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Evaluation of antiprogestin activity of a progesterone analog 14 - hydroxy progesterone in albino rats (*Rattus norvegicus albinus*)

B. SATHEESH KUMAR[1], M. KRISHNA REDDY [2], M. S. K. PRASAD [1] AND A. V. N. APPA RAO[3]
[1]Department of Biochemistry, Kakatiya University, WARANGAL(A.P.) INDIA
[2]Department of Zoology, University College, Kakatiya University, WARANGAL(A.P.) INDIA
[3]University College of Pharmaceutical Sciences, Kakatiya University, WARANGAL(A.P.) INDIA

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Progesterone is important for initiation and maintenance of pregnancy (hormone of pregnancy).The discovery that in the absence of progesterone, pregnancy cannot be initiated or maintained provided the basis for a massive effort of synthesis of antiprogestin molecules to develop emergency contraceptive pills. By the consequences, till date few progesterone antagonists (antiprogestins) have been synthesized which bind to and block the progesterone receptor. However, none of the antiprogestins are "pure" antiprogestins, having marked antiglucocorticoid properties as well, especially at higher doses. Thus the search for the "ideal" progesterone antagonist continues. The main objective of the present work is to evaluate antiprogestin activity of progesterone analogue, 14 β- hydroxy progesterone. A pregnane glycoside, carumbelloside-I (3-O-beta-D-glucopyranosyl-(1→6)-beta-D-glucopyranosyl-3beta,14beta-dihydroxypregn-5-en-20-one) was isolated from the n-butanolic extract of Caralluma umbellata (Asclepiadaceae) and was modified to a structural analog of progesterone, 14 β- Hydroxy progesterone (14β- OHP) in just two simple steps. Anti-implantation and abortifacient activities of the compound have been studied to evaluate its antiprogestin activity. To study the anti-implantation effect, pregnant wistar strain albino rats were administered with 14β-OHP (daily dose of 5 mg/Kg body weight) intraperitoneally from the day 1 to day 7 of pregnancy. On day 10, laparotomy was performed under light ether anesthesia. The uteri were examined to determine the number of implantation sites. The abdomens were sutured and the animals were allowed to go on term. The number of young born at term was also recorded. The abortifacient activity was studied in another group of pregnant rats. The animals were treated with 14β- OHP (5 mg/Kg body weight daily) intraperitoneally from day 8 to 12 of pregnancy. They were sacrificed on day 19 of pregnancy. Both horns of uterus were observed for the number of live fetuses. The compound showed about 44.23% anti-implantation and 62% of abortifacient effect at their corresponding dose levels tested. The results indicated the antiprogestin activity of 14 β- hydroxy progesterone in albino rats.

Key words : 14β- OHP, Anti-implantation, Abortifacient, Antiprogestin and Contraceptive

INTRODUCTION

Emergency contraceptive pills (ECPs) are an important option for women who have recently had unprotected intercourse and who do not want to become pregnant. Hormonal and non hormonal drugs are being used as ECPs which prevent ovulation or fertilization and possibly post fertilization (implantation of blastocyst).

The Combined Oral Contraceptive Pill (COCP), often referred to as the birth-control pill, or simply "the Pill", is a combination of an estrogen and a progestin, taken orally to inhibit normal female fertility (FDA, 1997). Progestin Only Pills (POP) are the contraceptive pills (mini pills) which contain synthetic progestins only and do not contain estrogen. However currently used estrogen-progestin combination oral contraceptives are undergoing continuing epidemiologic study because of their possible role in increasing the risk of early-onset breast cancer (Chlebowski *et al*., 2003). Other possible side effects for which questions persist include alteration in lipid profiles and, for older women who smoke, cardiovascular effects as well as possible thromboembolic events.

Synthetic antiprogestins such as mifepristone (RU 486), Onapristone (ZK 98.299) and Liloprestone (ZK 98.734), intercept progesterone action (antagonists) at the molecular level of receptor binding and have the potential to terminate early pregnancy (Puri and Van Look, 1991).Mifepristone (RU-486) is widely used to terminate the pregnancy in earlier stage (Rang *et al.,* 1997). It is a synthetic antiprogestin used as an abortifacient at large single dose (about 300mg) in the first two months of pregnancy and in smaller single dose (10 mg) as an emergency contraceptive (Piaggio *et al*., 2003). Mifepristone can be used as a regular contraceptive at 2 mg daily to prevent ovulation (Chabbert-Buffet*et al.*,

2005). Recently, treatment with mifepristone has emerged as the most effective hormonal method with very low side-effects and higher efficacy than the standard Yuzpe regimen (combined pill) (WHO, 1999).

Potent and specific progesterone antagonists have been sought for many years. To date some progress had been made in developing such compounds (Dore *et al.*, 1986), but all of the described progesterone antagonists also show at least some degree of glucocorticoid antagonism (Mao *et al.*, 1992). Thus the search for the "ideal" progesterone antagonist continues.

Since the antiprogestins show anti-implantation and abortifacient effect, the main objective of the present work is to evaluate anti-implantation and abortifacient effect of 14β- OHP in albino rats.

MATERIALS AND METHODS

Preparation of 14 -Hydroxy progesterone (14 -OHP):

Caralluma species are rich source of pregnane glycosides which structurally resemble progesterone. To date many pregnane glycosides have been isolated from these species. The plants Caralluma umbellata have been collected from Chandragiri hills, Tirupathi, Chittoor district, Andhra Pradesh. The plant has been identified by Prof. V. S. Raju, Taxonomist, Department of Botany, Kakatiya University, Warangal. Voucher specimen of plant is being maintained in the herbarium of the University College of Pharmaceutical Sciences, Kakatiya University, Warangal.

Fresh whole plants were chopped, crushed and then macerated with ethyl alcohol to get ethanolic extract. The ethanolic extract was fractionated with different solvents of increasing polarity like toulene, diethyl ether, ethyl acetate, butanone and n-butyl alcohol. The n- butyl alcohol fraction was subjected to chromatographic separation to isolate Carumbelloside-I (Lee-juian *et al.*, 1994). The Carumbelloside-I (3-O-β-D-glucopyranosyl-(1→ 6)-β-D-glucopyranosyl-3β,14β-dihydroxypregn-5-en-20-one) was hydrolyzed by enzyme β- glycosidase (emulsin) prepared from almonds to get genin of Carumbelloside-I. The chemical name of the genin is 14 hydroxy – 14 β – pregn – 5 – en – 3, 20-diol. The genin was converted to 14β-Hydroxy progesterone by Oppenauer Oxidation (Fig. 1). The identity of 14β-Hydroxy progesterone was established by recording its ^{1}H, ^{13}C NMR and mass spectra. The data tallied with the values reported in the literature (Templeton *et al.*, 1987). Female albino rats (Wistar strain) were brought from National Institute of Nutrition (NIN), Hyderabad. All animals were housed in standard cages in uniform lighting and at room temperature. Animals were fed on balanced diet as suggested by NIN, Hyderabad.

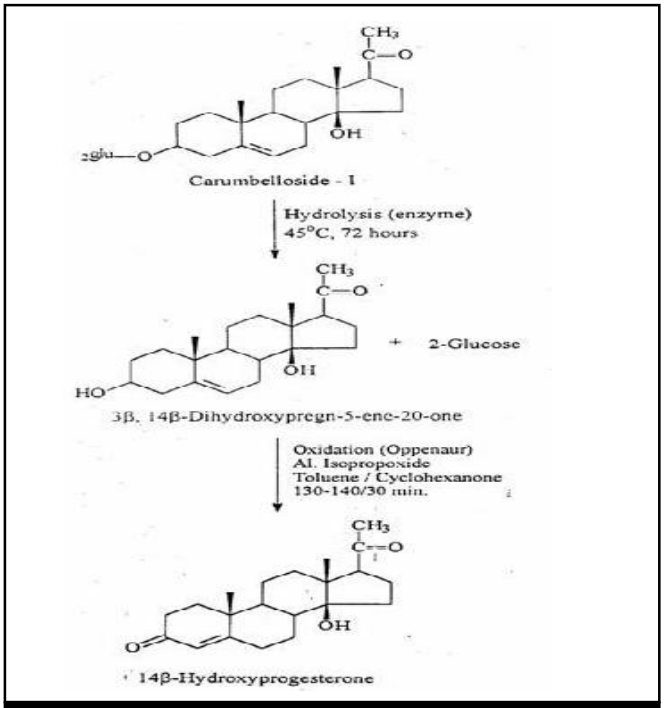

Fig. 1 : Schematic presentation of 14 -Hydroxy progesterone preparation

Study of anti-implantation and abortifacient activity:

Female Wister strain albino rats of proven fertility weighing about 200-225 g with at least three regular estrous cycles were caged with male rats of proven fertility in the ratio of 2:1 in the evening of proestrus. Mating occured during the estrus phase at night. The vaginal smears were examined in the following morning. Successful mating was ascertained by the presence of thick clumps of spermatozoa in their vaginal smear or presence of copulatory (vaginal) plug. Following mating, these animals were separated and that day was designated as day 1 of pregnancy. The pregnant rats were randomly assigned to four groups each containing six animals. The four groups were divided into two separate sets each containing two groups (control and treatment).

Anti-implantation activity:

The animals of first set were used for anti implantation study. The animals of treatment group of this set were treated with 14 β- Hydroxy progesterone at a dose level of 5 mg /Kg body weight intraperitoneally for 7 days consecutively starting from the first day of pregnancy. Whereas the control group animals were received vehicle only (1% CMC). On day 10 of pregnancy, the rats were anaesthetized and a small incision was given in the lower

abdomen. The number of implantation sites was counted and the number of young born at term was also recorded (Khanna and Chaudhary, 1968). The results are shown in Table 1. The anti implantation effect was determined by the percentage change of number of implantation sites as compared to control group.

Anti-implantation effect (%) = (A-B)/A X 100

A= no of implantation sites in control group

B= no of implantation sites in treatment group

Abortifacient activity:

The animals of another set were used for abortifacient study. Animals of treatment group were treated with14 β- Hydroxy progesterone at a dose level of 5 mg /Kg body weight intraperitoneally from 8 th – 11 th day of pregnancy. Whereas the control group animals were received vehicle only (1% CMC). On the day 19 th of pregnancy the animals were sacrificed by cervical dislocation and the number of alive fetuses were counted and recorded (Wong *et al*., 1987). The abortifacient effect was determined by the percentage change of number of live fetuses as compared to control group.

Abortifacient effect (%) = (A-B)/A X 100

A= no of live fetuses in control group

B= no of live fetuses in treated group

In both the studies the body weights of animals were recorded on daily basis. The results are shown in Table 2.

Statical analysis:

The results were expressed as mean ± SD (Standard Deviation). The level of significance was determined by student's t-test. A probability level less than 5% ($p<0.05$) was considered statistically significant difference between test and control groups for measured values.

Results and Discussion

The results obtained from the present investigation are summarized below:

Anti-implantation activity:

Administration of the 14β- OHP at the immediate postcoitus period (1-7 days) resulted in failure of 2 out of the 6 rats used in anti-implantation group to conceive. A significant ($p<0.05$) decrease (44.23%) in average number of treatment group was found as compared to the control group (Table 1). The body weights similarly increased as the pregnancy progressed confirming the continuous growth and development of uterine contents. In the treatment and control group the average number of implantation sites were 9.33 and 4.83, respectively but the average number of litters delivered on full term were 8.83 and 3.83, respectively. This study indicates that the compound shows good anti-implantation activity.

Abortifacient activity:

In comparison with control group, the treatment group shows a significant ($p<0.05$) decrease (62.0%) in number of live fetuses (Table 2). It indicates that the 14β- OHP shows strong abortifacient activity.

Administration of the 14β- OHP resulted in complete abortion of fetuses in 2 out of the 6 rats used in treatment group. For the treatment and control group the average number of live fetuses was 8.33 and 3.17, respectively. A significant ($p<0.05$) decrease in average no. of live fetuses in treatment group was recorded as compared to the control group. Presence of red spots in the horns of uterus indicated the resorptions of fetuses.

A reduction in body weights was observed in abortifacient group as compared to the control group. It indicates the decrease in uterine contents which may be attributed to abortifacient effect of test compound.

These results suggest that the progesterone analog 14β- OHP produced abortifacient and anti - implantation effect in albino rats which indicate the anti-progestin effects. Since the compound 14β- OHP is a structural analog of progesterone it may show affinity to progesterone receptor and may act as competitive inhibitor of progesterone. Mifepristone is a competitive inhibitor

Table 1 : Anti-implantation effect of 14-β-hydroxy progesterone in albino rat

Treatment (Dose)	Body wt. of animals (g)		No. of pregnant / treated rats	Average no. of implantation sites	Anti- implantation effect (%)	Average no. of young/litters
	Day 1	Day 10				
Control (1% CMC)	212±4.28	240±5.73	6/6	9.33±1.62	5.08	8.83±1.11
Treatment (5 mg/Kg)	215±3.19	236±6.27	4/6	4.83±1.81	44.23*	3.83±1.45

All values are mean±SD of six animal observations,

*indicate significance of value at P<0.05 with control

Table 2 : Abortifacient effect of 14-β-hydroxy progesterone in albino rats.

Treatment (Dose)	Body wt. of animals (gr)		No. of live Fetuses in individual rats	Average no. of live fetuses	Abortifacient effect (%)
	Day 1	Day 10			
Control (1% CMC)	218±4.05	270±5.76	9, 6, 8, 9, 7, 11	8.33±1.75	Nil
Treatment (5 mg/Kg)	209±3.92	255±6.12	5, 0, 3, 7, 0, 4	3.17±2.78	62.0*

All values are mean±SD of six animal observations,
*indicate significance of value at P<0.05 with control

that acts both at progesterone and glucocorticoid receptors. It is a weak partial agonist with predominantly antagonistic activity to progesterone. The available data in the present study indicate that the 14β- OHP may possess antiprogestin activity. However, it is possible that the14β- OHP may act in a similar way like mifepristone.

Conclusion:

The results of this study indicate that the14β- OHP causes anti-implantation and abortifacient effect in rats. This indicates the antiprogesterone activity of 14β- OHP. Since the compound is an analog of progesterone the compound may probably bind to and block the progesterone receptor like other antiprogestin molecules (e.g.: mifepristone). Hence, it might be exploited to prevent unwanted pregnancy and control the population explosion. However, these findings need further investigation at increased dose levels for dosage optimization, determination of mode of action, safety and reversibility of the compound to qualify it as an "ideal" antiprogestin.

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