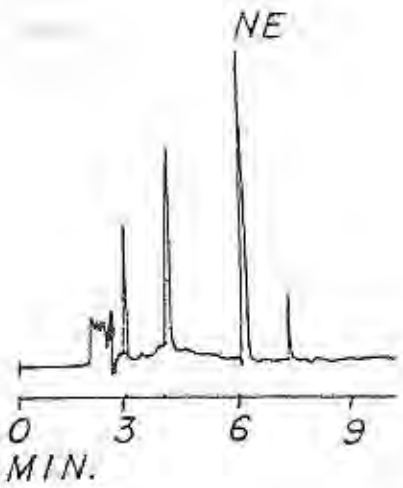

Fig.2. HPLC determination of norethindrone (NE) in plasma. Conditions : column, Zorbax ODS (250 mm x 4.6 mm, 5 um); mobile phase, 70% methanol,1, ml/min; detector, UV, 240 nm.

A comparison of plasma levels of NE after oral or rectal administration of either the tablet, capsule or rectal suppository is shown in Fig. 2. and table 1. The levels of NE, at various time intervals, were highest following rectal administration as compared with oral one. However, at end of 12 hr there were no significant differences among the three formulations used in the present study.

Moreover, no significant differences in the plasma levels of NE were found after administration of either the formulated tablet or the formulated capsule.

Pharmacokinetics parameters:

Table 2, shows that AUC of NE during the first 12 hr of drug administration was the least by the tablet form and highest by rectal suppository. Moreover, AUC between zero to infinity was significantly and insignificantly higher in the suppository and the capsule groups, respectively, when compared to the tablet group. No significant differences were found in the AUC between 12 to infinity, t1/2, Ka, Ke, or Tmax. Cmax of NE by rectal route was significantly more than by the oral route of administration.

DISCUSSION

Norethindrone is widely used in contraceptive formulations and also used in the hormone replacement therapy and yet few pharmacokinetics studies of NE have been carried out[10]. The present study reveals that while NE was absorbed into the circulation by all three formulations, the AUC (bioavailability) and the Cmax (maximum plasma concentration) of the rectal suppository was higher than the oral formulations. The disappearance of NE from the circulation (t1/2) generally followed a biphasic pattern by both routes of administration. The differences in half-lives between both routes were not statistically significant indicating that the clearance (rate of metabolism) of NE is independent of the route of administration.

NE is known to be absorbed almost completely when administered orally. After its absorption through the gastric mucosa, NE would enter the hepatic portal circulation which results in the loss of about 36% of the dose and reducing bioavailability[11,12]. The pharmacokinetics of NE like those of other contraceptive steroids, show a very wide variation between different subjects. In 1987, Yong-en et al.,[10] found that the t1/2 and AUC of 1 mg of NE, given orally to women, were 7.6 hr and 53.6 ng.h/ml, respectively, but there was a 3 to 5 fold variation in the range of these parameters. Although variations between subjects in the first-pass effect will affect the amount of the dose reaching the systemic circulation in a biologically active form and hence account for some of the variability in some of the pharmacokinetic parameters such as bioavailability, it will not be responsible for the wide variability in other parameters such as the elimination half-life[14]. Different factors are known to affect the metabolism of drugs but the major determinant appears to be genetic factors[11].

In our study, the rectal absorption of NE exceeded the oral values which may reflect partial avoidance of hepatic first-pass metabolism of NE after rectal delivery. The superior rectal vein, perfusing the upper part of the rectum, drains into the portal vein and subsequently into the liver. On the other hand, the middle and inferiorrectal veins drain the lower part of the rectum and venous blood is returned to the inferior vena cava[13]. Therefore, drugs absorbed by rectum will be deliverd preferentially to the systemic circulation by passing the liver and avoiding first pass metabolism. Substantial research efforts have been directed towards the development and optimization of rectal drug formulation. In the majority of such studies, the AUC, Cmax and Tmax were the parameters of primary interest. The AUC is considered to represent the extent of absorption, whereas Cmax and Tmax are often regarded as indicators of the rate ofabsorption[1,13].

In the present study, the bioavailability of NE from either hard gelatin capsule or PEG suppository administration was higher than the tablet one indicating that the incorporation of NE with PVP 44,000 to form a coprecipitate gave higher dissolution rate than the tablet which may contain the NE in a free form.

In conclusion, the rectal route offers the possibility of reducing the effective dose of NE. This advantage results because the drug is absorbed to the systemic circulation and circumvents the first-pass effect of hepatic metabolism. Formulation of NE with PVP 44,000 in either rectal suppository base or hard gelatin capsule may enhance the bioavailability.

Table 1. Plasma norethindrone concentrations (ng/ml) after rectal and oral administrations

Time (hr)	Tablet	Capsule	Suppository
0.5	7.65 ± 1.1	7.55 ± 0.9	10.48 ± 1.2*
1	10.53 ± 1.3	9.50 ± 1.9	14.55 ± 1.0*
2	8.83 ± 1.1	8.70 ± 2.5	11.60 ± 1.0*
4	7.50 ± 1.3	8.65 ± 1.0	9.73 ± 1.2*
6	5.70 ± 1.3	7.40 ± 1.1	8.15 ± 0.9*
8	3.55 ± 1.3	4.28 ± 1.1	6.08 ± 0.8*
12	2.93 ± 0.9	3.45 ± 1.2	3.20 ± 0.9*

Values are mean ± SD (n=4).
*Significantly different at $p<0.05$ from the tablet using student's "t" test.

Table 2. Pharmacokinetic parameters of 2.5 mg norethindrone by oral and rectal administrations

	Tablet	Capsule	Suppository
$t_{\frac{1}{2}}$ (hr)	6.54 ± 1.77	9.11 ± 4.64	5.996 ± 0.80
Auc_{0-12} (ng\ml\hr)	67.06 ± 3.85	76.9 ± 2.97*	93.9 ± 3.25*
$Auc_{12-\infty}$ (ng\ml\hr)	19.18 ± 7.47	36.30 ± 24.9	26.33 ± 12.07
$Auc_{0-\infty}$ (ng\ml\hr)	86.20 ± 11.3	122.2 ± 44.3	120.3 ± 15.2*
Ka (hr)	2.18 ± 0.39	2.66 ± 1.04	2.78 ± 1.26
Ke (hr)	0.14 ± 0.026	0.11 ± 0.054	0.133 ± 0.033
C_{max} (ng\ml)	11.03 ± 1.0	10.23 ± 0.62	14.55 ± 1.0*
T_{max} (hr)	1.37 ± 0.19	1.32 ± 0.17	1.24 ± 0.30

Values are mean ± SD (n=4).
*Significantly different at $p<0.05$ from the tablet using student's "t" test.

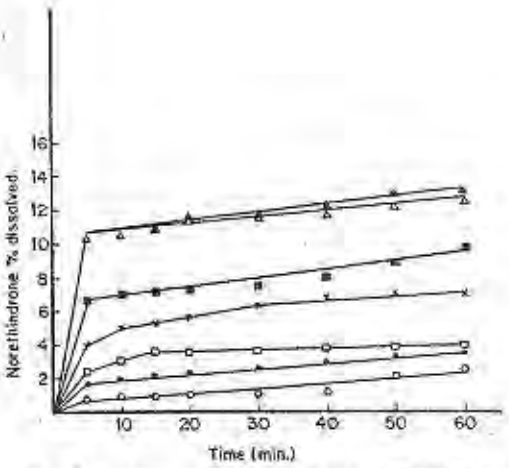

Fig.(1): Dissolution profile of norethindrone PVP 44,000 coprecipitate at different ratios: (□) 1:1, (x) 1:3, (■) 1:4, (△) 1:8, (▲) 1:12, (•) 1:8 physical mixture (○) drug alone.

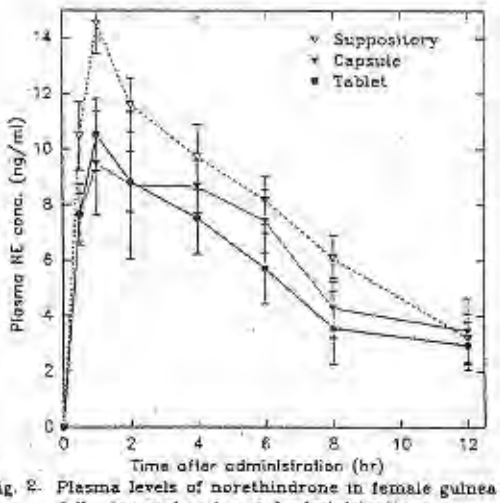

Fig. 2. Plasma levels of norethindrone in female guinea pigs following oral and rental administrations

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استخدام كروماتوجرافيا السوائل تحت الضغط العالى
فى تقدير نوراثيندرون فى بلازما الدم بعد تعاطيه بالفم والشرج ودراسة حركة الدواء

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فى هذا البحث تم دراسة حركية الدواء للعقار فى اثنى عشر من اناث الخنازير السليمة صحيا بعد ابتلاع جرعة واحدة من العقار فى صورة مترسبات موضوعة فى كبسولات جيلاتينية صلبة وأخرى محضرة فى أقماع من البولى ايثيلين جليكول وابتلاع اقراص من نفس العقار الموجود فى الأسواق.

وقد تحققت هذه الدراسة باستخدام ٣ مجموعات من الخنازير كل مجموعة مكونة من ٤ حيوانات فقط وكل مجموعة أخذت جرعة واحدة من الكبسولات والأقراص أو استخدمت الأقماع. وقد تم سحب كميات من بلازما الدم لمدة ١٢ ساعة على فترات منتظمة وبذلك تم تقدير كمية العقار فى بلازما الدم باستخدام كروماتوجرافيا السوائل تحت الضغط العالى.

وقد اثبتت النتائج أن العقار يمتص سريعا باستخدام الأشكال الصيدلية المستخدمة ولكن اقصى تركيز للعقار فى البلازما والاتاحة الحيوية كانت بالغة الارتفاع باستخدام الاقماع عن طريق الشرج ووجد انه لا توجد اهمية كبيرة فى الفروق فى فترة نصف الحياه فى البلازما (الوقت) ثابت ومعدل الامتصاص والتخلص والوقت اللازم للوصول لاقصى تركيز فى البلازما قد لوحظت الفروق بين التعاطى بالفم والشرج وهذه النتائج تقترح أن استخدام طريق الشرج بالاقماع يفضل لانه يقلل من كمية الجرعة الفعالة التى تستخدم فى علاج التعويض الهرمونى والمناعة للحمل.

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