Knox Van Dyke, Zuguang Ye, and Richard Rossan

A COMBINATION OF TETRANDRINE (TT) AND CHLOROQUINE (CQ) EFFECTIVELY TREATS CQ RESISTANT FALCIPARUM MALARIA IN AOTUS MONKEYS

In 2010-219 million malaria cases/year

- 1. This created 1.3 million deaths with 65% of deaths in children under 15 years of age.
- About 125 million pregnant women are at risk each year and in Sub-Saharan Africa maternal malaria is associated with 200,000 estimated infant deaths /year.
- There are 219 million cases/year worldwide .

Malaria Endemic in Equator

- Malaria is endemic in a broad band around the equator- in areas of Central and South America, many parts of Asia, and much in Africa; in Sub-Saharan Africa 85-90% of malaria fatalities occur.
- Every year , 125 million international travelers visit these countries and more than 30,000 get the disease.
- It is usually found in rural areas rather than cities.

Drugs for Malaria

- 1. Originally quinine extracted from the bark of the chinchona tree was the best antimalarial drug.
- 2. World war II- chloroquine, primaquine and quinacrine were used extensvely
- 3. Vietnam-resistance to chloroquine
- US Army had a major development program to develop new antimalarial. I developed the antimalarial drug screening system used worldwide today –mefloquine and halofantrine were found in our screening system.

Prophylaxsis of Malaria

- 1.The most important antimalarial drug from the points of cost and effectiveness is chloroquine = GOLD STANDARD
- 2.Over the last 60 years of use, chloroquine resistance has spread over almost the entire tropical belt.
- 3. Chloroquine is still used a great deal.
- 4.Where CQ use has stopped for some time-CQ is useful again as an antimalarial

Mechanism of Chloroquine's Action

- 1. CQ is absorbed by the parasitized red cell
- 2. It goes through the membrane surrounding the parasite-the parasitopherous membrane
- 3.It goes through the membrane of the parasite.
- 4. It enters the food vacuole
- 5. It inhibits the parasites's heme polymerase causing heme toxicity-killing the parasite

CQ Resistance Mechanisms

- 1. multiple drug resistance (MDR) is a 170 Kd glycoprotein membrane exit pump utilizing ATP/ATPase. The glycoprotein for falciparum malaria is PfMDR. CQ is pumped from food vacuole.
- 2. PfCRT is the chloroquine resistance transporter . It is a chloride channel linked to proton translocation. CQ does not enter if resistance transporter is mutated or damaged.
- Some parasites have only 1. Some may have only 2 and some parasites have both 1 and 2.

Using Tetrandrine(TT) and CQ

- 1. We found that using both tetrandrine and chloroquine together- the power of CQ was increased 43 fold.
- We know that tetrandrine inhibits the MDR mechanism
- We know that tetrandrine is antimalarial without CQ resistance
- If tetrandrine binds the PfCRT, which it almost certainly does, synergism probably occurs from inhibition of multiple mechanisms.

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 blocker accomplishes this feat. We know tetrandrine stimulates MDR-ATPase like (V).
- 2. Tetrandrine is a calcium channel blocker which reverses PfMDR exit pump like (V).
- 3. Tetrandrine inhibits PfMDR blocking CQ exit

Mechanisms of Tetrandrine(T)

- 1. T inhibits the MDR exit pump by stimulating MDR- ATPase and depleting ATP causing energy loss and shutting off pump.
- 2. T inhibits the nf-kappa b transcription factor responsible for MDR pump production. This action inhibit s CQ's exit from food vacuole.
- 3. T overcomes the effect of the mutations of the PfCRT and therefore allows CQ to enter parasite food vacuole thus killing it.
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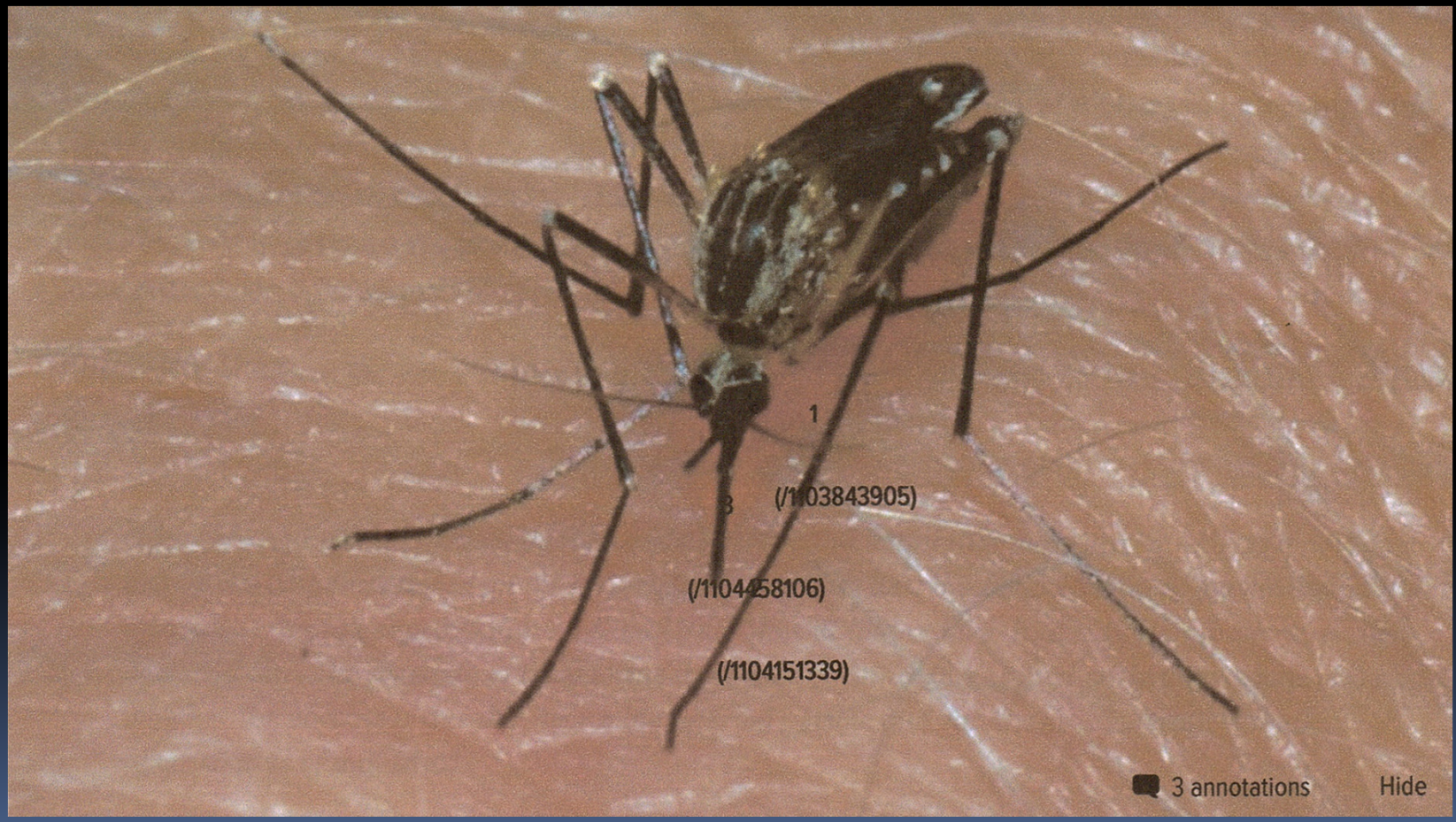
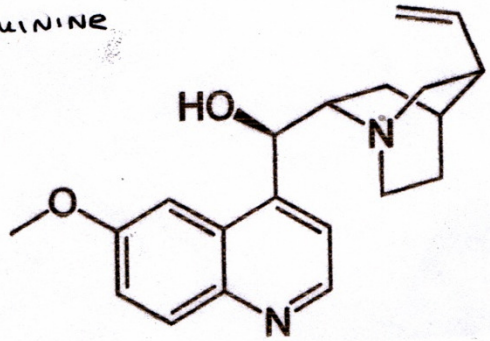
Anopheles Mosquito



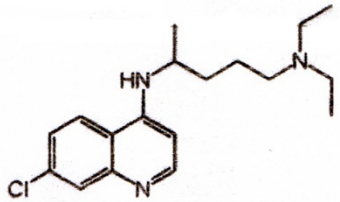
Aotus Monkey

Anti-Malarial Drugs

Quinine

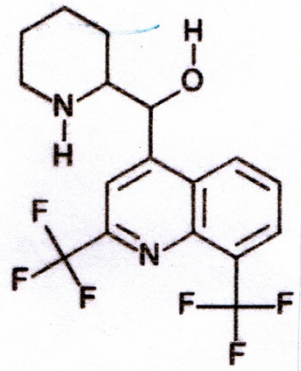


Chloroquine



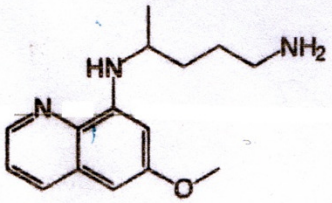
Systematic (IUPAC) name

(*RS*)-*N*′-(7-chloroquinolin-4-yl)-*N*,*N*-diethyl-pentane-1,4-diamine



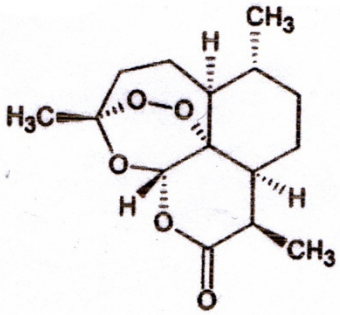
Mefloquine

Primaquine

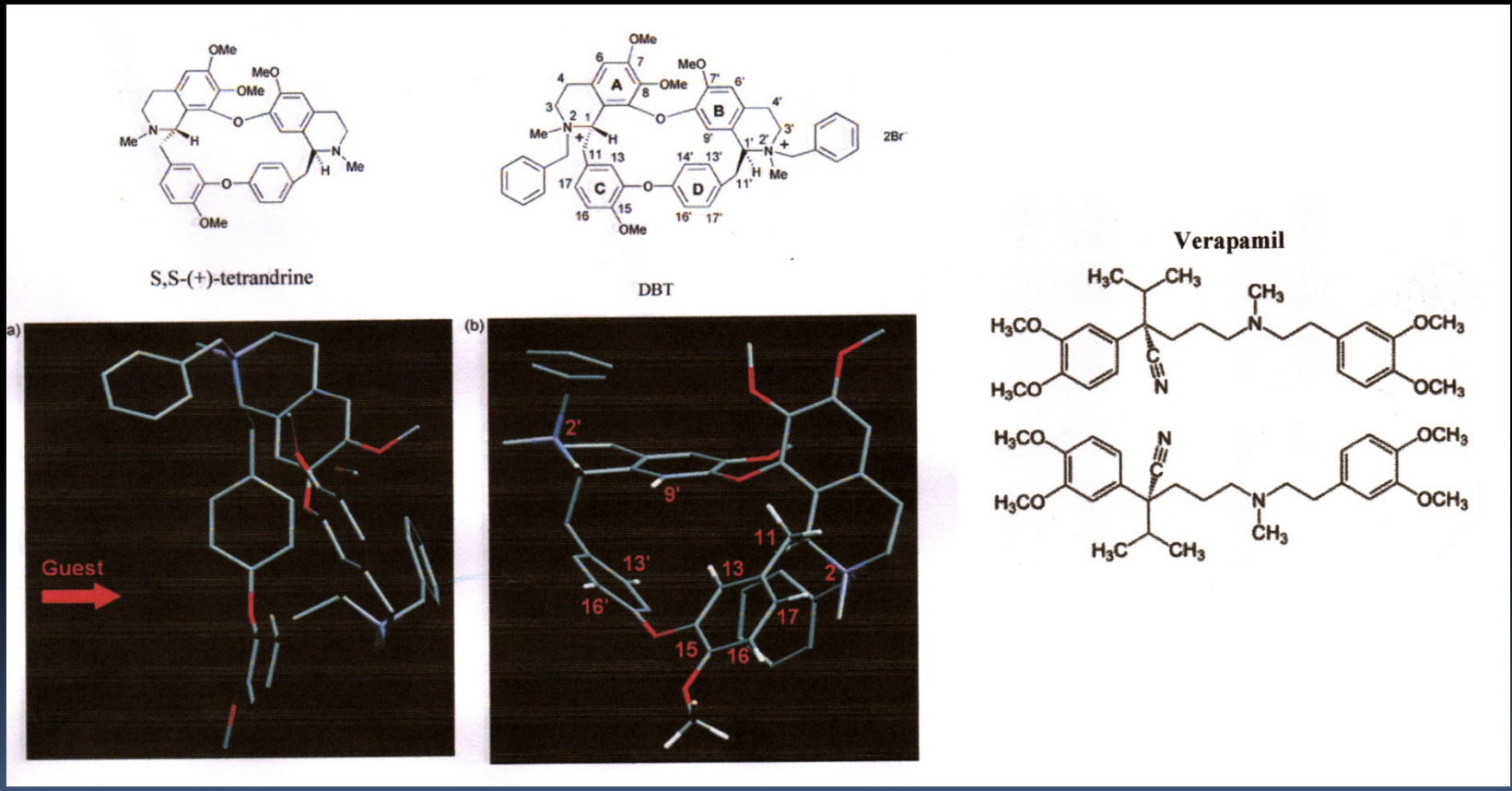


Systematic (IUPAC) name

Artemisinin



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

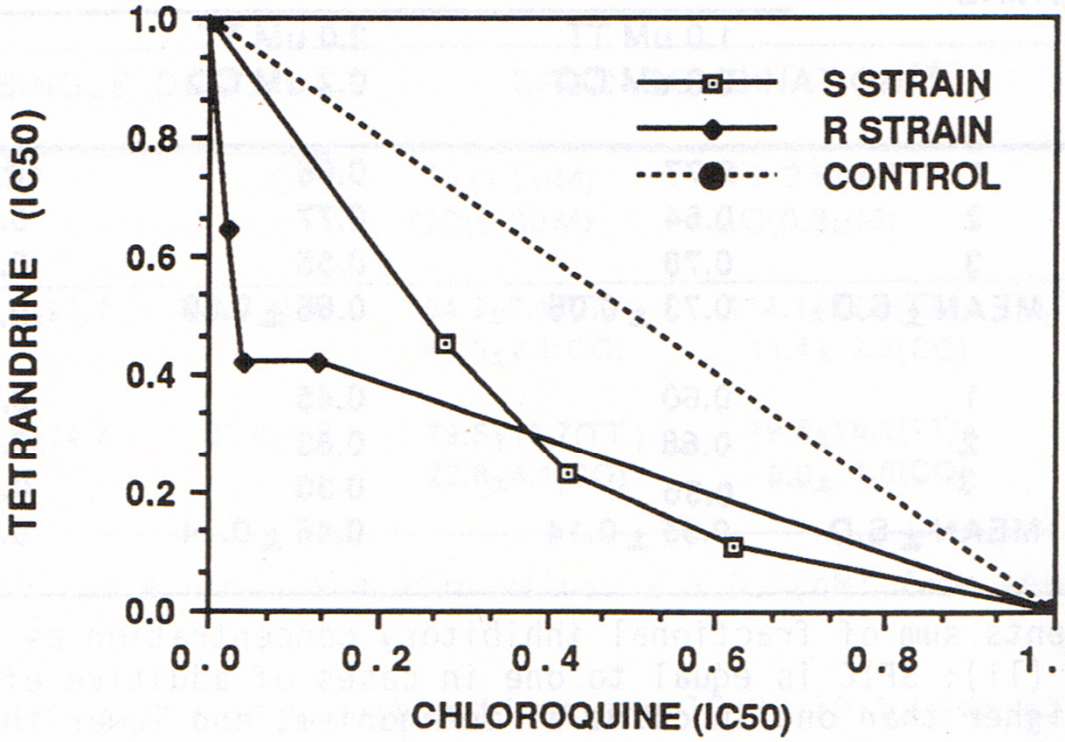
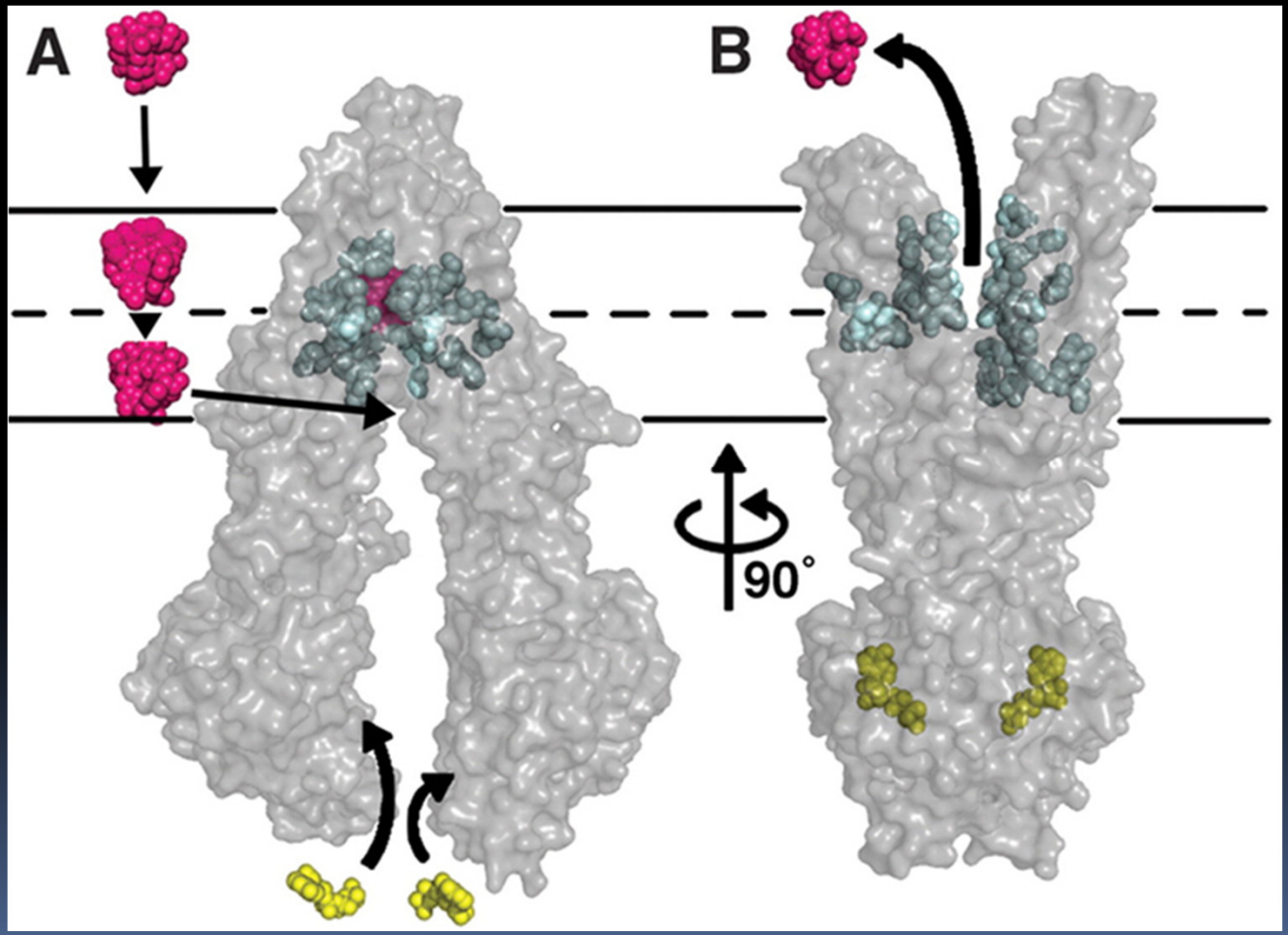


Figure 1. Synergistic effect of tetrandrine and chloroquine on CQ-sensitive (S strain) and CQ-resistant (R strain) P.falciparum in vitro.

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=(++).

Conclusions-CQ+TT

- The combination of CQ and TT produces a synergistic effect on CQ resistant falciparum malaria.
- It occurs because the combination inhibits both multiple drug resistance and affects the chloroquine resistance transporter.
- Toxic verapamil inhibits both mechanisms as well. It is likely that both mechanisms are inhibited by hydrophobic binding –Tetrandrine does the same and both drugs inhibit calcium channels but TT exerts little toxicity.

Acccomplishments since 1966

- 1.Developed the first antimalarial drug screen
- 2.Development of malarial purine salvage and pyrimidine "de novo" synthesis
- 3.After screening 10,000 drugs –mefloquine and halofantrine were recognized
- 4.First recognition of 43 fold synergism between chloroquine(CQ) and tetrandrine (TT) against P. falciparum chloroquine resistance
- 5. CQ andTT effective in CQ res. Aotus monkeys