Article

Effect of Artesunate Treatment on Some Brain Biomolecules and its Behavioral Implication

Ekong MB [1], Igri AO [2], Ekanem TB [3]

Abstracts

Background: Artesunate (AS) is an artemisinin antimalarial drug used as a single drug or in combination with other antimalarials. **Objective:** This study was to find its effect on some brain biomolecules and behavioural activities in Wistar rats. **Methods:** Forty adult male Wistar rats weighing between 150-180g were divided into four groups of A, B, C and D with 10 animals each. Group A served as the control that received tap water, while groups B, C and D served as the experimental groups that received 2.85mg/kg (therapeutic dose-TD) and 5.71mg/kg (high pharmacologic dose-HPD) of AS per day for 3 days, and 2.85mg/kg (long duration therapeutic dose -LDTD) of AS per day for six days respectively. Half of the dose was administered twelve hourly (twice a day), and twelve hours after the last treatments, behaviour test using the 'open field maze' was carried out. Immediately after, the animals were sacrificed with chloroform anaesthesia and the whole brain removed and weighed. Whole brain homogenates were used to determine brain total protein (TP), triacylglycerol (TAG) and cholesterol (CH).Data were analyzed statistically by ANOVA and Tukey-Kramer Multiple Comparative Test as applicable. **Results:** There were no difference ($p<0.05$) between the experimental groups and the control group in the anthropometric parameters and behavioural activities. In the brain biomolecules concentration, TP was lower in concentration in the HPD group, TAG was lower in concentration in the LDTD group, while the HPD and LDTD groups had lower CH concentration compared to the control. In all the parameters studied no difference was found between the TD group and the control. **Conclusion:** AS at recommended dose may not affect some behaviour and brain biomolecule concentration, unlike when taken in excess of dose and or time. Even at these doses/time there may have been no behavioural manifestation.

Key words: Artesunate, Brain, Anthropometry, Behavior, Biomolecules, Rats

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Introduction

Antimalarial drugs currently in use for the management of malaria in humans display quite extensive and varying activities against various strains of *Plasmodia* and preferential activities on one or more stages of the parasite's development[1]. Even at this, the parasites have evolved a way of getting round the potency of most of these drugs[2-4].

The potency of the artemisinins and its derivatives makes them highly effective in combating malaria in the world[5-7]. They act rapidly on the parasites and do not remain in the blood stream for long[8], therefore most of the parasites are destroyed before the drug concentrations drop to sub-therapeutic levels, reducing the chances that the parasites will form resistance[9]. In clinical trials, artesunate (AS), a derivative of artemisinin, has been reported to destroy cancer cells[10], and also reduces proliferation, interferes in DNA replication and cell cycle and, enhances apoptosis[11].

It is documented that AS and other derivatives of artemisinin cause significant toxic effects. In the nervous system, they cause damage to the brain stem and other centers involved predominantly in auditory processing and vestibular reflexes[12-16]. Sedative effects, lowered movement of synchronism, analgesia, muscle relaxation, tremor and convulsion have been reported[17]. These effects are usually due to the release of free radicals from its endoperoxide bond[18], and free radicals have been implicated in a lot of adverse effects in different parts of the body[18-20].

The effects of this drug as it affects the different parts of the brain usually range from the alteration in the biochemical activities to cellular damage and behavioural manifestation[13-17]. The biochemical activities involve the alteration in the biomolecules concentration and activities within the tissues, while the behavior of animals as affected by endogenous and exogenous agents like drugs may only be appreciated using specialized apparatus such as the open field, Morris water and elevated plus mazes. When studying locomotion, exploratory and anxiety as combined parameters, the open field maze is usually employed[21,22]. Hence, this study aimed to check the effect of AS on some behavioral activities and some biomolecules in the brain of Wistar rats.

Methods

Forty adult male Wistar rats weighing between 150-180g procured from the animal house of the Department of Anatomy were handled in accordance with the International regulation governing the use of laboratory animals. The animals were randomly assigned into four groups (A, B, C, and D) of ten animals each. Group A served as the control, while groups B, C and D were the experimental. Two packets of standard AS drug were procured from a reputable pharmacy in Calabar. Each packet contained twelve 50mg blistered tablets of AS. Clean tap water was used as vehicle to dissolve the drug and the mg/kg body weights equivalent to a physiologic man was calculated as the therapeutic dose. The control group received a placebo of tap water, while the experimental groups received different doses of AS, all by oro-gastric tubes. The animals were treated twelve hourly (twice daily). The treatment is as seen in Table – I.

Table I: Schedule of the drug administration

Group	Dosage per day of AS	Duration (days)
A	Control	3
B (TD)	*2.86mg/kg	3
C (HPD)	*5.71mg/kg	3
D (LDTD)	*2.86mg/kg	6

n = 10, *The drug was administered twice daily. The dose per day is the sum of the treatment in a day (morning and evening). Therefore, half of these values were administered per treatment

TD = Therapeutic Dose, HPD = High Pharmacologic Dose, LDTD = Long Duration Therapeutic dose

The apparatus used for the open field test' was constructed of white plywood of 72×72cm with 36cm walls. One of the walls was clear Plexiglas, so the animals will be visible, and the floor lined with clear Plexiglas. Blue lines were drawn on the floor with a marker and this was visible through the clear Plexiglas floor. These lines divided the floor into sixteen 18×18cm squares. A central square of 18×18cm was drawn in the middle of the open field. The maze was located in a 1.8×4.6m test room lit by a 60-Watt red lamp for a background lighting[21,22].

Rats were carried to the test room in home cages and were handled by the base of their tails at all times. Each rat was placed in the proximal right-hand corner of the maze and allowed to explore the apparatus for five minutes. After the five minute test, the rat was returned in its home cage and the open field was cleaned with 70% ethyl alcohol and permitted to dry before introduction of the next rat. Behavior was scored manually, and each trial was recorded for latter analysis using a video camera positioned above the apparatus. The counting was done manually.

The following activities were carried out: frequency of line crossing; frequency of central square entry (CSF), central square duration (CSD); frequency of rearing; frequencies of stretch-attend (SA); urination and defecation[21,22].

Immediately after the behaviour test, the animals were sacrificed using chloroform anaesthesia. Their brains were immediately removed, blotted dry on filter paper and weighed using a Mettler p163 balance. They were homogenized in cold 0.25m STKM (sucrose tris-KCl-MgCl) buffer. The homogenates were then used to estimate total protein (TP), triacylglycerol (TAG) and cholesterol (CH). The homogenization procedure involved rinsing the brain in cold 0.25m STKM buffer, crushing in mortar and homogenization in cold 0.25m STKM buffer. The homogenates were washed into a volumetric flask and made up to 15ml using STKM buffer. Aliquots of the homogenates were spurned using a centrifuge. The supernatant of the aliquots were then used for TP estimation using Biuret kit, TAG estimation using GPO- PAP kit and CH estimation using CHOP-PAP kit methods.

Statistical analysis using a one-way analysis of variance (ANOVA) was used to compare the group's mean for the body and brain weights, open field parameters and brain biomolecules, for treatment and their interactions. Thereafter post-hoc test using Tukey-Kramer Multiple Comparative Test was carried out to find the level of significance at $p<0.05$. All the results were expressed as mean ± standard error of mean.

Results

There was no significant ($p = 0.0857$) difference in the body weights of the animals in the experimental groups compared to the control, as well as no difference among the experimental groups. There was no significant ($p = 0.4942$) difference in the body weights of the animals in the experimental groups compared to the control, as well as no difference among the experimental groups. This is as seen in Table – II.

Table II: Summary of the body and brain weights of the control, TD, HPD and LDTD groups

Groups	Body weight (g)	Brain weight (g)
A(Control)	181.30±3.49	1.49±0.01
B (TD)	163.80±3.12^{NS}	1.48±0.01^{NS}
C (HPD)	165.40±8.24^{NS}	1.45±0.04^{NS}
D (LDTD)	175.10±5.04^{NS}	1.46±0.01^{NS}

Result are presented as mean ± standard error of mean, n = 10, NS = Not significantly different from the group A at $p < 0.05$, TD = Therapeutic dose, HPD = High pharmacologic dose, LDTD = Long duration therapeutic dose

There was no urination in all the groups, hence the reason it was not represented on Table – III.

There was no significant difference ($p = 0128$) between the experimental groups and the control, but the LDTD group was significantly ($p < 0.01$) lower than the TD group. This is as seen in Table – III.

Table III: Summary of behavioral activities of the control, TD, HPD and LDTD groups

Groups	TLA	CSF	CSD	SA	DEF
A (Control)	68.0±7.85	1.20±0.49	2.85±1.34	2.00±0.60	1.20±0.80
B (TD)	72.2±5.43^{NS}	1.80±0.53^{NS}	2.05±0.59^{NS}	1.62±0.45^{NS}	2.80±0.49^{NS}
C (HPD)	91.4±7.87^{NS}	1.60±0.54^{NS}	3.98±1.49^{NS}	1.80±1.20^{NS}	1.80±0.53^{NS}
D (LDTD)	89.4±6.31^{NS}	1.80±0.57^{NS}	4.26±1.54^{NS}	0.60±0.27^{NS}	0.20±0.13^{b}

Result are presented as mean ± standard error of mean, n = 10, NS = Not significantly different from group A (control), b = Significantly different from B at $p < 0.01$, TD = Therapeutic dose , HPD = High pharmacologic dose, LDTD = Long duration therapeutic dose, TLA = Total locomotor activity, CSF = Central square frequency, CSD = Central square duration, SA = Stretch attend, DEF = Defecation

The HPD group had a significantly lower ($p = 0.0046$) TP concentration compared to the control and the TD groups. There were no significant difference between the TD, and LDTD groups and the control group. The LDTD group was significantly lower ($p < 0.05$) than the TD, HPD and the control groups, while there was no difference between the other experimental groups and the control. The HPD and LDTD groups were significantly lower ($p < 0.05$) than the control group, though there was no difference between these groups and the TD group. There was however no difference between the TD group and the control. This is as seen in Table – IV.

Table IV: Summary of brain biomolecules estimates in the control, TD, HPD and LDTD groups

Group	TP	TAG	CH
A (Control)	2.14±0.02	120.29±4.65	228.41±3.07
B (TD)	2.16±0.04^{NS}	114.10±4.53^{NS}	210.64±3.49^{NS}
C (HPD)	1.79±0.06^{*,b}	131.11±4.92	195.24±5.09***
D (LDTD)	2.04±0.13^{NS}	89.25±2.46 ***,b,c	195.56±6.69 ***

Result are presented as mean ± standard error of mean, n = 10, NS = Not significantly different from group A (control), * = Significantly different from A at $p<0.05$, *** = Significantly different from A at $p < 0.001$, c = Significantly different from C at $p < 0.001$, á = Significantly different from B at $p < 0.05$, TD = Therapeutic dose, HPD = High pharmacologic dose, LDTD = Long duration therapeutic dose, TP = Total protein, TAG = Triacylglycerol, CH = Cholesterol

Discussion

AS is a water-soluble form of artemisinin, and is effective as an antimalarial agent. In this study, AS effects on some brain biomolecules and behavior were investigated. The anthropometric parameters revealed insignificant ($p=0.0857$, 0.4942) difference between the treatment groups and the control in the body and brain weights respectively. This implies that the AS treatment at these doses and time did not affect the body and brain weights of the animals. This is consistent with a previous report[23].

The behavior of animals requires special attention and specialized study, because we may not appreciate most of these different behaviours exhibited ordinarily. Hence, the use of the open field maze for locomotion, exploratory and anxiety related behaviours. TLA measures locomotor activity, exploration and anxiety, CSF and CSD measure exploration and anxiety, while SA, urination, and defecation, measure anxiety[21-25]. High frequencies of TLA, CSF and CSD, indicate increased locomotion, exploration and decreased anxiety, whereas the high frequencies of SA, urination, and defecation, indicate increased anxiety.

In this study, there was no difference between the experimental groups and the control group in TLA, CSF, CSD and SA, while there was no urination in all the groups. This indicates that the behaviours of the animals were not altered after treatment with AS. The LDTD group had a significant reduced defecation than the HPD group. Hall et al[24] described defecation and urination as indices of anxiety in rodents. Bindra and Thompson[26] later reported that urination and defecation does not measure anxiety effectively, and this was supported by Lister[27]. Hence, we did not rely on urination and defecation as parameters for behavioural study. These results indicate that at these doses and time, AS did not increased or decreased locomotion and exploratory behaviours, and did not have anxiogenic nor anxiolytic effects. It is reported that natural occurring locomotor activation and inhibition may differ in its underlying mechanisms from similar behaviours induced by drugs[28]. Our result is at variance with Odo et al[29] who reported decreased locomotor and exploratory behaviours with AS.

Biomolecules are the components of every cells of the animal's body whose alteration in

concentration may result in transient to drastic damage to the cells that may ultimately affect large tissue areas of the body[30]. In the brain, this process may be irreversible, and this is often fatal. In this study, some biomolecules concentrations in whole brain homogenate were investigated. The TP was significantly ($p=0.0046$) lower in the HPD group compared to the control and TD groups. It is reported that micromolar concentration of AS is required for toxicity to mammalian cells[20], and protein is the working molecule or building blocks of all cells[31], therefore toxicity of AS may result in depletion of protein concentration as seen in this study. This is in line with Ekong et al[23] who reported a significant decrease in brain TP concentration of the experimental group treated with 17.50/5.71mg/kg of amodiaquine (AQ) and AS combination. There was no difference in TP concentration between the LDTD group and the control. A previous report indicates that AS does not remain in the body for long because it is rapidly metabolized and excreted[8]. This may have been the reason for the AS not affecting the TP of the LDTD group. Disruption in TP concentration may result in neurotransmitter depletion, neurodegeneration and disruption of signaling pathway and ion channels that may ultimately result in irreversible damage to the brain.

TAG concentration was significantly ($p<0.05$) lower in the LDTD group compared to the control, TD and HPD groups. AS releases oxygen free radicals from its endoperoxide bond and this have been implicated in lipid peroxidation[18]. The peroxidation of lipid may have resulted in depletion of TAG in the LDTD group, since the drug was administered longer than the normal duration. This is consistent with Ekong et al[23] who reported decreased TAG concentration in the group treated with 8.75/2.86mg/kg of AQ and AS combination for six days. The HPD group had significant ($p<0.05$) higher TAG concentration compared to the TD group. Increased AS concentration may have resulted in physiologic response by the brain to counter the peroxidation of the lipid leading to increased synthesis of lipid by the smooth endoplasmic reticulum. This is consistent with Ikeda et al[32] who reported increased brain TAG on rats subjected to hypoxia.

CH concentration was significantly ($p<0.05$) reduced in the HPD and LDTD groups compared to the control group. Peroxidation of lipid may have resulted in this reduction[18]. This is consistent with Ekong et al[23] who reported decreased CH concentration in groups treated respectively with 17.50/5.71mg/kg and 8.75/ 2.86mg/kg of AQ and AS combination for three and six days. It has been documented that cellular depletion of CH reduces sensitivity of signal enzymes to capacitance Ca^{2+} entry[33]. Thus, the depletion of the store of CH may limit the activities of the brain since lipids play a significant role in myelination, signal transmission, membrane formation, and determine the entry and exist of signaling molecules and ions[34]. Depletion of these lipids might result in alteration of these functions that may result in catastrophic implications.

In both brain biomolecule concentration and behavioural activities, the TD group was not different from the control group. This may be that the drug treatment may not have adverse effects at recommended dose. Behavioural activities are usually the manifestation of biochemical and cellular processes, which if drastic predisposes one to elevated or depressed behaviour. Increased behavioural activities are mostly due to activation of the brain which later manifests as excitation of the central neurons and an increased in cerebral metabolism. This was not the case in this study and may imply that the biomolecule changes may have been transient. Genovese et al[35] reported neurotoxicity in the absence of deficits in behavioural performance in rats using arteether, another derivative of artemisinin.Our study revealed that changes were only observed in the biomolecule

concentration of the HPD and LDTD groups treated with 5.71mg/kg and 2.86mg/kg of AS per day for 3 and 6 days respectively. These doses were more than the recommended doses and time of the drug.

Conclusion

We then conclude that AS may not be harmful at its recommended dose, since its effect was not different from the control in the anthropometric parameters, behavioural activities and biomolecule concentration studies. Thus, AS at recommended dose may not affect some behaviour and brain biomolecule concentration, unlike when taken in excess of dose and or time. Even at these doses/time there may have been no behavioural manifestation.

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