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Malaria Chemotherapy and Drug Resistance

David Sullivan, MD

Eradication Attempts

- Hookworm 1909-1924: Sanitation and drug treatment; no reduction in 15 yrs.
- Yellow Fever 1915-1930s: Mosquito control; discovery of natural reservoir in jungle animals ceased efforts.
- Yaws 1940-1960: Mass screening and penicillin; continued spread after best efforts.
- Malaria 1955-1975: DDT & chloroquine; \$2 billion spent; insecticide resistance & African failure of DDT ceased efforts.
- Smallpox 1967-1977: **Successful** vaccine heat stable and 99% effective as single dose; no hidden carriers.
- Dracunculiasis 1986-?: 3.5 million to 100,000; Sudan - 605 of current cases; Nigeria increase; surveillance and water control
- Polio 1985-?: Gone from western hemisphere; problematic in Congo, Sudan, and Afghanistan; vaccination- and surveillance-based control.

If Not Eradication, Then Control!

"At this time one must question the wisdom of even contemplating another eradication effort until the two eradication programs now in progress have been successfully concluded, until the lessons from those programs have been digested and until the savings from polio eradication have begun to be realized.

Furthermore, there is at this time no candidate disease for which there is a sound scientific basis for eradication, for which the epidemiologic feasibility of doing so is clear and for which there is a reasonable expectation of political commitment by both afflicted countries and those expected to provide the added resources."

DA Henderson

Malaria Control

Vaccines

"Stimulate the Phagocytes. Drugs are a Delusion " GB Shaw, *The Doctor's Dilemma* 1906

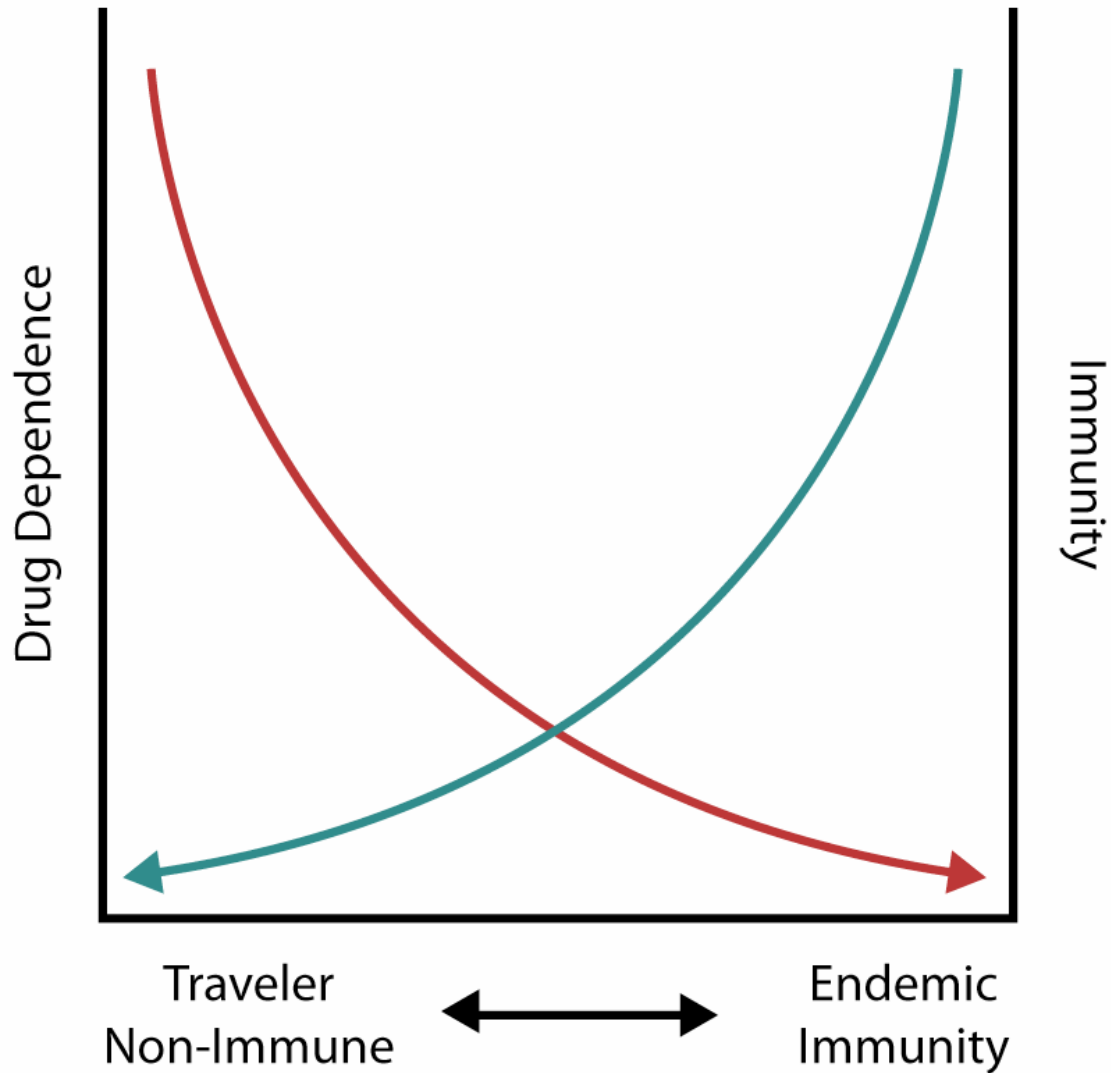
Bednets

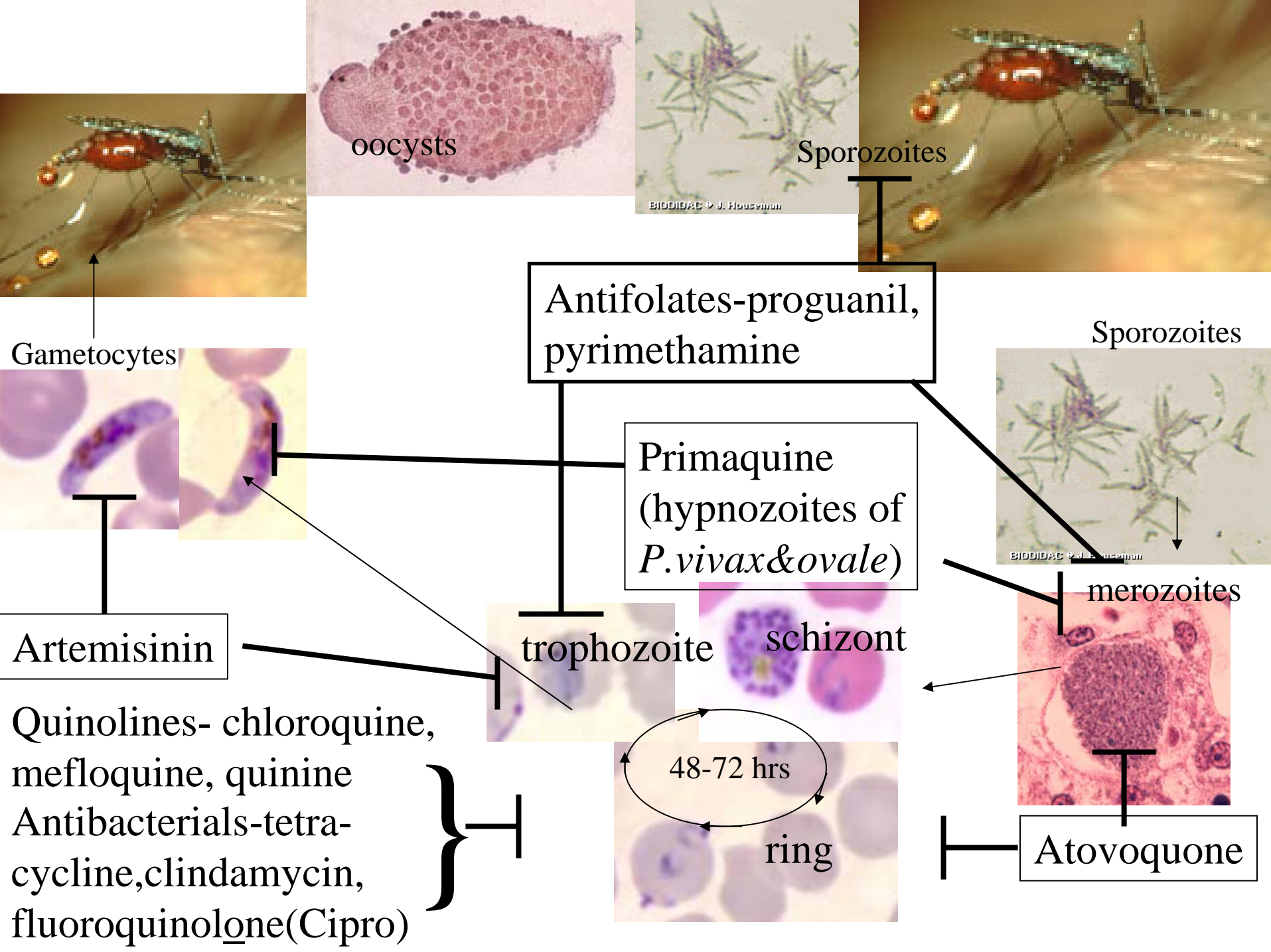
"I myself have been infected with malaria only once in spite of nineteen years' of service in India and thirteen subsequent 'malaria expeditions' to warm climates; and I attribute this to my scrupulous use of the bed net." Ronald Ross *Studies on Malaria* 1928.

Chemotherapy

"We must learn to shoot microbes with magic bullets." Paul Ehrlich in *Microbe Hunters* Paul de Kruif 1926

Drug Dependence is Related to Immune Status





Classification of Antimalarial Agents

1. Tissue Schizontocidal Drugs
 - a. Causal Drugs: Eliminate liver stage from initiating erythrocytic stage
 - b. Hypnozoitocidal: Radical cure of exo-erythrocytic hypnozoites of *P. vivax* and *P. ovale* after treatment of acute erythrocytic phase
2. Blood Schizontocidal Drugs
3. Clinical Cure: Fast action on erythrocytic stages- artemisinin and quinolines
4. Suppressive Therapy: Slower suppressive action on erythrocytic stages
5. Gametocytocidal Drugs: Destroy sexual erythrocytes preventing transmission to mosquito
6. Sporontocidal Drugs: Prevent formation in mosquito of oocyst and sporozoites
7. Chemoprophylaxis
 - a. Causal: Eliminate liver stage from initiating erythrocytic stage
 - b. Clinical or Also Suppressive: Eliminates development of merozoites in erythrocytes.

Five Broad Groups Based on Mechanism of Action

1. Quinoline: Inhibits heme crystallization
2. Artemisinin: 1. Binds heme iron and or iron and generates oxygen radicals. 2. Damages SERCA Ca⁺⁺ P-ATPase
3. Antifolate: Inhibit DNA synthesis
4. Atovaquone: Collapses mitochondrial membrane potential
5. Antibacterial: ribosome inhibition-tetracycline, clindamycin and erythromycin, DNA gyrase inhibition-fluoroquinolones

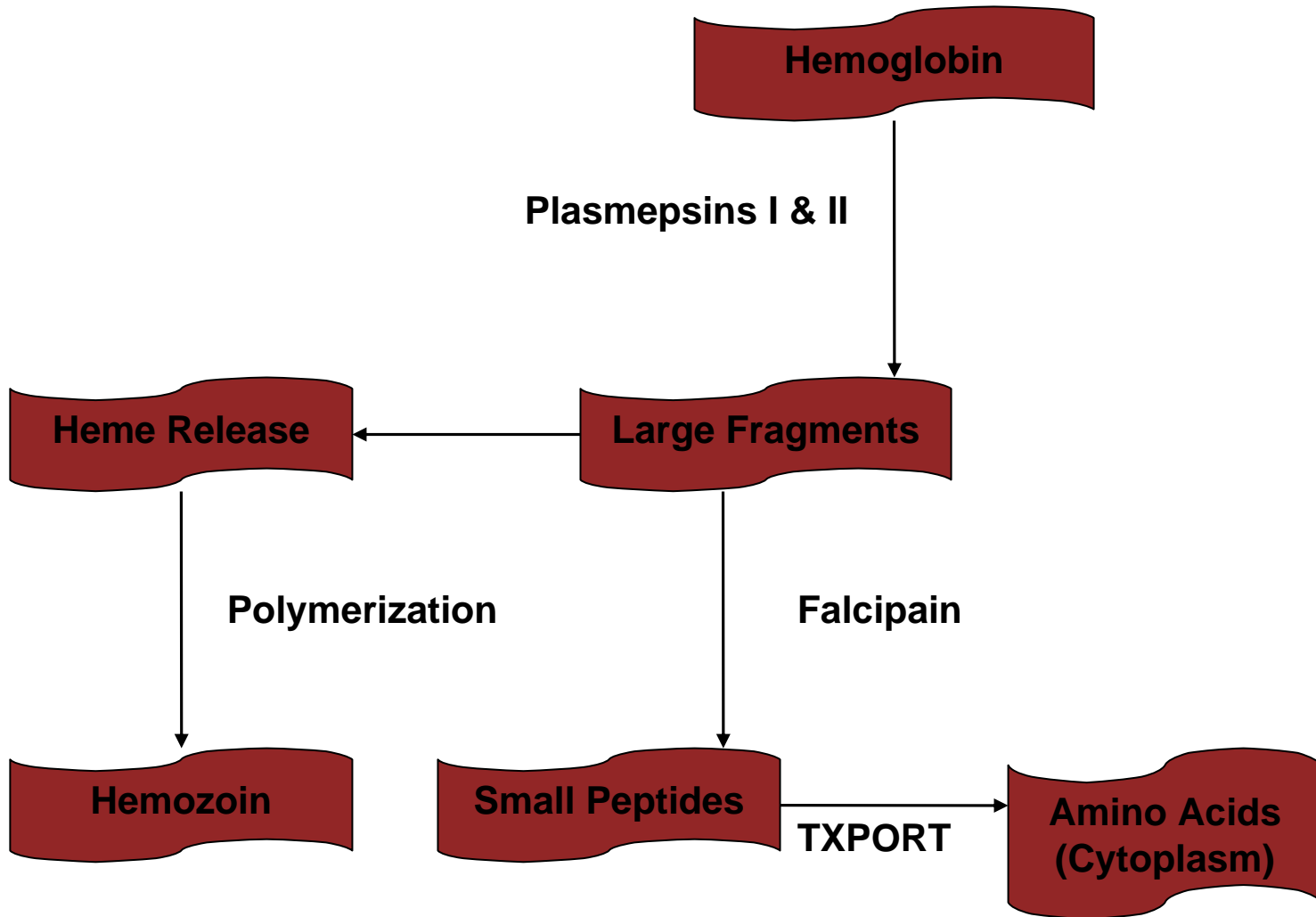
***Plasmodium* Specialized Organelles**

- Digestive vacuole physiology or the Plasmodium iron problem
- Specialized lysosome for degrading hemoglobin and making malarial pigment
- Mitochondria-lack of active TCA cycle with 99% energy from glycolysis
- Apicoplast-chloroplast origin, makes heme and fatty acids and has prokaryote ribosomes

Ehrlich: specific receptors in the parasite with higher avidity for drug than those present in host or ideally lacking in host. He developed arsenicals and methylene blue for the treatment of sleeping sickness and syphilis.

Perkins: In 1826. while trying to make quinine from coal tar, made aniline dye-mauve, which spawned dye industry and later stains for bacteria. Prontosil was made for its color fastness.

Plasmodium Hemoglobin Degradation

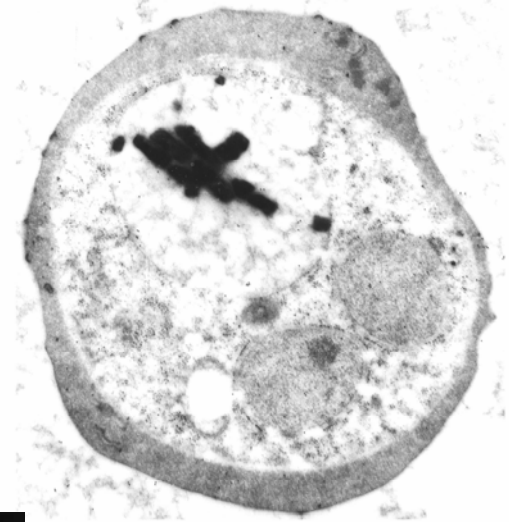


The Matter With Iron and Heme Metabolism

P. falciparum invades a host cell with 20 mM heme iron that can make O^2 radicals in an acidic vacuole.

P. falciparum lacks heme oxygenase and has to synthesize its own heme.

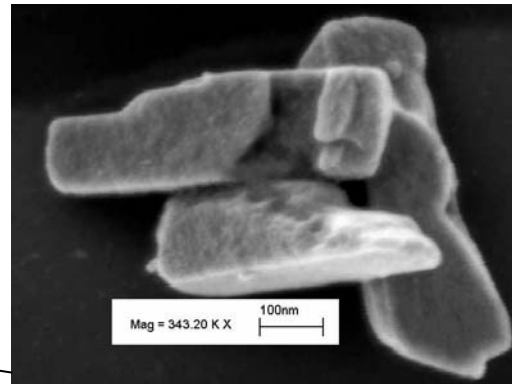
Iron chelators kill the parasite.



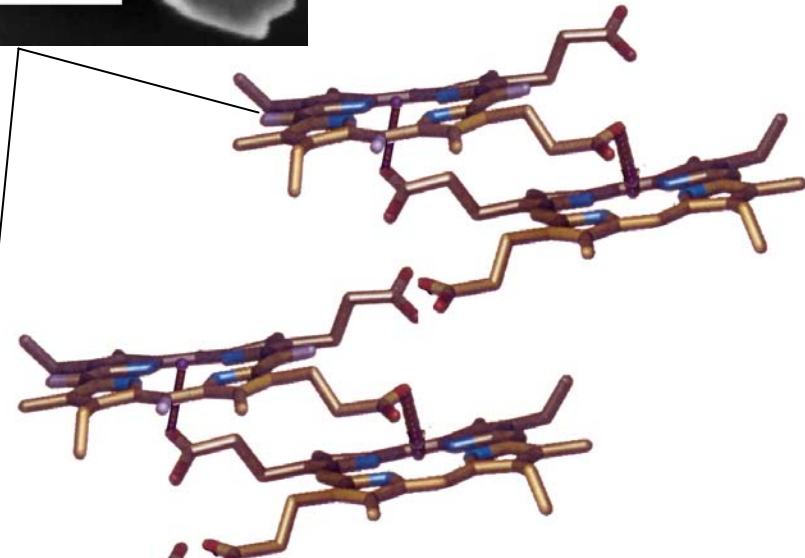
Ring



Troph-Schizont



Head to tail dimer of adjacent hemozoin hemes



Formation of Hemozoin Detoxifies Reactive Heme By Coordinate Iron-Oxygen Bond.

Quinoline Inhibition of Heme Crystallization

1. Stage specificity for killing of parasites actively degrading hemoglobin
2. Hyper- concentration to millimolar levels in the digestive vacuole from nanomolar levels in plasma
3. Digestive vacuolar swelling as early morphologic effects of quinoline treatment

Previous theories postulated that the chloroquine-induced disruption of heme crystallization results either from binding of drug to heme with sequestration of monomeric substrate or from direct interaction of chloroquine with a crystallization protein.

The molecular mechanism of action of quinoline inhibition of heme crystallization was unanswered.

Quinoline mechanism of action: still not fully resolved

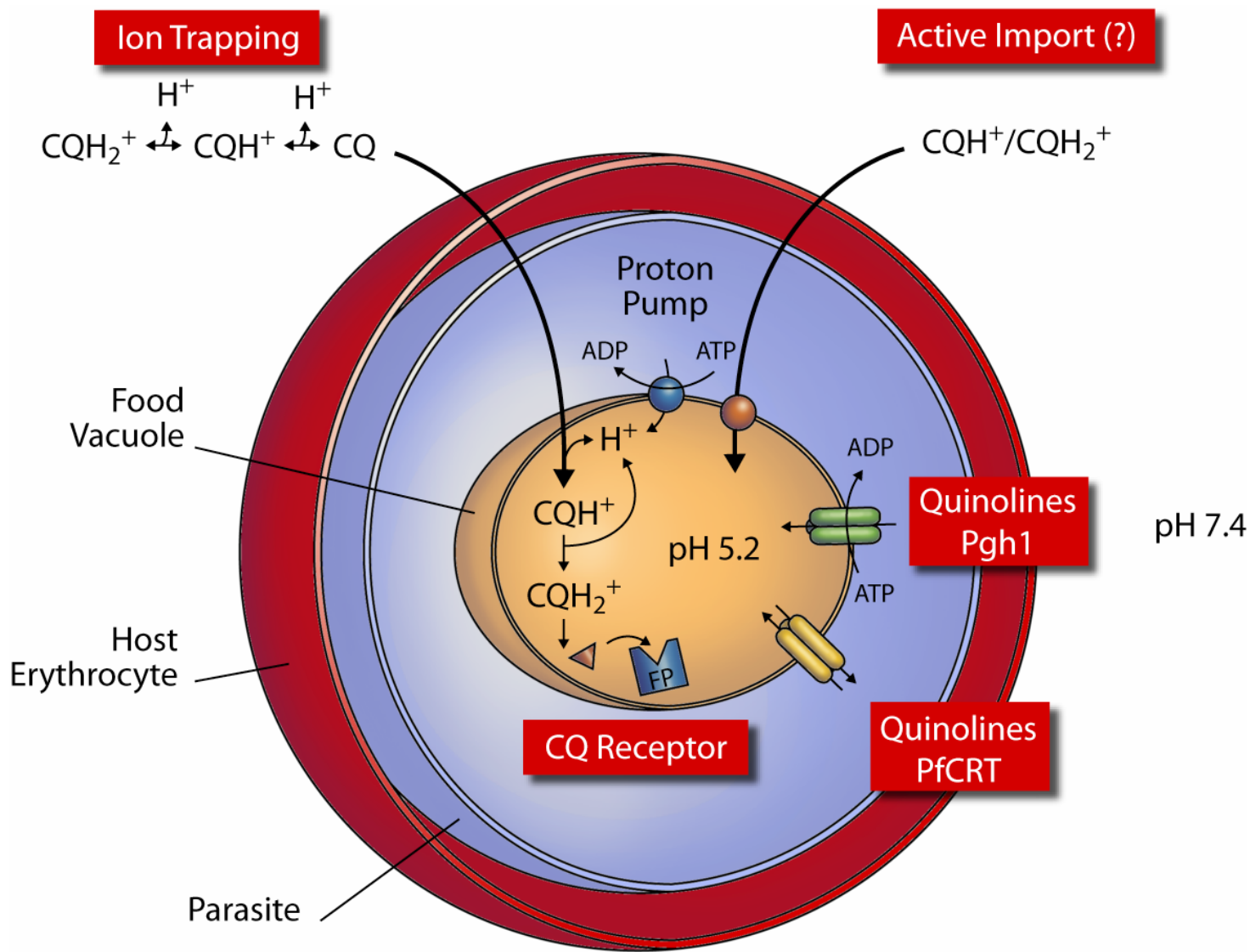
Quinolines copurify with heme crystals in subcellular fractionations

Quinolines reversibly inhibit heme crystallization in time, temperature dependent and pH dependent fashion.

The membrane Pfchloroquine resistant transporter is on digestive vacuole membrane.

Debate still ongoing if quinoline acts in digestive vacuole or cytosol? How much heme is uncoupled from crystallization? Is a single head to tail heme dimer toxic like monomeric heme?

Drug Targets



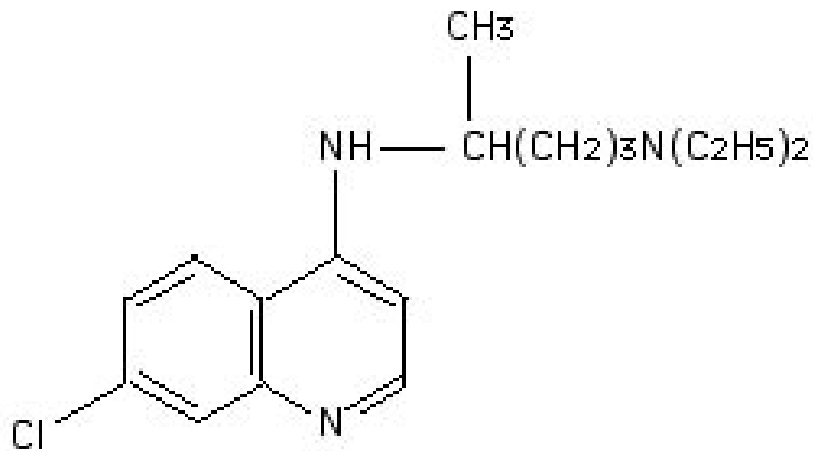
4-Aminoquinolines - Chloroquine

1. Chloroquine

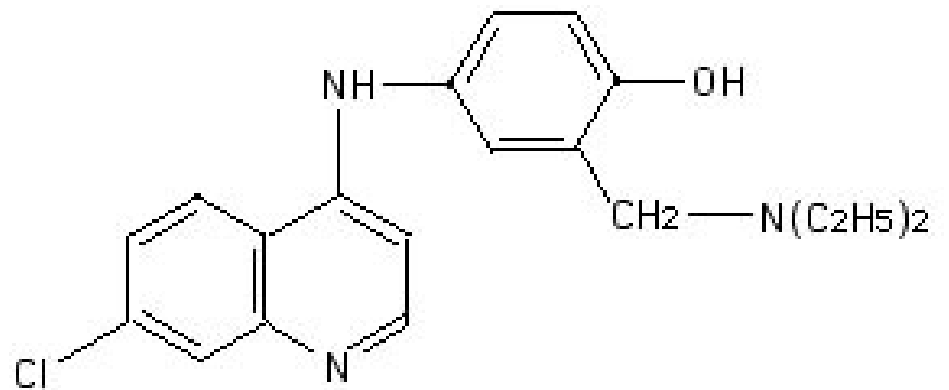
- a. More rapidly acting than quinine and much less toxic.
- b. Has a much longer half-life than quinine
- c. Used for amebic hepatitis and rheumatologic disorders.
- d. Principal metabolite desethylchloroquine has approximately equal antimalarial activity.
- e. Antipyretic effect as well as an antimalarial.
- f. Side Effects: Cinchonism; pruritus in dark-skinned people can be dose limiting; very rare acute neuropsychiatric syndrome; residents on long term prophylaxis should have vision checked after 5 years on drug; diazepam is antidote for poisoning.

2. Amodiaquine

- a. More active against chloroquine resistant isolates but more toxic
- b. Side effects: 1 in 2000 develop agranulocytosis (no white blood cells); serious hepatotoxicity can occur; cinchonism and pruritus also.



CHLOROQUINE



AMODIAQUINE

Quinine & Quinidine

1. Quinine

- a. General protoplasmic poison
- b. Bitter powder from bark of cinchona tree used as flavoring and treatment of night cramps as well as an antimalarial
- c. Not a potent antipyretic but has this effect
- d. Very complex to synthesize and still obtained from natural sources
- e. Secondary alcohol group essential for function
- f. Side effects: Cinchonism symptom complex of tinnitus (ringing in the ears); high tone hearing impairment; nausea, dysphoria, and vomiting; can affect cardiac electrical rhythm by prolonging repolarization; blindness and deafness are common following self-poisoning (8 g orally is fatal), but are rare in malaria treatment; most important side effect is stimulation of pancreatic beta cells to cause hyperinsulinemic hypoglycemia; does not cause premature labor and actually lessens contractions in malarious women that are treated; associated with blackwater fever but mechanism is unclear and association is not absolute

2. Quinidine

- a. dextrorotatory diastereoisomer of quinine. A more active antimalarial but more cardiotoxic
- b. Side effects: effect on heart, effect on pancreas, and deafness

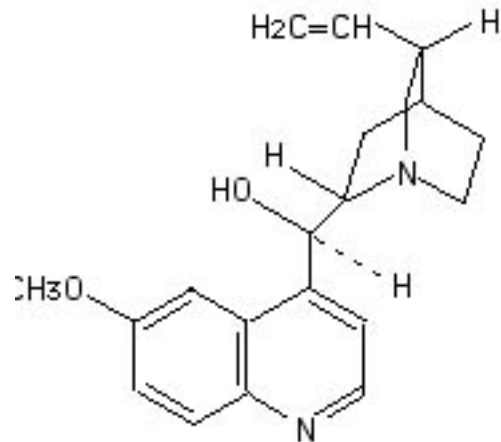
Mefloquine and Halofantrine

- **Mefloquine**

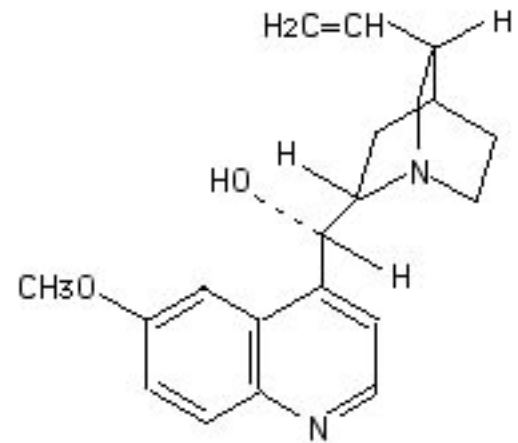
- a. Used as a 50:50 racemic mixture of the erythroisomers.
- b. Very insoluble in water
- c. Side Effects: Most people vomit after first loading dose for treatment. As chemoprophylactic, 1:15,000 incidence of acute and self-limiting neuropsychiatric reactions (nightmares, convulsions and psychosis). Ten times incidence higher with treatment.

- **Halofantrine**

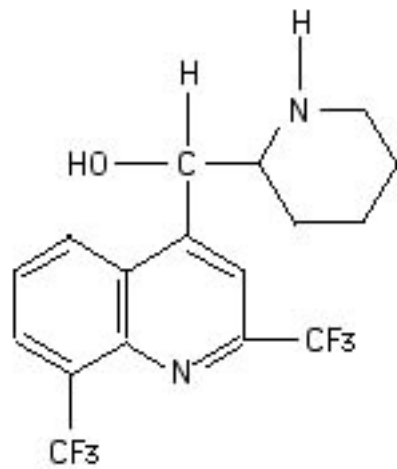
- a. More potent than quinine or mefloquine
- b. Side Effects: well-tolerated, diarrhea at high doses. Sudden death from cardiac causes potentiated with previous mefloquine treatment.



QUINIDINE



QUININE

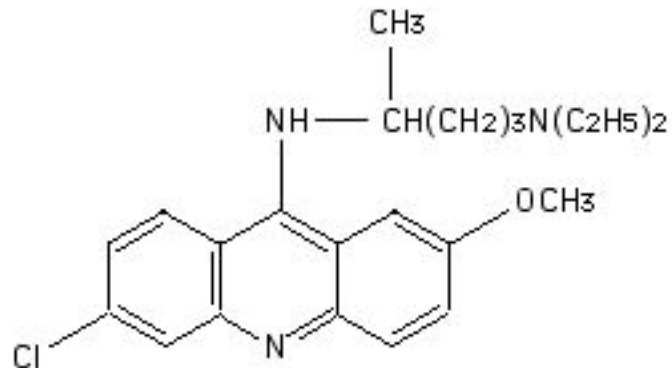


MEFLOQUINE

Acridine

Mepacrine: same side chain as chloroquine. More toxic but used in WWII before chloroquine. Still used for giardiasis.

Side effects: people turn yellow and have skin eruptions and mental disturbances

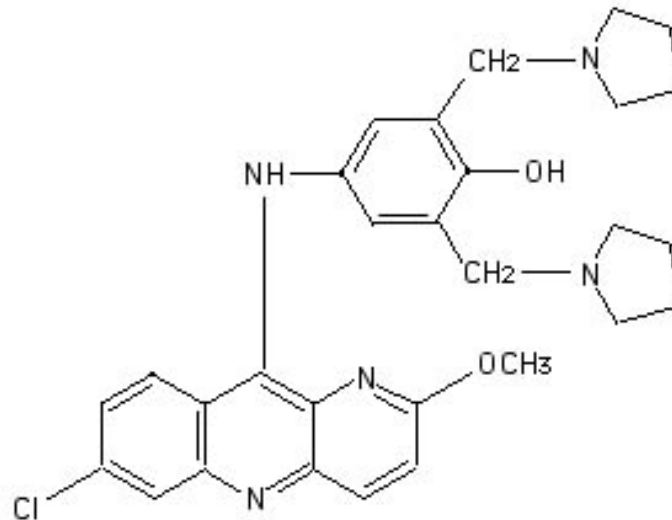


QUINACRINE

Benzonaphthyridines

Pyronaridine: Synthesized in China as reminiscent of Mepacrine and Amodiaquine. Effective against multi-drug resistant plasmodium.

Side effects: headache, dizziness, gastrointestinal distress, and heart abnormalities.



PYRONARIDINE

8-Aminoquinolines

1. Primaquine

- a. Only drug class that kills hypnozoites of *P. vivax* and *P. ovale*.
- b. Intermediate resistance of *P. vivax* to primaquine is appearing.
- c. Ineffective against asexual blood forms of *P. falciparum*.
- d. Kills gametocytes of all species of malaria including *P. falciparum*.
- e. Mechanism of action: Possible metabolite interferes with mitochondrial function.
- f. Side effects: Hemolysis in persons with G6PD deficiency (an antioxidant enzyme). Nausea, vomiting, and GI upset are common, especially if more than 30 mg is taken

2. Tafenoquine (WR 238,605)

1. more than 10 times more active as hypnozoitocidal drug and has blood schizontocidal activity and gametocidal activity.
2. Early report in Australian military that it causes a keratitis.
3. Drug studies still ongoing.

Artemisinin

1. Sesquiterpene lactone extracted from herb called sweet wormwood (*Artemisia annua*).
2. Rapidly effective against malaria parasites including multiresistant strains.
3. 4 log drop in parasite counts.
4. May become most important treatment for drug-resistant malaria.
5. No reported resistance.
6. Artemisinin suppositories
7. Artemether dissolved in peanut oil and given as intramuscular injection.
8. Artesunate: tablets or IV formulation
9. Pharmacokinetics: $t_{1/2}$ of 1-3 hrs
10. Side effects: No clinical toxicity seen in thousands of people. Can have neurotoxicity in animals. Anecdotal reports of embryo toxicity in rats and mice and should be used with caution during pregnancy.

The Numbers

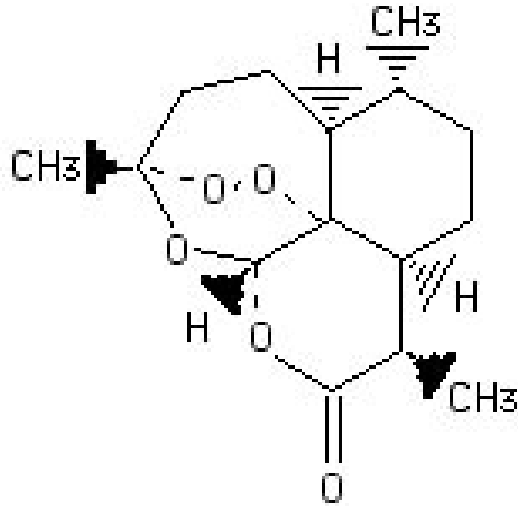
70 kg person X @70 mL/kg = 4.9 L of blood @ 5 L = 5×10^3 ml = 5×10^6 μ L

5×10^6 RBCs per μ L of blood

2.5×10^{13} RBCs

1% parasitemia = 1 in 100 iRBCs = 2.5×10^{11} parasites

Artemisinin reduces by 4 logs parasite biomass with each asexual cycle.
This is the most rapidly acting antimalarial.

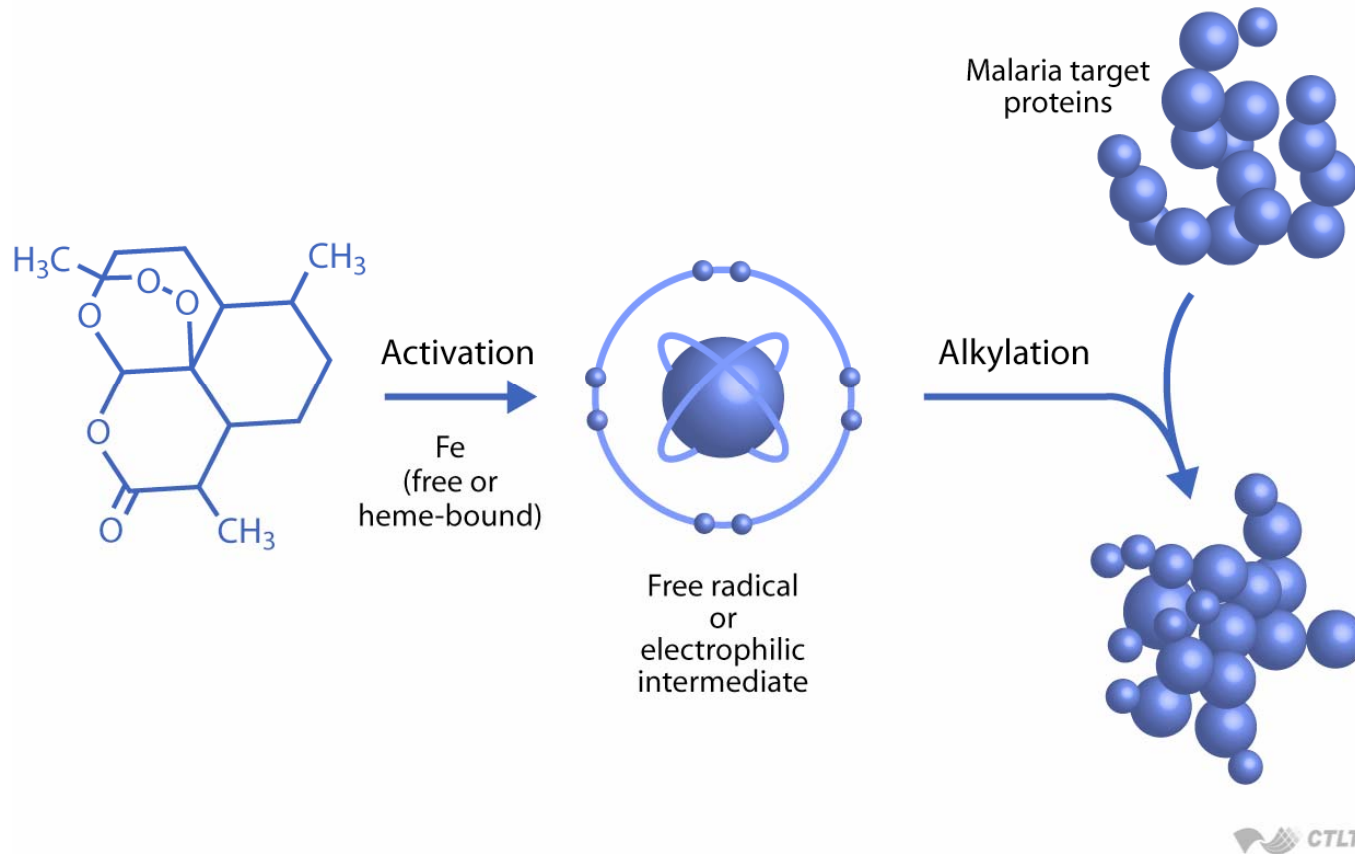


Endoperoxide bridge
generates single
oxygen radical

ARTEMISININ

Old “where the money is” theory: artemisinin makes oxygen radicals in digestive vacuole where lots of oxygen and heme coexist.

Activation and Targeting of Parasite Proteins



Target proteins

1. Artemisinin increases lipid damage (Berman Adams 1997)
2. Histidine-rich protein or translationally controlled tumor protein TCTP covalent heme-art-protein adducts
3. Does not prevent heme crystallization even though binds heme
4. inhibits hemoglobin proteases in presence of heme
5. Binds and inhibits calcium transporter PfATPase6

New fact-artemisinin
inhibits calcium ATPase
pump PfATP6 in parasite.
Iron chelator decreases
effect.

Nature 424:957-61 2003

Eckstein-Ludwig & Krishna

Dihydrofolate Reductase Inhibitors

Diaminopyrimidines

1. Pyrimethamine

- a. Used in combination with long-acting sulfonamides.
- b. Inhibits all stages except for late-stage gametocytes and hypnozoites.
- c. More potent than chloroguanide and has longer half life.
- d. Side effects: Generally safe and well-tolerated with mild bone marrow depression in persons with folate deficiency. Toxicity of combination with sulfonamides due to sulfonamides.

2. Trimethoprim

- a. (Bactrim) in combination with sulfamethoxazole.
- b. Effective treatment for uncomplicated malaria.

Inhibition of Dihydrofolate Reductases by Pyrimethamine and Trimethoprim

Concentration (nM) for 50% inhibition of DHFR

Inhibitor	Mammalian Rat liver	Bacterial <i>E. coli</i>	Protozoal <i>P. berghei</i>
Pyrimethamine	700	2500	0.5
Trimethoprim	260,000	5	70

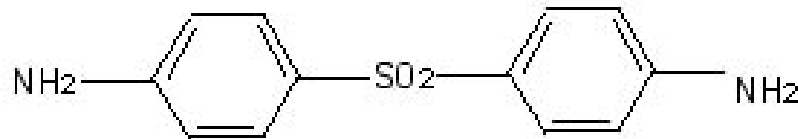
Adapted from Ferone, Burchall & Hitchings, 1969

Dihydrofolate Reductase Inhibitors

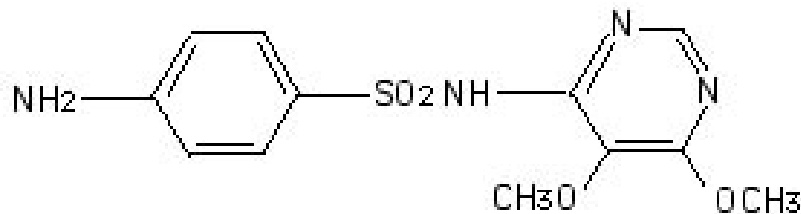
Diaminopyrimidines (cont.)

3. Sulfadoxine

- Long half-life up to 120-200 hours
- Combination with pyrimethamine 20:1 known as Fansidar valuable in curative treatment of resistant strains as well as prophylactic.
- Clears as rapidly as chloroquine, but no antipyretic effect.
- Side effects: Stevens-Johnson syndrome. Fatal reactions as high as 1:18,000-26,000 for chemoprophylaxis. Aplastic anemia and agranulocytosis can occur.



DAPSONE

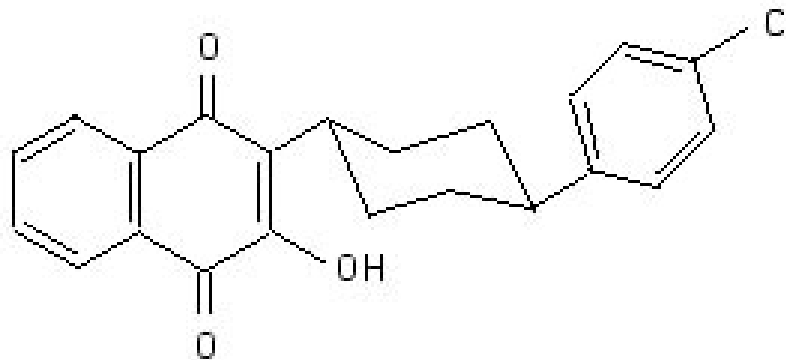


SULFADOXINE

Naphthoquinolines

1. Atovaquone:

- a. First used in the 1940's but rapid resistance when used alone.
- b. Combination with tetracycline or proguanil will circumvent this.
- c. In combination with dapson, it is a very promising casual prophylactic.
- d. Currently in combination with proguanil as Malarone.



ATOVAQUONE

Antibacterials

1. Tetracycline

1. Blocks 30s ribosome in bacteria.
2. Useful in combination therapy with quinine for multiple-resistant organisms.
3. Slow action.
4. Useful chemoprophylaxis in areas with multiresistant *Plasmodium*.
5. Side effects: Sun sensitivity; damages bones and teeth in fetus and young children.

2. Clindamycin

1. Blocks 50s ribosomes.
2. Treats mild malaria and can work against quinoline-resistant strains.
3. Azithromycin and erythromycin are similar and inhibit 50s ribosomes by a slightly different mechanism.

3. Fluoroquinolones

1. Inhibit DNA gyrases
2. Slight antimalarial activity.

Multiple-Drug Therapy: A forgotten lesson

Artemisinin/quinolines

Atovoquone/proguanil or dapsone

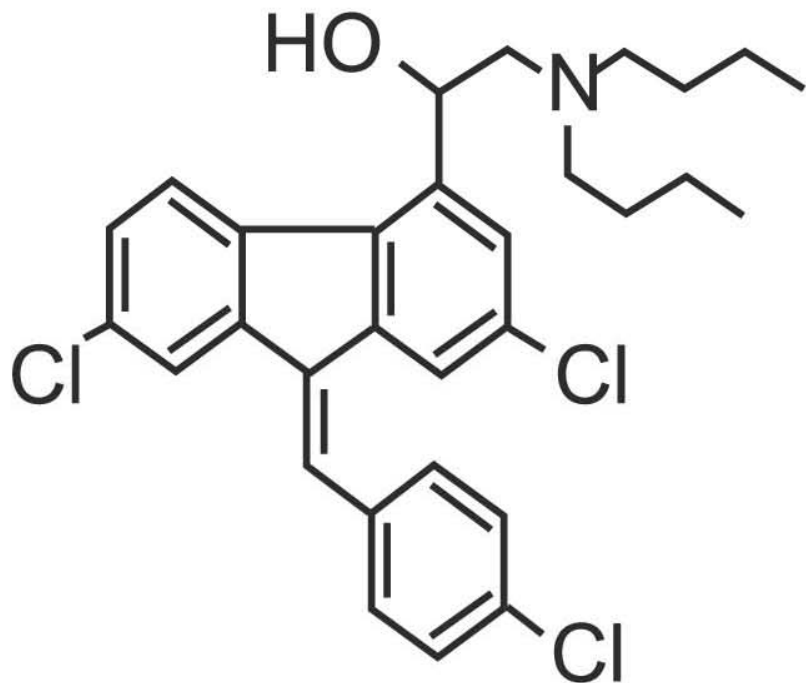
Quinolines/tetracycline

Adjunctive Therapy

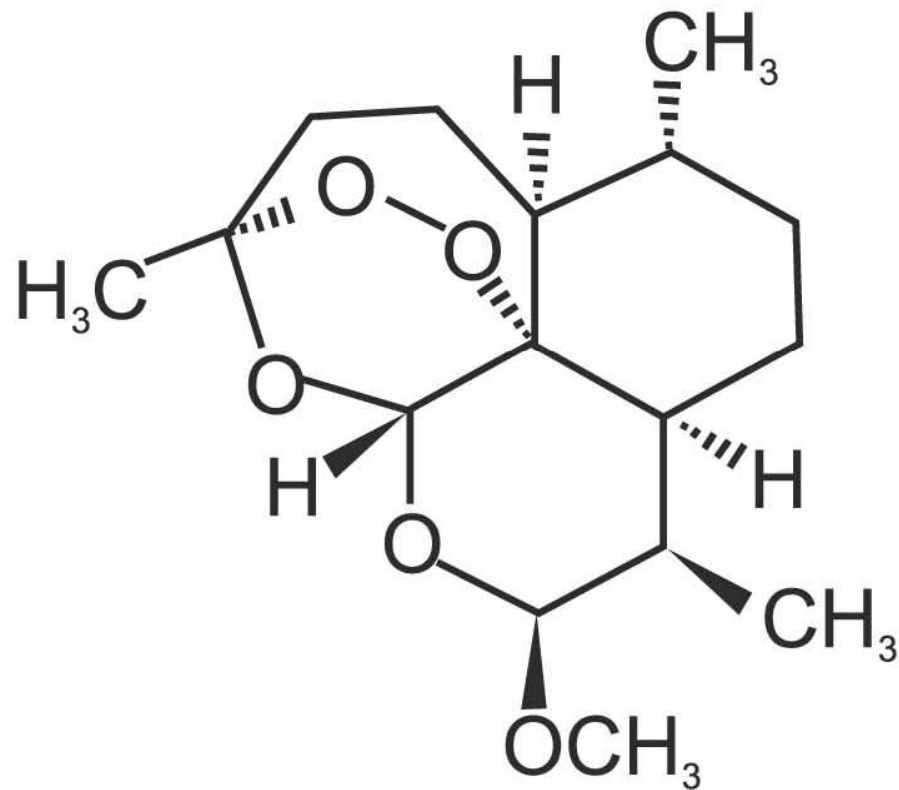
Steroids: Worsened outcome in large study of cerebral malaria patients.

Iron Chelation: Can only be given as continuous infusion but hastens recovery in cerebral malaria patients and does hasten clearance of parasitemia. Iron chelation is bacteriostatic and inhibits growth but does not kill mammalian cells. In culture, iron chelation kills *Plasmodium*.

Lumefantrine



Artemether



COARTEM

UNIQUE TARGETS FOR ANTIPLASMODIAL DRUGS

1) Unique essential metabolic pathway

Web page for *Plasmodium* metabolic pathways.

<http://sites.huji.ac.il/malaria/>

2) Inhibitor with differential activity- parasite vs. host

3) Cure disease

Rational drug development is identifying unique targets present in *Plasmodium* but not host cells and are essential for maturation.

1) Proteases involved in hemoglobin degradation

2) Orotic acid dehydrogenase activity in mitochondria

3) Phospholipid biosynthesis inhibitors

4) Every newly discovered unique *Plasmodium*

enzyme

Gametocytidal Activity

1. Gametes degrade hemoglobin for 48 hrs before becoming relatively metabolically inactive for the rest of 28-day life.
2. Chloroquine and pyrimethamine/sulfadoxine both clear gametocytes within 28 days. Chloroquine enhances infectivity before clearance while Pyr/sulfa decreases infectivity. Chloroquine-treated uninfected nonimmune serum enhanced infectivity of gametocytes, while Pyr/sulfa decreased infectivity. Hogg, B et al *Am J Trop Med Hyg* 58:176-182 1998.
3. Mature gametocytes are metabolically resting and the quinolines and anti folates do not effect them.
4. **Primaquin, tafenoquine and artemesinin are most effective at killing mature gametocytes.**

Sporontocidal Activity

- Proguanil and pyrimethamine clearly are.
- The sulfa drugs have varied effects.
- Primaquine given in filter feeds does not work, but it is effective when given to host (need metabolite).

Drug Regimens

1. Chemoprophylaxis
2. Curative
3. Intermittent presumptive therapy (pregnancy, disease prevention in children)
4. Population treatments

Chemoprophylaxis Principles

1. Avoid infection.
2. No drug is perfectly effective; all have risks. 20% of all people taking chemoprophylaxis report some effect.
3. Prophylactic drugs like chloroquine, mefloquine, and tetracycline are not designed to eliminate hypnozoites of *P. vivax* or *P. ovale*. Primaquine can be given for radical cure if exposure is substantial (>6 months).
4. Start chloroquine one week early to build up therapeutic doses. In emergency travel, may take chloroquine on two consecutive days.

Chemoprophylaxis Principles (cont.)

5. Continue drugs for 4 weeks (mefloquine 2 weeks) after leaving malarious area so that schizontocidal concentrations are present when merozoites emerge from liver. Mefloquine and Maloprim are started early to watch for side effects. Alternative is to bring drugs along for standby treatment of fevers for individuals who are pregnant, young children or people with allergies.
6. Chloroquine, quinine, pyrimethamine. and proguanil are safe during pregnancy. Sulfonamides are safe at term. Mefloquine is still under evaluation, but probably safe. Malorone is category C, probably safe but some minimal animal toxicity with atovoquone.
7. Chemoprophylaxis of children in endemic area has been shown to reduce mortality (25%) but is not widely done.

P. falciparum

Is a

Medical

emergency

Initial Management Decisions

- Oral vs. Intravenous chemotherapy
 - Severity of disease
- Correct Drug
 - Resistance ?
- Correct dose
 - mg/kg of salt or base
- Response to therapy
 - Parasite clearance time
 - Fever clearance time
- Check the glucose
- Hypoglycemia from parasite or drugs

Oral vs. Intravenous Chemotherapy

- Signs of End Organ Involvement
 - Pulmonary edema
 - Renal failure
 - Coma
 - Severe anemia-transfusion lifesaving
- Unable to Tolerate Oral Medications
- Parasitemia over 5%

If yes to any of the above, then IV chemotherapy.

Delay Equals Bad Outcome

Delay from onset of symptoms to medical presentation

Delay from presentation until consideration of malaria

Delay from consideration to microscope diagnosis

Delay from diagnosis until start of therapy.

Delay of reassessment (ICU care; hypoglycemia; staff not as familiar with IV Quinidine).

Pregnancy and Drugs

Chemoprophylaxis

- continuous provision of antenatal chemotherapy to prevent parasitemia. Chemoprophylaxis during first pregnancy does not reduce immunity in subsequent pregnancies.
- Chloroquine, amodiaquine, proguanil, mefloquine

Intermittent presumptive treatment (IPT)

- Full treatment doses at intervals to reduce disease.
- 2-3 intervals at scheduled visits.
- sulfadoxine-pyrimethamine

Treatment During Pregnancy

1. IV quinine
2. Chloroquine
3. SP
4. Artesunate combinations
5. Quinine and clindamycin
6. Amodiaquine

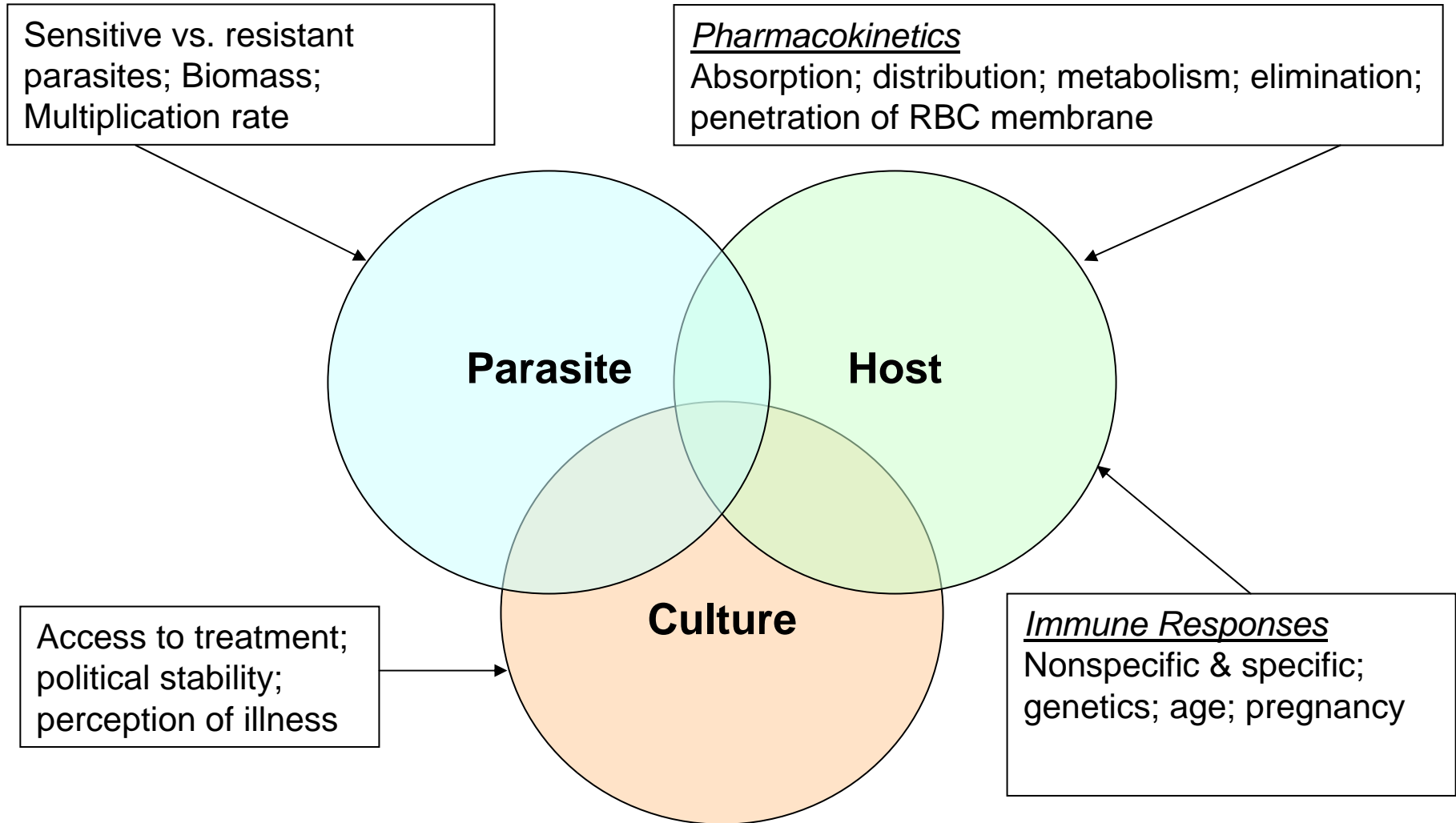
What Is Drug Resistance?

The ability of a parasite strain to survive and/or multiply despite administration & absorption of a drug given in doses equal to or higher than those usually recommended but within the limit of tolerance of the subject. (World Health Organization, 1986)

Why Don't Antimalarial Drug Treatments Always Work?

- Wrong Diagnosis
- Incorrect choice of drugs
- Sub-optimal regimen (dose, schedule, duration)
- Non-compliance
- Sub-optimal absorption (nausea, diarrhea, vomiting, malabsorption)
- Idiosyncratic pharmacokinetics
- Poor quality drugs
- Resistance of the pathogen to the drug

Antimalarial Drug Resistance: Social, Economic, Host, and Parasite



Mechanisms of Resistance: Quinolines

- Resistant parasites accumulate less drug. (period)
- Strains can be sensitive to one quinoline (quinidine) and resistant to another (chloroquine).
- Theories propose drug increased efflux vs. decreased drug influx.
- Pf Multi Drug Resistant (MDR) gene does not correlate with CQR, but mostly with MFQR.
- PfCRT-Positional cloned to chromosome 7. No homology to other pumps.
- Biochemical changes in parasite pH from 7.1 to 7.3 distinguishes sensitive from resistant parasites, respectively. *J Cell Biol* 140:335-345

Antimalarial drug resistance mutations

Low- to intermediate-level resistance High-level resistance

P. falciparum

Chloroquine CRT; 76 CRT; 76 and other mutations,
MDR;86, and other undefined gene products

Mefloquine, halofantrine, lumefantrine

MDR; amplification of wild-type allele

Pyrimethamine DHFR; 108 then 51 and 59

DHFR; 108 + 51 + 59 + 164

Cycloguanil, Chlorcycloguanil DHFR; 16 + 108

DHFR; 108 + 51 + 59 + 164

Atovaquone No low-level resistance documented Cytochrome
b; 133 ± 280

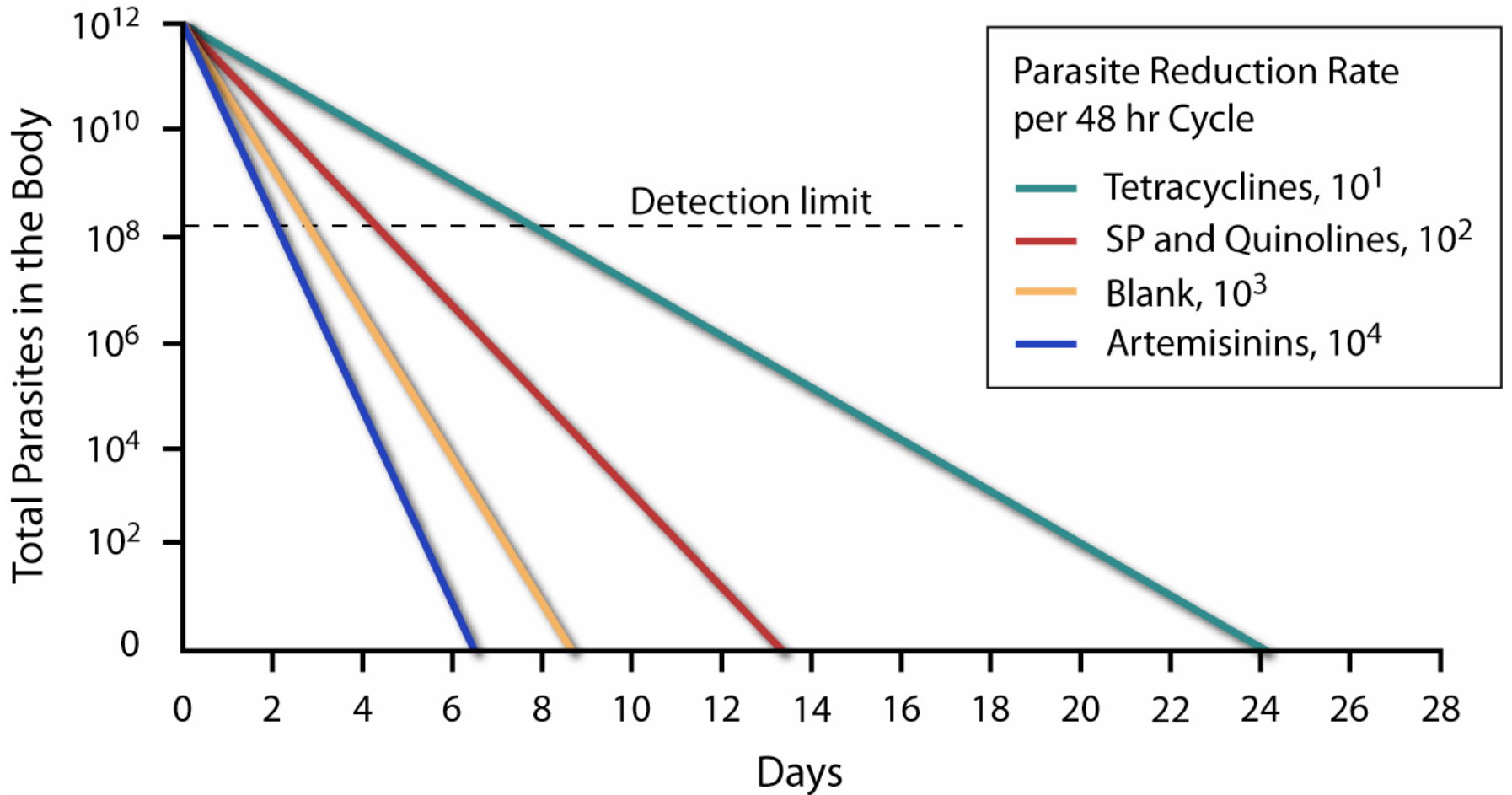
Sulfonamides and sulfones DHPS; 436, 437, 540, 581, 613A

Artemisinin and derivatives No resistance

P. vivax Pyrimethamine DHFR; 117 + 58 DHFR; 117 + 58 +
59 + 61 + 13

Atovaquone mutation on cyt B

How Fast Do Drugs Kill Parasites?



Adapted by CTLT from White 2004 J. Clin Invest 113:1084-1092

