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SELECTIVE ANTIMALARIAL ACTIVITY OF TETRANDRINE AGAINST CHLOROQUINE RESISTANT PLASMODIUM FALCIPARUM

Zuguang Ye* and Knox Van Dyke

Department of Pharmacology and Toxicology
West Virginia University, Health Sciences Center
Morgantown, WV 26506

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Summary. Antimalarial activity of tetrandrine was studied using a continuous in vitro culture of Plasmodium falciparum. Experimental results showed that tetrandrine has potent antimalarial effect on both chloroquine sensitive and resistant strains of Plasmodium falciparum. Interestingly, tetrandrine is about three times more potent against the chloroquine resistant strain than it is against the sensitive strain based on their IC_{50} values, which were 5.09×10^{-7} M for the sensitive strain and 1.51×10^{-7} M for the resistant strain. In addition, reversal experiments revealed that tetrandrine cannot reverse chloroquine-resistance, although it nas verapamil-like, calcium-channel-blocker activity.

Owing to the resistance of Plasmodium falciparum to commercially available antimalarials, novel drugs are needed. Plants provide therapeutic substances for malaria, eg. quinine and other cinchona alkaloids which are useful drugs against resistant malaria. Recently, Chinese scientists have shown that qinghaosu (artemisinin-QHS), isolated from Artemisia annua, provides rapid antimalarial action against both chloroquine (CQ) resistant and sensitive malaria (1). The use of continuous in vitro culture of P. falciparum allows an efficient search for antimalarial compounds from plants (1-4). The purpose of this work is to report on a new potent antimalarial called tetrandrine (TT). It is an alkaloid (Fig. 1), which is isolated from Stephania tetranda S. Moore used for centuries in traditional Chinese medicine as an antirheumatic or analgesic agent. In 1976, TT was found to possess antisilicotic action and has been used clinically to treat silicosis (5,6). TT also possesses Ca^{++} channel blocking activity (7) and has been shown to produce a negative inotropism (8), shortening of the cardiac action potential

*Zu-guang Ye is a visiting scholar from the Department of Pharmacology, Institute of Chinese Materia Medica, Academy of Traditional Chinese Medicine, Beijing, China.

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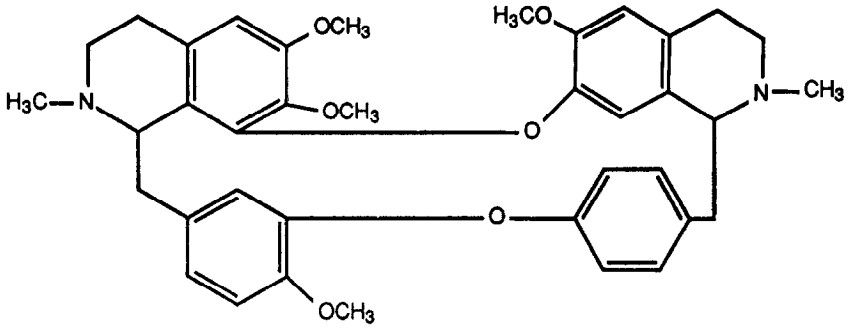

Figure 1. Structure of tetrandine.

(9), block of contraction in K^+-depolarized smooth muscle (10), hypotensive effect in normotensive and hypertensive rats (11) and lowering of blood pressure in dogs (12). Consequently, TT is said to exhibit a verapamil-like inhibition of Ca^{++} channels (12).

Recently, Milhous and coworkers indicated that racemic verapamil reverses CQ resistant falciparum malaria (14). We demonstrated that (+)-verapamil, devapamil and gallopamil have similar activity without significant calcium channel inhibition (15). The above findings have led us to study whether tetrandrine can reverse CQ resistance of P. falciparum.

Materials and Methods

TT was kindly supplied by Dr. Vincent Castranova, ALOSH/NIOSH. Because it is insoluble in water at pH=7, several drops of 1.0 N hydrochloride acid were added to dissolve it in water, and then the pH of this solution was adjusted to 6.2 with 1.0 N NaOH. Thus, a stock solution ($1x10^{-2}$ M) of TT was produced. After sterilization by filtration, serial dilutions of TT were made using the complete medium with 10% pooled human A^+ serum, 25 mM HEPES and 25 mM Na_2HCO_3.

Chloroquine diphosphate, which was a gift from the Winthrop-Sterling Drug Company, Renssalaer, New York, U.S.A., was dissolved in phosphate buffer (pH=7.2), diluted and sterilized similar to TT.

Two strains of P. falciparum were used in these studies: the CQ-sensitive FCMSU1/Sudan strain donated by Dr. J.B. Jensen and a CQ-resistant Indochina (W2) strain provided by Dr. W.K. Milhous. Both parasite strains were cultured in our lab for over one year using the candle jar method (16). For a given experiment, 4-day-old Petri dish cultures with 5-10% parasitemia were diluted with medium containing sufficient nonparasitized human erythrocytes type A to obtain a culture with a final hematocrit of 1.5% and parasitemia of 0.5-1.0%.

Antimalarial activity of a drug was assessed by the method of Desjardins et al (17). Flat-bottomed plastic 96-well microtiter plates were used with each well of the microtiter plates containing a total volume of 250 ul of preparation. Each well contained (1) 25 ul of complete medium with or without the drug to be tested; (2) 175 ul of either the parasitized culture or nonparasitized human erythrocytes as a control; and (3) 25 ul of complete medium with or without TT. After incubation of the plates in a candle jar for 24 hr at 37°C and 25 ul of [2,8-^{3}H] adenosine (0.5 uCi) were added to each well. Plates were incubated at 37°C for an additional 18 hr.

Post-incubation, well contents were harvested onto small disks of fiberglass filters using a Bellco semiautomated cell harvester. The filters were washed five times with distilled water and each disk was removed and placed into a glass scintillation vial containing 10 ml of liquid scintillation fluid (18). Radioactivity was counted using a Packard Tri-Carb scintillation spectrometer Model 2425. Concentration-inhibition curves were plotted and the IC_{50} of a drug was calculated using an Apple II E computer. Duplicate wells were prepared for each concentration of drugs. Experiments were repeated a total of three times.

Results

The values given in Tab. 1 verify that FCMSU1/Sudan is a CQ sensitive strain, while W2 is a CQ resistant one. The IC_{50}s for CQ in the FCMSU1/Sudan and W2 strains are 3.34×10^{-8} and 1.14×10^{-7} M respectively. The difference in CQ sensitivity between the two strains is more than three fold.

TT was very toxic to both sensitive and resistant strains of *P. falciparum in vitro* and its IC_{50}s for the two strains are 5.09×10^{-7} M and 1.51×10^{-7} M respectively. As seen in Tab. 1 and Fig. 2, TT is 3.4 fold more potent against the resistant strain of *P. falciparum* than sensitive strain.

In order to test whether TT can reverse the drug resistance exhibited by the CQ-resistant strain of *P. falciparum*, effects of the combination of TT and CQ on both sensitive and resistant strains of the parasite were observed through using the method reported by Martin and coworkers (14). Concentrations of 5×10^{-9} and 1×10^{-8} M of TT were selected to be combined with CQ for reversal

TABLE 1

EFFECT OF TETRANDRINE ON CHLOROQUINE-SENSITIVE AND -RESISTANT STRAIN OF P.FALCIPARUM IN VITRO

DRUGS	TRIALS	IC_{50} OF DRUGS ($\times 10^{-8}$ M)	
		S* STRAIN	R* STRAIN
TETRANDRINE	1	50.16	17.84
	2	56.58	14.15
	3	45.98	13.31
	MEAN $\pm$ SD	50.91 $\pm$ 5.34	15.10 $\pm$ 2.41**
CHLOROQUINE	1	2.95	14.35
	2	3.29	10.84
	3	3.78	9.03
	MEAN $\pm$ SD	3.34 $\pm$ 0.42	11.41 $\pm$ 2.70**

* S and R strain: chloroquine-sensitive (FCMSU1/Sudan) and -resistant (W2) strain of P.falciparum.
**$P<0.01$ as compared with the IC_{50} for sensitive strain.

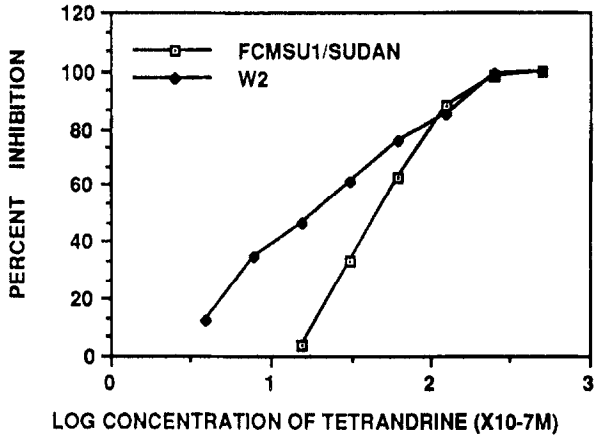

Figure 2. Effect of tetrandrine on chloroquine sensitive (FCMSU1/Sudan) and resistant (W2) strains of falciparum malaria in vitro.

experiments. Based on previous experiments, these concentrations were not toxic to parasite growth. As seen in the Tab. 2, TT at concentration of 5 x 10^{-9} M had no reversal of CQ-resistance, because the IC_{50} of CQ combined with TT (at 5 x 10^{-9} M) was found to be larger than that of CQ alone, regardless of strain.

TABLE 2

EFFECT OF COMBINATION OF TETRANDRINE AND CHLOROQUINE ON P.FALCIPARUM

DRUGS*	TRIALS	IC_{50} OF CQ AGAINST P.FALCIPARUM (10^{-8} M)	
		S STRAIN**	R STRAIN**
CQ	1	2.72	6.98
	2	2.89	6.90
	3	2.02	11.54
	MEAN ± SD	2.54 ± 0.46	8.47 ± 2.66
CQ+T1	1	2.89	6.80
	2	2.85	9.51
	3	3.35	14.40
	MEAN ± SD	3.03 ± 0.03	10.23 ± 3.85
CQ+T2	1	2.20	5.52
	2	2.15	5.37
	3	1.76	6.50
	MEAN ± SD	2.04 ± 0.24	5.79 ± 0.61

* CQ: chloroquine; T1 and T2: tetrandrine at the concentration of 5 x 10^{-9} M and 1 x 10^{-8} M respectively.
** S and R strain: chloroquine sensitive (FCMSU1/Sudan) and resistant (W2) strain.

However, when the concentration of TT was increased to 1 x 10^{-8} M, the IC_{50} of CQ decreased to 2.04 $\pm$ 0.24 x 10^{-8} M (sensitive strain) and 5.79 $\pm$ 0.61 x 10^{-8} M (resistant strain). CQ IC_{50} values were decreased by 1 x 10^{-8} M TT by 20% and 32% respectively as compared with the IC_{50}s of CQ alone (2.54 $\pm$ 0.46 x 10^{-8} M for sensitive strain and 8.47 $\pm$ 2.66 x 10^{-8} M for resistant strain), although those differences are not statistically significant.

Discussion

Although the research on TT began as early as the 1930's (19), most reports of TT activity focused on its actions on the cardiovascular system or its antiinflammatory activity. To our knowledge, TT has not been reported to have antimalarial action. However, we found that TT has a potent antimalarial effect on both CQ sensitive and resistant strains of *P. falciparum in vitro*. Its antimalarial activity against the sensitive strain of the parasite is less than QHS (20). However, its antimalarial potential against the resistant strain is most promising and worthy of further study.

Zeng et al (12) showed that about 44% of whole blood TT was associated with erythrocytes. In addition, its half-life in dogs was 88 minutes, which is approximately three times greater than that found for QHS (21). Experiments indicated that TT is sparingly toxic to animals and humans (6,22). These features should prove beneficial in man particularly in the lesser doses used for antimalarial activity.

Interestingly, TT exhibited more antimalarial potential against the CQ resistant strain than the sensitive strain of *P. falciparum in vitro*. Since TT appears to be more selective for the CQ-resistant parasite, it might somehow react with the actual mechanism involved in confering resistance. Recently, we have provided evidence that CQ resistance possibly resembles multiple drug resistance in cancer cells and might be associated with a similar 155-170 Kd protein as shown by photoaffinity labeling with ^{3}H azidopine (unpublished data). It is possible that this protein actually causes the drug to concentrate in the resistant cell. It is know that 155-170 Kd protein is present in three to five times more concentration in the CQ resistant compared to CQ sensitive parasite (unpublished data).

Furthermore, TT is a dibenzyl isoquinoline derivative and, therefore, displays some similarity to known 4- or 8-aminoquinoline antimalarials. The chemical similarity also might somehow contribute to its antimalarial activity.

TT has been used widely in China in the treatment of hypertension, particularly in the therapy of hypertensive crisis since it exerts potential pharmacological activities on the cardiovascular system (23-25). Based on the characteristic features of its pharmacological action, TT has been referred to as a verapamil-like Ca^{++} channel blocker (26). However, it did not reverse CQ

resistance of P. falciparum, because TT increased antimalarial efficacy of CQ against both CQ resistant and sensitive strains of the parasite, while verapamil with the reversal activity enhances schizonticidal effect of CQ against the resistant strain only (14). Therefore, we can conclude that the increase of CQ antimalarial activity by TT was either additive or synergistic in the interaction of the two compounds.

Recent research indicates that the reversal of CQ resistance by verapamil and its derivatives is not linked to calcium channel inhibition (15). Reversal of resistance apparently is dependent on a lipophilic binding of the drug by the 155-170 Kd protein. Therefore, it is not surprising that TT has no resistance reversal activity, even though it is active as a calcium entry inhibitor.

We believe that TT may be a very useful antimalarial because its action appears to be more selective toward the CQ resistant strain of P.falciparum and therefore when combined with CQ, TT might prevent the emergence of CQ resistance.

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