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Short communication

Effects of Artesunate on some biochemical parameters in pregnant albino Wistar rats challenged with lethal strain Plasmodium berghei NK65: Appreciating the activities of artemisinin drugs on key pregnancy hormone balance

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ABSTRACT

In humans, malaria in pregnancy can cause serious maternal and foetal morbidity and in extreme untreated cases, foetal mortality occurs. The therapeutic approach to curbing this malaise is the administration of an effective and/or combinations of anti-malaria medicaments. Acute or chronic administration of some of these drugs, however, gives rise to some adverse medical conditions including reproductive dysfunction, especially in pregnancy. Studies aimed at the hormonal interplays following administration of these drugs in pregnancy have been limited due to too few appropriate animal models. In this experiment, pregnant albino rats were infected with rodent parasite, *Plasmodium berghei* on the 5th day of gestation, following which biochemical changes, specific for pregnancy maintenance were monitored in the blood of test rats. We observed that infecting the pregnant rats with *P. berghei* negatively impacted the measured biological parameters (hormones) compared to unchallenged controls. The observed effect was however retreated following oral administration of 3 mg/kg body weight, qDay of Artesunate until the 17th day of gestation. Findings, therefore, suggest that Artesunate is an effective therapeutic agent in pregnancy, demonstrated by the restoration of the hormonal changes occasioned by the parasitic infection.

1. Introduction

As a leading cause of death worldwide, especially in Africa, malaria remains a global health challenge with grave health and economic implications (Sachs and Malaney, 2002). Anaemia caused by *Plasmodium falciparum*, the causative agent of human malaria leads to increased morbidity and mortality of foetuses (Guyatt and Snow, 2001; Snow et al., 2005). Lack of effective treatment of this malaise, occasioned by multi-drug resistance (MDR) of malaria parasite has posed a serious threat to global medical research and therapeutics. MDR, therefore, has created a dire need for novel pharmaceuticals. One of such novel drugs is artemisinin and its analogues. Artesunate is a membrane targeting semi-synthetic analogue of artemisinin capable of forming free radicals (Cui and Su, 2009; Lee et al., 2010). Artemisinins are generally more effective when used in combination therapies to combat *plasmodium falciparum* (Majori, 2004; Tall et al., 2007). Near a decade ago, serious health concerns were being raised over the use of anti-parasitic drugs including artemisinin and its derivatives (Adam et al., 2009; Dellicour et al., 2007). Similarly, there is an alarming artemisinin misuse and/or abuse especially in

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Africa, thus the emerging artemisinin resistance (Noedl et al., 2008; Talisuna et al., 2012; White, 2010). Documented evidence link the use (or misuse) of artemisinin to various toxicological effects including suppression or disruption of normal hormonal functions and other pathophysiological dysfunctions (Nontprasert et al., 2002; Olumide and Raji, 2011). *Plasmodium berghei* is a unicellular protozoan that targets predominantly non-human hosts. We considered it a practical pathogenic laboratory species for understanding the humoral pathophysiology of *Plasmodium falciparum*, the causative agent of human malaria. In this communication, we evaluated the effects of Artesunate (a derivative of artemisinin) on certain biochemical parameters of pregnant rats infected with lethal strain *P. berghei* NK65, albeit, on a short-term basis especially after re-medication to subvert relapse.

2. Materials and methods

2.1. Animals, drugs and parasites

Adult female Wistar rats were obtained from the Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, and the research animal unit of the Department of Veterinary Physiology, Biochemistry and Pharmacology, Michael Okpara University of Agriculture, Umudike, Nigeria. They were acclimatised for 2 weeks with ad libitum feed and water (Animal welfare, 2007). After which they were mated and confirmed pregnant 8 days later with a drop of serum from clotted blood collected from a snip-cut on the tail of the rats and read off using a pregnancy (Hicks Sleek PT-05) test kit (data not shown). Artesunate was obtained as tablets (GlaxoSmithKline Pharmaceutical Company, Nigeria) while the *P. berghei* NK65 parasites were kindly donated by Dr Ikenna Eze of the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka.

2.2. Challenge with Plasmodium berghei

Pregnant Wistar rats (n = 10) were infected with previously maintained lethal strain *Plasmodium berghei* strain NK65 (Waki et al., 1982) by intraperitoneal inoculation with 3×10^7 parasitized rat erythrocytes on Day 4 (or 12) of gestation, a method modified from Odetola and Basir (1980). Infected animals were shared into 2 groups (A and B), with a 3rd pregnant group (n = 5) that was neither challenged nor treated (C). Group A animals served as negative control and received normal saline. Group B animals were treated orally with 3 mg/kg body weight of Artesunate four times daily (qDay) for an initial 3 days, and then stopped for 5 days before administering prophylactic dose of 3 mg/kg qDay for another 5 days to mimic administrations aimed at subverting relapse created by *P. falciparum* resistance by some medical practices in Nigeria (data not shown). All animals were allowed free access to pelletized rat food (Vital Feed®) and water *ad libitum*, except for 2 h restriction to food prior to drug administration, done before 10:00 h. After the treatment period, animals (dams and pups) were sacrificed by cervical dislocation, 2 days prior to the anticipated time of delivery (Day 19) and blood collected from the dams only. Blood was allowed to clot, centrifuged and serum used for biochemical analysis. The dead pups were harvested and weighed.

2.3. Hormonal assay

Serum from each female rat was assayed for serum testosterone (ST), luteinizing hormone (LH) and follicle-stimulating hormone (FSH), progesterone(Pro), estriol(E-ol) and prolactin(PL) levels were measured using the enzyme-immunoassay (E.I.A.) techniques (Raji et al., 2005), carried out using appropriate E.I.A. kits (Immunometrics, London, UK). The optical density was read using a spectrophotometer (Jenway, 6300 spectrophotometer) at wavelengths between 492 and 550 nm.

2.4. Data and statistical analysis

Data (n = 5) obtained were presented as mean ± SEM and analysed using one-way analysis of variance (ANOVA) and posthoc comparisons were carried out using either Dunnett's *t*-test or Tukey's test (where appropriate) on GraphPad Prism version 4.05. Values of $P < 0.05$ were considered significant in the study.

3. Results

Fig. 1A–F is the graphical representation of our results. We find a positive correlation between the Artesunate treatment and the biochemical parameters examined in pregnant rats. Our results show that infection with the *P. berghei* pathogen significantly decreased the serum concentration of luteinizing hormone (LH) compared to uninfected control (from 0.13 ± 0.01 to 0.09 ± 0.05 μg/l) by day 15, an effect that reversed following oral administration of artesunate causing an up-regulation in the serum hormonal concentration to 0.17 ± 0.04 μg/l. Similarly, there was a decreased FSH concentration from 0.18 ± 0.04 μg/l in the untreated animals to 0.12 ± 0.03 μg/l fifteen days' post infection, the drug showed a trend towards reversing this, although not significant ($P > 0.05$).

We did not observe any variation in testosterone concentrations in response to the infection and/or treatment. Progesterone, the pregnancy hormone, was up-regulated significantly by day 15 post-infection in the plasmodium-challenged untreated group to 16.11 ± 2.51 ng/ml as against the non-infected untreated group. Artesunate administration day-15 post-infection also caused a decline in serum progesterone levels of test animals to 14.13 ± 2.47 ng/ml. Although the estriol concentration was significantly suppressed by the parasitic infection from day 6 until day 15 compared to un-infected control, artesunate doesn't seem to have an effect on its overall concentration. A similar trend was observed for serum prolactin concentration on day 15; even though artesunate had a down-regulation effect in prolactin levels by day 6 in pregnant rats similar to the un-challenged condition.

4. Discussion and conclusion

4.1. Discussion

This study intends to throw additional lights to information simulating human malaria in pregnancy condition in laboratory animals and monitoring the interplay between the plasmodium disease and changes in hormonal levels. In our study, we found systematic decrease and increase of these hormones as result of the infection which correlates strongly with the fundamental rise in abortions and stillbirths among sub-Saharan African women, where malaria is endemic (Sachs and Malaney, 2002). Progesterone, a pregnancy support and maintenance hormone was increased despite parasitemia in the third trimester (day 15) when progesterone synthesis has been reportedly highest (Agoreyo and Onwegbu, 2015; Gibori et al., 1977; Morishie et al., 1973), and becomes a score of placental viability and readiness for labour. Although quiescent in actual pregnancy, the sharp decline in LH levels in the untreated rats, was consistent with previous studies in which increases in LH were observed for the initial 8 h of pregnancy, thereafter a decline, before a latter trend upwards in LH-levels from day 18 to 23 of pregnant rats (Linkie and Niswender,