Aqueous extract of Cryptolepis sanguinolenta enhance cytochrome P450 1A isozyme activity in presence of Atesunate

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Abstract

The clinical response to drugs may be affected by the simultaneous administration of other drugs that modify the pharmacokinetics and the disposition profile of medications. Drug metabolism via the cytochrome P450 (CYP) system has emerged as an important determinant in the occurrence of several drug-drug interactions. In our earlier studies, Cryptolepis sanguinolenta was reported to cause sub therapeutic blood levels of dihydroartemisinin of artesunate which could lead to decreased effectiveness, and possibly resistance as a result of herb-drug interactions. However, the role of metabolism in such herb-drug interaction has not been studied. Aim: The present study was therefore aimed at determining the effect of Cryptolepis sanguinolenta administration on the metabolism of artesunate. Methods: Reconstituted freeze dried Cryptolepis sanguinolenta was administered in drinking water at the therapeutic dose of 36 mg/kg body weight for two weeks, followed by a single oral dose of artesunate (150 mg/kg body weight). All the animals were sacrificed by cervical dislocation after 24 hr. Liver was excised and microsomes prepared and activities of PNPH, AmD and NOD were determined as a measure of CYP2E1, CYP2B1 and CYP1A activities respectively. Results: Concurrent administration of C. sanguinolenta and artesunate significantly enhanced the activity of CYP1A.

Index terms— Co-current administration, Cryptolepis sanguinolenta, metabolism, cytochrome P450, artesunate.

1 Introduction

he clinical response to drugs may be affected by the simultaneous administration of other drugs that modify the pharmacokinetics and the disposition profile of medications 1 . Many drugs affect CYP enzymes Authors ? : Department of Biochemistry, Cell and Molecular Biology, Faculty of Science, P. O. Box LG4, University of Ghana, Legon-Accra, Ghana. E-mail : aocloo@ug.edu.gh Authors ? : Department of Biochemistry, Cell and Molecular Biology, Faculty of Science, P. O. Box LG4, University of Ghana, Legon-Accra, Ghana.E-mail : maxi2g2@yahoo.com Authors ? : Department of Biochemistry, Cell and Molecular Biology, Faculty of Science, P. O. Box LG4, University of Ghana, Legon-Accra, Ghana.E-mail : Adjimani@ug.edu.gh Authors ?: Centre for Scientific Investigation Research into Plant Medicine, P. O. Box 73, Mampong-Akuapeng, Ghana. E-mail : aasittie@hotmail.com by either inducing the biosynthesis of CYP isoenzymes or by directly inhibiting the activity of the CYP isoenzymes, thus affecting the metabolism or clearance of the other drugs 2 . Inhibition of enzyme activity may result in higher concentrations and/or prolonged half-life of the substrate drug, which enhances the potential for toxic side effects. The clinical significance of a specific drug interaction depends on the degree of accumulation of the substrate and the therapeutic window of the substrate 3 . Enzyme induction on the other hand, is associated with an increase in enzyme activity. Fo drugs that are substrates of the isoenzyme induced, the effect is to lower the concentration of these substrates. The clinical consequence of the presence of an inducing agent and the resultant decrease in concentration of the substrate may mean a loss of efficacy 4 .

1 INTRODUCTION

Secondary plant metabolites share the biotransformation pathways of most chemicals and synthetic drugs. Consequently, this leads to a competitive inhibition of metabolic enzymes if herbal products are administered with drugs that are metabolized by the same enzymes 5 . For instance, studies have shown that St John's wort causes induction of drug metabolizing enzymes, including CYP2C9 which can lead to, for example, potential loss of efficacy of warfarin and phenprocoumon; CYP2C19 and CYP1A2 which can also lead to potential loss of efficacy of theophylline 6 .

In our earlier studies, Cryptolepis sanguinolenta was reported to cause sub therapeutic blood levels of dihydroartemisinin of artesunate which could lead to decreased effectiveness, and possibly resistance as a result of herb-drug interactions 7 . However, the role of metabolism in such herb-drug interaction has not been studied. To this end, the present study was aimed at of six. They were fed on standard laboratory chow diet obtained from Ghana Agro Food Company (GAFCO), Tema, Ghana and water ad libitum and acclimatized for a week.

One group (No drug) received water throughout the study. A second group (Cs ext) was administered reconstituted freeze dried Cryptolepis sanguinolenta in their drinking water at the therapeutic dose (36 mg/kg) (CSRPM, unpublished data) for two (2) weeks and single oral dose of the extract at the end of the two weeks, a third group (Artesunate) was given water for two weeks and as a single dose of Artesunate (150 mg/kg based on the recommended dosage in humans) after two weeks and a fourth group (Cs + Artesunate) received the reconstituted extract for two weeks and single oral dose of artesunate at the end of the two weeks. Studies were conducted in accordance with internationally accepted principles for laboratory animal use and care.

The reconstituted sample was prepared by obtaining a powdered dried root (360 g) of Cryptolepis sanguinolenta from the Centre for Scientific Research into Plant Medicine, Mampong, Ghana. This was boiled in 6 liters of water for 45 min and cooled. The resultant extract was filtered through a cotton wool, pre-frozen and lyophilized into powder using a freeze dryer (EYELA, Tokyo Rikakikai Co LTD, Japan). The extract was then stored in a cool dry place, in a desiccator at room temperature and was reconstituted in sterilized distilled water before administration. All the animals were sacrificed by cervical dislocation after 24 h of final drug administration and liver was excised, weighed, hepatic microsomes prepared and activity of cytochrome c reductase was determined in addition to activities of PNPH, AmD and NOD as a measure of CYP2E1, CYP2B1 and CYP1A activities respectively.

Hepatic microsomes of control (no drug) and treatment groups were prepared from tissue homogenate (20 % w/v) according to method of Lake 8 as modified by Anjum et al 9 by CaCl 2 precipitation of the post-mitochondrial fraction and centrifugation at 27,000 g for 15 min and the resulting microsomal pellet was washed in a 0.1M Tris buffer, pH 7.4 containing 0.15 M KCl and centrifuged at 27,000 g for 15 min in a highspeed Avanti J-E refrigerated centrifuge (Beckman Coulter Inc, USA). The microsomes were stored in aliquots of 1 ml at -40 ?C.

The microsomal protein contents were then determined spectrophotometrically at 595 nm by the Bradford method 10 using bovine serum albumin (BSA) as standard.

Cytochrome c Reductase activity was determined as described previously 11 . The reaction mixture included 250 ?l of 5 mg/ml cytochrome c, 631 ?l of 4 mg/ml microsomal sample and made up to 2.3 ml with 0.1 M tris-HCl buffer, pH 7.4. The reaction was initiated by the addition of 250 ?l of 2 % (w/v) NADPH solution. A reference blank with similar composition as the reaction mixture, except that the NADPH was replaced by an equal volume of 0.1 M Tris buffer pH 7.4 was prepared and absorbance at 550 nm was recorded every minute for 3 min against the reference blank and the NADPH -cytochrome c reductase activity was calculated as described previously 11 .

Para-Nitrophenol Hydroxylase (PNPH) activity was determined by the method of Reinke and Meyer 12 . The reaction mixture consisted of 10 ?l of 1.4 mg/ml para-Nitrophenol (PNP), 5 ?l of 5 mM MgCl2, 776 ?l of 4 mg/ml microsomal sample and made up to 1.0 ml with 50 mM Tris buffer, pH 7.4. This was incubated at 37 ?C for 3 min and 10 ?l of 50 mM NADPH was added to initiate the reaction and was again incubated for a further 10 min at 37 ?C, after which 0.5 ml of 0.6 M HClO 4 was added to stop the reactions. A blank with similar composition as the reaction mixture, except that the microsomal sample was replaced with an equal volume of distilled water and incubated for 3 min at 37 ?C.

Aminopyrine -N -Demethylase (AmD) activity was measured by determining the production of formaldehyde 13 . The reaction mixture included 0.2 ml of 50 mM semicarbazide HCl, 0.8 ml of 2.5 mM NADPH and 0.5 ml 20 mM aminopyrine. The mixture was incubated at 37 ?C for 2 min, in a shaking water bath. The enzyme reaction was initiated by adding 0.5 ml of diluted microsomal fraction (containing 4 mg/ml protein) to yield a final concentration of 1 mg/ml protein in the reaction mixture. The incubation was continued at 37 ?C for a further 30 min. Aminopyrine was substituted for equal volume of distilled water in the blank which contained other constituents of the reaction mixture. The reaction was stopped by adding 0.5 ml of 25 % w/v ZnSO4, thoroughly mixed and kept on ice for 5 min. To the mixture, 0.5 ml of saturated Ba(OH) 2 was added and centrifuged for 5 min at maximum speed, using the Denly bench centrifuge (BS 400, England), after a second round of mixing and cooling on ice. To 1 ml of the supernatant, 2 ml of Nash reagent (prepared from a mixture of 30 g ammonium acetate, 0.4 ml acetyl acetone and then made up to 100 ml with distilled water) was added and incubated at 60 ?C for 30 min after tightly capping the test tubes. The solutions were extensively cooled and the absorbance read at 415 nm against the blank. A standard curve over the range of 0 -0.1 mM formaldehyde was prepared using distilled water. The standard was subjected to the same treatment with the Nash reagent

as the supernatant. The specific activity was determined as the formaldehyde formed per incubation time per total protein (in reaction mixture) in nmol/min/mg. The 4-Nitroanisole -O -Demethylase (NOD) activity was determined by measuring the 4-nitrophenol produced **??**4 . The reaction mixture included 1 ml of 2 mM NADPH and an equal volume of microsomal dilution (containing a 4 mg/ml protein) and incubated at 37 ?C for 2 min in a shaking water bath. The enzyme reaction was initiated with 10 ?l of 500 mM 4 -Nitroanisole and incubated again for 15 min. A blank was prepared as the reaction mixture except that the microsomal proteins were denatured at 100 ?C. The enzyme reaction was terminated with 1 ml of ice -cold 20 % w/v TCA and allowed to stand on ice for 5 min.

2 III.

3 Statistical Analysis

The results were expressed as mean ± standard error of the mean (SEM). Significance of the difference between the control and test values was evaluated using analysis of variance (ANOVA). This was done using the computer programme 'Statistical Package for Social Sciences (SPSS), version 16.0. P -Value less than 0.05 ($p < 0.05$) was taken as the significance level.

IV.

4 Results

The effect of drug and herb administration on rat hepatic microsomal protein content is as shown in table 1. The hepatic microsomal protein content of all the treatment groups decreased compared to the control group (no drug). Even though C. sanguinolenta administration resulted in a significant decrease (46.4%, $p = 0.017$), the extract did not cause a significant change in microsomal protein content in presence of artesunate (Table 1).

Figure **??** shows the effect of concurrent administration of C. sanguinolenta and artesunate on rat hepatic microsomal cytochrome c reductase activity. All the treatments resulted in a decrease in cytochrome c reductase activity relative to the control (no drug). C. sanguinolenta treated and C. sanguinolenta-artesunate treated groups showed 18.1% ($p = 0.97$) and 10.7% ($p = 1.0$) decrease in the enzyme activity, respectively. Artesunate treated group showed a higher but nonsignificant decrease (40.2%) in the cytochrome c reductase activity. The C. sanguinolenta extract did not significantly alter the cytochrome c reductase activity in presence of artesunate.

The effect of concurrent administration of C. sanguinolenta and artesunate on para-Nitrophenol Hydroxylase (pNPH) activity as measure of CYP2E1 activity is as shown in Figure **??**. All the treatment groups showed a slight but non-significant ($p > 0.05$) increase in enzyme activity except C. sanguinolenta treated group which did not change the enzyme activity compared to the controls (no drug). The activities in Artesunate treated and C. sanguinolenta-artesunate treated groups increased by 19.5% and 20.5% respectively, but this was non-significant ($p > 0.05$) compared to the control group (no drug). Again the concurrent administration of the extract did not significantly alter the activity of CYP2E1 in presence of Artesunate.

Figure **??** shows the effect of concurrent administration of C. sanguinolenta and artesunate on rat hepatic microsomal Aminopyrine N-Demethylase (AmD) activity as measure of CYP2B1 activity. Again adminsitartion of C. sanguinolenta did not significantly alter CYP2B1 activity in presence of Artesunate though artesunate alone caused a slight but non-significant decrease (16.1%, $p > 0.05$) compared to the control group (no drug). However, the extract alone significantly increased the activity of the isozyme.

The effect of concurrent administration of C. sanguinolenta and artesunate on rat hepatic microsomal 4-Nitroanisole -O -Demethylase (NOD) activity as measure of CYP1A is shown in Figure **??**. Artesunate administration caused a significant increased in the CYP1A enzyme activity (150.9% increase, $p = 0.037$) relative to the controls (no drug) and concurrent administered with C. sanguinolenta caused a further significant increase in the enzyme activity relative to both the artesunate alone treated group (22.6%, $p = 0.028$ increase) and the control (207.7%, increase, $p = 0.012$). Thus, administration of the extract caused a significant increase in activity of the isozyme in presence of Artesunate.

V.

5 Discussion

The clinical response to drugs may be affected by the simultaneous administration of other drugs that modify the pharmacokinetics and the disposition profile of medications 1 . Artemisinin and its derivatives (artemether, artesunate, arteether, deoxyartemisinin and dihydroartemisinin) have been reported to undergo hepatic metabolism 15 . These classes of drugs are found to affect several principal CYP enzymes. Although the metabolic changes are usually moderate, in several cases such effects are shared by all five endoperoxides suggesting a class effect 16 . In the present study, the PNPH activity did not change significantly after treatment with artesunate in the absence or presence of extract. This suggests that artesunate is probably not a substrate of CYP2E1.

Artesunate slightly decreased (16.1%) the activity of AmD, though not significantly relative to the no-drug controls (Fig **??**). In the presence of C. sanguinolenta, however, artesunate did not affect the activity of AmD although the extract alone significantly increased the enzyme activity (Fig **??**). This suggests a slight inhibitory

effect of artesunate on CYP2B1. CYP2B1 of rats is analogous to CYP2B6 in humans 17 . Contrary to the findings in the present study, the hepatic metabolism of artesunate has been reported to be partially mediated by CYP2B6 in humans 16 . The exact mechanism of induction is not known. However, the enzyme might be induced via an increase in mRNA expression and/or activation of existing enzymes. Also, CYP2B proteins are reported to be down-regulated in primary cultures of rat hepatocytes by two independent mechanisms in response to lipopolysaccharide (LPS): NO-independent mRNA suppression at lower concentrations and NO-dependent protein suppression at higher concentrations 18 . The significant increase in the activity of AmD after C. sanguinolenta treatment suggests that the extract is probably a substrate of CYP2B1 (Fig **??**). It was therefore expected that the activity of CYP2B1 after pre-treatment with C. sanguinolenta before artesunate would at least be comparable to C. sanguinolenta alone treated group.

On the contrary, the AmD activity after administration of artesunate in the presence of C. sanguinolenta decreased (35.7%, $p < 0.05$) relative to the C. sanguinolenta treated group **??**Fig 3). This observation therefore suggests that whereas C. sanguinolenta possibly induces the expression of CYP2B1 mRNA, artesunate probably inhibit the activation of the proteins. The apparent inhibitory effect of artesunate observed in the present study as compared to other studies could be due to species variations and probably concentration effect of artesunate. Artesunate was given as a single oral dose (150 mg/kg) unlike the earlier studies where multiple doses were given.

Artesunate administration increased the NOD activity significantly relative to the no-drug controls in the present study (Fig 4) and upon concurrent administration with C. sanguinolenta and artesunate, there was a further significant increase (22.6%, $p = 0.028$) in the enzyme activity relative to the artesunate treated group and 207.7% ($p = 0.012$) compared to the no-drug controls. This suggests that artesunate is a possible substrate of CYP1A and also tend to have an enhanced activity in the presence of the extract. C. sanguinolenta could be a possible allosteric modulator of CYP1A in presence of Artesunate. Artemether and dihydroartemisinin (DHA) have been reported to be metabolized in vitro by CYP1A, CYP2B6, CYP2C19 and CYP3A4 18 . [16] Thus activation of CYP1A after administration of artesunate agrees with the earlier findings by Navaratnam et al. 18 since the DHA was the major metabolite of artesunate and also artemisinin and its derivatives undergo class effect when it comes to metabolism via CYP enzymes 16 .

The CYP mediated metabolism of artesunate was enhanced as a result of induction of CYP1A by Cryptolepis sanguinolenta. The enhanced activation of CYP1A after concurrent administration of C. sanguinolenta and artesunate (Fig **??**) probably explains the observed decrease in the AUC (a measure of the bioavailability in blood) and half life (T1/2) with the corresponding increase in elimination rate and clearance as indicated in our earlier publication. [7] However, it is possible that esterase activity might play a role in the metabolism of artesunate/dihydroartemisinin (DHA) though measures such as the use of anticoagulant containing tubes for collection of whole blood and storage at -80 ?C were put in place to reduce such possible effect.

6 VI.

7 Conclusion

The present study shows that metabolism of DHA, the major metabolite of artesunate, was significantly enhanced after concurrent administration of C. sanguinolenta and artesunate. This observation suggests that the effectiveness of artesunate after coadministration of C. sanguinolenta and artesunate may be affected as a result of rapid flush out of the drug leading to sub-therapeutic levels, a major factor for drug resistance.

8 VII. Acknowledgement

1 2 3 4

1

	Experimental Group	No Drug	Cs ext	Artesunate	Cs ext + Artesunate
	Microsomal Protein Content (mg/ml)	0.09 ± 0.01	0.05 ± 0.01*	0.06 ± 0.02	0.06 ± 0.02
Cyt. c Reductase Activity	(nmol/min/mg protein)	2.46			
		No Drug	Cs ext	Artesunate	Cs ext + Artesunate
		Animal Treatment			

Aqueous extract of Cryptolepis sanguinolenta aqueous at concentration of 36 mg/kg body weight was administered for two weeks after which a single artesunate (150 mg/kg body weight) was admi

[Note: Fig.1: Effect of concurrent administration of C. sanguinolenta and artesunate on rat hepatic microsomal cytochrome]

Figure 1: Table 1 :

8 VII. ACKNOWLEDGEMENT

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