Malaria is a mosquito-borne disease of humans and animals caused by protozoa of the genus Plasmodium.

MALARIA-WHY CAN'T WE SAVE CHILDREN AND PREGNANT WOMEN? MULTI-PRONGED ATTACK ON MALARIAL INFECTIONS USING NUTRITION AND DRUGS

Early Development of Antimalarial Drug Screening

- Initially we attempted to use tritiated thymidine or uridine incubated with parasitized cells as a drug screen. The nucleic acids of the malarial parasite failed to be labeled.
- Then we used tritiated adenosine to label parasite DNA and RNA. In 1967, we developed the first method to screen antimalarial drugs. Parasitized red cells are incubated for a period of minutes at 37 C. in another set of tubes drugs are added. The reaction is stopped with acid and the extent of labeled DNA/RNA polymer can be compared forming the basis of drug screening.

Why don't pyrimidines label the parasitized DNA/RNA?

- In collaboration with Dr George Tremblay – URI we found that malarial parasites make pyrimidines "de novo". They do not used preformed pyrimidines.
- Therefore thymidine and uridine are not used to make nucleic acids per se and therefore they will not label parasite nucleic acids when added to parasitized cells

Is adenosine used to make DNA/RNA of the parasite?

- We developed a method to free the parasite from its host cell erythrocyte,
- Therefore free parasites can be used to study the entire purine salvage network during incubation. We found that the parasite did not use adenosine directly but oxidatively deaminated adenosine to inosine. Then it cut off the sugar to create hypoxanthine and xanthine which it took up into the parasite for manufacturing of nucleotides
- Nucleotides were incorporated into DNA/RNA

Using anionic exchange HPLC we separated the entire tritiated purine salvage pathway

- We demonstrated the entire purine salvage pathway of malarial parasites for the first time.
- We showed that these free parasites can make ATP via glycolysis proving that they are actually alive.

Screening system finds Halofantrine and Mefloquine

- Using our screening system and working for the United States Army at West Virginia University Department of Pharmacology we screened 10,000 drugs.
- We found Halofantrine and Mefloquine both of which were commercially developed

Chloroquine Resistance was the Major Problem during the Vietnam War

- No one knew how resistance to chloroquine occurred nor did anyone have a method to treat it.

Milhouse at Walter Reed found verapamil could reverse chloroquine resistance in falciparum malaria. But it was a high dose effect that would cause cardiac arrest if used in such high doses.

Usually scientists search for new drugs if resistance occurs

- There were no effective ways at combatting the problem of chloroquine resistance
- But we knew that chloroquine resistance was partly due to a multiple drug resistance ATP driven pump and that verapamil reversed the pump.
- This is the first hint at solving the chloroquine resistance problem.

Another piece of the CQ resistance Puzzle found

- Scientists at NIH found there was a Plasmodium falciparum chloroquine resistance transporter (PFCRT) associated with the parasite food vacuole.
- Further verapamil inhibits this PFCRT mechanism.
- Now the question is –Could we find a drug which inhibits both mechanisms without the toxicity of verapamil ?

The discovery of tetrandrine /Chloroquine combinations

- In 1989 professor Zuguang Ye and myself made a startling discovery.
- We found a bisbenzylisoquinoline drug named tetrandrine which when combined with chloroquine causes a 43 fold synergistic boost to the effect of chloroquine using chloroquine resistant human malaria-Vietnam stain.

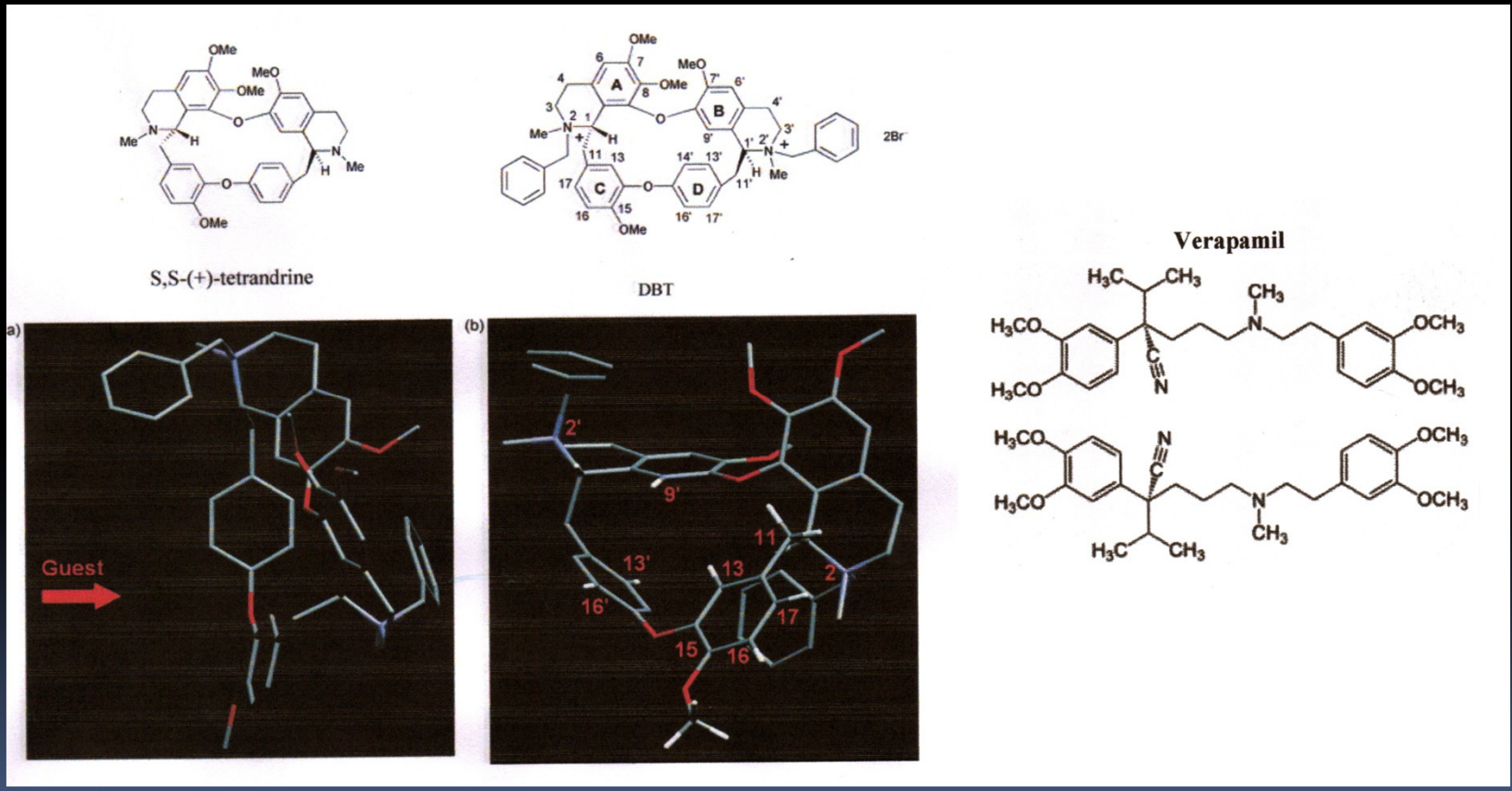
Use of tetrandrine and chloroquine in CQ resistance

- 1. It is likely that tetrandrine reverses chloroquine resistant falciparum malaria involving PfCRT because verapamil(V) ,a calcium channel inhibitor accomplishes this feat.
- 2. Tetrandrine is a calcium channel inhibitor which reverses PfMDR exit pump similar to (V).
- 3. The two chloroquine resistance mechanisms work in similar ways
- 4. Both Tetrandrine and Verapamil are hydrophobic.

Parasite Food Vacuole-CQ

- 1. Parasite food vacuole concentrates CQ using PfCRT. Mutant PfCRT is damaged by mutation and apparently causes CQ to exit food vacuole
- 2.CQ resistant parasite has multiple drug resistance exit pumps on food vacuoles (PfMDR) which cause the CQ to pumped out- also creating CQ resistance
- 3. The combination of 1. and 2. creates resistance in most CQ resistant strains.

Tetrandrine & Verapamil



Combination of CQ & TT

Table 1

IC_{50} (nM) OF TT AND CQ FOR EACH DRUG ALONE AND IN COMBINATION*

MALARIA***	SINGLE DRUG		DRUG COMBINATION**		
	TT	CQ	TT(1.0uM) CQ(0.3uM)	TT(2.0uM) CQ(0.2uM)	TT(3.0uM) CQ(0.1uM)
S STRAIN	498.1±93.7	26.7±3.8	54.9±7.1(TT) 16.5±2.1(CQ)	114.1±23.0(TT) 11.4± 2.3(CQ)	223.3±38.6(TT) 7.4± 1.3(CQ)
R STRAIN	197.5±24.7	185.8±4.9	79.5±13.7(TT) 23.8±4.1(CQ)	79.5±16.1(TT) 8.0± 1.6(CQ)	124.6± 9.6(TT) 4.2± 0.3(CQ)

*The data in the table above are mean values ± S.D. (nM) from three experiments except where noted.

**Ratios of TT/CQ in the drug combinations are 10:3, 10:1, and 30:1, respectively.

***S and R strains represent CQ-sensitive (FCMSU1/Sudan) and resistant (w2) strain of P. falciparum, respectively.

Berenbaum Plot of CQ & TT

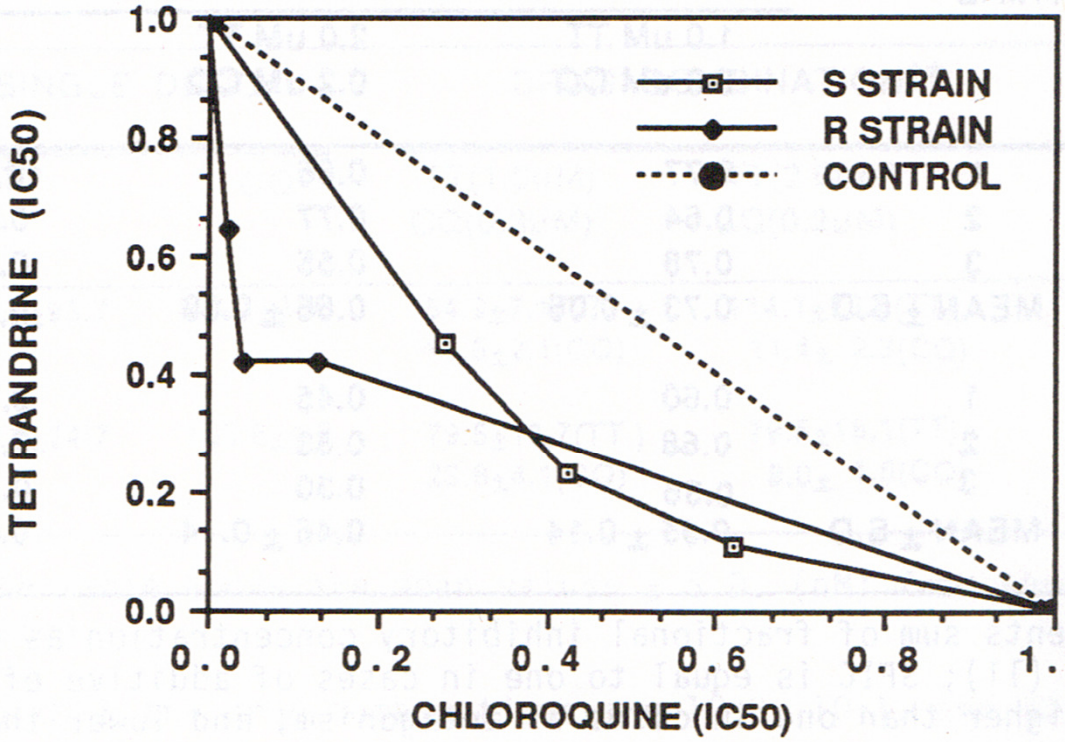
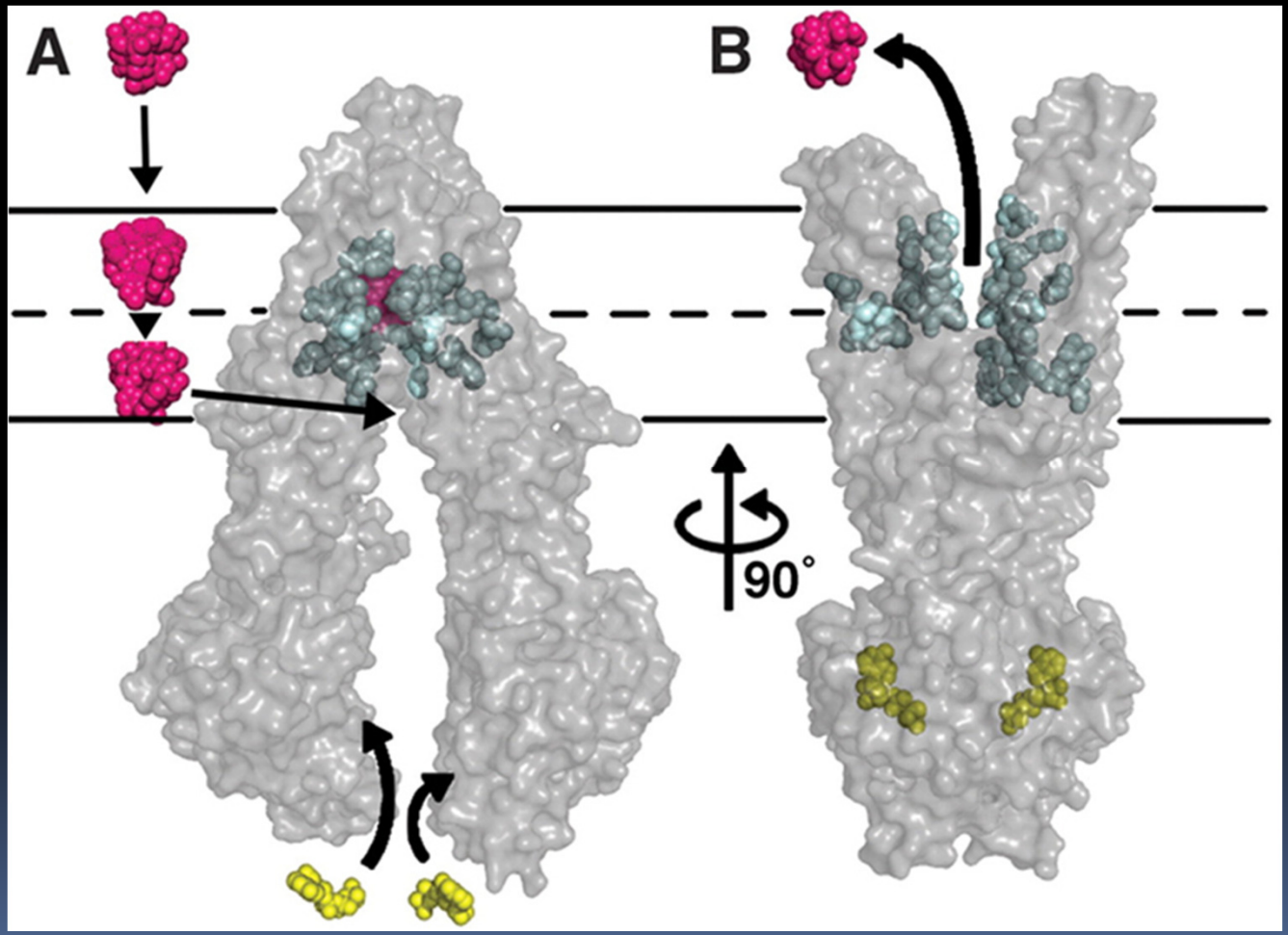


Figure 1. Synergistic effect of tetrandrine and chloroquine on CQ-sensitive (S strain) and CQ-resistant (R strain) P.falciparum in vitro.

MDR Drug Pump



Effect of CQ & TT Against CQ Resistant Malaria in Aotus Monkey

Table 1 Effect of the combination of tetrandrine and chloroquine against infected chloroquine-resistant *Plasmodium falciparum* malaria in *Aotus* monkeys

Expt. no.	Monkey no.	Dose[a] (Mg/kg)	Parasites cleared	Days from initial dose to parasite clearance	Days from final dose to recrudescence	Re-treating-post recrudescence (mg/kg)	Cured
1	025	15(tt)	+			30 (tt) [d]	(++)
		20(cq)		16	23	20 (cq)	
	055	15(tt)	+			30(tt) [d]	(++)
		20(cq)		8	33	20(cq)	
	016	15(tt)	+			30 (tt) [d]	(++)
		20(cq)		8	13	20 (cq)	
2	705	30(tt)	+			60 (tt) [d]	(++)
		20(cq)		7	9	20 (cq)	
	706	60(tt)	+			120 (tt) [d]	(++)
		20(cq)		7	14	20 (cq)	
	698[b]	-	-				
CQ[c]	520	20(cq)	-				
	432	20(cq)	-				
	349	20(cq)	-				
	544	20(cq)	-				
	631	20(cq)	-				

a tt and cq represent tetrandrine and chloroquine respectively.
b This monkey as a non-treated control died within 2 weeks after inoculation with the parasite.
c Monkeys were treated with CQ alone.
d Monkeys were cured after two week combinational cq and tt treatment=(++).

Duke Scientists find children with malaria deficient in L-arginine

- It has been found that children heavily infected with malaria are deficient in L-arginine
- Arginine is needed to produce large amounts of nitric oxide in macrophages which when reacted with oxygen produces a major toxin to the parasite.
- Infected children produce ADMA a NO synthesis inhibitor of nitric oxide synthase 2

Nitric Oxide Generated by 3 Pathways

- In order to generate sufficient NO one must
- 1. Have large amounts of L-arginine in diet
- 2. Have large amounts of L-citrulline in the diet. Excellent sources are watermelon
- 3 Have considerable amounts of nitrate in diet. Excellent sources are spinach, beets , dark green lettuce

Combining Chloroquine, Tetrandrine and continuous generation of Nitric oxide

- If a child or pregnant women utilizes sufficient chloroquine, tetrandrine and the three sources to generate sufficient nitric oxide continuously, chloroquine resistant malaria can be conquered.
- This will occur because this combination kills the parasite in multiple ways and stimulates the immune system of children and women.