

# Progress against Malaria, the Role of Antimalarial Drugs, and Their Use in Pregnancy

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## Disclosure Slide

The speaker has no financial or other interests which pose a conflict of interest.



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# 1. Malaria



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## Two Most Important Species of *Plasmodium* that Cause Malaria in Humans

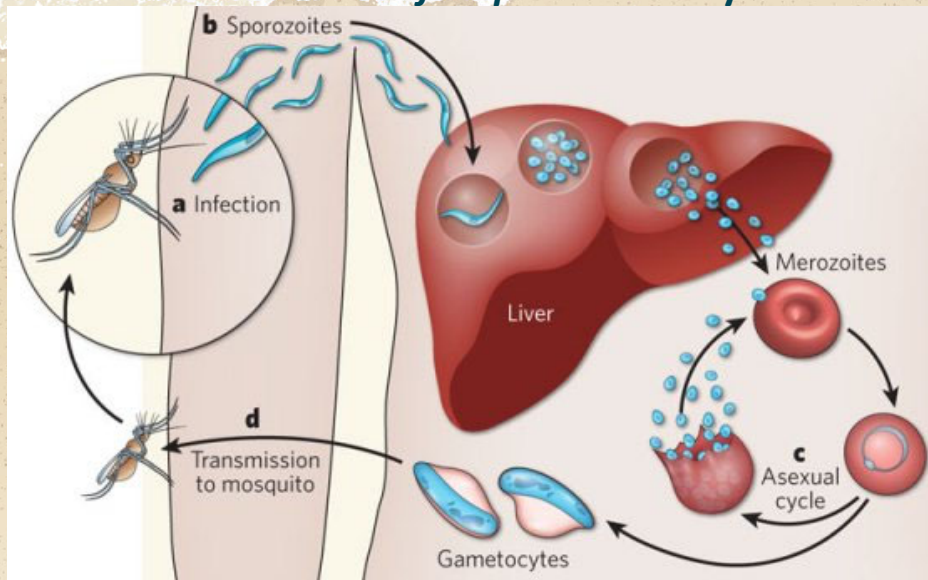
- *Plasmodium falciparum*
  - Can infect all red blood cells (RBCs)
  - Extensive adhesion of infected RBCs (IRBCs) to the walls of blood vessels, RBCs, and platelets
    - Sequestration in the microvasculature
    - Avoids passage through the spleen
  - Causes the most severe disease and the most deaths
- *Plasmodium vivax*:
  - Selectively infects reticulocytes and the youngest 1/7<sup>th</sup> of red blood cells
  - Less sequestration



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## *Plasmodium falciparum* Life Cycle



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## Important Features of Blood Stages of *P. falciparum*

- Hemoglobin degradation leading to the generation of free heme
- Some of the heme formed into hemozoin (malaria pigment)
- When the schizonts burst to release new merozoites, they also release hemozoin, cytokines and other toxic factors
  - Fever, chills, impaired consciousness
- Most antimalarials act primarily on the blood stages, e.g.:
  - Agents that block the formation of hemozoin
  - Agents that react with heme

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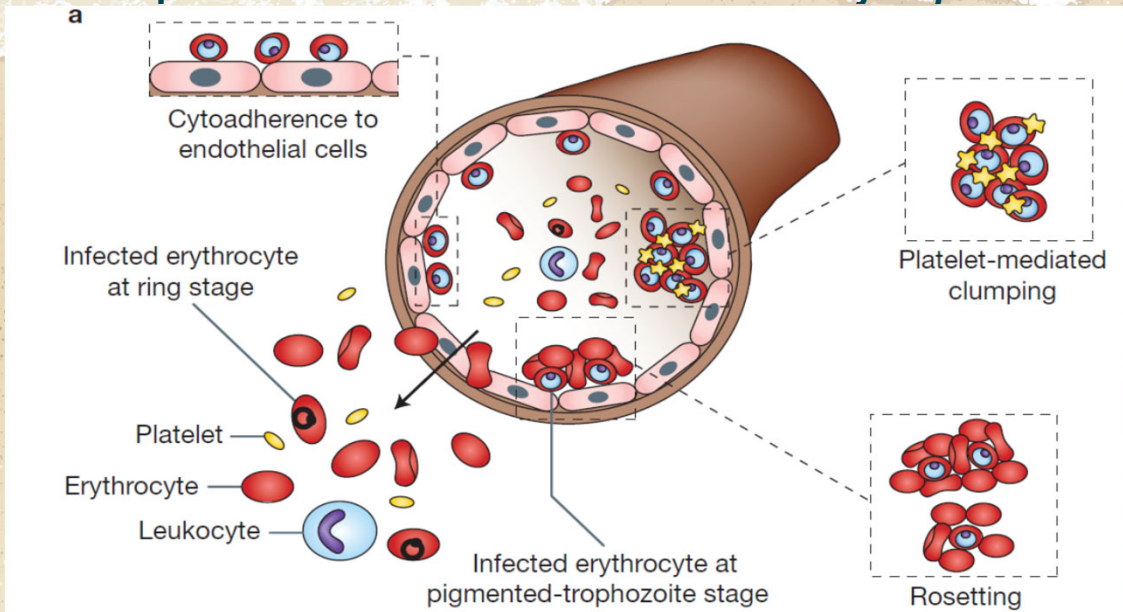
## Adhesion and Sequestration

- *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) receptors
  - Form clusters (knobs) on the cell surface of the IRBC
  - Multiple binding domains, most common:
    - Except for pregnant women:
      - The cysteine-rich interdomain region 1 (CIDR1) of PfEMP1 binds to:
        - » the host CD36 glycoprotein receptors
    - Additional binding in pregnant women:
      - The Duffy binding-like (DBL)<sub>γ</sub> domain of PfEMP1 binds to:
        - » Chondroitin sulfate A (CSA) proteoglycans in the intervillous space of the placenta and on the surface of the syncytiotrophoblast
- Infected RBCs are PfEMP1-specific (e.g. not bind to both CD36 and CSA)
  - Can be switched at each new asexual blood stage cycle allowing the parasite to adapt to new conditions (e.g. pregnancy)



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## Sequestration in Microvasculature with *P. falciparum*



(from Rowe et al., 2009)

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## Microcirculatory Obstruction and Immunity

- Sequestration leads to:
  - Microcirculatory obstruction
  - Impaired tissue perfusion leading to local hypoxia
  - Impact on bone marrow + increased removal of circulating red blood cells
    - Leads to anemia (e.g. ~7-32% decreases in hemoglobin/hematocrit in uncomplicated malaria)
- Leukocytes are attracted to the sites of sequestration and contribute to the congestion.
- Immunity:
  - Antibodies develop that block the binding of infected RBCs to the host receptors on the endothelium, other RBCs and platelets.



Based on Ian Clark et al., 2006

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## Placental Malaria and Immunity

- Infected RBCs that can bind to chondroitin sulfate A proteoglycans develop rapidly
  - Become sequestered in the placenta
  - Leads to decreased uterine artery blood flow and low birth weight
  - Most pronounced with first pregnancy
    - Diminishes with subsequent pregnancies due to antibodies that block the binding to chondroitin sulfate A proteoglycans
- Immunity obtained prior to first pregnancy does not protect against placental malaria

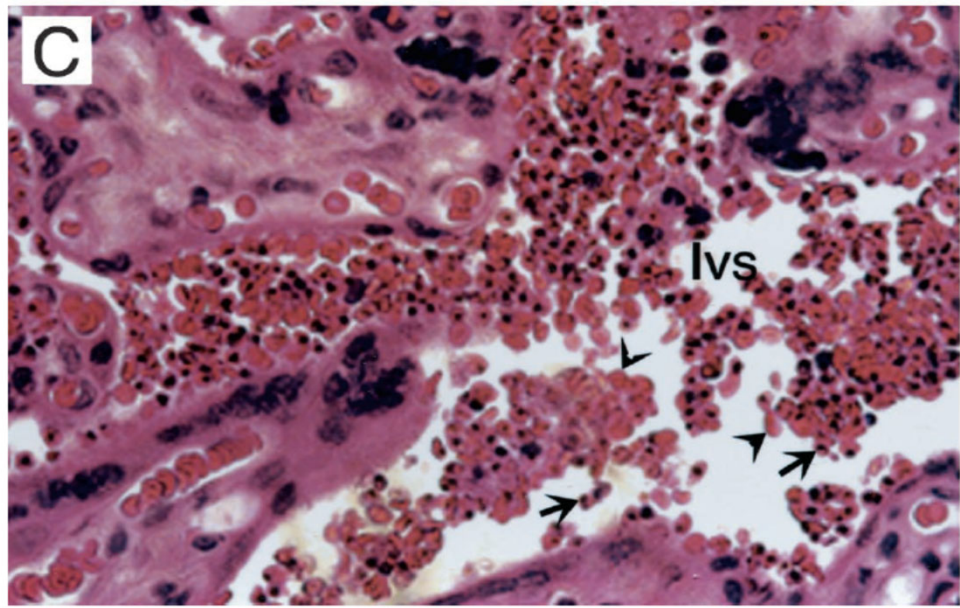


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## Sequestration in the placenta

-  
Ivs: intervillous space  
→: IRBCs  
➤: RBCs



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From Muthusamy et al, 2004

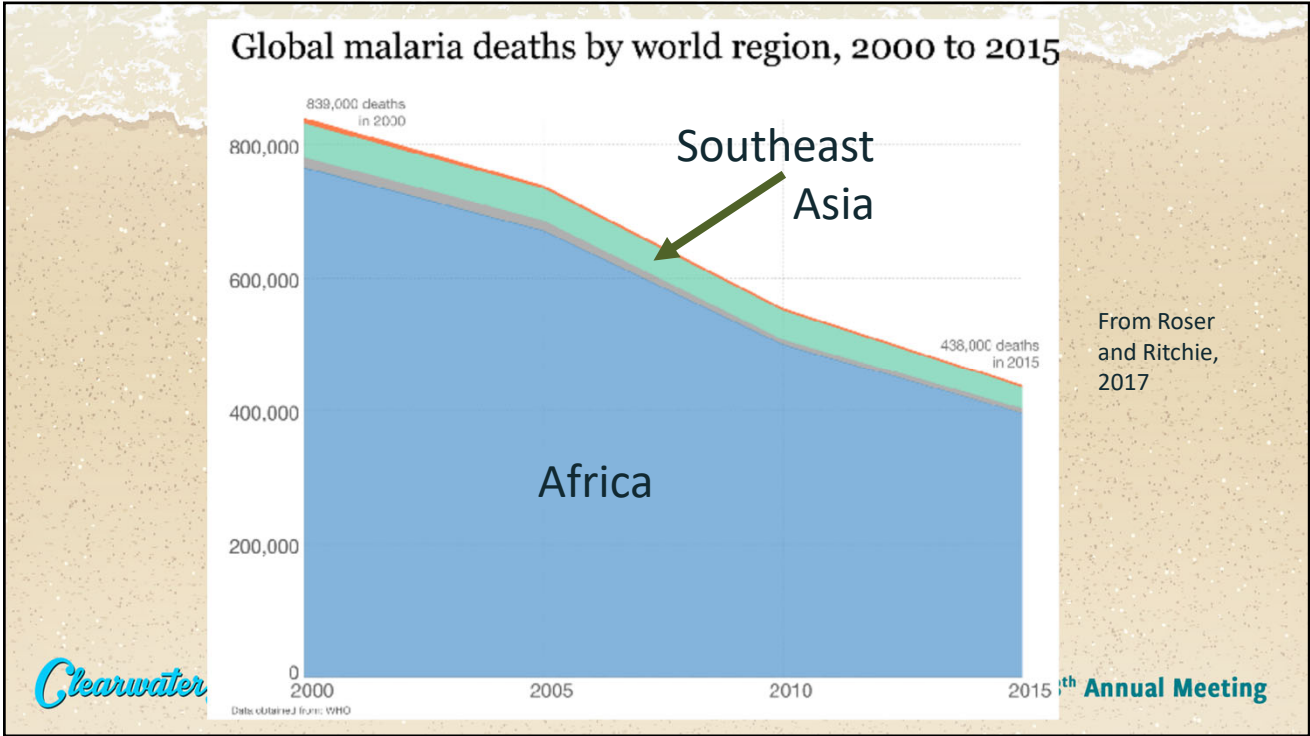
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## Malaria-Related Mortality

- Most sensitive populations (due to lack of immunity):
  - Children
  - Pregnant women
- On the decline

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- ### Causes of the Decline in *P. falciparum* in Africa
- Causes of the decline in prevalence in *P. falciparum* in Africa between 2000 and 2015:
    1. Insecticide-treated bed nets (68% responsible)
    2. Artemisinin-based combination therapy (19% responsible)
      - Combinations of an artemisinin and 1 or 2 non-artemisinin partner drugs
        - Known as ACTs
      - To be discussed further
    3. Indoor residual insecticide spraying (13% responsible)
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## Notes on Discussion of Studies

- Pregnant animal studies:
  - Most data from studies of the separate antimalarials, not from ACTs.
- Exposure ratios (“safety margins”):
  - Exposure at developmental no effect level (dNOEL) in animals/exposure at therapeutic dose in humans
  - AUC and Cmax ratios when possible
  - Human equivalent dose (HED) ratios
    - Calculated HED based on 2005 FDA Guidance, e.g.:
      - Divide rat dose by 6.2;
      - Divide rabbit dose by 3.1.



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## 2. Quinine



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## Quinine

- Used as a treatment for malaria since the 1600s
- The 1<sup>st</sup> WHO Guidelines for the Treatment of Malaria in 2006 recommended quinine, together with clindamycin (where available), for malaria in the first trimester.
  - Therapeutic dosage regimen: 10 mg/kg quinine twice daily for 7 days (about 1 g/day).
- Quinine and other quinoline antimalarials are thought to act by inhibiting the formation of hemozoin



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## Quinine in Pregnancy

- Quinine previously used to cause contraception and abortion and to induce labor.
- Embryoletality observed in rabbits, chinchillas, mice and dogs at HED ratios of 0.4 to 1.6.
- Brain and/or inner ear abnormalities:
  - Rabbits, chinchillas and guinea pigs at HED ratios of 0.025 to 2;
  - Humans: 37 case reports of single doses of 1 to 6 g p.o. quinine in the first trimester causing congenital anomalies of the inner ear, eyes and brain.
- Clinical studies involving about 1000 first trimester women treated for malaria have not revealed any effect on congenital anomalies.



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### 3. Artemisinin-Based Combination Therapy (ACTs)



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#### History of Artemisinins

- Extracts of the plant sweet wormwood (*Artemisia annua*) had been used effectively against malaria since at least the 4<sup>th</sup> century A.D.
- In 1972, the active substance (now called artemisinin) was isolated by a group in China.
- It was fast-acting, highly efficacious and, for decades, resistance was slow to develop.

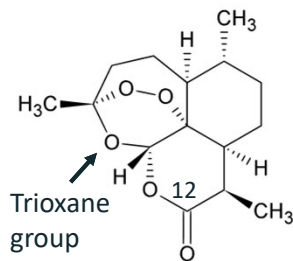


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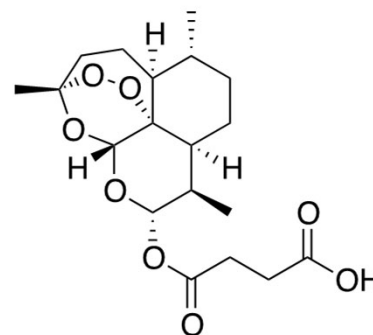


## Structures of Artemisinins

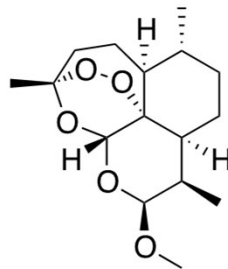
- 1,2,4-Trioxanes
- Three 12-carbon derivatives are commonly in ACTs
- Dihydroartemisinin (DHA) is the primary metabolite for artesunate and artemether (artesunate is a prodrug for DHA)



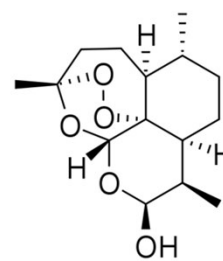
Artemisinin (12-carbon ketone)



Artesunate (succinyl ester)



Artemether (methyl ether)



Dihydroartemisinin (12-C-hydroxy)

## The Role of Heme in the Antimalarial Activity of Artemisinins

- Artemisinins react with heme:
  - The endoperoxide group of artemisinins is reduced by an electron contributed by the ferrous iron of heme to form carbon-based radicals that can alkylate proteins.
  - The extent of heme-mediated alkylation activity is highly correlated with *in vitro* antimalarial activity.
- Within *P. falciparum* parasites, artemisinins alkylated more than 40 proteins involved in multiple metabolic pathways in multiple parts of the cell:
  - Digestive vacuole
  - Mitochondria
  - Cytoplasm



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## History of Artemisinin-Based Combination Therapy (ACTs)

- 2001: The World Health Organization (WHO) recommended combinations of drugs
- 2006: First “WHO Guidelines for the Treatment of Malaria”:
  - Recommended artemisinin-based combination therapy (ACT) as one of the types of combinations.
    - Artemisinins reduce parasite numbers by 10,000-fold in each asexual cycle (~48 h) compared with 100- to 1000-fold for other antimalarials.
    - Artemisinins are eliminated rapidly and, when combined with a slowly eliminated antimalarial, the course of effective treatment is 3 days



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## ACTs (continued)

- 2010:
  - 2<sup>nd</sup> edition of the WHO Guidelines
  - These 5 ACTs strongly recommended for uncomplicated malaria for all except women in the first trimester:
    - Artemether + lumefantrine
    - Artesunate + amodiaquine
    - Artesunate + mefloquine
    - Artesunate + sulfadoxine + pyrimethamine
    - Dihydroartemisinin + piperaquine (2015)
- Artemisinins were not recommended for 1<sup>st</sup> trimester due to animal findings.
- WHO is now in the process of changing to a recommendation to use ACTs for uncomplicated malaria in the first trimester.



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## 4. Artemisinin Partner Drugs in Pregnancy



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### Developmental Toxicity and Exposure Ratios for Quinoline Partner Drugs

Quinoline	First Trimester Exposures		Effects	Rat			Rabbit			
				Exposure ratio at dNOEL			Exposure ratio at dNOEL			
	Infected	Prevention		HED	C <sub>max</sub>	AUC	Effects	HED	C <sub>max</sub>	AUC
Mefloquine	79	1194	ED, M <sup>a</sup>	0.13	-	-	ED	0.08	-	-
Amodiaquine	11	0	↓FW	0.5	0.17	0.11	-	0.7	1.0	1.1
Piperaquine	8	0	↓FW	0.17	-	0.3	-	1.4	-	0.15

<sup>a</sup>Also mice, HED ratio = 0.07

ED: Embryonic deaths; M: Malformations ↓FW: Decreased fetal weight

- Mefloquine was a selective embryotoxicant in rabbits and mice.



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## Sulfadoxine + Pyrimethamine

- Both are inhibitors of the biosynthesis of tetrahydrofolate
  - Sulfadoxine is a sulfonamide that inhibits dihydropteroate synthetase
  - Pyrimethamine is an inhibitor of dihydrofolate reductase (DHFR)
- Some antifolate agents are thought to cause malformations at therapeutic doses in humans, for example, aminopterin and methotrexate
- Pyrimethamine with or without sulfadoxine caused:
  - Embryo deaths and malformations in rats (HED ratio  $\cong$  0.1; AUC ratio  $\cong$  0.02) and
  - Embryo deaths in rabbits (HED ratio  $\cong$  4)
- Currently, artesunate + sulfadoxine + pyrimethamine has been eliminated as an ACT for use in the first trimester.



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## Embryofetal Development Studies of Lumefantrine

- Rat:
  - Decreased maternal body weight gain and litter size at 1000 mg/kg/day
  - At dNOEL (300 mg/kg/day): HED ratio = 2.5
- Rabbit:
  - Decreased maternal food consumption at 1000 mg/kg/day
  - No developmental effects at any dose
  - At dNOEL (1000 mg/kg/day): HED ratio = 17; AUC ratio = 3.5; Cmax ratio = 4.6
- Most favorable profile among all of the non-artemisinin ACT partner drugs
- Artemether + lumefantrine is the ACT with the most first trimester human data (no adverse effects seen in >500 patients)



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## 5. Artemisinins in Pregnancy



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### Embryofetal Developmental Toxicity Studies of Chlorproguanil, Dapsone and Artesunate (CDA)

- Artemisinins and ACTs were in development by 1995.
- In 2001, a Product Development Team (including GSK) formed to develop an ACT
  - Chlorproguanil, Dapsone and artesunate (CDA).
- The team sponsored embryofetal development studies of CDA
- Remarkable embryotoxicity was seen with CDA which triggered studies of artesunate alone.



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## Embryofetal Developmental Toxicity Studies of Artesunate

- At all doses in both rat and rabbit EFD studies (with dosing throughout organogenesis):
  - No maternal toxicity observed
  - Cardiovascular and skeletal malformations
  - Increased embryo death, e.g.:
    - Rats - at 16.7 mg/kg/day: 21 of 24 litters with total litter loss (0 in control)
    - Rabbits - at 12 mg/kg/day: 3 abortions (none in control), 2 does with total litter loss (none in control) and a 14% postimplantation loss (6% in control).
- GSK then conducted a series of studies to investigate the embryotoxicity of artemisinins which was then followed by additional studies elsewhere.



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## Contributors

### GSK:

Tacey White  
Susan Laffan  
Lorrie Posobiec  
Paul Bushdid  
Estella Cox

### GSK (Cont):

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Howard Solomon  
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SNBL-USA:  
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### Charles River:

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### Novartis:

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Maureen Youreneff



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## Findings in Subsequent Studies of Artesunate in Rats

- Embryotoxicity is due to a depletion of circulating primitive erythroblasts
  - Leading to marked embryonic anemia.
- Most sensitive period: GD 10 to 14.
- Affected erythroblasts were characterized by an intracellular accumulation of insoluble deposits of hemosiderin (an iron storage complex)
  - Revealed by Prussian Blue staining
- Only maternal effects detected were decreases in reticulocyte count which were correlated with incidences of embryo death.



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## Class Effect of Artemisinins in Rats

- Following a single oral dose to rats on GD 10:

	Control	39 $\mu\text{mol/kg}$ Artesunate	39 $\mu\text{mol/kg}$ DHA	65 $\mu\text{mol/kg}$ Artemether	65 $\mu\text{mol/kg}$ Arteether
Group size	23	25	25	25	25
Total litter loss	0	12	7	11	15
% Postimplantation Loss	5.7	69.7	55.4	68.5	73.6
% Ventricular septal defect (Litter Mean)	0	31.0	36.9	58.3	31.3



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## Artesunate Studies in Cynomolgus Monkeys

- EFD Study: Dosing on GD 20 to 50:
  - At 30 mg/kg/day, 6 of 15 embryos died between GD 30 and 40 and dosing was discontinued.
    - Three live embryos collected on GD 26, 32 and 36 had a marked reduction in embryonic erythroblasts, thin cardiac walls and enlarged atria.
  - At 12 mg/kg/day, 6 of 15 embryos died between GD 30 and 45.
  - 4 mg/kg/day: dNOEL
  - Reticulocyte counts on GD 31 compared to GD 19:
    - 4 of 9 females in the 30 mg/kg/day group and 1 of 11 in the 12 mg/kg/day had >90% decreases in reticulocyte count
- Follow-up study with dosing for 3 days (GD 29 to 31) or 7 days (GD 27 to 33):
  - At 12 mg/kg/day, there was no developmental toxicity.



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## Comparison of Artemisinin Exposure Ratios (Oral Studies)

Species	Dosing Period	Exposure Ratio	Artesunate	Artemether	DHA
Rat	GD10	Rat HED ratio at ~equivalent highly embryotoxic dose	0.6	1.0	0.75
		Estimated AUC Ratio at dNOEL (parent + DHA)	0.03	1.0	-
Rabbit	GD 7-19	HED Ratio	<0.4 (at dLOEL)	2.5 (at dNOEL)	-
Monkey	GD 27-33	HED Ratio at dNOEL	1.0	-	-
		AUC Ratio at dNOEL	0.02	-	-



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## In vitro Studies of Artesunate and DHA

- DHA in rat whole embryo culture (WEC):
  - The target was the circulating primitive erythroblasts
  - DHA induced apoptosis of circulating erythroblasts which is suggestive of an effect on the mitochondria
  - An increasing number of Type 1, primitive proerythroblasts were introduced into circulation and were apparently not very sensitive to DHA
- Cultures of blood cells from GD 12 and 14
  - Fluorochrome (BODIPY)-labeled artesunate was localized to the mitochondria
  - Mitochondria lost activity during a 2-h incubation period (MitoTracker Red CMXRos)



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## Autoradiography Studies of Artesunate in Rats

- GD 18:
  - Accumulation of radioactivity in sites of high heme content:
    - The bone marrow and spleen among maternal tissues
    - Fetal blood and liver concentrations were 3.8- to 8.8-fold higher than maternal blood levels at all timepoints (1, 6 and 24 hours postdose).
- GD 11:
  - Erythroblasts were only embryonic tissue labeled



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## First Trimester Exposures to Artemisinins and ACTs in Women Treated for Malaria

	1 <sup>st</sup> Trimester Exposures
Artesunate alone	
• Oral	99
• IV/IM	58
ACTs (Oral)	
• Artemether + Lumefantrine	540
• Artesunate + Mefloquine	71
• Artesunate + Amodiaquine	40
• Artesunate + Sulfadoxine + Pyrimethamine	88 (77 uninfected)
• Dihydroartemisinin + Piperaquine	16
Total	912

- No convincing evidence of adverse effects on pregnancy



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## Caveats about Clinical Studies

- Clinical studies presented here:
  - Include retrospective studies, studies with no internal comparison groups, and sometimes case reports
  - No follow-up beyond birth
- Interpretations of negative clinical studies:
  - EMEA, 2008:
    - N = 300 1<sup>st</sup> trimester exposures → <10-fold increase in overall malformations
    - N = 1000 1<sup>st</sup> trimester exposures → <2-fold increase in overall malformations
  - Dellicour, 2017 (overall background rate 0.9% malformations):
    - N = 1180 1<sup>st</sup> trimester exposures → <2-fold increase in overall malformations
    - N = 10,748 1<sup>st</sup> trimester exposures → <2-fold increase in a specific malformation with a background rate of 1/1000



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## 6. Artemisinin's Mechanism of Embryotoxic Activity



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### The Effects of the Inhibition of Heme Biosynthesis

- The last three steps of heme biosynthesis occur in the mitochondrion
  - The last step is the insertion of ferrous iron ( $\text{Fe}^{++}$ ) into protoporphyrin IX
    - Ferrochelatase (Fech)
- Fech forms an oligomeric mitochondrial inner membrane complex with:
  - The iron importer protein mitoferrin-1 (Mfrn1)
  - The ATP-binding cassette transporter protein (Abcb10)
  - Facilitates the importation of ferrous iron for heme biosynthesis.
- Mitoferrin-1 and Abcb10 are required for normal heme biosynthesis.
  - Mouse mitoferrin-1 or Abcb10 knockouts:
    - Embryos with severe anemia, midgestation death and, with the Abcb10 knockout, hemopoietic cells stained positively for Prussian blue



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## The Effects of the Inhibition of Heme Biosynthesis (Cont.)

- Effects of a heme biosynthesis inhibitor (S-53482) in rats on GD 12:
  - Anemia
  - Embryoletality
  - Thin ventricular walls
  - Ventricular septal defects, and
  - The accumulation of Prussian Blue-stained deposits in circulating erythroblasts.
- Conclusions:
  - Inhibition of heme biosynthesis causes artemisinin-like effects



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## Hypothesis: Artemisinins Inhibit Heme Biosynthesis by Alkylating Ferrochelatase

- Artemisinins localize to the mitochondria within the circulating erythroblasts
- Artemisinin is activated by reaction with heme to form carbon-centered free radicals
  - Short lifespan (a few nanoseconds)
  - Can move about 8-14 Å (heme diameter ~60 Å)
- Thus, the artemisinin, the ferrous iron within heme, and a polypeptide target must all be in close proximity.
- Heme is close to polypeptide in the active site of ferrochelatase
- Hypothesis: Artemisinins can access the active site and react with heme there

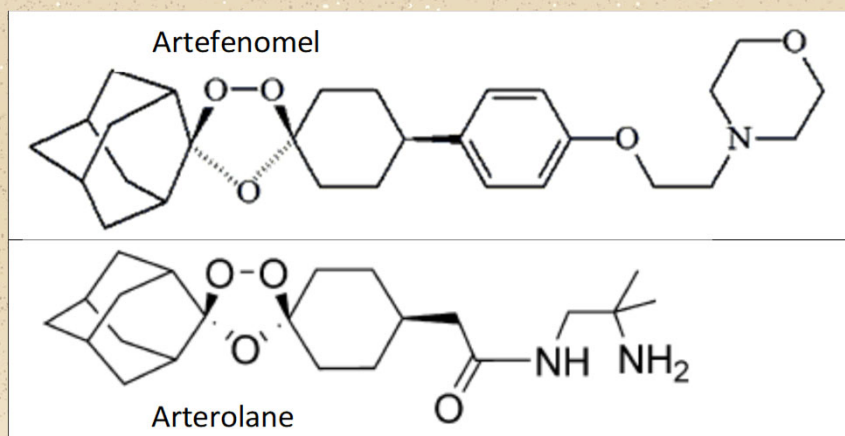


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## Trioxolane Antimalarials

- 1,2,4-Trioxolane 5-membered ring with endoperoxide group
- 1 or 2 bulky side groups
- Blood half-lives:
  - Arts: 0.5–2 h
  - Arterolane: 3.3 h
  - Artefenomel: 25–30 h



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## Embryotoxicity of Artefenomel and Arterolane

- Rat whole embryo culture (WEC):
  - Both artefenomel and arterolane caused DHA-like effects
  - “In vitro safety margin” (NOEL in WEC/IC<sub>50</sub> vs *P. falciparum in vitro*):
    - Arterolane: 12-fold greater than DHA
    - Artefenomel: 259-fold greater than DHA
- Single-dose study on GD 12 – artesunate and artefenomel:
  - Artefenomel:
    - Artesunate-like effects including embryonic anemia
    - AUC ratio ~100-fold greater than artesunate
- Bulky side groups restrict embryotoxic activity but not antimalarial activity
  - The two can be separated

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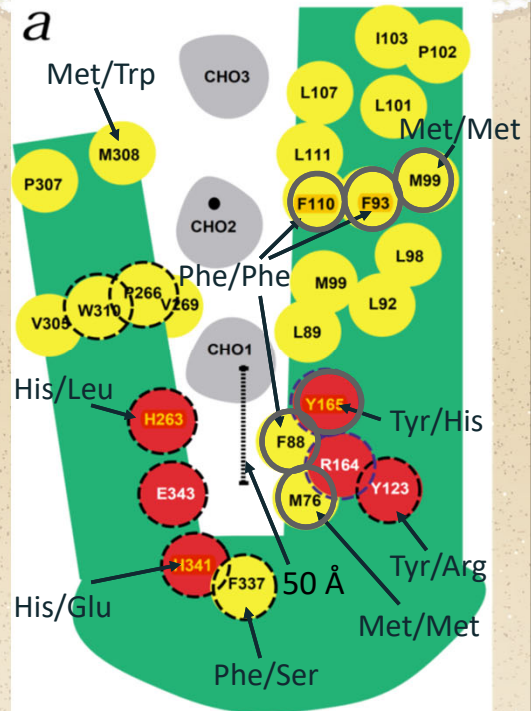
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## Human/Mouse Fech Active Site - Possible Targets

- Protoporphyrin IX + Fe<sup>2+</sup> → Heme B
- Residues with C=C or Met:
  - Mouse: 6 (solid circles)
  - Humans: mouse 6 + 5 more
- Hypothesis: DHA can access this active site more readily than artefenomel
  - Yellow – hydrophobic residues
  - Red – hydrophilic residues



From Wu et al., 2001



## 7. Discrepancy between Animal and Human Results for Antimalarials and Some Possible Explanations



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## The Findings from Animal Developmental Toxicity Studies Have Not Predicted the Human Findings

- There have been a significant number (>300) of recorded exposures in the first trimester of women with malaria for these 5 therapies:
  - Quinine
  - Mefloquine
  - Sulfadoxine + pyrimethamine
  - Artesunate alone or in an ACT
  - Artemether + lumefantrine
- All have exposure HED ratios <1 in at least one species and three (pyrimethamine, artesunate and artemether + lumefantrine) have AUC ratios  $\leq 1$  in at least 1 species
- With the possible exception of the administration of quinine to uninfected women, there is no good evidence of adverse effects in the first trimester in humans.



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## Possible Reasons for Different Animal and Human Results

- Animal Model Study Design
  - Treatment throughout organogenesis compared to therapeutic regimen of 3 days (approximately 1/17<sup>th</sup> of the human period of organogenesis)
- Physiological differences between animals and humans
  - Animal targets may not exist or be altered in humans
  - For artemisinins, the longer duration of primitive erythroblast production in the visceral yolk sac in humans (about 4 weeks)
    - May allow the embryo to recover from a 3-day depletion of circulating erythroblasts
    - In rats, it seems that the progenitor cells in the VYS (Type I or earlier) are not sensitive to artemisinins



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## Possible Reasons for Different Animal and Human Results - Sensitive Periods and Periods of Clinical Exposures

- Artemisinins
  - Based on animal studies, it is estimated that the putative sensitive period for in humans could be between:
    - Postconception day 21 to 23 (heartbeat)
    - Postconception days 60 to 70 (shift from primitive to definitive erythroblasts)
  - But, if there is a sensitive period, it could be much shorter
- Issues with studies:
  - First trimester exposures lumped together.
  - Most first trimester exposures occurred late in the first trimester.
  - May have included few exposures during sensitive period(s)



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## Animal Models Not Infected with Malaria – Does Malaria Protect Against Artemisinin Toxicity? - Bone Marrow

- More severe reticulocytopenia in healthy people than in people with malaria:
  - Uninfected: 5 of 6 groups had 60-75% decreases in reticulocytes
  - Patients: Mean decrease in 12 groups was 0 to 34% (most slight or no reduction)
- Causes:
  - Artemisinins accumulate in infected RBCs : ↑ >150-fold (vs uninfected)
  - Microcirculatory obstruction
- Volume of distribution: Healthy – 3.02 L/kg; infected 1.33 L/kg
  - Drug in plasma and RBCs ↑
  - Other tissues: ↓
- Interpretation: Reduction in bone marrow exposure → reduced reticulocytopenia



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## Animal Models Not Infected with Malaria – Does Malaria Protect Against Artemisinin Toxicity? - Embryo

- Sequestration in placenta leads to ↓ uterine artery blood flow and low birth weight
- Artemisinins accumulate in infected RBCs >150-fold (compared to uninfected)
- Sequestered RBCs that trap artemisinins could form a partial barrier that retards the transfer of artemisinins to the embryo.
  - Quinine and amodiaquine also become highly concentrated in infected RBCs
- Important since:
  - Pregnant women without malaria would be at increased risk due to artemisinins
  - In large parts of tropical Africa, malaria treatment is based on fever alone
    - In one study, 63% of 18,000 treated patients were uninfected



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## Backup Slides



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## Topics to be Covered Today

1. Malaria
2. Quinine
3. Artemisinin-Based Combination Therapy (ACTs)
4. Artemisinin Partner Drugs in Pregnancy
5. Artemisinins in Pregnancy
6. Artemisinins' Mechanism of Embryotoxic Activity
7. Discrepancy between Animal and Human Results for Antimalarials and Some Possible Explanations



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### *P.* *falciparum* Life Cycle

Female Anopheles Mosquito	Human Host	
	Bloodstream	Liver (Hepatocytes)
Sporozoites →	Sporozoites →	Grow, multiply into schizonts that each releases ← 1000's of merozoites
↑	↑	<b>Red blood cells</b>
↑	↓	<u>Asexual reproduction (~48-hr cycle):</u>
Zygotes (diploid)	↘	→ Merozoites
↑	→	→ Ring-stage (seen on smears)
↑	→	→ Pigmented-trophozoites
↑	↗	→ Schizonts (multiple nuclei) → <b>Knobs, Hemozoin</b>
↑	↑	→ 16 to 32 new merozoites/schizont
Male and female gametocytes mate	Merozoites	→ Red cells burst
	← Male and female gametocytes	← New merozoites, hemozoin and other toxic factors
		Causes the clinical symptoms of malaria (fever, chills, impaired consciousness)
		<u>Sexual Reproduction:</u> Merozoites →
		← Male and female gametocytes

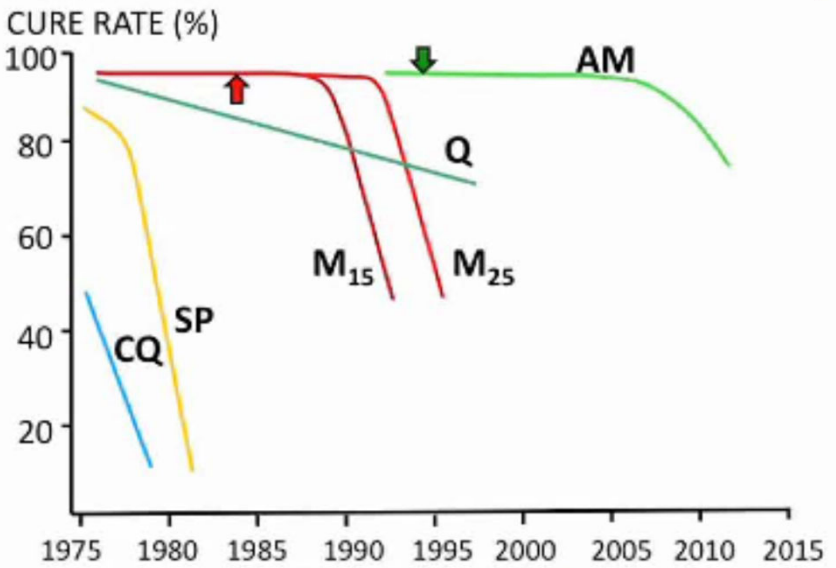


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## Antimalarial Drugs and Resistance

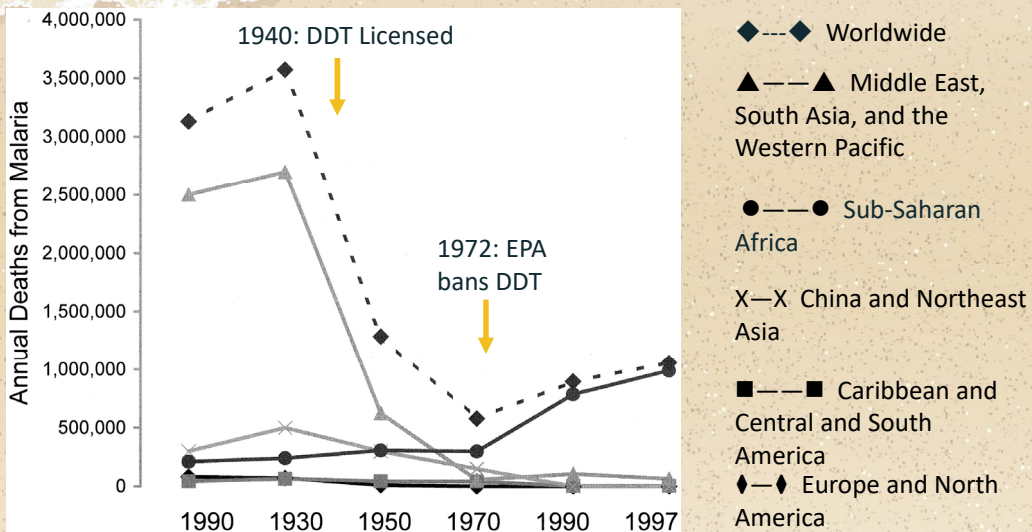
CQ = chloroquine  
 SP = sulfadoxine + pyrimethamine  
 M<sub>15</sub> = 15 mg mefloquine  
 M<sub>25</sub> = 25 mg mefloquine  
 Q = quinine  
 AM = artesunate + mefloquine



From Nosten, 2017

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## Malaria mortality in the 20th century



Richard Carter, and Kamini N. Mendis (2002). Clin. Microbiol. Rev. 2002;15:564-594

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**Proportions of estimated malaria deaths in 15 countries with nearly 80% of malaria deaths globally in 2016 (from WHO)**

Country	%	Country	%
Nigeria	24	Ghana	3
the Congo	11	Uganda	3
Burkina Faso	6	Angola	3
India	6	Kenya	2
Mali	5	Cameroon	2
Tanzania	4	Guinea	2
Niger	4	Chad	2
Mozambique	3	Others	20



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**Uncomplicated and Severe Malaria**

Uncomplicated	Non-specific Mild anemia? Fever
Severe (falciparum only)	Severe breathing difficulties Low blood sugar Severe anemia
Cerebral	Cerebral symptoms, such as coma



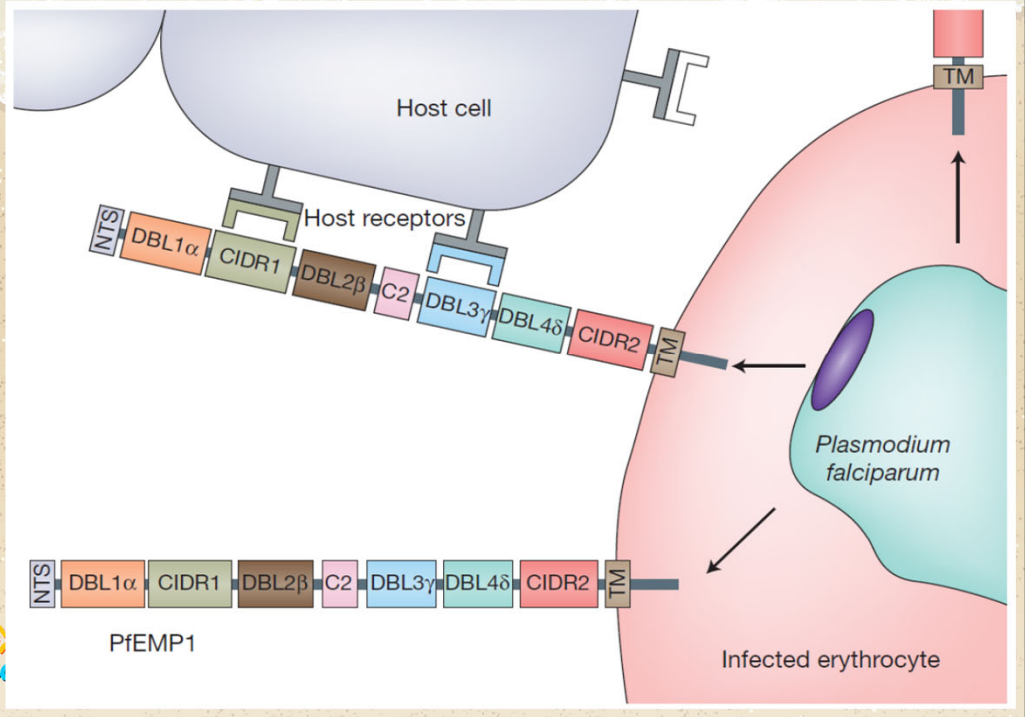
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## PfEMP1 Variant on IRBC Surface

(from Rowe et al., 2009)

Clearwater Beach



## Studies of Effects of DHA on Human Erythroid Cells

Source	Cells	Inducer	[DHA]
Finaurini et al., 2010	Peripheral blood CD34+ cells	20 ng/mL recombinant human stem cell factor	0.5 or 2 $\mu$ M
Finaurini et al., 2012	K562 leukemia cell line	30 $\mu$ M hemin (ferric heme) or 0.5 mM butyric acid	0.5 or 2 $\mu$ M
Parapini et al., 2017	K562 leukemia cell line	Hemin (30 $\mu$ M)	2 $\mu$ M

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## Effects of DHA on Cultured Human Erythroid Cells

- DHA inhibited cell proliferation and delayed erythroid differentiation
  - With CD34+ cells, the effects occurred at the pro- and basophilic erythroblast stages;
  - With K562 cells, DHA-induced apoptosis was also observed (as was observed with rat whole embryo culture).
- In studies of Fech gene expression in cultured K562 cells, DHA inhibited the expression of the Fech gene



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## Quinine and Miscarriage Rate

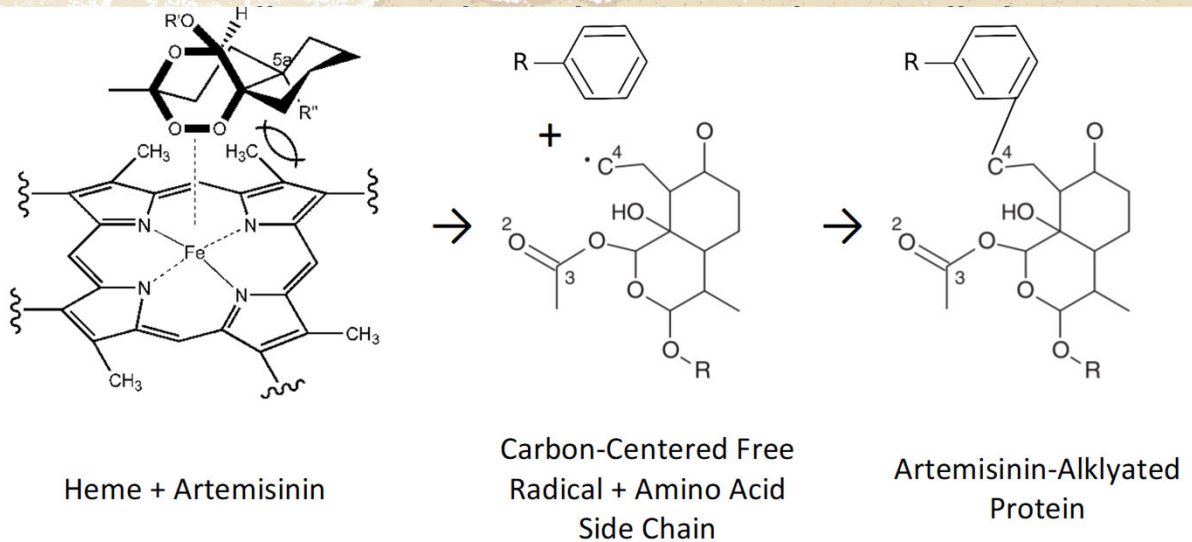
- Malaria causes miscarriages and there is evidence that quinine reduced the miscarriage rate for women in the first trimester with malaria.
- In one study, the miscarriage rate was higher with quinine than with artemether + lumefantrine.
- This could be because artemether + lumefantrine is more effective as an antimalarial than quinine, i.e. the miscarriage rate depends on the effectiveness of the antimalarial treatment.



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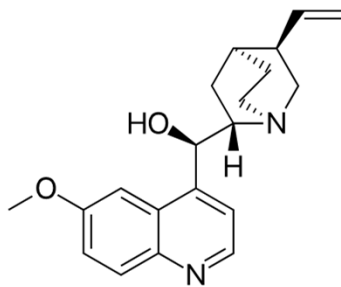
## Heme-Mediated Reductive Scission of the Endoperoxide



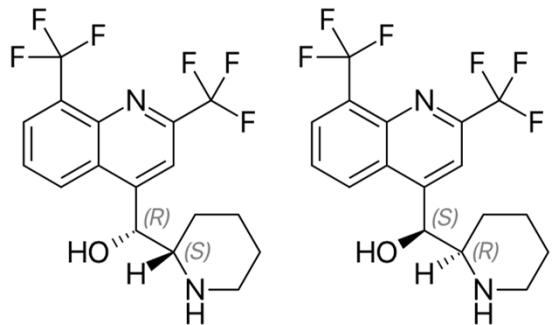
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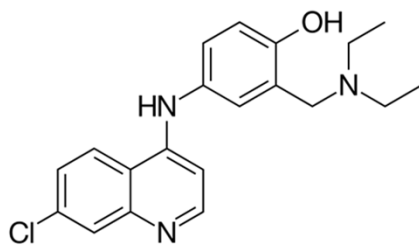
## Structures of Quinolines



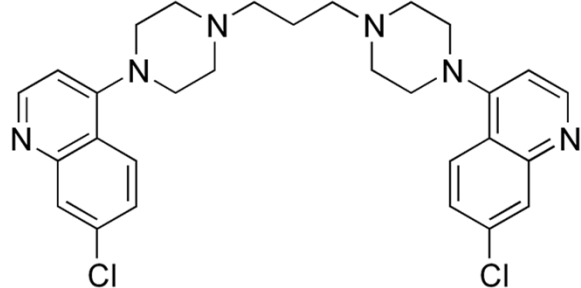
Quinine



Mefloquine (racemic mixture)



Amodiaquine

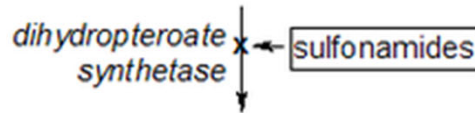


Piperaquine

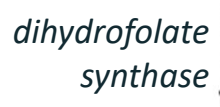
Clearwater

## Synthesis of Tetrahydrofolate

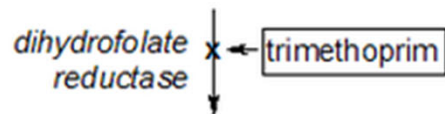
dihydropteroate diphosphate + p-aminobenzoic acid (PABA)



**dihydropteroic acid**



**dihydrofolic acid**



**tetrahydrofolic acid**

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## Toxicokinetic Studies of Artesunate in Rats on GD 11

- Rapid conversion of parent to DHA:
  - Following a single oral dose of 17 mg/kg artesunate, the  $T_{max}$  for parent was 0.3 hr and the AUC for DHA was about 8-fold that for the parent compound, artesunate.
- DHA appears to be the proximate embryotoxicant:
  - At oral and intravenous threshold doses for 100% embryoletality (17 and 1.5 mg/kg, respectively), the AUC for DHA was the only kinetic parameter that was similar for the two routes (147 and 219 ng.h/ml, respectively)

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## Antimalarials with Significant First Trimester Exposures

Antimalarial	First Trimester Exposures			Exposure Ratios at dNOEL ≤1 (treatment thru organogenesis except as noted)	
	Malaria		Total	HED Ratio	Kinetic Ratio
	Infected	Uninfected			
Non-Artemisinins					
• Quinine	>989	176	>1165	Rat = 0.8	
• Mefloquine	79	1194	1273	Rat = 0.13 Rabbit = 0.08	
• Sulfadoxine +Pyrimethamine	120	184	304	Rat = 0.1	Pyr: Rat C <sub>max</sub> = 0.02 Pyr: Rat AUC = 0.002
Artemisinins					
• Artesunate Alone or in ACT	279	77	356	D10: Rat <0.6 Rat <0.24 Rabbit <0.4 D27-33: Monkey = 1.0	D10: Rat AUC = 0.032 D27-33: Monkey = 0.02
• Artemether (+Lumefantrine)	553	43	596	D10: Rat = 1.0 Rat = 0.15	D10: Rat AUC = 1.0



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## Comparison of Artefenomel to Artesunate in Rats

- With dosing throughout organogenesis:
  - 100% total litter loss at 100 and 200 mg/kg/day (with maternal effects)
  - Cardiovascular defects at 40 mg/kg/day (with maternal effects)
- Single dose on GD 12:
  - Artesunate: Total litter loss and 21% postimplantation loss at 7 mg/kg
  - Artefenomel: 16% Postimplantation loss at 200 mg/kg
  - Both: Pale embryos, decreased erythroblast density, decreased reticulocyte counts



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## Comparison of Artefenomel to Artesunate in Single Dose Study in Rats on GD 12 (Cont.)

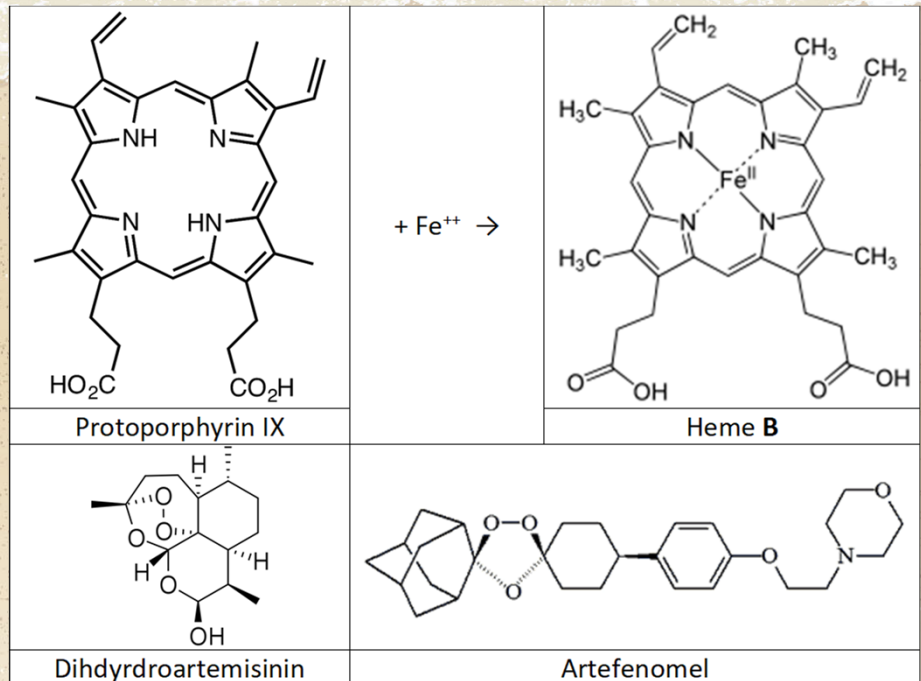
	Control	mg/kg Artesunate		mg/kg Artefenomel		
		7	14	50	100	200
Pregnant females	11	10	11	11	11	11
↓ Maternal body weight gain	-	-	-	+	+	+
Stat sig ↓ in fetal weight (%)	-	7.5	-	-	7.5	11.3
Total litter loss	0	<b>1</b>	<b>9</b>	0	0	0
% Postimplantation loss	4.5	<b>21.4</b>	<b>89.9</b>	3.2	6.4	<b>15.8</b>
Embryos (litters) examined GD 13	26 (2)	-	27 (2)	-	-	39 (4)
Number (%) of pale embryos	0 (0)	-	<b>27 (100)</b>	-	-	<b>8 (21)</b>
% Decrease retic count - on GD 13	-	-	<b>36</b>	-	-	-
- on GD 14	-	-	-	-	<b>16</b>	<b>28</b>

Embryos examined histologically – pale embryos had decreased density of red cells



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**Hypothesis:  
DHA is able to  
squeeze into  
the Fe<sup>2+</sup> active  
site much  
better than  
artefenomel**





## Possible Reasons for Different Animal and Human Results

### – Differences between uninfected animals and pregnant women with *falciparum* malaria

- Artemisinins accumulate in infected RBCs (>150-fold more radiolabeled DHA accumulated in infected RBCs than in uninfected RBCs)
- Pregnant women have extensive sequestration of infected RBCs in the microvasculature and in the intervillous space in the placenta which could retard the transfer of drug to the embryo and other tissues
  - The volume of distribution in uninfected subjects was 2.3-fold greater than in infected patients
- Not as much drug is reaching the tissues in infected women



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## Possible Reasons for Different Animal and Human Results

### – Uninfected Animal Models - Artemisinins and Embryotoxicity

- Sequestration of IRBCs in the placenta is sufficient to cause decreased uterine artery blood flow and low birth weight.
- It is hypothesized that malaria protects against artemisinin-induced embryotoxicity due to the sequestration of IRBCs in the placenta and the accumulation of artemisinin in these sequestered IRBCs, thus forming a partial barrier that retards placental transfer.
- This could be important since, in large parts of tropical Africa, malaria treatment is based on fever rather than microscopic confirmation of parasitemia and many pregnant women without malaria are exposed to antimalarials.



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Possible Reasons for Different Animal and Human Results

– Uninfected Animal Models

- Artemisinin and Reticulocytopenia

- Uninfected human subjects consistently have large decreases in reticulocyte count in response to artemisinins whereas malaria patients do not
  - 5 of 6 groups of healthy people treated with therapeutic doses of artemisinins showed large mean decreases in reticulocyte count (62, 60, 75, 47, and 69%);
  - In 12 groups of patients with malaria, the mean decreases were 0–34%.
- Sequestration of IRBCs in the bone marrow is sufficient to retard RBC production.
- It is hypothesized that malaria protects against artemisinin-induced decreases in reticulocyte count due to the sequestration of IRBCs in the bone marrow microvasculature and the accumulation of artemisinin in these sequestered IRBCs, thus retarding its transfer to the bone marrow.



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Possible Reasons for Different Animal and Human Results –

Uninfected Animal Models –

Quinine and Embryotoxicity

- For quinine and the artemisinins, most of the first trimester exposures from clinical studies were in infected women whereas the animals in developmental toxicity studies were not infected
- When trophozoite-infected RBCs were incubated in 40 nM quinine, the intracellular concentration of quinine (relative to extracellular) increased to 150 to 300-fold the concentration in the medium within 30 minutes (uninfected RBCs did not).
  - At least part of this accumulation is due to wild type forms of the *Plasmodium falciparum* Chloroquine Resistance Transporter (PfCRT) which are located in the membrane of the digestive vacuole and pump quinine into the vacuole.
- The volume of distribution of quinine is lower in infected people.
- Perhaps the failure to see congenital anomalies of the inner ear, eyes and brain in first trimester women treated for malaria with quinine is due to the sequestration of infected RBCs in the placenta and accumulation of quinine in these IRBCs forming a partial barrier that retards the placental transfer of quinine.



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## Antimalarial Activity In vitro (IC<sub>50</sub>s) and WEC NOELs for Artesunate, Artefenomel and Arterolane

	Bulky Side Groups	IC <sub>50</sub> (ng/ml) vs <i>P. falciparum</i>	WEC NOEL (ng/ml)	<i>In vitro</i> "Safety Margin"	Ratio to DHA
DHA	0	0.28	10	35	1
Arterolane	1	0.43	175	409	12
Artefenomel	2	0.55	5000	9063	259

- Artefenomel and arterolane embryos were pale and anemic after 48 hours of culture



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## Comparison of Artefenomel to Artesunate in Single Dose Study in Rats on GD 12

- Both agents caused:
  - Increased postimplantation loss
    - Artesunate
      - Total litter loss in 1 of 10 litters at 7 mg/kg and 9 of 11 at 14 mg/kg
    - Artefenomel
      - 16% at 200 mg/kg with effects on maternal body weight
  - Decreases in reticulocyte count which were correlated with the number of resorptions at 14 mg/kg artesunate and 200 mg/kg artefenomel;
  - Pale embryos on GD 13/14 which corresponded histologically to reduced erythroblast density.



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## Comparison of AUC Ratios for Single Dose in Rats on GD 12

Compound	Dose mg/kg	Developmental Effects	AUC	AUC Ratio <sup>a</sup>
Artefenomel	50	None	38.4 µg·h/ml	2.9
	100	7.5%↓ Fetal weight	71.4 µg·h/ml	5.3
	200	↑Postimplantation loss	97.7 µg·h/ml	7.3
Artesunate	7	Total litter loss; ↑postimplantation loss	69.4 ng·h/ml DHA Eq	0.058

<sup>a</sup>Ratio of AUC at designated dose in rats to the AUC at the therapeutic dose in humans.

- The AUC ratio for artefenomel was approximately 100-fold better than artesunate



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## Interpretations of Artefenomel Studies

- It is largely possible to separate the antimalarial activity of artemisinin from the embryotoxic activity.
- The lesser embryotoxicity of artefenomel could be due to the steric hindrance of the bulky side groups blocking its access to the active site of ferrochelatase.



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