Review

Clinical and non-clinical safety of artemisinin derivatives in pregnancy

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A B S T R A C T

Malaria in pregnancy is a clinically wasting infectious disease, where drug therapy has to be promptly initiated. Currently, the treatment of this infection depends on the use of artemisinin derivatives. The World Health Organization does not recommend the use of these drugs in the first trimester of pregnancy due to non-clinical findings that have shown embryolethality and teratogenic effects. Nevertheless, until now, this toxicity has not been proved in humans. Artemisinin derivatives mechanisms of embryotoxicity are related to depletion of circulating embryonic primitive erythroblasts. Species differences in this sensitive period for toxicity and the presence of malaria infection, which could reduce drug distribution to the fetus, are significant to the risk assessment of artemisinin derivatives treatment to pregnant women. In this review we aimed to assess the results of non-clinical and clinical studies with artemisinin derivatives, their mechanisms of embryotoxicity and discuss the safety of their use during pregnancy.

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1. Introduction

Malaria is a severe infectious disease that remains a major public health challenge in endemic regions, including countries from South and Central America, Africa and Asia. This disease is caused by parasites of the Plasmodium genus and transmitted by Anopheles mosquitoes. The most severe form of malaria is triggered by P. falciparum. It is estimated that annually more than 3 billion people are at risk of contracting malaria and 400,000 deaths are recorded as a result of this disease [1].

During pregnancy, malaria can be a clinically wasting condition. Its complications are remarkable in pregnant women, such as severe anemia and cerebral malaria, and their offspring face the possibility of stillbirths, miscarriages or low birth weight [2]. Antimalarial drug therapy during pregnancy has to be promptly initiated, making the safety of currently available drugs and their combination for mothers and their babies a research topic of paramount relevance. Moreover, due to the risk of malaria to the mother and the fetus, the World Health Organization (WHO) recommends chemoprophylaxis for pregnant women living in high-intensity transmission areas [3]. Nevertheless, based on non-clinical data, there are restrictions about malaria treatment for pregnant women, especially in the first trimester [4].

Currently, there are few drugs available for the treatment of malaria. Parasites, particularly P. falciparum, have become resistant
2. Artemisinin and its derivatives

Artemisinin is extracted from the leaves of the *A. annua* L. (Fig. 1a), and has been used in China for 2000 years as an antipyretic, referred to as qinghao [10,11]. It was discovered, purified and identified in 1972 [12]. Artemisinin is a sesquiterpene lactone containing an endoperoxide bridge, which is believed to be necessary for antimalarial activity (Fig. 1) [13]. Artemisinin is an extremely active antimalarial to treat uncomplicated and severe malaria [6].

The artemisinin content in dried leaves is between 0.06–2% and it is poorly soluble in water or oil [14]. The artemisinin low bioavailability and poor pharmacokinetics properties are the major drawbacks of its use. After its discovery, several semisynthetic derivatives were identified including the dihydroartemisinin (DHA) which is more potent and chemically stable than artemisinin (Fig. 1b) [15]. Water-soluble (artesunate and artemolate) and oil-soluble (artemether and arteether) artemisinin derivatives were synthetized and developed (Fig. 1b), and they are known as first generation endoperoxides [14]. After administration, these derivatives are metabolized in DHA (Fig. 1c). The antimalarial effect of the artemisinin derivatives results primarily from DHA, which disappears from plasma with a half-life of approximately one hour [16]. Artemisinin derivatives are currently the most important class of antimalarial drugs [3].

Efforts to find more metabolically stable artemisinin derivatives are ongoing and have highlighted the second generation of endoperoxides, including 10-(alkylamino)-artemisinins, as artesimone and artemside [10]. They are called second generation because they are more stable and potent than the others derivatives [14]. As only endoperoxide bridge is required for antimalarial activity, significant efforts have been focused on identification of fully synthetic artemisinin-like peroxides with a simple and cheaper synthesis, as for example trioxanes and diterpenes peroxides with antimalarial activity. However, these newly developed semi-synthetic and synthetic derivatives are still undergoing development [10].

Artemisinin and derivatives are rapidly effective and well tolerated but, due to the fact they have short half-life, monotherapy is not recommended to avoid resistance [6,11,14]. Therefore, artemisinin derivatives are administered combined with others antimalarial to increase efficacy and adherence to the treatment [6,18]. The use of artemisinin-based combination therapies (ACTs) for uncomplicated malaria has enabled a reduction of treatment from 7 (artemisinin monotherapy) to 3 days, avoiding recrudescence [3,6].

The most common ACTs used for malaria treatment are artether-lumefantrine, artesunate-amodiaquine, artesunate-mefloquine and artesunate-sulfadoxine-pyrmethamine [10,19]. ACTs for treatment of *falciparum* malaria reduce load gametocytes, reducing retransmission, but this effect is incomplete without the inclusion of primaquine, which is a known gametocytocide [3].

The exact mechanism of the antimalarial activity of the artemisinin derivatives remains controversial. Within the malaria parasitized erythrocyte, hemoglobin is degraded by a series of protease enzymes of parasite to release peptides and amino acids required for its development and to create space within its digestive vacuole, increasing the amount of free iron and heme [20]. One possible mechanism of action suggests that artemisinin derivatives, into the parasitized erythrocyte, accumulate and release free radicals through loss of endoperoxide bridge by iron or heme, which kills the parasite [21]. Recently, Wang et al. [22] showed that heme, rather than free ferrous iron, is predominantly responsible for artemisinin activation.

The artemisinin activation generates carbon-centered radicals which are highly reactive and can covalently bind to several proteins and alkylate them impairing their function [20]. These radicals
alkylate heme and form heme-drug adducts which have been verified by in vitro and in vivo studies [23–26]. This nonpolymerizable heme may accumulate and produce toxic reduced oxygen species being toxic to the malaria parasites [27]. Furthermore, it was demonstrated that artemisinin can covalently bind to more than one hundred parasite proteins, many of these proteins are important for parasite survival, such as a known artemisinin target P/ATP6, a sarcoplas/endo/oplasmic reticulum Ca2+–ATPase (SERCA) enzyme [21,22].

Artemisinin derivatives kill the intraerythrocytic forms, and their action on the young stages of Plasmodium prevents the development of pathological complications [10]. The artemisinin derivatives are more toxic to parasitized erythrocyte, which absorbs better the drug, being 100 times more concentrated in infected erythrocytes as compared to uninfected erythrocytes [22,28].

Currently, malaria therapy depends on the use of artemisinin derivatives and on the ACTs. In Southeast Asia, development of artemisinin-resistant strains of P. falciparum put at risk the benefits obtained by the reduction of malaria mortality achieved with the inclusion of this treatment since the year 2000; however, these drugs are still the best choice for falciparum malaria [3]. Additionally, artemisinin derivatives are being investigated as anticancer agents as well because they inhibit angiogenesis and cell growth in several neoplastic cell models [29]. These compounds have also demonstrated efficacy in the treatment of schistosomiasis and fasciolasis and in animal models of Clonorchis infection [30,31].

3. Non-clinical developmental toxicity studies with artemisinin derivatives

Since artemisinin derivatives were discovered and emerged as highly effective antimalarial to treat multidrug resistant parasites, their developmental toxicity has been evaluated. Initial studies conducted in mice, rat, rabbit, guinea pig and hamster demonstrated embryolethality, resorptions and whole litter resorption after administration of artemether, arteunate and DHA by different routes and range of doses between gestational days (GD) 5–18 [32,33]. Malformations were first reported by Li (1988) [34] who evidenced umbilical hernia and rib defects on fetuses after parenteral administration to pregnant rats of 26 mg/kg of arteunate from GD 6 to GD 8 (cited by Clark et al. [7]).

Many groups have described teratogenic effects of artemisinin derivatives in animals as mainly cardiovascular and skeletal defects, which happen in association with increased resorptions (Table 1). Malformations on cardiovascular system consist in wall heart, ventricular septal and great vessel defects. The skeletal malformations include long bones, scapulae and ribs defects. These drugs seem to be more embryo-lethal than teratogenic and the doses that induce these effects were close to each other in rats and rabbits [7].

The WHO concludes that all artemisinin derivatives were developmental toxicants showing embryolethality or teratogenicity in animals [35]. Embryo toxic effects of these antimalarial drugs have been subsequently confirmed in different species, including monkeys (Table 1).

These embryo toxic effects, as well as their antimalarial activity, seem to be a “class effect” depending on the presence of endoperoxide bridge [36]. However, artearon (also called O2Z77), a fully synthetic artemisinin derivative in phase III of clinical trials [37], showed a better safety margin than artemisinin and DHA in a comparative study using rat whole embryo culture (WEC) [36]. This brings light to the possibility of keeping apart antimalarial activity from embryo toxic effect.

Artemisinin derivatives monotherapy is not allowed due to emergence of resistance (WHO, 2015). A lot of ACTs have been produced as fixed-dose combination to reduce monotherapy inadequate use [3]. Besides that, non-clinical reproductive toxicity studies with ACTs are very scanty (Table 2). The combination of arteunate-dapsone-chlorproguanil showed classic embryo effects attributable to arteunate treatment alone with no additive effect to its toxicity [7]. Interestingly, Boareto and co-workers showed that arteunate and mefloquine co-administration reduced embryo toxic effects of arteunate treatment alone by mechanisms not yet elucidated [9,38]. It makes evident the importance to assess ACTs potential of developmental toxicity. This could help finding less harmful options for the treatment of malaria during pregnancy.

Additionally, long-term effects on offspring after prenatal antimalarial treatment with artemisinin derivatives were also poorly evaluated. Our group evaluated the effects of in utero exposure to oral arteunate treatment (7 mg/kg – GD 14–20) to rats and evidenced a reduction on sperm count and daily sperm production on male offspring and did not find any effect on female offspring [39]. Changes on sperm count were previously mentioned by WHO and raised concerns about the interference with male reproductive system [35]. A recent study also reported a reduction on sperm number and viability in adult rats exposed to 35 mg/kg of arteunisin for 7 days accompanied by testes and epididymis histopathological alterations [40]. The effects of these drugs on reproductive system remains to be better evaluated.

4. Clinical studies in pregnancy with artemisinin derivatives

It is difficult to compile data from clinical studies because of the huge heterogeneity of methodologies, different dose regimens and standard protocols for treatment of severe or uncomplicated malaria. Many pregnant women are not aware about their own pregnancy and are inadvertently exposed to ACTs to treat malaria episodes, and the miscarriages, which happen especially in this period, may not be detected. The majority of studies are with several ACTs but there were a few studies with monotherapy or both. Another problem is the absence of precise information about gestational time of exposure and different ways to define that. Moreover, the endemic malarial regions have other socio-economic issues, such as the prevalence of many other disorders during pregnancy and a precarious health attendance [53]. Most of clinical trials with antimalarial drugs exclude pregnant women, which makes it difficult to identify adverse effects in this group [54].

The use of artemisinin derivatives is allowed for treatment of malaria during pregnancy in the second and third trimesters. Clinical studies have demonstrated that the use of ACTs in the second and third trimesters is safe for both pregnant women and their babies [55–57]. Over 4000 pregnancies did not show any adverse effects when exposed to these drugs in those trimesters [3]. The same safety information is provided by non-clinical studies on late gestation [44].

The lack of clinical studies and the results from animal studies led WHO to recommend that ACTs not be used in the first trimester of pregnancy [54]. However, the use of artemisinin derivatives, mainly arteunate, is allowed to treat severe malaria in the first trimester due to high rates of mortality, stillbirth and miscarriage [3]. The effective treatment of malaria during pregnancy is evidenced by the prevention of low birth weight caused by this infection [55]. The available information about artemisinin derivatives exposure in the first trimester of pregnancy is in briefed on Table 3.

To this date, no studies in humans can relate exposure to artemisinin derivatives to increased risk of miscarriage, stillbirth
### Table 1
Non-clinical developmental toxicity studies of artemisinin derivatives isolated treatment.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Species</th>
<th>Route</th>
<th>Dosage (mg/kg/day)</th>
<th>Period of treatment</th>
<th>Embryofetal viability</th>
<th>Developmental toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A5</td>
<td>Rat</td>
<td>Oral</td>
<td>6/10/15</td>
<td>GD 7–19</td>
<td>Increased post-implantation losses (including fetal litter loss)</td>
<td>Reduced fetal body weight, skeletal and cardiovascular defects [7].</td>
</tr>
<tr>
<td>A5</td>
<td>Rabbit</td>
<td>Oral</td>
<td>5 to 12</td>
<td>GD 6–17</td>
<td>Increased post-implantation losses at 12 mg/kg</td>
<td>Skeletal and cardiovascular defects on highest dosage [7].</td>
</tr>
<tr>
<td>DHA</td>
<td>Rat</td>
<td>In vitro WEC</td>
<td>0.01 to 2 μg/mL</td>
<td>GD 9.5–11.5</td>
<td>No effect on embryo viability</td>
<td>Exposure for 48 h at 0.05 μg/mL and above led to pale yolk sac, reduced number of erythroblast and inhibition of angiogenesis underlying embryo anatomical abnormalities and cell death [41].</td>
</tr>
<tr>
<td>DHA</td>
<td>Rat</td>
<td>Oral</td>
<td>7.5 and 15</td>
<td>GD 9.5 and 10.5</td>
<td>Increased embryo death on GD 13–20</td>
<td>Mainly skeletal and cardiovascular malformations in surviving fetuses from litters with embryo deaths (C-section on GD 20). Cell death areas and erythroblasts alterations on embryos histological examination before GD 20 [48].</td>
</tr>
<tr>
<td>A5</td>
<td>Rat</td>
<td>Oral</td>
<td>17</td>
<td>GD 10 or 11</td>
<td>77% of embryo death on GD 14</td>
<td>Pale yolk sac, reduction in crown-rump length, erythroblasts depletion and heart abnormalities [42].</td>
</tr>
<tr>
<td>ATM</td>
<td>Rat</td>
<td>IP</td>
<td>1.5, 7 and 15</td>
<td>GD 0–7</td>
<td>No alterations</td>
<td>No effects on fetal body weight and growth rate of offspring until 5 weeks post-natal [43].</td>
</tr>
<tr>
<td>A5</td>
<td>Rat</td>
<td>Oral</td>
<td>10,17 and 30</td>
<td>GD 9–16 single or multiple days</td>
<td>Embryolethality was seen after treatment with single or multiple doses on GD 10–14</td>
<td>GD 10–14 was the sensitive period for cardiovascular malformations and skeletal defects. GD 10 was the most sensitive day for these teratogenic effects. These malformations appeared on litters with partial resorption [44].</td>
</tr>
<tr>
<td>ART</td>
<td>Rat</td>
<td>Oral</td>
<td>7, 35 and 70</td>
<td>GD 7–13, GD 14–20</td>
<td>100% of embryolethality at two highest doses on GD 7–13, and at 70 mg/kg on GD14–20</td>
<td>It was demonstrated a reduction on sperm number on male offspring after in utero exposure to 7 mg/kg/day on GD 14–20 [39].</td>
</tr>
<tr>
<td>DHA</td>
<td>Frog</td>
<td>In vitro</td>
<td>0.01 to 0.5 μg/mL</td>
<td>24 to 72 or 120–168 hpf</td>
<td>No effect on embryo viability</td>
<td>Exposure for 48 h above 0.05 μg/mL showed pale heart with less primitive red blood cells inside and at the highest concentrations (1 and 0.5 μg/mL) major abnormalities, mainly in the heart, have occurred [45].</td>
</tr>
<tr>
<td>A5</td>
<td>Monkey</td>
<td>Oral</td>
<td>4, 12 and 30</td>
<td>GD 20–50</td>
<td>Embryolethality was seen at 12 mg/kg and 30 mg/kg after 12 days of treatment</td>
<td>Surviving embryos showed reduced erythroblasts number, cardiomyopathy and slight reduction on long bones length [46].</td>
</tr>
<tr>
<td>A5</td>
<td>DHA</td>
<td>Oral</td>
<td>15 A5 11.1 DHA 19.4 ATM 20.3 ATE</td>
<td>GD 10</td>
<td></td>
<td>It was observed the same pattern of developmental toxicity for all derivatives: cardiovascular and skeletal abnormalities [47].</td>
</tr>
<tr>
<td>ATM</td>
<td>ATE</td>
<td>Oral</td>
<td>3.5 and 7</td>
<td>GD 0–6, GD 7–14</td>
<td>100% resorptions at 7 mg/kg administrated on GD 7–14</td>
<td>Reduction on fetal body weight and retarded skeletal development but no skeletal malformations were reported when the treatment was in the organogenesis period [48].</td>
</tr>
<tr>
<td>A5</td>
<td>ATM</td>
<td>IP</td>
<td>10/20 A5 8/16 ATM 15/30 ATE</td>
<td>GD 14–20</td>
<td>Whole litter resorption at higher doses</td>
<td>Fetuses exposed to A5 and ATE (10 and 15 mg/kg respectively) presented shortened bones (humerus, femur, tibia, fibula) and a reduction on fetal weight, crown-rump and tail length [49].</td>
</tr>
<tr>
<td>AA</td>
<td>A5</td>
<td>Oral</td>
<td>35/48 A5 17.2–101 AA μmol/kg</td>
<td>GD 12</td>
<td>Embryolethality at higher doses</td>
<td>No external alterations [50].</td>
</tr>
<tr>
<td>A5</td>
<td>AA</td>
<td>Oral</td>
<td>15 and 40</td>
<td>GD 9–11</td>
<td>Embryolethality including whole litter resorptions at 30 mg/kg</td>
<td>Higher incidence of deficient ossification and skeletal malformations, mainly abnormalities on limb long bones and scapulae [38].</td>
</tr>
<tr>
<td>A5</td>
<td>AA</td>
<td>Oral</td>
<td>15 and 40</td>
<td>GD 9–11</td>
<td>Embryolethality</td>
<td>Reduced erythroblasts, pale yolk sac and histopathological abnormalities on embryos mainly on liver and heart [9].</td>
</tr>
<tr>
<td>AA-EE</td>
<td>AA</td>
<td>Oral</td>
<td>2, 4 and 8</td>
<td>GD 6–15</td>
<td>Increased post-implantation losses at 8 mg/kg</td>
<td>Increased visceral and skeletal variations at 4 and 8 mg/kg and reduced female fetal weight at 8 mg/kg [51].</td>
</tr>
<tr>
<td>AA-EE</td>
<td>Rat</td>
<td>Oral</td>
<td>100, 200 and 300</td>
<td>GD 8–19</td>
<td>The highest dose reduced fetal viability</td>
<td>External malformations were observed at highest dose [52].</td>
</tr>
</tbody>
</table>

The studies are presented in chronological order.
A5: artesunate; GD: gestational day; DHA: dihydroartemisinin; WEC: whole embryo culture; ATM: artemether; IP: intraperitoneal; ART: artemisinin; FETAX: Frog embryo teratogenesis assay-xenopus; hpf: hours post-fertilization; ATE: arteether; AA: artelinic acid; AA-EE: A. annua ethanolic extract.
and congenital anomalies as summarized on Table 3. In view of these data, there are a few subjects exposed to these drugs in the first trimester. Some adverse effects are reported, such as miscarriage or a few congenital abnormalities, which are not clearly caused by ACTs. These effects are usually correlated with malaria infection or their incidences are not higher than local population rates. It suggests that more studies are required to verify the safety of ACTs in the first trimester of pregnancy.

5. Artemisinin derivatives mechanisms of embryotoxicity

The first insights about how these drugs cause toxic effects on embryo development around 10 years ago from in vivo and in vitro studies which remark pale yolk sac and reduced number of erythroblasts on rat embryos [8,41,42]. These effects were subsequently confirmed (Table 1). White et al. [42] demonstrated that artesunate induces embryonic erythroblasts death and that the depletion of these cells is not restored, which reduces oxygenation through fetal tissues causing its embryotoxic effects.

The critical period for embryo toxic effects of artemisinin derivatives was recognized as GD 10–14 in rats, and within this short time GD 10 was the most sensitive period for teratogenicity and GD 11 for embryolethality [44]. This sensitive period corresponds to timing when primitive erythroblasts are prominent on fetal circulation in rats produced by yolk sac islands [73]. It corroborates depletion of erythroblasts involvement with the embryo toxic effects observed. Indeed, it was demonstrated by in vitro studies that DHA causes defective and arrested cell division in embryonic erythroblasts followed by apoptosis [74].

Artemisinin derivatives' mode of antimalarial activity is not fully elucidated. It is proposed that these drugs exert their effects by multiple mechanisms, which is very common for herbal products [75]. It has been shown that these drugs need to be activated before their action and this generates ROS. The activation is supposed to be mediated by heme or ferrous iron generating carbon-centered radicals. Activated artemisinin derivatives may damage the parasites directly through oxidative stress and/or interfere with proteins or mitochondrial functions (reviewed by Pandey and Pandey-Rai [75]).

The heme, ferrous iron and oxidative stress involvement with their antimalarial effects may be correlated to the toxic effects to erythroblasts. Erythroblasts and reticulocytes are cells with high heme synthesis in which ferrous iron is required. Both of these cells are artemisinin toxicity targets in animals [76]. Indeed, it was demonstrated that higher concentrations of radiolabeled artesunate were present in tissues involved in hemoglobin synthesis or degradation, such as blood and liver in the fetus [76,77].

Mitochondria are a target for artemisinin derivatives and may be involved in erythroblasts and reticulocytes toxicity due to their role on cell death [76]. An herbicide inhibitor of heme synthesis (S-53492) affects mitochondrial function and increases iron deposits on embryonic erythroblasts leading to a pattern of embryo effects similar to that observed for artemisinin derivatives [78,79]. Since iron is toxic for erythroblasts and also plays a role on the activation of artemisinin derivatives, it is likely that mitochondrial damaged is involved in embryo toxic effects of these drugs [42].

Besides antimalarial actions, artemisinin compounds have emerged as an anticancer option. There are several mechanisms whereby they can act on cancer cells, such as inhibition of angiogenesis or cell differentiation and cell death [29]. The antiangiogenic effect of artemisinin was demonstrated by an in vitro assay using embryonic stem cells. This effect was attributed to oxidative stress, down-regulation of hypoxia-inducible factor-1α (HIF–1α) and vascular endothelial growth factor (VEGF) [80]. It is worth to remember that many anticancer drugs are teratogens and some teratogens became useful tools for anticancer therapy including thalidomide and arsenic [81].

Overall, embryo toxic mechanisms of artemisinin derivatives have probably multiple targets as it happens for their antimalarial [22] or anticancer effects [29]. The better understanding of these mechanisms of embryotoxicity will help to develop new strategies to obliterate this developmental toxicity.

6. Embryotoxicity differences from non-clinical and clinical data

The non-clinical information that circulating primitive erythroblasts are the main target for embryotoxicity of artemisinin derivatives [44,74] emphasizes the importance to consider species
<table>
<thead>
<tr>
<th>Drug</th>
<th>Route</th>
<th>Total dose*</th>
<th>Exposure</th>
<th>Pregnant women</th>
<th>Effects observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS</td>
<td>Oral</td>
<td>840 mg</td>
<td>Treatment of recrudescent infections – inadvertently exposed (GW 3–12)</td>
<td>15</td>
<td>There were 20% (3/15) of spontaneous abortion, which was not considered different from the rate in the general population. 8 followed babies were normal until one year of life [58].</td>
</tr>
<tr>
<td>AS-PSD</td>
<td>Oral</td>
<td>200 mg AS Single dose</td>
<td>Preventive (Accidentally)</td>
<td>77</td>
<td>There were 5% of babies exposed (of 119 in any trimester) with physical abnormalities after birth, including umbilical hernia and undescended testis, but this was not statistically different from unexposed fetuses. No abortions, stillbirths or infant deaths were related to treatment [59].</td>
</tr>
<tr>
<td>ASb</td>
<td>Oral - AS</td>
<td>777 mg – AS 307 mg – ATM</td>
<td>Treatment of confirmed cases most of all as re-treatment (GW 3–12)</td>
<td>44</td>
<td>Abortion rate was 18.9% within the community range (12.3%); All infants were born externally and neurologically normal [60].</td>
</tr>
<tr>
<td>ATM</td>
<td>IM</td>
<td>480 mg</td>
<td>Treatment after failure of chloroquine or quinine (GW 10)</td>
<td>1</td>
<td>There was no abortion, stillbirth or any congenital malformation [62].</td>
</tr>
<tr>
<td>ATM-PSD</td>
<td>IM – ATM</td>
<td>Not reported</td>
<td>Treatment after failure of uncomplicated cases (GW 6–12)</td>
<td>62</td>
<td>Most of the women enrolled received ATM injections and had normal babies followed until one year after birth [63].</td>
</tr>
<tr>
<td>ATM-LM</td>
<td>Other oral</td>
<td>Not reported</td>
<td>Treatment of episodes of fever most of all uncomplicated by diagnostic tests (GW – LMP until 12)</td>
<td>156</td>
<td>The treatment with AL did not enhance perinatal mortality or impair infant neurodevelopment. The incidence of malformations was 6.9% (mostly umbilical hernia) which was not higher than the incidence reported for the area. There was 4.5% of abortion after AL exposure which is not higher than spontaneous abortion rate data [64]. No effects on perinatal mortality or infants development until one year in a prospectiv cohort study [65].</td>
</tr>
<tr>
<td>ASd</td>
<td>Oral</td>
<td>930 mgd</td>
<td>Treatment of a single episode of malaria (GW &lt; 14 weeks)</td>
<td>64</td>
<td>Two miscarriages were reported which was considered a low rate and probably not higher than the incidence in the population. All babies were normal [66]. There was a slightly higher rate of abortion, perinatal mortality, stillbirth and premature delivery after treatment (in all trimesters) that could not be distinguished from the effects of acute malaria itself. No adverse effects to fetuses or newborns [67]. 24 miscarriages (31%) in the group exposed to AS which was not different from quinine or chloroquine treatment. No other adverse effect was related to treatment [68].</td>
</tr>
<tr>
<td>AS</td>
<td>IV-AS</td>
<td>630 mg – DHA in ACT</td>
<td>Treatment of severe malaria (GW not reported)</td>
<td>18</td>
<td>There were 5 cases of miscarriage in women that received DHA-PQ (62.5% of 8 patients) compared with 2.6% (1/38) which received quinine [69]. There were 12.3% of miscarriage/stillbirth but the ACT was not associated with increased risk of any adverse pregnancy outcome. There were 0.6% of congenital anomalies which was not higher than global prevalence [70].</td>
</tr>
<tr>
<td>ATM-LM</td>
<td>Oral</td>
<td>Not reported</td>
<td>Treatment of malaria, it was not mentioned if there were confirmed cases or the severity (GW 3–12)</td>
<td>172</td>
<td>There was no increased risk of miscarriage in confirmed exposure (133 women). No fetal outcome was evaluated [71].</td>
</tr>
<tr>
<td>Any ACT</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Inadvertent treatment of malaria confirmed or not (most of all GW 6–12)</td>
<td>299</td>
<td>There was no increased risk of miscarriage, even when it was considered just the exposure on embryo-sensitive window. There was also no increased risk of any major congenital malformations in comparison to quinine [72].</td>
</tr>
</tbody>
</table>

The studies are presented in chronological order. AS: artesunate; ATM: artemether; PSD: pyrimethamine-sulfadoxine; MQ: mefloquine; CL: chloroquine AT: atovaquone; PG: proguanil; LM: lumefantrine; GW: gestational weeks; LMP: last menstrual period; DHA: dihydroartemisinin; PQ: piperaquine; IV: intravenous. IM: intramuscular.

* The mean total dose was reported by the authors or corresponds to the amount of all dosage/day received per woman, if it was described in mg/kg it was considered as a person of 70 kg.

** Artesunate or artemether were administrated alone or in combinations, different dose regimens were used and they were classified together as primary or re-treatment (43 and 57% of patients on first trimester, respectively).

† It was considered the dosage recommended for an adult at the label of Coartem® (Novartis), since the authors mentioned that its recommendations was followed.

‡ AS was administered as monotherapy to 21 women and the others were exposed to different ACTs, one with DHA and all other combinations were with AS. The authors mentioned that the women received a range from 12 to 16 mg/kg as total dose of artesunate according to the severity of malaria.

§ In this number it is considered confirmed and unconfirmed exposed women.

¶ 183 women received any artesinin derivative alone (AS) or in different combinations to initially treat a falciparum malaria episode in the first trimester and 129 received an artesinin derivative (mostly AS or AS-CL) in the first trimester after quinine failure. In this number can be included data from other previously published studies in the same area [60,61,68].
Fig. 2. Differences between non-clinical and clinical studies about the safety of artemisinin derivatives use during pregnancy. The presence of malaria infection may imply in more activation of artemisinin derivatives (ART) by heme, which is provided mainly by parasite hemoglobin digestion, generating carbon-centered free radicals that are highly reactive molecules [20,27]. These radicals can covalently bind to several parasite proteins alkylating them [22] and consequently being less available to pass to the embryo. In agreement with this hypothesis, it was shown that ART are more concentrated inside infected erythrocytes (IE) than in non-infected erythrocytes (NIE) [22,28]. The mechanism of embryotoxicity of ART involves the depletion of circulating embryonic primitive erythroblasts [44,74]. These cells are produced over approximately two days and are circulating from gestational day (GD) 10–14 in rats [44,83]. These target cells are formed over a longer period of time, from 3 to 6 weeks of gestation, and they are circulating from 4 to 9 weeks of gestation in humans [76,83]. Thus, considering a short treatment period (3–7 days [3]) in this estimated sensitive window for humans, the ART damaged cells may be replaced by newly formed primitive erythroblasts and the consequences to the fetus could be not as dire.

In mammals primitive erythroblasts are formed in the blood islands of the yolk sac and are the first red blood cell produced by the embryo. The progenitors of these cells in rodents are present in the yolk sac for around 48 h and primitive erythroblasts are released into circulation (around GD 8.5 in mice and GD 10 in rats) where they proliferate by cell division until the liver starts definitive erythropoiesis [around GD 13 in mice and GD 14.5 in rats] [74,82,83]. The sensitive period for developmental toxicity of artemisinin derivatives in rats coincides with the period when this susceptible embryonic cell population is circulating [44,74].

In humans primitive erythroblasts are formed by the yolk sac between 3–6 weeks of gestation and are circulating in the embryo around 4–9 weeks of gestation (reviewed by [82]), this latter is the supposed sensitive period for toxicity to artemisinin derivatives [76]. Thus, if these sensitive cells are formed over a longer period of time, which means longer than the typical treatment period (3–7 days [3]), then damaged cells would be replaced by newly formed cells, and the consequences might not be as dire [84]. Supporting this idea recent findings indicate that less mature forms of primitive erythroblasts (type I and pre-type I, such as the cells found in the yolk sac) are less sensitive to DHA, than circulating type II or III primitive erythroblasts [74]. This hypothesis, which were raised by Clark [84], could explain why in non-human primates, treatment with greater than 12 mg/kg of artesunate for 12 days was necessary to induce embryolethality and teratogenicity [46]. Therefore, dose and time of exposure may be differently relevant for humans. However, it should be taken in account that during nine months of pregnancy a women can get sick in endemic areas more than once.

All developmental non-clinical studies with artemisinin derivatives until now have been conducted in non-infected animals. Meanwhile, almost all clinical studies have been conducted in malaria infected pregnant women or unconfirmed cases of women exposed without knowing their pregnancy. Due to obvious ethical issues they do not have non-treated infected groups as well as non-infected treated pregnant women as controls. There is scanty information about the exposure to these drugs during the first trimester of pregnancy because of WHO use restriction [3]. These are some of the difficulties to compare data from non-clinical and clinical studies and to define the risks for pregnant women and their fetuses.

Could the parasite changes on the host alter artemisinin derivatives toxicity? It was demonstrated less toxicity of artesunate in infected than in non-infected animals [85]. As reviewed by Clark [76] the reduction on reticulocytes caused by artemisinin derivatives was also lower in malaria patients. Nevertheless, there is no information about this reduction on embryo toxic effects but it is reasonable to think that it could happen [76].
Furthermore, experimental malaria infection alters amino acid transplacentl transfer and drug transporters [86,87]. This can alter kinetics of antimalarial drugs and needs to be considered on ACTs toxicity evaluation. The effects of pathophysiological changes on embryotoxicity of artemisinin derivatives have not been evaluated so far. These alterations underlying malaria during pregnancy could be one reason for the differences on data from developmental toxicity between non-clinical and clinical studies about developmental toxicity of artemisinin derivatives.

Some possible theories for the differences in sensitivity for embryo-fetal developmental toxic effects of artemisinin derivatives observed in non-clinical and clinical studies are summarized in Fig. 2. Briefly, if the human embryos are susceptible to the toxic effects of these drugs but the time of exposure is shorter than the period when the target cells (primitive erythroblasts) are being formed, it should be expected that these cells can be restored without too harmful effects for the fetus. Furthermore, the higher concentration of artemisinin derivatives in infected erythrocytes [28], and perhaps more activation and consequently bind to parasite proteins [22], could lead to less distribution of drug to the embryo. Taken together, the consequences for the fetus of pregnant infected women treated with artemisinin derivatives could be significantly different from the ones observed in experimental uninfected animals.

Finally, until now there is no evidence about embryo toxic effects of these drugs in humans. Large clinical trials and the appropriate evaluation of inadvertent exposure in the first trimester of pregnancy are highly necessary. This will bring concise information about the safety of artemisinin derivatives in the critical sensitive period for toxicity. Besides that, there are many difficulties to assess information in pregnant women, thus, non-clinical studies with infected animals might help evaluate the effects of the infection and treatment together.

7. Conclusions and perspectives

There are a lot of gaps between non-clinical and clinical studies about developmental toxicity of artemisinin derivatives. Studies have several differences and it is very difficult to compare them and define the potential risks of the use of these drugs in the first trimester of pregnancy. Besides that, the risks of malaria in pregnancy seem to be higher than the ACTs adverse effects, and the effective treatment of the pregnant women is important to prevent malaria recrudescence. The mechanisms of embryo toxicity are not completely understood, but might not be so relevant for humans, considering the short time of treatment (3–7 days) compared with the longer period of target cell formation in the human embryo (~3 weeks). Apparently malaria infection might alter artemisinin derivatives activation and trap these drugs into the parasited erythrocyte, altering its distribution to embryo and consequently its embryo toxic effects. Further experimental studies using infected animals could be used to test this latter hypothesis. Large clinical trials and pharmacovigilance of pregnant women who receive these medications are essential. Thus, more information is needed to definitively allow artemisinin derivatives use during the whole pregnancy period.

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