

Full Length Research Paper

# Changes in oestrous cycle of adult female wistar rats treated with artemether

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Artemether is an ante malaria that has proved rapidly effective. It has replaced chloroquine and quinine in many parts of the world. This is an experimental study designed to examine the effects of artemether on oestrous cycle of lower primates since this drug is highly consumed by females in child bearing age. Twenty four cyclic female rats were randomly divided into three groups A, B and C all administered 10 mg/kg body weight of rat for 24, 48 and 96 h, respectively intramuscularly. Estrous cycles were established by vagina smears taken daily and smears examined under the light microscope. Results of this study show prolongation of various phases of the oestrous cycle in many of the rats. This was however reversible and dose dependent. It was concluded that arthemeter treatment altered estrous cycles in rats, thus distortion of menstrual cycle should be borne in mind in patients taking this drug.

**Key words:** Artemether, estrous cycle, vaginal smear, *Rattus norvegicus*.

## INTRODUCTION

Artemether is a lipid soluble methylether of dihydroartemisinin. Artemisinin is a novel sesquiterpene lactone, extracted from the leaves of the shrub *Artemisia annua* and possesses an endoperoxide bridge which is a rare feature in natural products. The Endoperoxide Bridge is essential for its antimalarial activity.

Its chemical formula is 3R,5aS,6R,8aS,9R,10S,12R,12aR)-Decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4,3-j]-1,2-benzodioxepin. Its molecular formula is C<sub>16</sub>H<sub>26</sub>O<sub>5</sub> and its molecular weight is 298.4.

## Clinical pharmacology

Artemether is active against all Plasmodia including those which may be resistant to other antimalarials.

Artemether has very rapid schizontocidal activity. The schizontocidal activity of artemether is mainly due to destruction of the asexual erythrocytic forms of *P. falciparum* and *P. vivax*.

Artemether is concentrated in the food vacuole. It then splits its endoperoxide bridge as it interacts with haem,

blocking conversion to haemozoin, destroying existing haemozoin and releasing haem and a cluster of free radicals into the parasite. There is inhibition of protein synthesis during growth of trophozoites. There is no cross resistance with chloroquine.

Artemether is not active against hypnozoites, therefore, an 8- amino-quinoline derivative such as primaquine should be given sequentially after the combination in cases of mixed infections of *P. falciparum* and *P. vivax* to achieve hypnozoites eradication.

Artemether reduces gametocyte carriage. There is no rationale at present for using artemether for chemoprophylaxis.

## Pharmacokinetics

The drug is slowly absorbed from intramuscular injection. Peak plasma concentrations have been achieved in about 6 h after intramuscular injection of artemether. Artemether is hydrolyzed after administration to a biologically active metabolite, dihydroartemisinin. Dihydroartemisinin accounts for most or all of clinical antimalarial activity.

Total protein binding is 95.4%. The drug is rapidly and extensively metabolised in the liver.

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In animal studies, unchanged artemether has not been detected in both faeces and urine due to its rapid and high first-pass metabolism, but several metabolites (unidentified) have been detected in both faeces and urine.

The elimination half-life is approximately 1 h, but following intramuscular administration, the elimination phase is prolonged because of continued absorption.

The elimination half life of dihydroartemisinin was approximately 2 h. Artemether has been reported to clear fever in severe falciparum malaria within 30 - 84 h.

## Indications

Artemether injection is indicated for treatment of severe and complicated malaria caused by *P. falciparum* both in adults and children in areas where there is multidrug resistance.

## Contraindications

Artemether is contraindicated in patients with hypersensitivity to artemether or other artemisinin compounds.

Artemether is not recommended in the first trimester of pregnancy because of limited data.

Intramuscular artemether and dihydroartemisinin tablets have proved rapidly effective and they have replaced chloroquine and quinine in China and many parts of the world (LI-GQ, 1994). In a study artemether caused abortion in 4% and still births in 3% of kaven pregnant women in Thailand (McGready, 1998).

Ovarian activity is initiated by LH after which the subsequent LH stimulation does not alter or affect it (Lipner, 1973). Its activity begins about 4<sup>th</sup> -5<sup>th</sup> day of age (Uilenbroek and Van der Linden, 1983). It is initiated through follicular growth independent of gonadotrophins (Peter and Byskor, 1973). At this period, ovarian histology show non-growing oocytes in groups and surrounded by the closest stromal cells, destined to differentiate and form the first set of granulosa cells (Byskot and Lintern-Moors, 1973).

The mean oestrous cycle in 4.4 days (Abiodun, 2002) or 4.5 days (Blandau, 1955). Proestrus lasts for 12 - 14 h, Estrous 25 - 27, Metestrus 6 - 8 h and Diestrus 55 - 57 h.

## MATERIALS AND METHODS

Twenty four rats weighing 150 - 180 g were acclimatized for one week in the animal house of the department of Anatomy and cell biology OAU Ile-Ife Nigeria.

Their oestrous cycles were established by daily vaginal smear taken with (Abiodun, 2002); Blandau (Blandau, 1955) Ashtwood separate pipettes containing physiological saline (0.9 NaCl). Under light microscope, smears were examined to determine the epithelia

cells predominantly present in the vaginal lavage.

## RESULTS

### Treatment on proestrous

Three groups of rats were treated with 10 mg/kg body weight of artemether at 1000, 1400 and 1800 h proestrous, respectively. All continued into oestrous the following day. Later there was prolongation of proestrous in many members administered 10 mg/kg body weight between 24 and 96 h (4 days).

### Treatment on oestrous

Dose of 10 mg/kg body weight of the drug was administered for two consecutive days to members of this group. About half of the members of the group had normal oestrous cycle after the first dose while the remaining had prolonged diestrus II before entering proestrous and oestrous.

### Treatment on diestrus I and II

10 mg/kg body weight of the drug was given to this group. It showed prolongation of Diestrus I in all the members varying from one to four days.

These photomicrographs are characteristic for early oestrous development after a period of anoestrus. The vaginal smear of control cases showed fewer cells than that shown in Figures 1 to 9.

## Examination of vaginal smears in the rat administered arthemeter

**Dioestrus:** At this stage of the oestrous cycle there is little material to be collected, and it consists mainly of traces of secretory material with cellular debris. There are few intact cells to be found. Some parabasal and intermediary cells may be observed. There are usually few leucocytes, if any, to be seen (Figure 1).

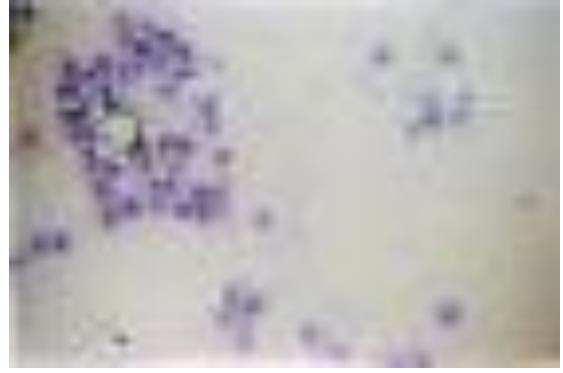
### Transition from dioestrus to pro-oestrus

There is considerably more mucus at this stage, often present as thick strands or discs. The histological picture is more complex and darker. Leucocytes are rarely seen in pro-oestrus. Most of the cells present are parabasal cells, often with an irregular or shrunken appearance, but intermediary cells may also be observed (Figure 2).

**Early pro-oestrus:** The smear becomes "cleaner", with less mucus. Parabasal cells appear more clearly, as do



**Figure 1.** showed the estrous cycle of rats treated with 10 mg/kg body weight arthemeter for 24 h, there is little material to be collected, and it consists mainly of traces of secretory material with cellular debris. There are few intact cells to be found. Some parabasal and intermediary cells were observed.



**Figure 2.** Showed photomicrograph of the proestrous stage of rats treated with 10 mg/kg body weight of arthemeter for 48 h, here, more mucus was observed at this stage, present as thick strands or discs. The histological picture is more complex and darker. Leucocytes. Most of the cells present are parabasal cells.

intermediary cells. Cell debris can still be observed.

**Pro-oestrus:** The smear is much lighter in colour and mucus is rarely seen. The cells are predominately intermediary cells, and parabasal cells are rare. Leucocytes are hardly ever observed (Figure 3).

#### The transition from pro-oestrus to oestrus

The smear is clear and dominated by cells. These consist of intermediary cells, superficial cells and anuclear (keratinised) cells.

**Oestrus:** The smear consists nearly entirely of keratinised superficial cells that lie singly in early oestrus.

**Figure 3.** This smear is much lighter in colour and mucus is rarely seen. The cells are predominately intermediary cells, and parabasal cells are now rare probably as a consequence of the treatment. Leucocytes were not observed.



**Figure 4.** Showed photomicrographs of rats treated with 10 mg/kg body weight of arthemeter for 72 h. A few intermediary cells with intact nuclei may occasionally be observed probably as a consequence the treatment and duration of administration.

They form groups as oestrus progresses and by the end of this stage of the cycle they can form large flakes (Figure 4). A few intermediary cells with intact nuclei may occasionally be observed.

#### The transition from oestrus to metoestrus

Although flakes of keratinised cells are still present, this stage is characterised by the presence of leucocytes and (to a lesser extent) intermediary cells.

**Metooestrus:** The picture at this stage of the cycle is dominated by leucocytes, often in large numbers, and intermediary cells (Figure 5).

As this stage progresses, more intermediary cells begin to appear. These are often small and dark. Parabasal cells can also be seen. However, larger intermediary cells and leucocytes are also present (Figure 6).



**Figure 5.** Photomicrograph of the smear of the metestrous stage rats treated with 10 mg/kg body weight arthemeter for 72 h leucocytes, are in large numbers, and intermediary cells.



**Figure 6.** As this stage progresses, more intermediary cells begin to appear. These are often small and dark. The figure showed smear of rats treated with 10 mg/kg body weight of arthemeter for 24 h, compared to figure 5 above, Parabasal cells were observed. However, larger intermediary cells and leucocytes are also present.

**The transition from metoestrus to dioestrus**

This stage is characterised by the reduction in cell numbers and the reappearance of mucus. Often in thin strands (Figure 7).

This picture shows a continuation of this process, as the mucus becomes progressively more apparent and cell numbers decline (Figure 8).

**Anoestrus:** The cellular picture at this stage resembles in many ways the transition from dioestrus to pro-oestrus, but the general picture is darker, the cells rarely appear intact and there is a lot of mucus and cellular debris. It is often at this stage that the rat's oestrous cycle may halt.

**The transition from pro-oestrus to oestrus**

Figure 9 shows a characteristic early oestrous development



**Figure 7.** Photomicrograph of rats treated with 10 mg/kg arthemeter for this stage is characterized by the reduction in cell numbers and the reappearance of mucus. Often in thin strands.



**Figure 8.** Photomicrograph of rats treated with 10 mg/kg arthemeter for 48 h metestrous as compared to 72 h. This stage is characterized by mucus, and it becomes progressively more apparent and cell numbers decline.



**Figure 9.** Photomicrograph of rats treated with 10 mg/kg arthemeter for 48 h metestrous as compared to group of rats treated with arthemeter for 24 h.

after a period of anoestrus. The vaginal smear may in some cases show fewer cells than in this picture (Figure 9).

Figures 1 to 9 show the observed daily vaginal smear taken on rats treated with 10 mg/kg body weight arthemeter intramuscularly.

## DISCUSSION

Oestrous cycle has been known to be disrupted by genetic, nutritional and endocrine factors. Several drugs affect oestrous cycle by acting at different levels of hypothalamic-pituitary axis or at ovarian level to inhibit ovulation (Astwood, 1939). In this present investigation, effect of arthemeter on oestrous cycle showed that it altered the oestrous cycle. His alteration is dose dependent and reversible thus when the drug is withdrawn the cycle returns back to normal. This is similar to observation on Halofanthrine studies (Psychopharmacology, 2000). Therefore distortion of menstrual cycle should be borne in mind in patient using it. Withdrawal of function thus causing delay in piestrous and metestrus. The drug should therefore be made in event of distortion. The delay in oestrous circle is probably due to the fact that arthemeter treatment inhibits the hormonal function probably while compromising cellular integrity and function. Influence of sex, estrous cycle, and drug-onset age on cocaine self-administration in rats (*Rattus norvegicus*). Katak et al in their work investigated the influence of sex, phases of the estrous cycle, and age of drug onset on cocaine self-administration. Adult male, adult female, and adolescent male rats (*R. norvegicus*) were evaluated using low fixed-ratio (FR) schedules of drug delivery with a single fixed cocaine unit dose or a range of cocaine unit doses with a single FR schedule. Sex differences in adults were observed for mg/kg consumption of the 3.0 mg/kg unit dose, with consumption being significantly less in estrus females than in males. Over the estrous cycle, mg/kg consumption of this unit dose was significantly less during estrus than during metestrus-diestrus. Differences due to age of drug onset were also observed, with mg/kg consumption of the 3.0 mg/kg unit dose being significantly less in adolescent males than adult males or adult females during metestrus-diestrus. In contrast, these various groups did not have significantly different mg/kg intakes of cocaine unit doses <3.0 mg/kg, nor did they significantly differ in the rates and patterns of responding and number of infusions earned as a function of FR schedule or unit dose of cocaine available. The role of sex, estrus cycle, and drug-onset age on cocaine self-administration appears to be minimal under these experimental conditions.

Experimental conditions that favor no sex or age differences in cocaine intake (1.0 mg/kg unit dose and low FR) may be useful for evaluating potential sex or age differences in the consequences of cocaine self-administration more reliably, as cocaine intake would not be an uncontrolled factor in comparison to this study, arthemeter administration was only observed in female wistar rats thus found to inhibit estrous cycle.

## RECOMMENDATION

It was recommended that studies on age, sex and after effects of loading dose of arthemeter should be subjected for future studies including hematological parameters.

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## REFERENCES

- Abiodun AA (2002). Effects of halofanthrine on Oestrous cycle, Ovulation and microscopic structure of the Ovary: Biosci. Res. Comm., 14(1): 11-14.
- Astwood EB (1939). Changes in weight and water content of uterus of the normal adult rat. Am. J. Physiol., 126: 162-170.
- Blandau RJ (1955). Ovulation in the living albino rat. Fertil. Steril., 6: 391-404.
- Byskot AG, Lintern – Moors S (1973): Follicle formation in the immature ovary; the role of the rete ovari J. Anat., 116: 207-217.
- Li-G, Guo XB, Fu LC (1994). Clinical trials of artemisinin and its derivatives in the treatment of malaria in China. Trans. Royal Soc. Trop. Med. Hyg., 88(1): 55-56.
- Lipner H (1973). Mechanism of mammalian ovulation. The handbook of Physioilogy, Endocrinology, 2(1): 409-437.
- McGready R, Cho T, Cho JJ (1998). Artemisinin derivatives in the treatment of falciparum malarial in pregnancy, Trans. Royal Soc. Trop. Med. Hyg., 92: 430-433.
- Peter SH, Byskor AG (1973). The effect of gonadotrophin and follicle growth initiation in neonatal mouse ovary. J. Reprod. Fertil., 35: 139-141.
- Psychopharmacology (Berl) (2000). Effects of sex and the estrous cycle on regulation of intravenously self-administered cocaine in rats, 152(2): 132-139.
- Uilenbroek J, Van DLR (1983). Changes in gonadotrophin binding to rat ovaries during sexual maturation. Acta Endocrinil. (Copenh), 103: 413-419.