Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 353 (2007) 177–181
BBRC
www.elsevier.com/locate/ybbrc

Antimalarial properties of green tea

Anna Rosa Sannella [a], Luigi Messori [b,*], Angela Casini [b], Franco Francesco Vincieri [c], Anna Rita Bilia [c], Giancarlo Majori [a], Carlo Severini [a]

[a] *Department of Infectious, Parasitic and Immunomediated Diseases, Vector-Borne Diseases and International Health Section, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy*
[b] *Department of Chemistry, University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino, Florence, Italy*
[c] *Department of Pharmaceutical Sciences, University of Florence, Via U. Schiff 6, 50019 Sesto Fiorentino, Florence, Italy*

Received 10 November 2006
Available online 11 December 2006

Abstract

We show here that a crude extract of green tea as well as two of its main constituents, *epigallocatechin-3-gallate* (EGCG) and *epicatechin gallate* (ECG), strongly inhibit *Plasmodium falciparum* growth *in vitro*. Both these catechins are found to potentiate the antimalarial effects of artemisinin without interfering with the folate pathway. The importance of these findings and their mechanistic implications are discussed in view of future therapeutic strategies.

Keywords: Malaria; Artemisinin; Green tea; Catechins; *Plasmodium falciparum*

Green tea has attracted large attention, recently, both in the scientific community and in the public opinion, for its pronounced health benefits towards a variety of disorders ranging from cancer to weight loss [1]. A clear link between the health benefits of green tea and the high content of catechins has rapidly emerged [1,2]; in particular, much emphasis has been given to the anticancer effects of these substances and to the investigation of the underlying biochemical mechanisms [3,4].

Appreciation of the health effects of green tea has longly been hindered by the low oral bioavailability of its polyphenolic catechins [5]. Moreover, absorbed green tea catechins are known to undergo rapid and extensive metabolic transformations [6]. These are most probably the reasons why no association has been reported so far between green tea consumption and its antimalarial effects despite the large use of this beverage in several Asian countries where malaria is endemic.

Catechins, the active substances of green tea, represent ca. 15% of plant dry weight [7]. The most important green tea catechins are (−)-epigallocatechin gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), and (−)-epicatechin (EC), altogether accounting for over 85% of the total flavonoid content. Notably, all these substances are strictly related to each other from the chemical and structural point of view as shown in Fig. 1. Among green tea catechins, EGCG is by far the most abundant (about 60% of total content; thus, one 240 ml cup of brewed tea may contain up to 200 mg EGCG).

In the frame of a larger research project aimed at evaluating the possible interactions, in malaria treatment, between artemisinins [8] and a variety of natural substances, we analysed the specific antiplasmodial properties *in vitro* of the main green tea catechins, either alone or in combination with artemisinins, against two distinct *Plasmodium falciparum* strains: 3D7, chloroquine sensitive (CQS), and FCR-1/FVO, chloroquine resistant (CQR). Also, the antimalarial effects of crude extracts of green tea were studied.

* Corresponding author. Fax: +39 055 4573385.
E-mail address: luigi.messori@unifi.it (L. Messori).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.12.005

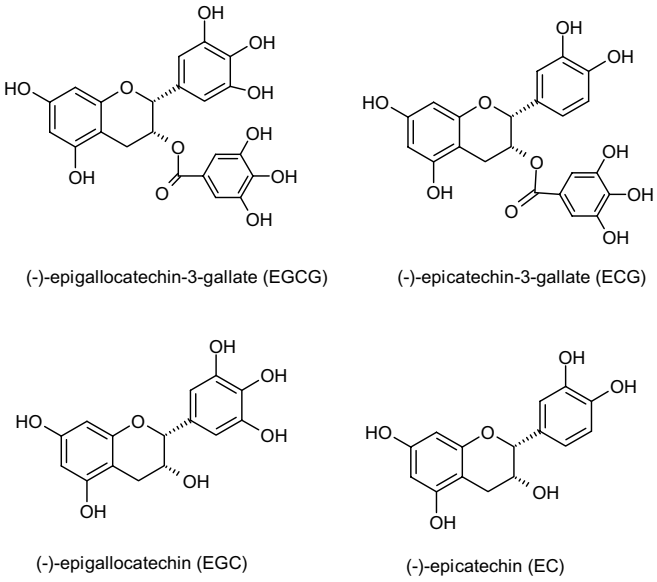

Fig. 1. Chemical structure of the main green tea catechins.

Materials and methods

Reagents. Artemisinin, tea catechins and folinic acid used in this study were from Sigma and green tea crude extract from Indena (extract from *Camellia sinensis* contains 60.0% of total polyphenols, >40.0% of EGCG by HPLC, and <0.1% of caffeine by HPLC).

Parasite maintenance. Cultures of *P. falciparum*, either 3D7 drug-sensitive (CQS) or FCR-1/FVO resistant to chloroquine (CQR), were grown *in vitro* in human red blood cells (O^+) as formerly described [9].

In vitro determination of antimalarial activity of tea catechins. Tea catechins were prepared as stock solutions in 95% ethanol and then diluted in complete RPMI medium (containing 10% human serum). In all tests, the concentration of ethanol was maintained at 0.02% and did not inhibit the growth of control cultures. The four individual tea catechins were presented at concentrations ranging between 1 and 250 μM. Growth inhibition was evaluated by a well-established enzymatic method based on lactate dehydrogenase activity of *P. falciparum* (pLDH) [10]. Two parasite strains of *P. falciparum,* 3D7 drug-sensitive (CQS) and FCR-1/FVO resistant to chloroquine (CQR), were used for the *in vitro* tests after culture syncronization by sorbitol [11]. The effects of tea catechins on cultured parasites were determined by light microscopy and pLDH activity, as previously described in detail [12]. Each assay was performed in triplicate, on three separate occasions.

Then *P. falciparum* growth inhibition was analysed as a function of added artemisinin, at sublethal doses, ranging from 2.5 to 40 nM, either in the presence (or in the absence) of 15 μM EGCG or of 2.5 μM ECG. The effects of artemisinin and catechins were assessed using isobologram analysis and by analysis of the sum of the fractional inhibitory concentrations (FICs) [13].

Folate pathway "rescue" experiment. The experiment was carried out by addition of folinic acid (5-formyltetrahydrofolic acid = FA, Sigma) 500 μg/ml to the growth medium; growth inhibition was evaluated with respect to a control as earlier described [14]. We also checked that such a concentration of FA, given alone, does not affect significantly *P. falciparum* growth.

Results

In vitro P. falciparum growth inhibition studies

The growth inhibition effects of the individual green tea catechins and of green tea crude extracts were measured on two *P. falciparum* strains, 3D7 drug-sensitive (CQS) and FCR-1/FVO resistant to chloroquine (CQR), according to the procedure reported in the experimental section. Relevant antiplasmodial effects were indeed found for EGCG and ECG, in terms of 50% inhibitory concentration (IC_{50}) (Fig. 2). The following values were determined: EGCG (3D7 $IC_{50} = 37.240$ μM, 95% CI = 26.41–67.68; FCR-1/FVO $IC_{50} = 31.381$ μM, 95% CI = 21.05–55.43) and ECG (3D7 $IC_{50} = 10.897$ μM, 95% CI = 4.321–18.41; FCR-1/FVO $IC_{50} = 7.237$ μM, 95% CI = 2.563–16.40), as

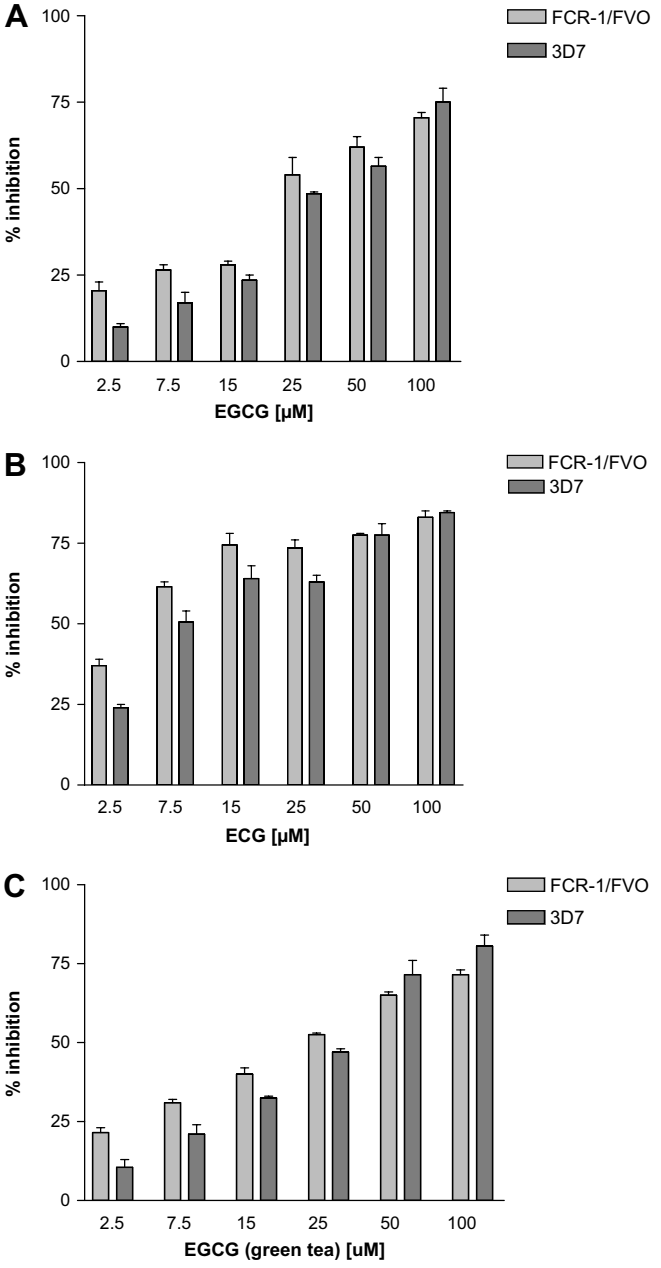

Fig. 2. Antiplasmodial activities of catechins. Percentage of inhibitions of 3D7 and FCR-1/FVO *P. falciparum* strains against increasing concentrations of EGCG (A), ECG (B) or EGCG from green tea crude extract (C), respectively. The data are expressed assuming no inhibition for untreated controls.

Table 1
IC_{50} values for EGCG, ECG, green tea extract, artemisinin (ART), ART/15 μM EGCG and ART/2.5 μM ECG against 3D7 and FCR-1/FVO *P. falciparum* strains

Plasmodium falciparum strains		
Name of compounds	IC_{50} (95% CI)	
	3D7	FCR-1/FVO
(−)-Epigallocatechin gallate (EGCG) μM	37.240 μM (26.41–67.68)	31.381 μM (21.05–55.43)
(−)-Epicatechin gallate (ECG) μM	10.897 μM (4.321–18.41)	7.237 μM (2.563–16.40)
Green tea (EGCG) μM	30.766 μM (26.03–37.14)	28.616 μM (20.82–41.12)
Artemisinin (ART) nM	14.084 nM (13.28–16.14)	12.008 nM (9.553–14.97)
ART/15 μM EGCG	8.433 nM (3.711–12.88)*	9.375 nM (1.2–16.58)*
ART/2.5 μM ECG	9.540 M (3.655–14.18)*	4.079 nM (0.08–11.39)*

* $P < 0.001$.

summarized in Table 1. Notably, both these catechins showed somewhat higher antiplasmodial effects on the CQR strain; ECG resulted in general more "active" than EGCG.

The IC_{50} values measured for EGC were far higher ($IC_{50} > 300$ μM) whereas EC turned out to be substantially ineffective ($IC_{50} > 500$ μM). Notably, the crude extract was found to produce appreciable growth inhibition effects that are well consistent with its EGCG content (the green tea crude extracts contains ~40% w/w of EGCG). IC_{50} values of 30.766 μM (95% CI = 26.03–37.14) on CQS strain and 28.616 μM (95% CI = 20.82–41.12) on CQR strain were determined (Fig. 2 and Table 1).

Subsequently, we analysed the possible pharmacological interactions between artemisinin and EGCG or ECG on both CQS and CQR parasite strains. Artemisinin is a well known, potent, and clinically established antimalarial drug, with an IC_{50} value falling in the low nanomolar range [8]. We wondered whether green tea catechins might potentiate its action; possible interactions among green tea catechins and artemisinin were evaluated through an established methodology relying on the so called "isobologram" analysis [13,15].

Using EGCG 15 μM and ECG 2.5 μM in combination with artemisinin, the sum of FICs was found to be 1 and 0.9 for the 3D7 CQS strain, respectively, and 1.2 and 0.68 for FCR-1/FVO CQR strain. The results of isobologram analysis are consistent with an essentially *additive effect* of both these catechins with artemisinin, as shown in Fig. 3; a sub-synergistic effect might be attributed to ECG on the CQR *P. falciparum* strain. Notably, the described additive effects are quite evident in the lower artemisinin concentration range.

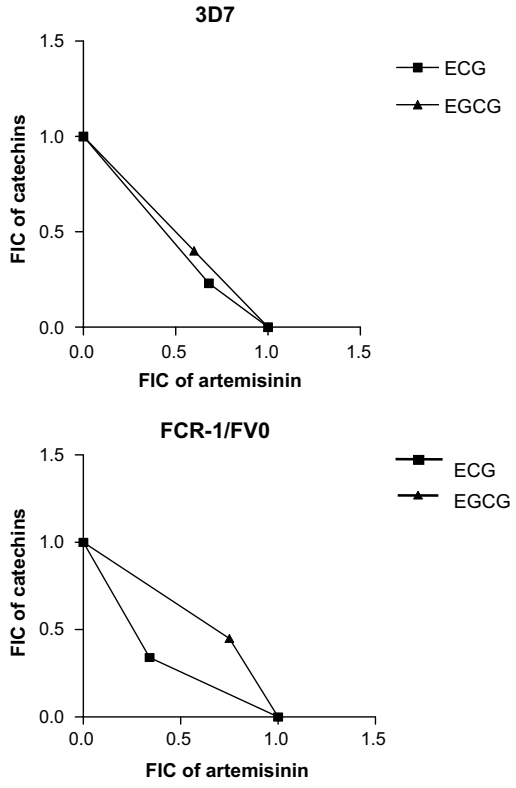

Fig. 3. Isobolograms showing the interaction between artemisinin and EGCG or ECG catechins when administered on 3D7 and FCR-1/FVO *P. falciparum* strains, respectively.

Mechanistic studies: the "folate pathway" hypothesis

The essential additivity of the effects caused by epicatechin and artemisinin, given in combination, suggests that these substances may interfere with *P. falciparum* metabolism at two distinct sites. Where this not the case, the reducing properties of epicatechins would be expected to antagonize—rather than increase—the biological effects of artemisinin, as previously found in the case of glutathione and other reductants [16]. Notably, recent studies directed to elucidate the mechanisms of their anticancer actions revealed that green tea catechins possess relevant antifolic effects as a consequence of direct inhibition of DHFR (dihydrofolate reductase) [17]. This observation is further supported by the evident chemical and structural analogy of tea catechins to some classical antifolic agents e.g. *trimethoprim* and *methotrexate*.

Since inhibition of folates represents a typical mechanism of action for a few clinically established antimalarial drugs [18,19], we hypothesized that such a mechanism might be operative also in the case of EGCG and of

structurally related catechins. This hypothesis was promptly tested in our laboratory through a simple experiment, carried out according to the procedure recently described by Djapa et al. [14]. In detail, we investigated whether administration of appropriate amounts of folinic acid, a well-known folate pathway "rescue" agent [14,20] might reverse the growth inhibition effects of EGCG. We found that addition of excess folinic acid (at 500 μg/ml) does not reverse appreciably the antimalarial activity of 100 μM EGCG. Indeed, comparable inhibition values were measuredin the two cases (76% EGCG + FA versus 79% EGCG alone), at variance with the case reported by Djapa et al. [14]. This observation suggests that the relevant antiplasmodial effects of EGCG are not a consequence of folate pathway inhibition but must arise from interference with a different biochemical pathway.

Discussion and conclusions

Establishing unambiguously the antimalarial effects *in vitro* of the crude extract of green tea as well as of some of its major polyphenolic constituents represents, in our opinion, a result of great interest. Indeed, these substances are very abundant and widespread in the malaria endemic countries, are cheap and easily accessible, safe and virtually lacking of systemic toxicity. Our results might pave the way toward the development of these substances into effective antimalarial agents. Moreover, the additive/sub-synergistic interaction observed between EGCG or ECG and artemisinin might be conveniently exploited to design new and/or more effective combination therapies.

Particular attention has been paid to the molecular mechanisms that might be at the basis of the observed *in vitro* antimalarial effects of these epicatechins. Despite the structural relatedness of catechins to classical antifolic agents and the reported ability of epicatechins to inhibit crucial enzymes of the folic acid pathway, a simple and direct *rescue* experiment, carried out in our laboratory, has shown that this mechanism is likely not to account for observed *P. falciparum* growth inhibition. Other mechanisms of action should be searched for.

Notably, it has recently been reported that C-3 gallic acid esters of catechins, namely ECG, EGCG, (−)-catechin gallate, and (−)-gallocatechin gallate, are potent inhibitors of three important enzymes (FabG, FabZ, and FabI) involved in the fatty acid biosynthesis of *P. falciparum* with IC_{50} values in the range of 0.2–1.1 μM while gallic acid and other similar phenolic compounds are devoid of any enzyme inhibitory activity [21]. Kinetic analysis using (−)-catechin gallate as model compounds revealed that FabG was inhibited in a noncompetitive manner. FabZ was inhibited competitively, whereas both compounds behaved as tight-binding noncompetitive inhibitors of FabI. It is well conceivable that interference with fatty acid biosynthesis may represent a primary mechanism to explain the observed *in vitro* growth inhibition effects [22].

In any case, our results unequivocally demonstrate that two green tea catechins, i.e. EGCG and ECG, and, accordingly, green tea crude extracts, strongly inhibit *P. falciparum* growth *in vitro*. Additionally, both catechins have been shown to potentiate, at least moderately, the antiplasmodial effects of artemisinin, when the latter is administered at sub-lethal doses. To the best of our knowledge, this is the first report of direct antimalarial effects for green tea crude extracts. Appropriate pharmaceutical strategies might now be devised to increase the low bioavailability of green tea catechins and to protect them against rapid *in vivo* metabolic transformations, in such a way to make them more amenable as antimalarial agents, either alone or in combination therapies.

Acknowledgments

The Ente Cassa di Risparmio di Firenze is gratefully acknowledged for generous financial support. We thank L. Gradoni and A. Savarino for help in data analysis.

References

[1] N.T. Zaveri, Green tea and its polyphenolic catechins: medicinal uses in cancer and noncancer applications, Life Sci. 78 (18) (2006) 2073–2080.
[2] J.V. Higdon, B. Frei, Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions, Crit. Rev. Food Sci. Nutr. (1) (2003) 89–143.
[3] S. Garbisa, S. Biggin, N. Cavallarin, L. Sartor, R. Benelli, A. Albini, Tumor invasion: molecular shears blunted by green tea, Nat. Med. 5 (11) (1999) 1216.
[4] I. Gouni-Berthold, A. Sachinidis, Molecular mechanisms explaining the preventive effects of catechins on the development of proliferative diseases, Curr. Pharm. Des. 10 (11) (2004) 1261–1271.
[5] H.H. Chow, I.A. Hakim, D.R. Vining, J.A. Crowell, J. Ranger-Moore, W.M. Chew, C.A. Celaya, S.R. Rodney, Y. Hara, D.S. Alberts, Effects of dosing condition on the oral bioavailability of green tea catechins after single-dose administration of Polyphenon E in healthy individuals, Clin. Cancer Res. 11 (12) (2005) 4627–4633.
[6] W.Y. Feng, Metabolism of green tea catechins: an overview, Curr. Drug Metab. 7 (7) (2006) 755–809.
[7] D.A. Balentine, S.A. Wiseman, L.C. Bouwens, The chemistry of tea flavonoids, Crit. Rev. Food Sci. Nutr. 37 (8) (1997) 693–704.
[8] P.M. O'Neill, G.H. Posner, A medicinal chemistry perspective on artemisinin and related endoperoxides, J. Med. Chem. 47 (12) (2004) 2945–2964.
[9] W. Trager, J.B. Jensen, Human malaria parasites in continuous culture, Science 93 (1976) 673–675.
[10] M.T. Makler, J.M. Ries, J.A. Williams, J.E. Bancroft, R.C. Piper, B.L. Gibbins, D.J. Hinrichs, Parasite lactate dehydrogenase as an assay for *Plasmodium falciparum* drug sensitivity, Am. J. Trop. Med. Hyg. 48 (1993) 739–741.
[11] C. Lambros, J.P. Vanderberg, Synchronization of *Plasmodium falciparum* erythrocytic stages in culture, J. Parasitol. 65 (1979) 418–420.
[12] C. Severini, M. Menegon, A.R. Sannella, M.G. Paglia, P. Narciso, A. Matteelli, M. Gulletta, P. Caramello, F. Canta, M.V. Xayavong, I.N.S. Moura, N.J. Pieniazek, D. Taramelli, G. Maiori, Prevalence of *pfcrt* point mutations and level of cloroquine resistance in *Plasmodium falciparum* isolates from Africa, Infect. Genet. Evol. 6 (2006) 262–268.

[13] M.C. Berenbaum, A method for testing for synergy with any number of agents, J. Infect. Dis. 137 (1978) 122–130.
[14] L.Y. Djapa, R. Zelikson, A. Delahodde, M. Bolotin-Fukuhara, A. Mazabraud, *Plasmodium vivax* dihydrofolate reductase as a target of sulpha drugs, FEMS Microbiol. Lett. 256 (1) (2006) 105–111.
[15] A. Bell, Antimalarial drug synergism and antagonism. Mechanistic and clinical significance, FEMS Microbiol. Lett. 253 (2005) 171–184.
[16] S.L. Smith, C.J. Sadler, C.C. Dodd, G. Edwards, S.A. Ward, B.K. Park, W.G. McLean, The role of glutathione in the neurotoxicity of artemisinin derivatives in vitro, Biochem. Pharmacol. 61 (4) (2001) 409–416.
[17] E. Navarro-Peran, J. Cabezas-Herrera, F. Garcia-Canovas, M.C. Durrant, R.N. Thorneley, J.N. Rodriguez-Lopez, The antifolate activity of tea catechins, Cancer Res. 65 (6) (2005) 2059–2064.
[18] A.C. Anderson, Targeting DHFR in parasitic protozoa, Drug Discov. Today 10 (2) (2005) 121–128.
[19] D.C. Chan, A.C. Anderson, Towards species-specific antifolates, Curr. Med. Chem. 13 (4) (2006) 377–398.
[20] D.S. Rosenblatt, V.M. Whitehead, N.V. Matiaszuk, A. Pottier, M.J. Vuchich, D. Beaulieu, Differential effects of folinic acid and glycine, adenosine, and thymidine as rescue agents in methotrexate-treated human cells in relation to the accumulation of methotrexate polyglutamates, Mol. Pharmacol. 21 (3) (1982) 718–722.
[21] D. Tasdemir, G. Lack, R. Brun, P. Rüedi, L. Scapozza, R. Perozzo, Inhibition of *Plasmodium falciparum* fatty acid biosynthesis: evaluation of FabG, FabZ, and FabI as drug targets for flavonoids, J. Med. Chem. 49 (2006) 3345–3353.
[22] Another recent study has suggested that EGCG might have antimalarial effects *in vivo* through interference with cytoadherence processes: M. Dormeyer, Y. Adams, B. Kramer, S. Chakravorty, M.T. Tse, S. Pegoraro, L. Whittaker, M. Lanzer, A. Craig, Rational design of anticytoadherence inhibitors for *Plasmodium falciparum* based on the crystal structure of human intercellular adhesion molecule 1. Antimicrob. Agents Chemother. (2006) 24–730.