Tetracycline Antibiotics in Malaria

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Abstract: The emergence and rapid extension of *Plasmodium falciparum* resistance to various antimalarial compounds has gradually limited malaria therapeutic possibilities available to clinicians and the choice of an adapted prophylaxis to travellers specific for their destinations. In this context, doxycycline constitutes an interesting alternative apart from its counter-indications, occurring primarily in children less than eight years old and in pregnant women. Already used successfully in the treatment of malaria in association with quinine in zones of multi-resistances, doxycycline has proven to be effective and well-tolerated in the prevention of malaria. Resistance to doxycycline has not been described until now. The listed prophylactic failures are primarily dependent on an incorrect observation. The mechanisms of action of doxycycline on the parasite are not completely elucidated. The identification of the molecular targets of doxycycline would allow the design of structural analogues that are more active and stable.

Key Words: Malaria, doxycycline, prophylaxis, treatment, antimalarial, antibiotics.

INTRODUCTION

The programme of malaria eradication, initiated in 1960 by the World Health Organization, failed in stable malaria areas, and the current situation remains alarming. Nearly 40% of the world population lives in endemic areas, with 300 to 500 million new infections and 1.5 to 2.7 million deaths occurring each year [1]. The vector control came up against the appearance of resistances in *Anopheles* to principal insecticides employed [2]. The vaccine prospects remain limited by the complexity of the biology of *Plasmodium falciparum*, its antigenic diversity, as well as the inconclusive clinical trials for vaccine candidates in terms of protection and duration of action [3]. The disease prevention and chemotherapy remain a major research focus in the antimalarial fight, and new molecules are constantly required to combat ever-emerging parasitic strains resistant to antimalarial compounds [4].

Chloroquine was introduced in 1940 and has played a dominant role in the chemoprophylaxis and the therapy of malaria. However, extension of chloroquine-resistance to the malaria endemic areas limits its use today [5]. The mechanisms of resistance of *P. falciparum* and the associated molecular markers are generally well-documented for the following antimalarial compounds [6]: chloroquine, mefloquine, halofantrine, cycloguanil, pyrimethamine, sulfadoxine and atovaquone. Cases of resistance to artemisinine and its derivatives used in association in the therapy of malaria have previously been described [7], with some isolates presenting decreased susceptibilities *in vitro* to arthemeter and artesunate [8-10]. The recent introduction of Malarone® (association of atovaquone-proguanil) into the chemoprophylaxis of malaria has already fallen victim to the appearance of resistances [11]. Some antibiotics used in the treatment or the prophylaxis of malaria include the macrolides and, in particular, azithromycin, which has been shown to be effective both *in vitro* [12] and clinically [13].

The tetracyclines, discovered in the beginning of 1940, are broad-spectrum antibiotics and have a wide range of potency on a number of bacteria, particularly intracellular bacteria, or protozoa such as *Plasmodium* [14] as well as lymphatic filariasis [15]. The first clinical use of tetracyclines (aureomycin or chlortetracycline) in the treatment of malaria occurred in 1950 [16]. In Mexico, in 1952 [17] and 1956 [18], Ruiz-Sanchez *et al.* successfully used terramycin (oxytetracycline) in the treatment of uncomplicated malaria to *P. falciparum* and *P. vivax* on a small series of patients (15 and 17, respectively). After the development of resistance to chloroquine in 1960, several studies carried out in 1970 [19, 20] supported the recommendations of the Center for Disease Control for using doxycycline in chemoprophylaxis of *P. falciparum* malaria. Currently, this molecule is used therapeutically in combination with quinine and in chemoprophylaxis in zones of multi-resistances, such as Southeast Asia. Lastly, doxycycline has been used as an initial therapy in chemoprophylaxis in chloroquine-resistance areas by the French military forces deployed in endemic malaria areas.

This review aims to present the pharmacological properties, the mechanisms of action and the activity on the parasite, the therapeutic and chemoprophylactic efficacy, the potential resistance, the tolerability, and the prospects for doxycycline in the treatment of malaria.

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PHARMACOLOGICAL PROPERTIES

Principal data concerning the pharmacokinetic parameters of doxycycline are summarized in Table **1**. Doxycycline is very quickly absorbed *per os* since it is detectable in blood 15 to 30 minutes after its uptake [21-23]. The majority of absorption is carried out in the duodenum [23] and is not modified by food, dairy products or cations. Conversely, didanosine, iron salts, gastro-intestinal topics, enzymatic inductive anticonvulsivants, alcohol and denutrition decrease its absorption. The bioavailability of doxycycline is nearly 95%. The maximum plasmatic concentration of doxycycline (Cmax) varies from 1.5 to 7 µg/ml and is usually reached in 3 hours, while its half-life varies from 14 to 26 hours [24]. Cmax values are higher in older people due to a reduced digestive elimination and are lower in teenagers for unknown reasons [25]. As much as 93% of doxycycline is associated with plasmatic proteins, and little accumulates in red blood cells where its concentration is 2.3 times higher than the corresponding culture medium [26]. Doxycycline has good tissue diffusion because of its high liposolubility and is not metabolized. Forty percent of doxycycline is excreted in three days in faeces by bile, and 30% in the urine.

Several studies were performed to determine the pharmacokinetic properties of doxycycline in healthy volunteers, but little was completed during infections. Only one study was carried out during the treatment of uncomplicated malaria in combination with quinine or artesunate [27]. In a series of 17 patients, the posology of 200 mg per day chosen empirically was determined to be too weak. The authors recommend an initial dose of 400 mg and a twice-daily administration of 200 mg doxycycline in order to maintain plasmatic concentrations at therapeutic rates during the treatment of malaria.

MECHANISMS OF ACTION

Cyclines are a family of antibiotics long known to inhibit protein synthesis of bacteria. Their mechanism of action was elucidated further at the molecular level when it was determined that they were fixed at the proteins S4, S7 and S9 of the small ribosomal subunit 30S and with various ribonucleic acids of the ribosomal RNA 16S [28-31], preventing the binding of aminoacyl transfer RNA to site A of the ribosome, thus blocking the elongation of the translation. However, these mechanisms of action for *Plasmodium* are much less clearly identified.

In 1976, a study [32] highlighted an inhibition of more than 95% of the protein synthesis by chlortetracycline on a cytosolic translation system *in vitro* of *Plasmodium knowlesi* (simian species of *Plasmodium are* also responsible for rare

Table 1. Pharmacokinetic Data of Doxycycline

Pharmacokinetic Parameters			Doxycycline
Bioavailability			95%
Tmax (hours)			2-4
Volume of distribution (ml/kg)			1451[a]
Serum half-life (hours)			14-26
Binding with plasmatic proteins			82-93%
Ratio [GR]/[plasmatic]			2.3[b]
Metabolisation			none
Half-life of elimination (hours)			3 days
Elimination			Urine and faeces
Cmax (µM)	Malaria prevention	Child	ND
		Adult	ND
	Malaria Treatment	Child	ND
		Adult [a]	3.17 (1.63-7.72)
	Volunteers	Adult	1.7-2[c]
AUC (µg.h/ml)	Malaria prevention	Child	ND
		Adult	ND
	Malaria Treatment	Child	ND
		Adult [a]	49.6 (25.1-140.1)
	Volunteers	Adult	12.7-40.1[d]

ND: no data, variable [a]: according to the weight and the age of the patient (n=17 patient, Newton, 2005), [b]: measurements carried out *in vitro* [26], [c]: Cmax (mg/l) with 100 mg of doxycycline *per os* [22], [d]: AUC (mg/L.h).
Cmax = peak plasma concentration, Tmax = time to Cmax and AUC = area under plasma concentration/time curve.

human infections in Borneo). However, the concentration used (10^{-4} M) was more than 10 times the dose used in therapies, and would probably be toxic in humans. Several later studies showed an action of the cyclines on the plasmodial mitochondrion at concentrations of 1 μM to 10 μM, which was similar to clinical concentrations. A study highlighted a synergy of *in vitro* action between the exposure time of the parasite to the tetracyclines and the increase in the oxygen content of the environment of *Plasmodium* [33], suggesting an action of the tetracyclines on mitochondrion. The latter is implied in the control of the oxidative stress and in the energy production of anaerobic *Plasmodium* [34]. According to three previous studies, cyclines would directly inhibit the mitochondrial protein synthesis [35-37], and would also decrease the activity of an enzyme, resulting in the dihydroorotate dehydrogenase being applied in the synthesis of *de novo* pyrimidines [38]. Doxycycline would inhibit the synthesis of nucleotides and the deoxynucleotides at *P. falciparum* [39], at a concentration much higher than those observed *in vivo* (200 μM). The *in vitro* exposure of *P. falciparum* to minocycline would also decrease the transcription of mitochondrial genes (subunit I of the cytochrome C oxidase and the apocytochrome b) and of plastid genes (subunit rpoB/C of the RNA polymerase), suggesting an activity on these two organelles [40]. One study showed that doxycycline would act specifically on the apicoplast of *P. falciparum* [41], and to a lesser extent, on the mitochondrion whose division is inhibited at the end of the cycle that the authors allot to the apicoplastic attack (the two organelles having common metabolic pathways). A parasite exposed to 1 μM of doxycycline for 20 hours presents during the following cycle (at 72 hours) an inhibition of the apicoplastic replication visualized by fluorescence confocale microscopy, electron microscopy and analysis of the parasitic transcriptome. Two recent studies confirm the specific action of the cyclines on the apicoplast of *P. falciparum* [42, 43]. However, another team did not note the inhibition of the plastid replication of different *Toxoplasma gondii,* a member of the Apicomplexan like *P. falciparum*, by submitting the parasite to 100 μM of tetracycline for 48 hours [44].

ANTIPLASMODIAL ACTIVITY

Activity on Sporogonie

The lack of an *in vivo* effect of the tetracyclines on the development of gametocytes (suggested by Ruiz Sanchez [17, 18]) is confirmed by a study performed in 1971 in the United States on healthy volunteers experimentally infected with *P. falciparum* or *P. vivax* [19], and treated by tetracycline or doxycycline. These molecules do not have any action on sporogonie in *Anopheles*, as they do not decrease the capacity of mosquitoes to become infected after blood feeding on carriers of gametocytes under treatment [20].

Activity on the Hepatic Forms

In 1972, Willerson *et al*. showed the following effect of minocycline on the hepatic stages of *P. falciparum*: the administration of 100 mg per day for 7 days, beginning one day prior to exposure to mosquito transmitted sporozoites, prevented malaria in four non-immune healthy volunteers [20]. Several studies performed *in vivo* on simian models (monkeys rhesus and chimpanzees) infected by *P. cynomolgi bastianellii*, *P. vivax* or *P. cynomolgi ceylonensis* showed that terramycin, minocycline or demeclocycline also had an activity on the hepatic forms [45-47]. In a murine model, doxycycline proves to also be effective on the hepatic stages of *P. berghei* and *P. yoelii yoelii* [48], as the administration of 1.4 mg of doxycycline simultaneously or three hours after the injection of sporozoïtes prevented the appearance of a parasitaemia in 100% of the rodents (n=10), while the untreated controls became infected.

However, a study carried out in the United States by the American army on non-immune healthy volunteers in 1994 [49] showed that doxycycline was only partially effective on the hepatic forms of *P. falciparum*. Of the twelve subjects who received 100 mg of doxycycline per day three days prior to an exposure to infected mosquitoes and during the six following days, four developed malaria. Moreover, the regular uptake of doxycycline did not alter the level of antibodies against preerythrocytic stages of *P. falciparum* [50]. These results justified the recommendation of the prophylactic schedule currently adopted with doxycycline, which imposes a daily dose of 100 mg and its continuation during four weeks after the return from endemic areas.

Activity on the Erythrocytic Forms

According to Geary *et al.* [51], the cyclines are equally active against the three asexual stages of blood development of *P. falciparum*. According to Dahl *et al.* [41], the old trophozoïtes and the young schizontes would be more susceptible to doxycycline than the young trophozoïtes and the old schizontes. There is a relationship between the amount and duration of exposure and the effect of doxycycline on the erythrocytic stages with an increased activity at the time of the second cycle, even after a short exposure to 1 μM during the first cycle (personal data not published). This action against the progeny of treated parasites rather than the parasites exposed to the drugs was termed ‘delayed death’.

The *in vitro* susceptibility of *P. falciparum* to doxycycline estimated by the inhibiting concentration 50% (IC_{50}) is about 5.1 μM [3.10-8.38] for multiresistant isolates (from Africa or Southeast Asia) and of 4.3 μM [2.90-6.38] for isolates from West Africa [52]. If we compare the values of the IC_{50} of doxycycline with those of the other antimalarial compounds that are below 1 μM, doxycycline appears to be much less active. A more recent study carried out on 71 Senegalese isolates (average IC_{50} of 11.3 μM [9.5-13.4] for doxycycline) resulted in a lack of correlation observed between the *in vitro* estimated IC_{50} of the isolates to doxycycline and arthemeter, chloroquine, quinine, amodiaquine, pyrimethamine or cycloguanil, suggesting an absence of cross resistance between these molecules [53]. A synergy of action does not exist between the cyclines and chloroquine, mefloquine or quinine [54]. On the other hand, a synergy of action appeared between doxycycline and atovaquone [55, 56] and between doxycycline and artemisinine [57, 58], despite previous studies that showed an additive effect [59, 60]. This justifies doxycycline therapeutic use in combination with a fast schizontocide. The distribution and range of doxycycline IC_{50} values were determined for 747 African isolates. A "triple normal" distribution was fitted to the data using a Bayesian mixture modelling approach. The values for all 747 isolates were classified into 3 components: a first

component A with an IC_{50} mean of 4.9 µM (± 2.1 µM), a second component B with an IC_{50} mean of 7.7 µM (± 1.2 µM), and a third component C with an IC_{50} mean of 17.9 µM (± 1.4 µM). The cut-off for reduced susceptibility to doxycycline *in vitro* was estimated by the geometric mean + 2 standard deviations of the IC_{50} values of the *P. falciparum* isolates associated with C component, that is to say 34.2 µM. Isolates with an $IC_{50} > 35$ µM are considered as isolates with reduced susceptibility to doxycycline *in vitro* (submitted publication).

EFFICACY OF DOXYCYCLINE

Malaria Treatment

All of the studies undertaken in 1950 [17, 18] and 1970 [19, 20, 61, 62] showed the efficacy of the cyclines in monotherapy in the treatment of uncomplicated *P. falciparum* and *P. vivax* malaria. However, in the event of infections with *P. vivax*, some relapses were observed [63] during the two to three months following the first clinical malaria episode or during the first month [64], evidencing an insufficient activity of the cyclines on the hypnozoïtes. Moreover, a radical cure was obtained only after a minimal duration of 7 days treatment of *P. falciparum* (with a dose of 200 mg per day of doxycycline), leading to the disappearance of the parasites in only four to five days on average and approximately 7 days for *P. vivax*. Because of the risk of evolution of uncomplicated *P. falciparum* malaria towards severe forms, the slow schizontocide activity of doxycycline is not suggested for use as cyclines in monotherapy. Conversely, their association with other antimalarial compounds in zones of multi-resistances (Southeast Asia and South America) were the subject of many studies [65-80], which are summarized in Table **2**.

The "standard" treatment of uncomplicated *P. falciparum* malaria in zones of multi-resistances relies on the association of quinine (30 mg/kg/day) to doxycycline (200 mg per day) for 7 days [81]. This treatment makes it possible to obtain a radical cure even in the event of reduction in the *in vitro* susceptibility of isolates to quinine [82]. This bitherapy has a therapeutic efficacy from 91 to 100% in zones of multi-resistances. All other associations tested are lower or equal, in terms of therapeutic efficacy, and are often more expensive, except the combination clindamycin-quinine, which would constitute an interesting alternative in the treatment of malaria in pregnant women and children aged less than 8 years to which the cyclines are counter-indicated.

Malaria Chemoprophylaxis

Currently, doxycycline is used in malaria chemoprophylaxis at 100 mg per day from the first day in endemic areas up to four weeks after return. This schedule, initially recommended by the WHO in 1985, was based on previous studies [19, 20]. The principal studies relating to the efficacy and the tolerance of doxycycline in malaria prevention are summarized in Table **3**. These studies were carried out on the following various populations followed for periods of at least 28 days after the halt of prophylaxis: semi-immune or immune subjects living in endemic areas [83-90] and non-immune travellers, mainly soldiers [91-97]. The results show efficacy from 91 to 99% in immune and semi-immune subjects, and from 95 to 100% in travellers. The majority of the failures observed in the malaria prophylaxis to *P. falciparum* were attributed to a maladjustment of posology confirmed by weak plasmatic concentrations in doxycycline [87], to the use of half dose [84] or to poor observance [92, 98]. However, true prophylactic failures exist. For example, two Australian soldiers presented a *P. falciparum* malaria two weeks after their return from New Guinea in spite of good observance (testified by plasmatic concentration of doxycycline) [93]. No *in vitro* chemosusceptibility test of the isolates to doxycycline was performed in these cases.

The major clinical studies of efficacy and tolerance of doxycycline in malaria prophylaxis against *P. vivax* are summarized in Table **4**. The results show efficacy from 83.1 to 98.7% in immune and semi-immune subjects, and from 52.7 to 100% in travellers. Doxycycline has poor efficacy in the prevention of *P. vivax* malaria relapses.

Resistance to Doxycycline

The mechanisms of resistance of bacteria to the cyclines and the implicated proteins were previously identified [14] and are summarized in Table **5**.

Efflux pumps were the most studied among the Tet proteins, encoded by genes belonging to the major facilitator superfamily, and include over 300 individual proteins [99]. All the *tet* efflux protein genes code membrane-associated proteins that export tetracycline from the cell. Export of tetracycline reduces the intracellular drug concentration and thus protects the ribosome within the cell. Recently, nucleotide sequence and transfer properties of two novel types of *Actinobacillus pleuropneumoniae* plasmids carrying the tetracyclines resistance gene *tet*(H) were discovered [100]. The weak accumulation of tetracycline inside the parasite [26], in spite of sequences homology between a putative transporter of *P. falciparum* and bacterial efflux pumps (Fig. **1**), may suggest the absence of this type of mechanism of resistance to the cyclines in *P. falciparum* (no X-ray crystallographic data are available about efflux pumps in order to predict and compare three-dimensional structures).

The *tet*X genes have been identified in anaerobic bacteria of the genus *Bacteroides*. TetX protein is a flavin-dependent monooxygenase conferring resistance to tetracycline antibiotics by degradation *in vitro* and *in vivo* [101]. No homology of sequence was found between the *tet*X gene and any gene of the genome of *P. falciparum*.

The existence of specific mutations in bacterial rRNA 16S gene can confer a resistance to tetracyclines to *Helicobacter pylori* by diminution of drug fixing to ribosomes [102].

Ribosomal protection proteins are cytoplasmic proteins that protect ribosomes from the action of tetracycline in a GTP-dependent way [103, 104], and display sequence similarity to translation elongation factors EF-G/EF-2 and EF-Tu/EF-1α [105]. Likewise, they possess four functional domains EFTu, EFTu D2, EFG-IV and EFG-C. Interestingly, *P. falciparum* possesses a *tet*Q GTPase family gene (PFL-1710c number access in Plasmodb database). The amino acid sequences of five bacterial protection proteins and the *P. falciparum* tetQ GTPase protein were aligned using the

Table 2. Clinical Studies Comparing the Association with Cyclines and other Therapeutics in the Treatment of *P. falciparum* Malaria

Study			Treatment	No. of Patients	Parasitic Clearance (Days)	Disappear-ance of the Fever (Days)	Efficacy (%) (Duration of the Follow-Up in Days)
Colwell, 1972	Adults	Thailand	**QT**	30	3.6	ND	**96.6** (28)
			QCq	36	3.2	ND	41.6[a] (28)
Chin, 1973	Adults	Thailand	Q[b]	10	2.1	3	75 (28)
			QT[b]	12	2.4	2.2	**66.7** (28)
			QTPy[b]	13	1.6	2.8	**66.7** (28)
Colwell, 1973	Adults	Thailand	**QT**[c]	32	2.4	4	**84** (28)
			QB[c]	31	2.7	3	81 (28)
Noeypatimanond, 1983	Adults Children	Thailand	TAm	51	4.1	ND	**96** (28)
Giboda, 1988	Adults	Kampuchea	Q	43	5.6	3.6	41.8[d] (12)
			QT	22	5.9	3.8	**100**[d] (12)
Looareesuwan, 1994	Adults	Thailand	**MT**	47	2.7	2	**94** (28)
			QT	46	3.1	2.6	**98** (28)
Looareesuwan, 1994	Adults	Thailand	**MD**	48	2.9	1.7	**96** (28)
			AD	49	2.7	1.6	**80** (28)
Metzger, 1995	Adults	Gabon	Q	37	2.2	2	38 (28)
			QCl	36	2.4	2	92 (28)
			QD	35	2.2	2	**91** (28)
Na-Bangchang, 1996	Adults	Thailand	AAz[e]	30	1.2	0.8	14.8 (28)
			AD[e]	30	1.3	1.1	**53.3** (28)
Looareesuwan, 1996	Adults	Thailand	**AtT**	25	2.8	1.3	**100** (28)
			AtP	24	3	3.5	100 (28)
			DTA	22	2.7	2.2	**91** (28)
			AtPy	13	2.6	2.5	77 (28)
Duarte, 1996	Adults Children	Brazil	**AT**[f]	88	ND	ND	**80** (28)
			QT[f]	88	ND	ND	**77** (28)
Bunnag, 1996	Adults	Thailand	**QT**[g]	46	3.7	3.1	**87** (28)
			QT[g]	40	3.7	3.1	**100** (28)
Pukrittayakamee, 2000	Adults Children	Thailand	Q	68	3.2	2.3	87 (28)
			QCl	68	3.3	2	100 (28)
			QT	68	3.2	1.5	**98** (28)
Taylor, 2001	Adults	Irian Jaya	Cq	30	2.8	1.7	22 (28)
			D	20	3.8	2.6	**64.7** (28)
			DCq	39	3.4	2.5	**90.9** (28)
Pukrittayakamee, 2004	Adults Children	Thailand	Q	30	3.3	2.6	84 (28)
			QT	30	3.4	1.4	**100** (28)
			QPr	29	3.2	2	72 (28)
			QPr	37	3.3	2.5	93 (28)
			With	23	2.9	1.4	90.5 (28)
			APr	27	2.6	1.3	84 (28)
Alecrim, 2006	Adults	Brazil	ArLu	28	1.9	ND	100 (6)
			QD	31	3.3	ND	**100** (6)

[a]: study performed in zones of chloroquine-resistance; [b]: quinine 7 days, quinine tetracycline 3 days and quinine tetracycline pyrimethamine 3 days; [c]: quinine 1 day, tetracycline 7 days and bactrim 5 days; [d]: susceptibility *in vivo* after 10 days of treatment; [e]: artesunate amount of load + doxycycline 5 days or azithromycin 2 days; [f]: artesunate + tetracycline 7 days and quinine 3 days + tetracycline 7 days; [g]: quinine 5 or 7 days + tetracycline 7 days.

Q: quinine; T: tetracycline; Cq: chloroquine; Py: pyrimethamine; B: bactrim; Am: amodiaquine; M: mefloquine; D: doxycycline; A: artesunate; Cl: clindamycin; Az: azithromycin; At: atovaquone; P: proguanil; Pr: primaquine; Ar: arthemeter; Lu: lumefantrine.

Table 3. Clinical Studies of Doxycycline Efficacy and Tolerance, Comparatively in *P. falciparum* Malaria Chemoprophylaxis

Study			Molecule Used	Proph Duration (Days)	No. of Patients	No. Case	Efficacy (%) (Average Duration of the Follow-Up Days)
Patients living in endemic area							
Pang, 1987	Children	Thailand	DOX Cq	44 37	95 93	5 31	**94.7** (63) 66.7 (63)
Pang, 1988	Children	Thailand	**DOX**[1] DOX[2] Vit B	97 107 86	77 77 80	2 2 16	**97.4** (150) **97.4** (150) NA (150)
Watanasook, 1989	Adults	Thailand	**DOX**[3] **DOX**[4] Mal	119 119 119	243 243 123	18 38 25	**92.6** (119) **84.4** (119) 79.7 (119)
Shanks, 1992	Adults	Thailand	P-Da Py-Da DOX	80 80 80	184 177 77	19 21 3	89.7 (80) 88.7 (80) **96.1** (80)
Weiss, 1995	Children	Kenya	Vit B Pr* DOX* M* Cq-P*	77 77 77 77 77	64 78 74 74 73	20 4 2 4 7	NA (98) 83 (98) **91** (98) 81 (98) 72 (98)
Ohrt, 1997	Adults	Irian Jaya	DOX* M* Placebo	87 91 48	67 68 69	1 0 53	**99** (87) 100 (91) NA (48)
Andersen, 1998	Adults	Kenya	DOX* Az[5]* Az[6]* Placebo	70 70 70 70	48 55 53 57	3 8 16 48	**92.6** (98) 82.7 (98) 64.2 (98) NA (98)
Taylor, 1999	Adults	Irian Jaya	DOX* Az[5]* Placebo	140 140 140	75 148 77	2 27 29	**96.3** (140) 71.6 (140) NA (140)
Travellers							
Rieckmann, 1993	Adults	New Guinea-New-Guinea	M **DOX** **DOX-Pr** **DOX-Cq**	28 42 21 84	40 60 69 125	0 0 0 0	100 (208) **100** (63) **100** (42) **100** (84)
Shanks, 1995	Adults	Somalia Kampuchea	**DOX**[7] **DOX-Cq**[8]	140 140	900 600	1 2	**99.9** (260) **99.7** (260)
Shanks, 1995	Adults	New Guinea-New-Guinea	**DOX-Pr**[9]	42	53	2	96.2 (322)
Baudon, 1999	Adults	Gabon and Central Africa	DOX Cq-P	150 150	171 270	5 26	**97.1** (210) 91.4 (210)
Schwartz, 1999	Adults	Ethiopia	M DOX Cq Pr	ND ND ND ND	25 19 8 106	0 1 3 4	100 (498) **95** (498) 62.2 (498) 96.2 (498)
Peragello, 2002	Adults	Eastern Timor	M **DOX**[10]	168 168	280 5860	0 94	100 (708) **98.4** (708)
Sonmez, 2005	Adults	Afghanistan	M DOX	84 84	414 986	0 0	100 (264) **100** (264)

NA: no appreciable; *: efficacy = 100 X [1 (rate of failure/rate of failure in the placebo group)]; [1]: doxycycline 100 mg/day or 50 mg/day according to weight's; [2]: doxycycline 50 mg/day or 25 mg/day according to weight's; [3]: doxycycline 100 mg/day; [4]: doxycycline 50 mg/day; [5]: azithromycin 250 mg/day; [6]: azithromycin 1000 mg/week; [7]: + primaquine with the return 15 mg/day 15 days; [8]: + primaquine with the return 15 mg/day 15 days; [9]: + primaquine 7.5 mg/day 5 days; [10]: + primaquine 7.5 mg 3 times/day 14 days with the return.
DOX: doxycycline; Cq: chloroquine; Mal: Maloprim® (pyrimethamine dapsone); P: proguanil; Py: pyrimethamine; Da: dapsone; Pr: primaquine; M: mefloquine; Az: azithromycin.

Table 4. Clinical Studies of Doxycycline Efficacy and Tolerance, Compared in *P. vivax* Malaria Chemoprophylaxis

Study			Molecule Used	Proph Duration (Days)	No. of Patients	No. Case	Efficacy (%) (Average Duration of the Follow-Up Days)
Patients living in endemic area							
Pang, 1988	Children	Thailand	**DOX**[1] DOX[2] Vit B	97 107 86	77 77 80	3 16 62	**96.1** (150) **79.2** (150) NA (150)
Watanasook, 1989	Adults	Thailand	**DOX**[3] **DOX**[4] Mal	119 119 119	243 243 123	28 41 64	**88.5** (119) **83.1** (119) 48 (119)
Shanks, 1992	Adults	Thailand	P-Da Py-Da DOX	80 80 80	184 177 77	3 22 1	98.4 (80) 87.6 (80) **98.7** (80)
Taylor, 1999	Adults	Irian Jaya	DOX* Az[5]* Placebo	140 140 140	75 148 77	1 1 27	**98.7** (140) 99.3 (140) NA (140)
Travellers							
Rieckmann, 1993	Adults	New Guinea-New-Guinea	M **DOX** **DOX-Pr** **DOX-Cq**	28 42 21 84	40 60 69 125	4 2 0 0	90 (208) **96.7** (63) **100** (42) **100** (84)
Shanks, 1995	Adults	Somalia Kampuchea	**DOX**[6] **DOX-Cq**[7]	140 140	900 600	2 6	**99.8** (260) **99** (320)
Shanks, 1995	Adults	New Guinea-New-Guinea	**DOX-Pr**[8]	42	53	9	**83** (322)
Schwartz, 1999	Adults	Ethiopia	M DOX Cq Pr	ND ND ND ND	25 19 8 106	13 9 1 1	48 (498) **52.7** (498) 87.5 (498) 99.1 (498)
Peragello, 2002	Adults	Eastern Timor	M **DOX**[9]	168 168	280 5860	0 191	100 (708) **96.7** (708)

NA: no appreciable; *: efficacy = 100 X [1 (rate of failure/rate of failure in the placebo group)] ; [1]: doxycycline 100 mg/day or 50 mg/day according to weight's; [2]: doxycycline 50 mg/day or 25 mg/day according to weight's; [3]: doxycycline 100 mg/day; [4]: doxycycline 50 mg/day; [5]: azithromycine 250 mg/day; [6]: + primaquine with the return 15 mg/day 15 days; [7]: + primaquine with the return 15 mg/day 15 days; [8]: + primaquine 7.5 mg/day 5 days; [9]: + primaquine 7.5 mg 3 times/day 14 days with the return.
DOX: doxycycline; Cq: chloroquine; Mal: Maloprim® (pyrimethamine dapsone); P: proguanil; Py: pyrimethamine; Da: dapsone; Pr: primaquine; M: mefloquine; Az: azithromycin.

Table 5. Mechanisms of Bacterial Resistance Mediated by *tet* and *otr* Genes

Efflux Pumps	Ribosomal Protection	Enzymatic	Unknown
tet(A), (B), (C), (D), (E)	*tet*(M)	*tet*(X)	*tet*(U)
tet(G), (H), (I), (J), (K)	*tet*(O)		*otr*(C)
tet(L), (V), (Y), (Z)	*tet*(Q), (S), (T)		
*tcr*3	*tet*(W)		
tet(30), (31)	*tet*		
otr(B)	*otr*(A)		
*tet*P(A)	*tet*P(B)		

multiple-sequence alignment program ClustalW [106]. As shown in Fig. (**2**), there is a great homology or identity in the sequences and the three-dimensional structures of these proteins (Figs. **3, 4** and **5**). *P. falciparum* may have the capacity to resist tetracycline in this manner.

The Swiss-prot accession numbers P02982, P23054, Q7BTS0, Q7BL39, A5JHJ7 and Q8I3U3 correspond respectively to tetA (*Escherichia coli*), tetB *(Bacillus subtilis*), tetC (*Francisella tularensis*), tetG (*Salmonella enterica*), tetY (*Aeromonas bestiarum*) and a putative transporter (*Plasmodium falciparum* 3D7). Residues are coloured according to their physical and chemical properties: hydrophobic = red, basic = pink, acidic = blue, hydrophilic = green.

Fig. (1). CLUSTALW alignment of five bacterial efflux pumps protein sequences and a putative transporter of *Plasmodium falciparum*.

Drug pressure with cyclines was performed in a murine model in *P. berghei* [107]. The administration of increasing minocycline doses to mice infected with 10^7 parasites after 86 successive passages over a 600-day period made it possible to obtain a "resistant" strain with IC_{50} = 600 mg/kg/day only six times higher than that of the susceptible starting strain (100 mg/kg/day). However, this resistance was unstable. After 16 additional passages without drug pressure in mice, the strain returned to its initial susceptibility level.

In spite of prophylactic failures with doxycycline, no resistance was observed in *in vitro* chemosusceptibility test. To date, there is no molecular marker associated with *P. falciparum* resistance to doxycycline. In addition, the therapeutic failures reported with association quinine-doxycycline

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CAA52967        ----------------------MKIINIGVLAHVDAGKTTLTESLLYNSGAITELGSVDKG 39
Q48791          ----------------------MKIINIGILAHVDAGKTTLTESLLYSSGAIKELGSVDSG 39
CAA69103        ----------------------MKIINLGILAHVDAGKTTLTESLLYTSGAIAEPGSVDKG 39
CAD20560        ----------------------MKIINIGILAHVDAGKTTLTESLLYASGAISEPGSVEKG 39
AAF01499        ----------------------MKIINIGILAHVDAGKTTVTEGLLYKSGAINKIGRVDNA 39
AAN36428        MFKRVYVYKNSFWKYKKRFFSKKLVNIGILAHIDAGKTTISEDILYQSKEIKVKGNINDQ 60

CAA52967        TTRTDNTLLERQRGITIQTGITSFQWENTKVNIIDTPGHMDFLAEVYRSLSVLDGAILLI 99
Q48791          TTKTDTMFLERQRGITIQTAITSFQRENVKVNIVDTPGHMDFLADVYRSLSVLDGAILLI 99
CAA69103        TTRTDTMNLERQRGITIQTAVTSFQWEDVKVNIIDTPGHMDFLAEVYRSLSVLDGAVLLV 99
CAD20560        TTRTDTMFLERQRGITIQAAVTSFQWHRCKVNIVDTPGHMDFLAEVYRSLAVLDGAILVI 99
AAF01499        TTTTDSMELERDRGITIRASTVSFNYNDTKVNIIDTPGHMDFIAEVERTLKVLDGAILVI 99
AAN36428        NTQLDFLKQERERGITIKSAYSCFEWNKIKVNLIDTPGHIDFSNETFISLCVLDKCIIVI 120

CAA52967        SAKDGVQAQTRILFHALRKMGIPTIFFINKIDQNGIDLSTVYQDIKEKLSAEIVIKQKVE 159
Q48791          SAKDGVQSQTRILFHALRKMNIPIIFFINKIDQNGINLPDVYQDIKDKLSDDIIIKQTVN 159
CAA69103        SAKDGIQAQTRILFHALQTMKIPTIFFINKIDQEGIDLPMVYQEMKAKLSSEIIVKQKVG 159
CAD20560        SAKDGVQAQTRILFHALRKMNIPTVIFINKIDQAGVDLQSVVQSVRDKLSADIIIKQTVS 159
AAF01499        SAKEGIQVQTKVIFNTLVKLNIPTLIFVNKIDRKGVCLDEIYTQIQEKLTSNLAIMQSVK 159
AAN36428        DSKEGVQIQTINIFRYIKEN-LPIYFFLNKMDINHIDIDSNFLSIKNRLTKKGLLITYPI 179

CAA52967        LYP--NMCVTNFTE-------------------------------------------------- 171
Q48791          LNL--KPYVIDYTE-------------------------------------------------- 171
CAA69103        QHP--HINVTDNDD-------------------------------------------------- 171
CAD20560        LSP--EIVLEENTD-------------------------------------------------- 171
AAF01499        IKDKGDFELTNVRDDKVI---------------------------------------------- 177
AAN36428        YENKKLKYILDIPSMVLYSYPHINYGQTFMYNSYFLKTLLEGSLYEKKLYSHFAMINPKP 239

CAA52967        ---------------------------------SEQWDTVIEGNDDLLEKYMSGKSLEALEL 200
Q48791          ---------------------------------PEQWETVIVGNDYLLEKYTIGKTLNIAEL 200
CAA69103        ---------------------------------MEQWDAVIMGNDELLEKYMSGKPFKMSEL 200
CAD20560        ---------------------------------IEAWDAVIENNDELLEKYIAGEPISREKL 200
AAF01499        ---------------------------------QSQIIEKLLDINDYLAEKYINGDVIAEKEY 207
AAN36428        DNLSKENKNKNNINIANVLNDDIIDYIIKKREEIIEILCDLDNTIEYKYLNNIPISYEHF 299

CAA52967        EQEESIRFHNCSLFPVYHGSAKNNIGIDNLIEVITN------------------------- 236
Q48791          EKEENERIQSCSLYPVYHGSAKNNIGIKQLIEVITS------------------------- 236
CAA69103        EQEENRRFQNGTLFPVYHGSAKNNLGIRQLIEVIAS------------------------- 236
CAD20560        AREEQQRVQDASLFPVYHGSAKNGLGIQPLMDAVTG------------------------- 236
AAF01499        NDVFLDEINNCNLYPVFHGSALKNIGIDELLFAITK------------------------- 243
AAN36428        KNSLSLCIDKKYIFPIFSGSALHSYGVHILLDYITHNEKGMEKGMEKGMEKGMEKGMEKG 359

CAA52967        ------------------------------------------------------------
Q48791          ------------------------------------------------------------
CAA69103        ------------------------------------------------------------
CAD20560        ------------------------------------------------------------
AAF01499        ------------------------------------------------------------
AAN36428        MEKGMEKGMEKGMEKGMEKGMEKGMENEMEMDMEKKMEKERQMNHNKQRTNNNTNETPFL 419

CAA52967        ----------KFYSSTHRGPSELCGNVFKIEY---------------------------- 258
Q48791          ----------KLFSPTQLNSDKLCGNVFKVEY---------------------------- 258
CAA69103        ----------KFYSSTPEGQSELCGQVFKIEY---------------------------- 258
CAD20560        ----------LFQPIGEQGGAALCGSVFKVEY---------------------------- 258
AAF01499        ----------YLPTKSYNTEDLLSAYVYKIDR---------------------------- 265
AAN36428        SYERINKDKYHIYQNKKCGNNSDMYHPYDITYNHNNLIKNTDVIKNNIIFNNKKALNSLY 479

CAA52967        ----------TKKRQRLAYIRLYSGVLHLRDSVRVS-EKEKIKVTEMYTSINGELCKIDR 307
Q48791          ----------SDDGQRLVYVRLYSGTLHLRDSVNIS-EKEKIKVTEMYTSINGELRQIDK 307
CAA69103        ----------SEKRRRFVYVRIYSGTLHLRDVIKIS-EKEKIKITEMCVPTNGELYSSDT 307
CAD20560        ----------TDCGQRRVYLRLYSGTLRLRDTVALA-GREKLKITEMRIPSKGEIVRTDT 307
AAF01499        ----------DEKSRKMTFLRVFSGNIRTRQDVYINGTEETFKIKSLESIMNGEIVKVGQ 315
AAN36428        KTIIFIFKLSNEKNGFNTFCKVFKGKLSKNTKLLNLRNKKTEIVKGIYKVKADRYITTNV 539

CAA52967        AYSGEIVILQNE-FLKLNSVLGDTKLLPQ-------------------------------- 335
Q48791          AEPGEIIILKNE-LLKLNNVLGDKKRLPH-------------------------------- 335
CAA69103        ACSGDIVILPND-VLQLNSILGNEMLLPQ-------------------------------- 335
CAD20560        AYQGEIVILPSD-SVRLNDVLGDQTRLPR-------------------------------- 335
AAF01499        VNSGDIAIISNANSLKIGDYIG--KKYDG-------------------------------- 342
AAN36428        LDTNDIGMVRGFENIILCDIICEKDDEKPSHNNNDFFSEDQTTDHNKQNNEYSHSNHIEN 599

CAA52967        ------------------------------------------------------------
Q48791          ------------------------------------------------------------
CAA69103        ------------------------------------------------------------
CAD20560        ------------------------------------------------------------
AAF01499        ------------------------------------------------------------
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(Fig. 2) contd….

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AAN36428        TLNELYHKSHKEDEKNHALHEGTYSQNNLFIGHNDLPPLSNIYKLLKDEIPNKQWLYFLK 659

CAA52967        --RKKIENPHPLLRTTVEPSKPEQREMLLDALLEISDSDPLLRYYVDSTTHEIILSFLGK 393
Q48791          --REILENPLPMLQTTIEPCKSVQREKLLDALFEISDSDPLLQYYVDTVTHEIVLSFLGE 393
CAA69103        --RKFIENPLPMLQTTIAVKKSEQREILLGALTEISDGDPLLKYYVDTTTHEIILSFLGN 393
CAD20560        --KRWREDPLPMLRTTIAPKTAAQRERLLDALTQLADTDPLLRCEVDSITHEIILSFLGR 393
AAF01499        --ILDIKIAQPALRASIKPCDLSKRSKLIEALFELTEEDPFLDCEINGDTGEIILRLFGN 400
AAN36428        SYKKRISKNIIVCTCAIEPKEYKKEKDLKNILKQICLEDNSILIFTD-KNNKLVIGSIGI 718

CAA52967        VQMEVISALLQEKYHVEIELKEPTVIYMERPLKNAEYTIHIEVPPNPFWASIGLSVSP-- 451
Q48791          VQMEVTCTLIQEKYHIEIETRKPTVIYMERPLKKSEFTIDIEVPPNPFWASIGLSVTP-- 451
CAA69103        VQMEVICAILEEKYHVEAEIKEPTVIYMERPLRKAEYTIHIEVPPNPFWASVGLSIEP-- 451
CAD20560        VQLEVVSALLSEKYKLETVVKEPSVIYMERPLKAASHTIHIEVPPNPFWASIGLSVTP-- 451
AAF01499        IQMEVIESLLKSRYKIDARFGELKTIYKERPKRNSKAVIHIEVPPNPYWASIGLSIEP-- 458
AAN36428        LNIEVIIDKIKNDYNIDIKTSPVEIIQKEYIQGYYENSIKKEMKVGSYISTIILGFVIKE 778

CAA52967        ------------------------------------------------------------
Q48791          ------------------------------------------------------------
CAA69103        ------------------------------------------------------------
CAD20560        ------------------------------------------------------------
AAF01499        ------------------------------------------------------------
AAN36428        KDEFIDISSYVQNVLKHEKISHFLSSEEGIKNNISNNKYNNNNKYNNNNKYNNNNKLNIS 838

CAA52967        ----------------------------------------------LPLGSGMQYESSVS 465
Q48791          ----------------------------------------------LPLGSGIQYESLVS 465
CAA69103        ----------------------------------------------LPIGSGVQYESRVS 465
CAD20560        ----------------------------------------------LSLGSGVQYESRVS 465
AAF01499        ----------------------------------------------LPIGSGLLYKTEVS 472
AAN36428        DNLDKDNNLLLYDDIRFEDNKKMYISTTNDDRQNYDEHHNINILDNMEIKESTEKDRKKN 898

CAA52967        LGYLNQSFQNAVMEGIRYGCEQGLYGWNVTDCKICFKYGLYYSPVSTPADFR-------- 517
Q48791          LGYLNQSFQNAVMEGIRYGCEQGLYGWKLTDCKICFKYGLYYSPVSTPADFR-------- 517
CAA69103        LGYLNQSFQNAVMEGVLYGCEQGLYGWKVTDCKICFEYGLYYSPVSTPADFR-------- 517
CAD20560        LGYLNQSFQNAVRDGIRYGLEQGLFGWNVTDCKICFEYGLYYSPVSTPADFR-------- 517
AAF01499        YGYLNNSFQNAVKDAVEKACKEGLYGWEVTDLKVTFDYGLYYSPVSTPSDFR-------- 524
AAN36428        YVYNNLKLGNSKSMYDTKGVKNWVHKYNDDHDKIYLEDNIKDHPHKQSIDDEPELLCDND 958

CAA52967        ------------------------------------MLAPIVLEQVLKKAGTELLEPYLS 541
Q48791          ------------------------------------MLAPIVLEQAFRKSGTELLEPYLS 541
CAA69103        ------------------------------------LLSPIVLEQALKKAGTELLEPYLH 541
CAD20560        ------------------------------------SLAPIVLEQALKESGTQLLEPYLS 541
AAF01499        ------------------------------------NLTPYVFWEALRKAGTEILEPYLK 548
AAN36428        DNDDNDNDDDNDDVDEYLLNFNYDTLFENSVTVHKDVLLYIDELKKMNKKKKNVYDNILN 1018

CAA52967        FKIYTPQEYLSRAYND------------------------APKYCANIVDTQLKNNEVIL 577
Q48791          FEIYVPQEYLSRAYND------------------------ASKYCANILNTKLKGNEVIL 577
CAA69103        FEIYAPQEYLSRAYHD------------------------APRYCADIVSTQVKNDEVIL 577
CAD20560        FILYAPQEYLSRAYHD------------------------APKYCATIETAQVKKDEVVF 577
AAF01499        YTVQVPNDFCGRVMSD------------------------LRKMRASIEDIIAKGEETTL 584
AAN36428        SCIISLKNCLSNGYHTNGNIINTEIIIKNLKIFDSSTTAVAKYACNHLYYEMIKKANIQI 1078

CAA52967        SGEIPARCIQEYRS-------DLTFFTNGRSVCLTELKG--YHVTTGEPVCQPR---RPN 625
Q48791          IGEIPARCIQEYRN-------SLTFFTNGRSVCLTELKG--YQVTNIKSAFQPR---RPN 625
CAA69103        KGEIPARCIQEYRN-------DLTYFTNGQGVCLTELKG--YQPAIGKFICQPR---RPN 625
CAD20560        TGEIPARCIQAYRT-------DLAFYTNGRSVCLTELKG--YQAAVGQPVIQPR---RPN 625
AAF01499        SGKIPVDTSKSYQS-------ELLSYSNGKGIFITEPYG--YDIYNDKPIINDIGNDNND 635
AAN36428        VNPLSLILIQTDEAYTGIIVKDLIQYRNGTIIQIMKNKESDFKLMKIYAIIPVKFTHNYS 1138

CAA52967        SRIDKVRYMFNKIT--------- 639
Q48791          NRIDKVRHMFNKINLH------- 641
CAA69103        SRIDKVRHMFHKLA--------- 639
CAD20560        SRLDKVRHMFQKVM--------- 639
AAF01499        SNKEGLRYLFQKQDEN------- 651
AAN36428        SILRSISSGHANFLMTFCGYKKC 1161
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The NCBI accession numbers AAF01499, Q48791, CAA52967, CAA69103, CAD20560 and AAN36428 correspond respectively to tetT (*Streptococcus pyogenes*), tetS (*Listeria monocytogenes*), tetM (*Neisseria meningitidis*), tetO (*Streptococcus pneumoniae*), tetW (*Butyrivibrio fibrisolvens*) and tetQ family GTPase putative (*Plasmodium falciparum* 3D7). Residues are coloured according to their physical and chemical properties: hydrophobic = red, basic = pink, acidic = blue, hydrophilic = green.

Fig. (2). CLUSTALW alignment of five bacterial ribosomal protection protein sequences and TetQ GTPase family putative protein of *Plasmodium falciparum*.

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AAN36428        TLNELYHKSHKEDEKNHALHEGTYSQNNLFIGHNDLPPLSNIYKLLKDEIPNKQWLYFLK 659

CAA52967        --RKKIENPHPLLRTTVEPSKPEQREMLLDALLEISDSDPLLRYYVDSTTHEIILSFLGK 393
Q48791          --REILENPLPMLQTTIEPCKSVQREKLLDALFEISDSDPLLQYYVDTVTHEIVLSFLGE 393
CAA69103        --RKFIENPLPMLQTTIAVKKSEQREILLGALTEISDGDPLLKYYVDTTTHEIILSFLGN 393
CAD20560        --KRWREDPLPMLRTTIAPKTAAQRERLLDALTQLADTDPLLRCEVDSITHEIILSFLGR 393
AAF01499        --ILDIKIAQPALRASIKPCDLSKRSKLIEALFELTEEDPFLDCEINGDTGEIILRLFGN 400
AAN36428        SYKKRISKNIIVCTCAIEPKEYKKEKDLKNILKQICLEDNSILIFTD-KNNKLVIGSIGI 718

CAA52967        VQMEVISALLQEKYHVEIELKEPTVIYMERPLKNAEYTIHIEVPPNPFWASIGLSVSP-- 451
Q48791          VQMEVTCTLIQEKYHIEIETRKPTVIYMERPLKKSEFTIDIEVPPNPFWASIGLSVTP-- 451
CAA69103        VQMEVICAILEEKYHVEAEIKEPTVIYMERPLRKAEYTIHIEVPPNPFWASVGLSIEP-- 451
CAD20560        VQLEVVSALLSEKYKLETVVKEPSVIYMERPLKAASHTIHIEVPPNPFWASIGLSVTP-- 451
AAF01499        IQMEVIESLLKSRYKIDARFGELKTIYKERPKRNSKAVIHIEVPPNPYWASIGLSIEP-- 458
AAN36428        LNIEVIIDKIKNDYNIDIKTSPVEIIQKEYIQGYYENSIKKEMKVGSYISTIILGFVIKE 778

CAA52967        ------------------------------------------------------------
Q48791          ------------------------------------------------------------
CAA69103        ------------------------------------------------------------
CAD20560        ------------------------------------------------------------
AAF01499        ------------------------------------------------------------
AAN36428        KDEFIDISSYVQNVLKHEKISHFLSSEEGIKNNISNNKYNNNNKYNNNNKYNNNNKLNIS 838

CAA52967        ----------------------------------------------LPLGSGMQYESSVS 465
Q48791          ----------------------------------------------LPLGSGIQYESLVS 465
CAA69103        ----------------------------------------------LPIGSGVQYESRVS 465
CAD20560        ----------------------------------------------LSLGSGVQYESRVS 465
AAF01499        ----------------------------------------------LPIGSGLLYKTEVS 472
AAN36428        DNLDKDNNLLLYDDIRFEDNKKMYISTTNDDRQNYDEHHNINILDNMEIKESTEKDRKKN 898

CAA52967        LGYLNQSFQNAVMEGIRYGCEQGLYGWNVTDCKICFKYGLYYSPVSTPADFR-------- 517
Q48791          LGYLNQSFQNAVMEGIRYGCEQGLYGWKLTDCKICFKYGLYYSPVSTPADFR-------- 517
CAA69103        LGYLNQSFQNAVMEGVLYGCEQGLYGWKVTDCKICFEYGLYYSPVSTPADFR-------- 517
CAD20560        LGYLNQSFQNAVRDGIRYGLEQGLFGWNVTDCKICFEYGLYYSPVSTPADFR-------- 517
AAF01499        YGYLNNSFQNAVKDAVEKACKEGLYGWEVTDLKVTFDYGLYYSPVSTPSDFR-------- 524
AAN36428        YVYNNLKLGNSKSMYDTKGVKNWVHKYNDDHDKIYLEDNIKDHPHKQSIDDEPELLCDND 958

CAA52967        ------------------------------------MLAPIVLEQVLKKAGTELLEPYLS 541
Q48791          ------------------------------------MLAPIVLEQAFRKSGTELLEPYLS 541
CAA69103        ------------------------------------LLSPIVLEQALKKAGTELLEPYLH 541
CAD20560        ------------------------------------SLAPIVLEQALKESGTQLLEPYLS 541
AAF01499        ------------------------------------NLTPYVFWEALRKAGTEILEPYLK 548
AAN36428        DNDDNDNDDDNDDVDEYLLNFNYDTLFENSVTVHKDVLLYIDELKKMNKKKKNVYDNILN 1018

CAA52967        FKIYTPQEYLSRAYND----------------------APKYCANIVDTQLKNNEVIL 577
Q48791          FEIYVPQEYLSRAYND----------------------ASKYCANILNTKLKGNEVIL 577
CAA69103        FEIYAPQEYLSRAYHD----------------------APRYCADIVSTQVKNDEVIL 577
CAD20560        FILYAPQEYLSRAYHD----------------------APKYCATIETAQVKKDEVVF 577
AAF01499        YTVQVPNDFCGRVMSD----------------------LRKMRASIEDIIAKGEETTL 584
AAN36428        SCIISLKNCLSNGYHTNGNIINTEIIIKNLKIFDSSTTAVAKYACNHLYYEMIKKANIQI 1078

CAA52967        SGEIPARCIQEYRS-------DLTFFTNGRSVCLTELKG--YHVTTGEPVCQPR---RPN 625
Q48791          IGEIPARCIQEYRN-------SLTFFTNGRSVCLTELKG--YQVTNIKSAFQPR---RPN 625
CAA69103        KGEIPARCIQEYRN-------DLTYFTNGQGVCLTELKG--YQPAIGKFICQPR---RPN 625
CAD20560        TGEIPARCIQAYRT-------DLAFYTNGRSVCLTELKG--YQAAVGQPVIQPR---RPN 625
AAF01499        SGKIPVDTSKSYQS-------ELLSYSNGKGIFITEPYG--YDIYNDKPIINDIGNDNND 635
AAN36428        VNPLSLILIQTDEAYTGIIVKDLIQYRNGTIIQIMKNKESDFKLMKIYAIIPVKFTHNYS 1138

CAA52967        SRIDKVRYMFNKIT--------- 639
Q48791          NRIDKVRHMFNKINLH------- 641
CAA69103        SRIDKVRHMFHKLA--------- 639
CAD20560        SRLDKVRHMFQKVM--------- 639
AAF01499        SNKEGLRYLFQKQDEN------- 651
AAN36428        SILRSISSGHANFLMTFCGYKKC 1161
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The three-dimensional structure of EFG of *Thermus thermophilus* was obtained from X-ray crystallography data and is available in the protein database (accession number 1ktv). EFG is dimeric and has two chains A and B with four functional domains: EFTu is coloured in red, EFTu-D2 in blue, EFG-IV in orange and EFG-C in magenta. This picture was realized with the software PyMOL version 0.98.

Fig. (3). Three-dimensional structure of Elongation Factor G of *Thermus thermophilus*.

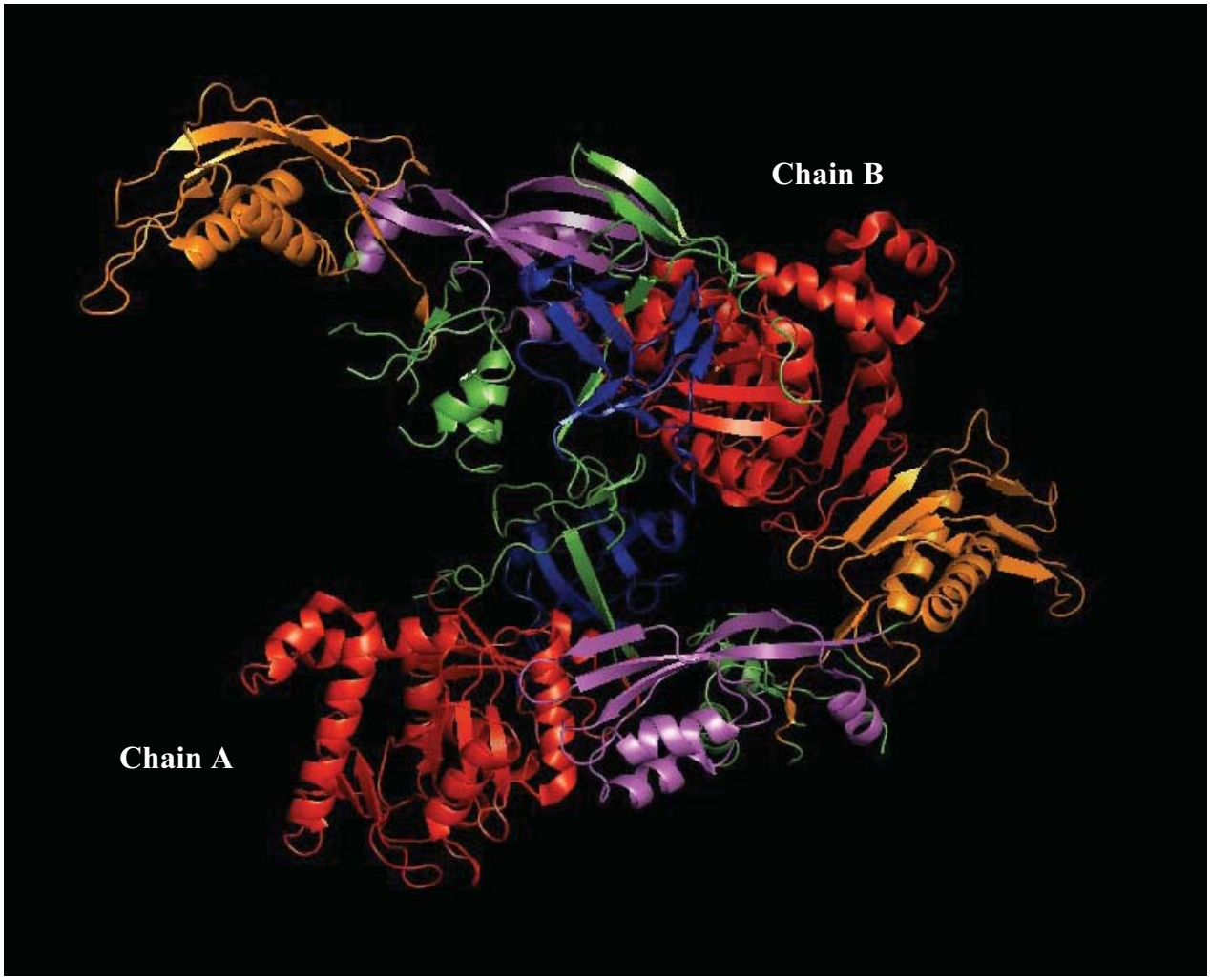
were never documented with respect to a resistance to doxycycline.

DOXYCYCLINE TOLERANCE

Doxycycline is counter-indicated in cases of allergies to cyclines, in pregnant women from the second quarter of pregnancy due to a risk of anomalies of the dental bud (no teratogenic effect was observed in animal models and some pregnant women treated with cyclines) and in children younger than eight years due to the risk of dyschromy and hypoplasia of dental enamel. Association with retinoid is also counter-indicated, as it can generate an intracranial hypertension. Association with anticoagulants (antivitamine K) is to be avoided because it may induce hemorrhagic accidents.

The side effects of cyclines are known in the treatment of the bacterial infections. Their tolerance is good in the long treatment course (several months up to five years) of acne at a dose of 250 mg of oxytetracycline twice a day, or 100 mg of minocycline twice a day [108, 109], and do not require any particular biological follow-up [110]. For malaria chemoprophylaxis, this may imply use of doxycycline for several months at low dose (100 mg per day). The following principal side effects reported in the literature are in order of frequency:

- digestive disorders (in 2.5 to 20% of the cases according to studies'). Rare esophagitis and oesophageal ulcerations [111, 112] are practically no longer reported since the introduction of the monohydrate formula is less ulcerogenic than the old monohyclate formula [113];
- sleep disorders (in 1.3 to 14% of the cases) and headaches (approximately 10%) are reported [97, 98, 114];
- cutaneous signs (in 0.6 to 8% of the cases according to authors') with urticarias, photosensitizations (the photo toxicity of doxycycline is a function of the dose, to 200 mg 50% of Caucasian subjects are photosensitized [115]), exfoliation, cutaneous rash [116], and sometimes photo-onycholysis [117];
- vaginal candidoses may occur in approximately 1% of the women [118];



The three-dimensional structure of TetW of *Butyrivibrio fibrisolvens* (NCBI accession number CAD20560) was obtained from the amino-acid sequence using the prediction three-dimensional structure software 3D-JIGSAW version 2.0. TetW is monomeric with four functional domains similar to EFG: EFTu is coloured in red, EFTu-D2 in blue, EFG-IV in orange and EFG-C in magenta. This picture was realized with the software PyMOL version 0.98.

Fig. (4). Three-dimensional structure of TetW protein of *Butyrivibrio fibrisolvens*.

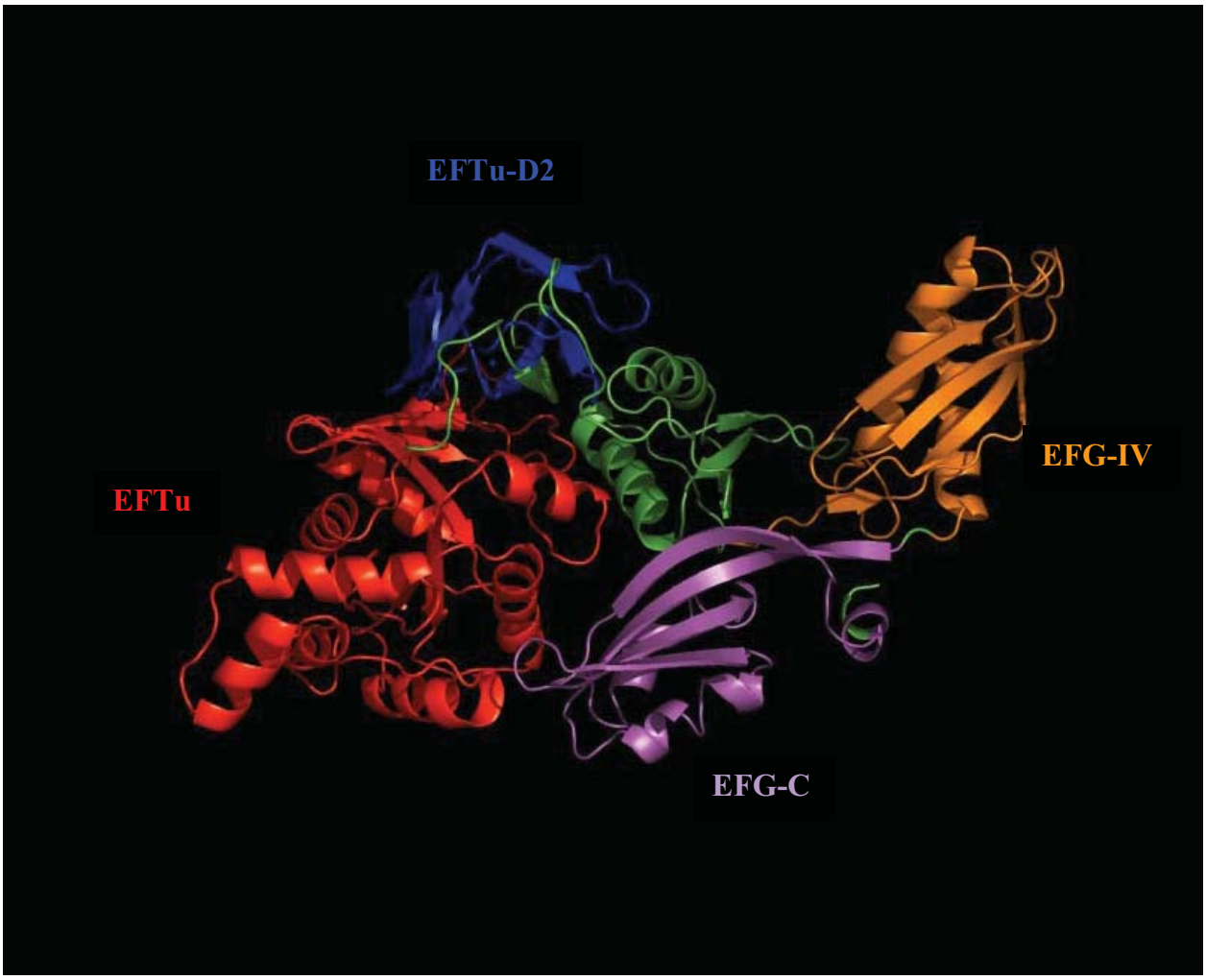
- two cases of intracranial hypertension were listed among patients without cardiovascular or neurological risk factors with doxycycline during a malaria chemoprophylaxis [119].

Thus, doxycycline tolerance is effective and equivalent to the majority of the other antimalarial drugs used in malaria prevention.

The use of an antibiotic for several months in malaria chemoprophylaxis may render the bacteria resistant to the cyclines (the molecular epidemiology and the bacterial mechanisms of resistance to the cyclines were largely described by Chopra in 2001 [14]). In 1988, the first publication of gastroenteritis to *Campylobacter jejuni* resistant to tetracyclines in American soldiers serving in Thailand [120]. A later study undertaken by the same team has shown that doxycycline in malaria chemoprophylaxis exposes fewer to the selection of bacteria resistance than to the acquisition of bacteria already resistant to the cyclines that were largely widespread in this country for a long time [121]. Only one other study was published regarding the risk of emergence of bacterial resistances to the cyclines [122] associated with their use in malaria chemoprophylaxis.

OUTLINES

The emergence and the rapid extension of the resistance of *P. falciparum* to principal antimalarial drugs available necessitate the search for new molecules. In the meantime, doxycycline constitutes an excellent molecule for the treatment of uncomplicated malaria in zones of multi-resistances but only in association with fast schizontocide like quinine or artesunate. Doxycycline's good tolerance and its efficacy were proven. Doxycycline is currently the only antimalarial drug for which no resistance of *P. falciparum* was described so doxycycline use should be expanded in particular in the prevention to *P. falciparum* malaria except in pregnant women and in children less than eight years old. The major limitation would probably be the poor compliance because of the short half life and the weak activity on hepatic stages which imposes a daily dose of 100 mg and its continuation during four weeks after the return from endemic areas. A better comprehension of the mechanisms of action of doxy-



The three-dimensional structure of TetQ GTPase putative of *Plasmodium falciparum* (NCBI accession number PFL1710c) was obtained from the amino-acid sequence using the prediction three-dimensional structure software 3D-JIGSAW version 2.0. This protein possesses two functional domains: EFTu is coloured in red and EFG-C in magenta. Amino-acids coloured in blue are the same of TetW. EFG-C seems to contain the two domains EFG-IV and EFG-C of TetW. The EFTu-D2 domain of TetW might exist in TetQ as the similarities are higher in this region between the two proteins. This picture was realized with the software PyMOL version 0.98.

Fig. (5). Three-dimensional structure of TetQ GTPase family protein of *Plasmodium falciparum* 3D7.

cycline on *P. falciparum* and the identification of its molecular targets would allow the design of more effective (IC_{50} around 1 nM) and stable structural analogues.

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