Food and Chemical Toxicology 49 (2011) 45–48

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Effects of co-administration of artesunate and amodiaquine on some cardiovascular disease indices in rats

J.O. Adebayo *, A. Igunnu, R.O. Arise, S.O. Malomo

Malaria Research and Toxicology Unit, Department of Biochemistry, University of Ilorin, Ilorin, Kwara State, Nigeria

ARTICLE INFO

Article history:
Received 2 March 2010
Accepted 16 September 2010

Keywords:
Antimalarial
Cardiovascular
Cholesterol
Heart
Toxicity

ABSTRACT

The effects of co-administration of artesunate and amodiaquine on some cardiovascular disease indices were investigated in albino rats (*Rattus novergicus*). The experimental animals were randomly divided into four groups: those administered distilled water (control), those administered artesunate (2 mg/kg body weight), those administered amodiaquine (6.12 mg/kg body weight) and those co-administered artesunate (2 mg/kg body weight) and amodiaquine (6.12 mg/kg body weight). The drugs were orally administered twice daily for three days after which the serum lipid profile, heart MDA content and heart ALP and ACP activities were determined. Artesunate significantly reduced ($P < 0.05$) total cholesterol and HDL-cholesterol concentrations in the serum with no significant effects ($P > 0.05$) on other parameters compared to controls. Amodiaquine, on the other hand, significantly reduced ($P < 0.05$) serum total cholesterol concentration while it significantly increased ($P < 0.05$) serum LDL-cholesterol and heart ACP activity compared to controls. Co-administration of artesunate and amodiaquine significantly reduced ($P < 0.05$) total cholesterol and HDL-cholesterol concentrations in the serum while significantly increasing ($P < 0.05$) serum LDL-cholesterol concentration, atherogenic index (LDL-C/HDL-C) and ACP activity in the heart compared to controls. The results obtained suggest that co-administration of artesunate and amodiaquine to patients with coronary heart disease should be with caution.

1. Introduction

Malaria is a vector-borne infectious disease caused by protozoa of the genus *Plasmodium*. It is widespread in tropical and subtropical regions, including parts of the Americas, Asia, and Africa. Each year, there are approximately 350–500 million cases of malaria, killing between one and three million people, the majority of whom are young children in Sub-Saharan Africa. Ninety percent of malaria-related deaths occur in Sub-Saharan Africa. Five species of the plasmodium parasite can infect humans; the most serious forms of the disease are caused by *Plasmodium falciparum* carried by female anopheles mosquito. Malaria caused by *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* causes milder disease in humans that is not generally fatal. A fifth species, *Plasmodium knowlesi*, causes malaria in macaques but can also infect humans. This group of human-pathogenic *Plasmodium* species is usually referred to as *malaria parasites* (Snow et al., 2005).

Malaria infections are treated through the use of antimalarial drugs, such as quinine or artemisinin derivatives. However, parasites have evolved to be resistant to many of these drugs thereby rendering them less effective over time. Thus, the increasing resistance of *P. falciparum* to antimalarial drugs is posing a major threat to the global effort to roll back malaria. The emergence of antimalarial drug resistance is dependent on the occurrence of a genetic change (mutation or gene complication) in a malaria parasite, which interferes with the parasite's susceptibility to a drug. A single mutation may be sufficient to confer almost complete resistance to some drugs such as atovaquone (Wellems and Plowe, 2001). Once a drug-resistant mutant has arisen, preventing spread of resistance is difficult. Spread is facilitated by the exposure of malaria parasites to sub-therapeutic levels of antimalarial drugs that kill sensitive parasites but allow parasites with a resistance mutation to survive and reproduce (Hastings et al., 2002).

Due to the increase in resistance of malaria parasites to conventional drugs, new therapeutic approaches have been developed. Prominent among these has been artemisinin-based combination therapy (ACT). The World Health Organization (WHO) has endorsed ACT as the "policy standard" for all malaria infections in areas where *P. falciparum* is the predominant infecting species (Timothy et al., 2005). Artemisinin has a very different mode of action compared to conventional antimalarials which makes it particularly useful in the treatment of resistant infections. However, in order to prevent the development of resistance to the drug, it is only recommended in combination with another non-artemisinin based therapy. Various ACTs are now available in Nigeria for the

* Corresponding author. Tel.: +234 7035487636.
E-mail address: topebayo2002@yahoo.com (J.O. Adebayo).

0278-6915/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2010.09.022

treatment of malaria, mostly caused by *Plasmodium falciparum*. Different laboratories in the country are now engaged in the evaluation of the toxic effects of ACTs. Ekanem et al. (2009) have reported that artequin (combination therapy comprising of artesunate and mefloquine) induced dose dependent reactive astrocytes formation in the hippocampus which may impair uptake of neurotransmitters and alter neuronal environment, thus altering hippocampul functions such as learning and memory. Obianime and Aprioku (2009) reported that the administration of artesunate/sulphadoxine/pyrimethamine and artesunate/amodiaquine combinations may cause some level of toxicity to prostatic/testicular structures. However, Morakinyo et al. (2009) reported that the administration of artemether-lumefantrine combination at the doses and durations employed in their study had no adverse effects on testicular functions in the rat.

Artesunate (a semi-synthetic derivative of artemisinin) and amodiaquine combination therapy which has been tested and proven to be effective in the treatment of malaria has been recommended for use (Nosten et al., 2000; Adjuik et al., 2002). Despite the effectiveness of artesunate–amodiaquine combination therapy in the treatment of malaria, it is not without side effects. Transient drug-induced fever seems to be the most frequent adverse effect (up to 25%) of artesunate when taken alone, while transient first-degree heart block appears to be a rare event (WHO, 1994). Amodiaquine has also been reported to have some cardiac side effects (Traebert and Dumotier, 2005). This work was therefore aimed at investigating the effects of co-administration of artesunate and amodiaquine on some cardiovascular disease indices, most of which were not considered by Obianime and Aprioku (2009), in uninfected rats.

2. Materials and methods

2.1. Animals and reagents

Twenty-four albino rats (*R. novergicus*) with an average weight of 150 g used for this study were obtained from the small Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. Artesunate and Amodiaquine were obtained from Saga laboratories, Vatwa Ahmedabad, India. All the other reagents used for this study were of analytical grade and were prepared in all glass-distilled water.

2.2. Animal handling and drug administration

The experimental animals were handled and used in accordance with the international guide for the care and use of laboratory animals (National Research Council, 1996). They were kept in standard laboratory conditions under natural light–dark cycle. The animals had access to diet and water *ad libitum* throughout the period of the experiment. The animals were randomly divided into four groups (of 15 rats each), which were designated A (control), B–D. The control rats in group A were administered distilled water orally for 3 days while rats in groups B and C were orally administered artesunate (2 mg/kg body weight) and amodiaquine (6.12 mg/kg body weight) twice daily for 3 days respectively. Artesunate (2 mg/kg body weight) and amodiaquine (6.12 mg/kg body weight) were co-administered twice daily to rats in group D for 3 days. The doses used in this study were the therapeutic doses of the two drugs, thus simulating a situation in which the ACT is used without any malaria infection. The drugs were administered as homogenous aqueous suspensions.

2.3. Sample preparation

At the end of the experimental period, venous blood was collected from the experimental animals according to the method of Narayanan et al. (1984). The serum was prepared by centrifuging the clotted blood samples at 3000 rpm for 5 min (Ogbu and Okechukwu, 2001) and collected by pippeting. The animals were also quickly dissected and the heart removed. The heart was suspended in ice-cold 0.25 M sucrose solution (1:5 w/v) and homogenized. The homogenates were kept frozen overnight to ensure maximum release of the enzymes (Ngaha et al., 1989).

2.4. Assay of biochemical parameters

Alkaline phosphatase (ALP, EC 3.1.3.1) and acid phosphatase (ACP, EC 3.1.3.2) activities were determined by the method of Ahamed and King (1959). Malondialdehyde level was obtained by the method of Varshey and Kale (1990). The total cholesterol concentration was assayed using Chod-PAP method reported by Fredrickson et al. (1967). HDL-cholesterol concentration was assayed using dextran as described by Albers et al. (1978). LDL-cholesterol concentration was assayed by polyvinyl sulphate method (PVS) as described by Assmann et al. (1984). The atherogenic indices were obtained by finding: (i) the ratio of serum LDL-cholesterol concentration to that of serum HDL-cholesterol concentration and (ii) the ratio of serum total cholesterol concentration to that of serum HDL-cholesterol concentration.

Table 1
Effects of co-administration of artesunate and amodiaquine on MDA concentration and the activities of heart alkaline and acid phosphatases in rat.

Treatments	Enzyme activities (IU/L)		MDA concentration (μmol/L)
	ALP	ACP	
Control	27.40 ± 5.94[a]	1059.40 ± 70.92[a]	38.50 ± 0.50[a]
Artesunate	32.20 ± 6.60[a]	1153.30 ± 66.33[a,b]	38.67 ± 0.47[a]
Amodiaquine	27.80 ± 4.09[a]	1225.60 ± 129.63[b]	39.33 ± 0.47[a]
Artesunate–Amodiaquine	29.40 ± 3.36[a]	1303.60 ± 155.61[b]	38.50 ± 0.50[a]

Each value is a mean of 5 determinations ± SEM. Values along the column with different superscripts are significantly different ($P < 0.05$).

2.5. Statistical analysis

All data are presented as mean ± standard deviation. The data from the various groups were compared for statistical significance using Duncan Multiple Range test according to Montgomery (1976). In all cases, probability level of 95% was taken as significant.

3. Results

The effects of co-administration of artesunate and amodiaquine on alkaline phosphatase activity, acid phosphatase activity and malondialdehyde concentration in rat heart are shown in Table 1. Drug administration in all the experimental groups (B–D) did not produce any significant effect ($P < 0.05$) on alkaline phosphatase activity in rat heart compared to control. On the other hand, administration of amodiaquine alone and its co-administration with artesunate significantly increased ($P < 0.05$) acid phosphatase activity in rat heart compared to control.

The effects of co-administration of artesunate and amodiaquine on total cholesterol concentration, HDL-cholesterol concentration, LDL-cholesterol concentration, and triacylglycerol concentration in rat serum are shown in Table 2. The results obtained showed that artesunate, amodiaquine and their co-administration significantly reduced ($P < 0.05$) total cholesterol level in the serum compared to control. Moreover, artesunate and its co-administration with amodiaquine caused a significant decrease ($P < 0.05$) in serum HDL-cholesterol concentration while amodiaquine alone did not have any significant effect ($P > 0.05$) compared to control. On the other hand, amodiaquine and its co-administration with artesunate significantly increased ($P < 0.05$) serum LDL-cholesterol concentration while artesunate alone had no significant effect ($P > 0.05$) compared to control. For all the treatments investigated, none had any significant effect ($P > 0.05$) on serum triacylglycerol concentration compared to control. The effect of co-administration of artesunate and amodiaquine on artherogenic index in rat serum is shown in Table 3. While artesunate had no significant effect ($P > 0.05$) on atherogenic index compared to control, amodiaquine and artesunate–amodiaquine combination significantly increased ($P < 0.05$) atherogenic index (LDL-C/HDL-C) compared to control.

4. Discussion

Alkaline phosphatase and acid phosphatase are majorly used as marker enzymes. Alkaline phosphatase is employed to assess the integrity of plasma membrane and endoplasmic reticulum (Wright