Effect Of Amodiaquine Plus Artesunate Combination On Some Macromolecules In The Brain Of Albino Wistar Rats

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Citation

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Abstract

Amodiaquine and artesunate (AQ+AS) combination is an artemisinin-base antimalarial drug with high efficacy. Reports on neurotoxic effects lead to this on study the effect of this drug combination on some macromolecules in the brain of Wistar rat. Twenty four adult Wistar rats weighing between 150-210g were equally divided into four groups of A, B, C and D. Group A served as the control and received distilled water. Groups B and C were treated with 8.75±2.86mg/kg and 17.50±5.71mg/kg of AQ+AS for three days respectively, while group D was treated with 8.75±2.86mg/k of AQ+AS for six days. Twelve hours after the last treatments, the animals were weighed and sacrificed. Their brains were removed and weighed and subsequently processed for bio-molecules estimations. The body weights of the control were not different significantly from the experimental groups. Though the brain weights varied significantly, the organ weight to body/brain weight ratio were not significantly different from the control. In the bio-molecules study, brain cholesterol, triacylglycerol and total proteins varied significantly. Based on the organ weight to body/brain weight ratio, the bio-molecules differences does not prove neurotoxicity at these doses since the differences may be attributed to the rat's size.

The study was carried out in the Laboratory of Biochemistry Department, Faculty of Basic Medical Sciences University of Calabar, Calabar-Nigeria

INTRODUCTION

Macromolecules are complex bio-molecules found in the cells and tissues of higher animals and these include; deoxyribonucleic acid, ribonucleic acid, polysaccharides, protein and lipids [1]. In the brain and spinal cord, lipids and proteins are important since they make up the bulk of the neuronal membrane, cytosol and organelles of the neurons, neuroglia and other connective tissues including myelin sheath.

Proteins and lipids are very necessary for proper functioning of each brain cell. Lipid functions in the replacement of damaged and worn membranes and as electrical insulators allow rapid propagation of depolarization waves along myelinated nerve [2,3]. The most abundant lipids being fatty acids and cholesterol (CH). Fatty acids are stored in the body as triacylglycerol (TAG) which constitutes the body's main caloric reserve, with CH being the precursor of all other steroids [3].

Substances taken into the body affect the brain bio-molecules in different ways. This includes increase in cholesterol and triacylglycerol [4,5,6,7], and decrease in lipids due to neurodegeneration [8]. Some of these effects may result from peroxidation of these macromolecules due to the presence of free radicals [9,10].

These free radicals are generated by both amodiaquine and artesunate (AQ and AS) [9,10], and these are antimalarial drugs manufactured in the form of a combination called Larimal (AQ+AS). These drug functions in the body as two different drugs having high efficacy [11,12,13,14].

AQ is a 4-aminoquinoline and AS is a water soluble hemi-succinate derivative of artemisinin. AQ acts by accumulating in the lysosomes of the parasites bringing loss of its function, and also binds to their nucleoproteins inhibiting the DNA and RNA polymerase [15], which end up generating free radicals [9]. AS act by heme-mediated decomposition of its endoperoxide bond to produce carbon-centered free radicals [10].

The actions of these drugs may affect the bio-molecules of the brain, hence we studied its effect on some brain bio-molecules of Wistar rats.

MATERIALS AND METHODS

Twenty-four adult Wistar albino rats weighing between 150-210g were divided into four groups of six animals each. Group A served as the control and the animals received distilled water, while groups B, C and D served as the experimental groups. Each packet of Larimal contains twelve blister tablets each of AQ Hydrochloride USP equivalent to AQ base (153.1mg) and AS (50mg). The drugs were administered in milligram per kilogram body weight (mg/kg) twice a day, three days for groups 2 and 3 animals, and six days for group 4 animals. These are as shown in Table 1. Twelve hours after the last administrations the animals were weighed before sacrificing by anaesthesia with chloroform and their brains removed, blotted dry on filter papers and weighed using a Mettler p163 balance. The brain of all the animals were homogenised in cold 0.25m STKM buffer. The homogenates were used to estimate; total proteins by Biuret kit method, cholesterol by CHOP-PAP kit method and triacylglycerol by GPO-PAP kit method.

Statistical analysis was carried out using one way analysis of variance (ANOVA), thereafter post-hoc test was carried out to find the level of significance at $p<0.05$. All the results are expressed as mean standard error of mean.

Figure 1

Table 1: Drugs administration

Group (n=12)	Dosage per day	Duration (day)
A	Distilled water	3
B	8.75+2.86mg/kg of AQ+AS	3
C	17.50+5.7mg/kg of AQ+AS	3
D	8.75+2.86mg/kg of AQ+AS	6

RESULTS

ANTHROPOMETRIC MEASUREMENTS

BODY WEIGHT

The control group weighed less than the group treated with 8.75±2.86mg/kg of AQ+AS for three days (B) and more than groups treated with 17.50±5.71mg/kg of AQ+AS (C) and 8.75±2.86mg/kg of AQ+AS (D) for three and six days respectively. These weights were however not significant. These are as seen in Fig. 1.

BRAIN WEIGHT

The control group weighed significantly ($p<0.01$) less than the group treated with 8.75±2.86mg/kg of AQ+AS for three days (B), but significantly ($p<0.001$) higher than the groups treated with 17.50±5.71mg/kg of AQ+AS (C) and 8.75±2.86mg/kg of AQ+AS (D) for three and six days respectively. These are as seen in Fig. 2.

ORGAN WEIGHT-BODY/BRAIN WEIGHT RATIO

There was no significant difference ($p<0.05$) in the organ-weight-body-brain weight ratio between the control and the experimental groups. These are as seen in Fig. 3.

BIO-MOLECULES ESTIMATIONS

CHOLESTEROL (CH)

The control group had significantly ($p<0.001$) higher level than the groups treated with 17.50±5.71mg/kg of AQ+AS (C) and 8.75±2.86mg/kg of AQ+AS (D) for three and six days respectively, but not significantly different from the group treated with 8.75±2.86mg/kg of AQ+AS for three days (B). These are as seen in Fig. 4.

TRIACYLGLYCEROL (TAG)

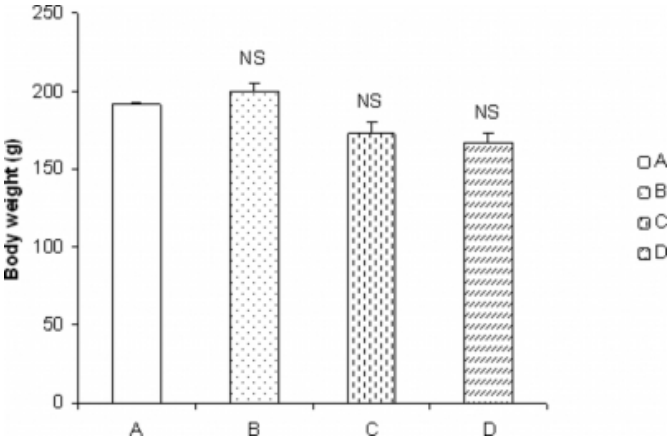
The control was significantly ($p<0.05$) higher than the group treated with 8.75±2.86mg/kg of AQ+AS for six days (B), but not different from the groups treated with 8.75±2.86mg/kg of AQ+AS (D) and 17.50±5.71mg/kg of AQ+AS (C) for three days. These are as seen in Fig. 5.

TOTAL PROTEIN (TBP)

The control group had significantly ($p<0.001$, 0.05) higher level than the groups treated with 17.50±5.71mg/kg of AQ+AS (C) and 8.75±2.86mg/kg of AQ+AS (D) for three and six days respectively, but not significantly different from the group treated with 8.75±2.86mg/kg of AQ+AS for three days (B). These are as seen in Fig. 6.

Figure 2

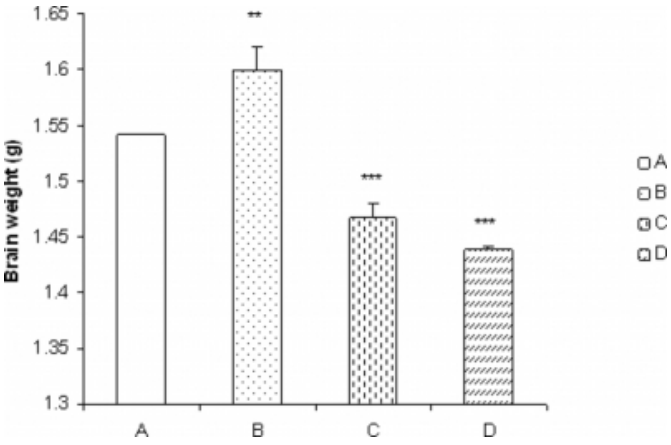
Figure 1: Body weights



Data are presented as mean±standard error of mean [NS] Not significantly different from the control (A)

Figure 3

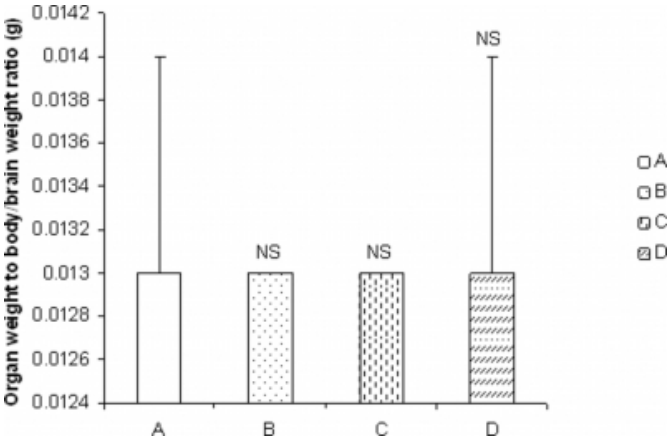
Figure 2: Brian weight



Data are presented as mean±standard error of mean
** Significantly different from control (A) at p<0.01
*** Significantly different from control (A) at p<0.001

Figure 4

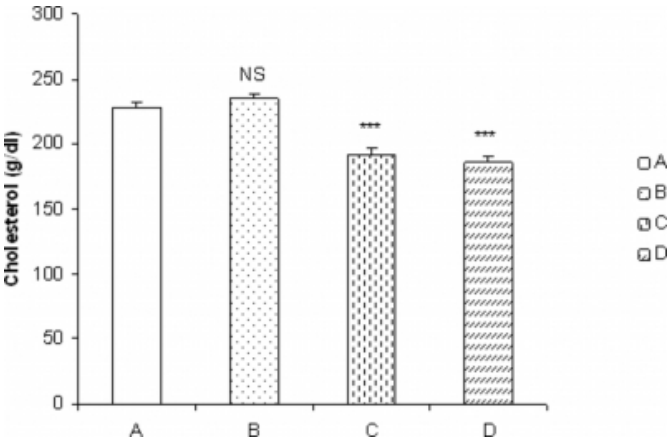
Figure 3: Organ weight to body/brain weight ratio



Data are presented as mean±standard error of mean [NS] Not significantly different from the control (A)

Figure 5

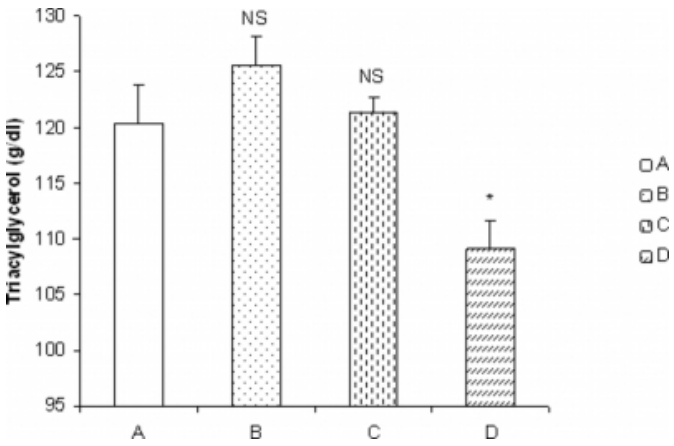
Figure 4: Cholesterol (CH)



Data are presented as mean±standard error of mean ***
Significantly different from the control (A) at p<0.001 [NS] Not significantly different from the control (A)

Figure 6

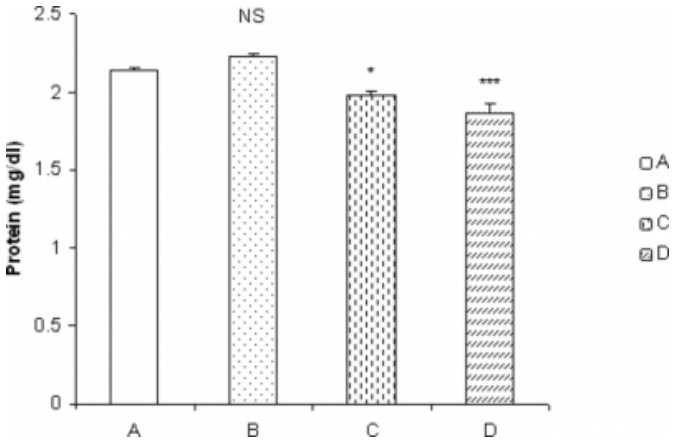
Figure 5: Triacylglycerol (TAG)



Data are presented as mean±standard error of mean * Significantly different from the control (A) at $p<0.05$ ^{NS} Not significantly different from the control (A)

Figure 7

Figure 6: Total Protein (TBP)



Data are presented as mean±standard error of mean * Significantly different from the control (A) at $p<0.05$ *** Significantly different from the control (A) at $p<0.001$ ^{NS} Not significantly different from the control (A)

DISCUSSION

The effect of amodiaquine+artesunate (AQ+AS) combination on some macromolecules in the brain of Wistar rats was carried out. Anthropometric results revealed a higher body and brain weights in the group treated with 8.75±2.86mg/kg of AQ+AS for three days, but a lower body and brain weights in the groups treated with 17.50±5.71mg/kg of AQ+AS and 8.75±2.86mg/kg of AQ+AS for three and six days respectively.

Analysis of organ weight in toxicology studies is an important end point for identification of potentially harmful effects of chemicals. Differences in organ weights between treatments groups are often accompanied by differences in body weight between these groups, making interpretation of organ weight differences difficult. Bailey et al [16] reported that analysis of organ weight and body/brain weight is predictive for evaluating the brain. In our study, the organ weight and body/brain weight ratio revealed no significant difference among the groups. This may be as result of the drug having no significant effect on the brain of the Wistar rats at these doses.

In the bio-molecules study, there was a lower level of brain cholesterol (CH), triacylglycerol (TAG) and total proteins (TBP) in the group treated with 8.75±2.86mg/kg of AQ+AS for six days, but a higher level of these bio-molecules in the group treated with 8.75±2.86mg/kg of AQ+AS for three days. This is consistent with the weight of the brain as discussed in this study. Increase in brain size also increases CH level which is an essential component of myelin in white matter [17] . But the formation of CH involves specific proteins which may also increase thereby adding to the total brain proteins [18] .

However brain TAG of the group treated with17.50±5.71mg/kg of AQ+AS for three days was not significantly different from the control, while CH and TBP where significantly ($p<0.001$, 0.5) lower. The lower levels of CH and TBP are consistent with the brain size in this study. The non consistency of TAG in the group treated with17.50±5.71mg/kg of AQ±AS for three days may be due to trauma caused by the treatment. Ikeda et al [4] reported increased brain TAG on rats subjected to hypoxia which later decreased on recovery. The drug may also have affected adrenocorticotropin hormone level whose increase level stimulates the synthesis of brain TAG [19] . Sun [20] had earlier reported marked alterations in acyl group compositions of major phosphoglycerides from whole brain homogenates in rat maintained on fatty acid deficient diet.

The drugs AQ±AS combination releases free radical and this usually result in lipid and protein peroxidation [9,10] . This drug combination did not really show these effects and hence may not be neurotoxic at these dosage and time as the difference seen may be attributed to the rat's size. Thus, this result encourages the use of the drug in malarial treatment as recommended by the manufacturers.

References

1. Murray RK. Biomolecules and biochemical methods. . In: Murray RK, Granner DK, Mayes PA, Rodwell VW, editors. Harper's Biochemistry. New York: Mecurant-Hill. 2000. pp. 6-14.
2. Koester J, Siegelbaum SA. Local signaling: passive electrical properties of the neuron. In: Kandel ER, Schwartz JH, Jessel TM, editors. Principles of Neural Science. New York, Elsevier. 2000. pp.140-149.
3. Mayes PA. Lipid of physiologic significance. In Murray RK, Granner DK, Mayes PA, Rodwell VW, editors. Harper's Biochemistry. New York: Mecurant-Hill. 2000. 160-171pp.
4. Ikeda M, Busto R, Yoshida S, Santiso M, Martinez E, Ginsberg MD. 1988. Cerebral phosphoinostide, triacylglycerol and energy metabolism during severe hypoxia and recovery. Brain Res 1998; 459(2): 344-350.
5. Lalitha T, Aarti T, Rohini P, Narayanasamy K, Ramakrishnan CV, Telang D. Alcohol exposure and undernutrition: effects on lipids metabolism and alcohol partitioning in rat brain regions in vitro. Med Alcohol Alcoholism 1990; 25(5): 477-482.
6. Ferrucci M, Busceti CL, Falleni A, Giorgi FS, Ruggieri S, Fornai F. Effects of methamphetamine on the cerebellar cortex: a preliminary study. Ann New York Acad Sci 2006; 1074: 149-153.
7. Hanz S, Fainzilber M. Retrograde signaling to injured nerve- the axon revisited. J Neurochem 2006; 99(1): 13-19.
8. Wang L, Schuster GU, Hultenby K, Zhang Q, Anderson S, Gustafsson J-?. Liver x receptors in the central nervous system: from lipid homeostasis to neuronal degeneration. Proct Natl Acad Sci, USA 2002; 99: 13878-13883.
9. Maggs JL, Tingle MD, Kitteringham NR, Park BK. Drug-protein conjugates-XIV. Mechanisms of formation of protein-arylating intermediates for amodiaquine, a myelotoxin and hepatotoxin in man. Biochem Pharmacol 1988; 37(2): 303-11.
10. Meshnick SR. Artemisinin: Mechanism of action, resistance and toxicity. Int J Parasitol 2002; 32(13): 1655-1660.
11. Ipca Laboratories Limited. 2004. 48, Kandivi Ind. Estate, Mumba; 400067.
12. Grandesso F, Hagerman A, Kamara S, Lam E, Checchi F, Balkan S et al. Low efficacy of the combination artesunate plus amodiaquine for uncomplicated falciparum malaria among children under 5 years in Kailahun, Sierra Leone. Trop Med Int Health 2006; 11(7): 1017-1021.
13. Martensson A, Stromberg J, Sisowath C, Msellem MI, Gil JP Montogomery SM et al. Efficacy of artesunate plus amodiaquine versus that of artemether-lumefantrine for the treatment of uncomplicated childhood Plasmodium falciparum malaria in Zanzibar, Tanzania. Clin Infect Dis 2005; 41(8): 1079-1086.
14. Guthmann JP, Ampuero J, Fortes F, van Overmeir C, Gaboulaud V, Tobback S, et al. Antimalarial efficacy of chloroquine, amodiaquine, sulfadoxine-pyrimethamine, and the combinations of amodiaquine + artesunate and sulfadoxine-pyrimethamine + artesunate in Huambo and Bie Provinces, Central Angola. Trans Royal Soc Trop Med Hyg 2005; 9I(7): 485-492.
15. O'Neill PM, Bray PG, Hawley SR, Ward SA, Park BK. 4-aminoquinolines-past, present, and future; a chemical perspective. Pharmacol Ther 1998; 77: 29-58.
16. Bailey SA, Zidell RH, Perry RW. Relationships between organ weight and body/brain weight in the rat: what is the best analytical endpoint? Toxicologic Pathology2004; 32(4): 448-446.
17. Zhang K, Sejnowski TJ. A universal scaling law between gray matter and white matter of cerebral cortex. Proct Natl Acad Sci, USA. 2002; 97: 5621-5626.
18. Schwartz JH, Westbrook GL. The cytology of neurons. In: Kandel ER, Schwartz JH, Jessel TM, editors. Principles of Neural Science. New York, Elsevier. 2000; 140-149.
19. Arnaud J, Nobile O, Boyer J. On the similarity of triacylglycerol and acetylcholesterol lipases in rat brain. J Neurochem 2006; 41(6): 1558-1562.
20. Sun GY. Effect of a fatty acid deficiency on lipids of whole brain, microsomes, and myelin in the rat. J Lipid Res 1972; 13: 56-62.

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