IN VITRO STUDIES ON THE MECHANISM OF ACTION OF TWO COMPOUNDS WITH ANTIPLASMODIAL ACTIVITY: ELLAGIC ACID AND 3,4,5-TRIMETHOXYPHENYL-(6'-O-GALLOYL)-O-b-D-GLUCOPYRANOSIDE

Dell'Agli M., 2° anno di corso del Dottorato in Scienze Farmacotossicologiche, Farmacognostiche e Biotecnologie Farmacologiche, XVI ciclo. Durata del dottorato in anni: 4. Sede di servizio: Dipartimento di Scienze Farmacologiche, Università di Milano.

Malaria remains a major problem of public health worldwide mainly due to the increase in parasite resistance to available drugs. The identification of new biochemical targets and the search for new antimalarial compounds is essential (1). Several drug target can be identified in the metabolism of intraerythrocytic parasites: one is the proteolytic cleavage or haemoglobin and another one is the detoxification of haem leading to the formation of malaria pigment or haemozoin (1,2). Proteinases are essential to a variety of viruses and other pathogenic microrganisms, including malaria parasites (1). In particular *Plasmodium falciparum* degrades haemoglobin in a digestive vacuole (DV) by means of aspartic proteinases (plasmepsin I-X) and one cysteine proteinase (falcipain) . Plasmepsins I and II appear to initiate cleavage of the haemoglobin tetramers, while falcipain digests the fragmented substrate (3).
It was previously shown that the methanolic extract of the bark of *Tristaniopsis calobuxus* Brongniart & Gris (Myrtaceae) inhibited parasite growth *in vitro*. From the extracts, two compounds with *in vitro* antiplasmodial activity were isolated and identified as ellagic acid and 3,4,5-trimethoxyphenyl-(6'-O-galloyl)-O-*b*-D-glucopyranoside (TMPGG) (4).
The present study was performed to investigate the mechanism of action of ellagic acid and TMPGG. Two hypotheses were taken into consideration: first, we analysed the ability of ellagic acid to interfere with the formation of β-haematin *in vitro*, using a spectrophotometric microtitre-based method recently developed (5). Secondly, since *T. calobuxus* bark extract was shown to inhibit other proteases (6), we investigated whether the compounds could inhibit plasmepsins, using recombinant plasmepsin II as target (7). Ellagic acid inhibited β-haematin formation in a dose-dependent manner. At the highest dose ellagic acid inhibited β-haematin formation by 65.6% whereas the inhibition by the reference compound chloroquine was complete. The IC_{50}s, namely the molar equivalent of test compound able to inhibit β-haematin formation by 50%, were 1.67 ± 0.81 and 5.42 ± 1.3 for chloroquine and ellagic acid, respectively. When tested on recombinant plasmepsin II, both ellagic acid and TMPGG inhibited the enzyme in a concentration dependent manner: IC_{50}s were 4.02 ±drslt 1.26 and 35 ± 8.74 μM for ellagic acid and TMPGG, respectively (mean ± sd). These doses are 10 fold higher than those needed to inhibit the parasite growth *in vitro* (0.5 μM and 3.2 μM for ellagic acid and TMPGG, respectively) (4). The potency ratio between the two compounds remained similar, equal to 10.
The further step of this study will be to investigate the mode of enzyme inhibition at the molecular level. HPLC-MS of the protein will be performed to obtain data as regards the binding of the inhibitor to the protein. Preliminary results have been obtained by analysis of the recombinant proenzyme and its activated form by HPLC-MS before and after trypsin digestion.
The molecular weight of proplasmepsin II was 43580 Da, which is very close to that calculated on the basis of the amminoacid sequence (43583 Da)(8). After 1 and 3 hours of activation, at pH 4,7 we obtained two forms of the activated protein, one with molecular weight 38185 Da and the other of 37093 Da. The quantitative proportion of the two forms was dependent of the time of activation.
These experiments will be repeated after incubation of the protein with ellagic acid.

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