Effect of selected anti-malarial drugs on the blood chemistry and brain serotonin levels in male rabbits

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Abstract: The effects of oral administration of sulfadoxine - pyrimethamine (SP), artesunate (A) and sulfadoxine - pyrimethamine - artesunate (SPA) on blood chemistry and brain serotonin in rabbits were investigated. Forty rabbits were divided into four groups of ten animals each. The group that served as the control received 2ml of distilled water while the other groups were received 1.25/25mg base/kg body weight of SP, 3.3mg/kg body weight of A and 1.25/25mg base/kg body weight of SP plus 3.3mg/kg body weight of A respectively by oral route daily for 3 days in a week for four weeks. At the end of each week of drug administration, three rabbits from each group were anaesthetized, blood was taken from the jugular veins using sterile needle and serum was extracted. The rabbits were sacrificed by decapitation; the liver and brain tissues were excised and homogenized. Total blood protein, cholesterol, triglyceride, albumin, creatinine and urea concentrations, creatine kinase, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, ALP activities were assayed using CX5 synchron autoanalyzer. The brain and liver serotonin levels were determined using high performance liquid chromatography (HPLC). There were no significant differences ($P \leq 0.05$) in the concentrations of serum albumin, urea, creatinine, cholesterol and triglyceride of rabbits administered SP, A and SPA for 4 weeks, except in serum total protein. No significant differences existed in the activities of AST, ALT and ALP, except in creatine kinase which was elevated in the control. The brain serotonin levels of rabbits administered SP, A and SPA were significantly higher as compared to the control throughout the duration of the study Data of the study indicate that oral administration of SP, A or SPA in rabbits do not affect blood chemistry, but affected brain serotonin levels and could alter some neural functions.

Keywords: Artesunate, sulphadoxine - pyrimethamine, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, ALP, serotonin

INTRODUCTION

Malaria ranks among the major health and development challenges of the world. The disease is prevalent in almost 100 countries, accounting for 40% of the world's population. It affects an estimated three to five hundred million people, causing more than a million deaths per year (Snow *et al,* 1992). It kills a child every 30 seconds and it has been reported that about 3000 children below the age of five years die daily (WHO, 1998). Although the disease is spread in most tropical countries, sub – Sahara Africa, with more than 80% of the world's malaria cases are the focus of most efforts in combating the disease due to the high morbidity and mortality rates.

Early diagnosis and effective treatment of the associated infections would be an added advantage in the Roll Back Malaria partnership (Kachur *et al.*, 2004). There is however an increase in the resistance of *Plasmodium falciparum* to the antimalarial agents which are frequently used such as chloroquine (CQ) and sulfadoxine - pyrimethamine (SP) (Hay *et al.,* 2004; WHO 2003; O'Dempsey 2000; Dorsey *et al.,* 2007; Kissinger 2000). As a result of the parasite resistance to first-line drugs, combination therapy was introduced and this has improved parasite clearance especially in a semi-immune population (Van Vugt *et al.*, 2000).

Countries experiencing resistance to malaria drugs were encouraged to change to the combination therapy. Thus the artemisinin based combination therapies (ACTs) came into use (White, 1999; Bloland *et al,* 2000; Nosten and Brasseur, 2002 and WHO, 2001). Artesunate and artemether are known to clear parasitaemia more than the combination therapy comprising of chloroquine and sulfadoxine - pyrimethamine (WHO, 1998). When these drugs are used in combination with other antimalarial, they would help to prevent the development of resistant strain. Such drugs include amodiaquine, sulfadoxine - pyrimethamine, mefloquine and clindamycin. There has been no confirmed evidence ad to the development of resistance of *Plasmodium falciparum* to either artemisinin or any of its derivatives.

There is risk of adverse effects associated with the use of the combination therapy. Though there has been report of neurotoxicity observed in the animals treated with artemisinin derivatives, there is no evidence that it exists in humans to date (Van Vugt *et al.,* 2000; Hien *et al*,

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2003; Sowunmi and Fashina 2001). The deaths of embryo and morphological abnormalities in early pregnancy have been observed in animals but no reported case has been observed in humans treated with artemisinin during the second and third trimesters.

Artemisinin and its derivative are reported to have low toxicological effects. Hence the observed toxicity in artemisinin combination therapy could be attributed to the drugs used in combination with the artemisinin (Nosten and White, 2007). Artemisin causes growth inhibition and apoptosis of cancer cells (Nam *et al,* 2006) and acute hepatotoxicity in guinea pigs (Nwanjo and Oze, 2007). Thus the study investigated the effects of artesunate - sulfadoxine - pyrimethamine combination on the biochemical parameters of rabbits when compared with effects of each of the drugs (artesunate and sulfadoxine - pyrimethamine)

MATERIALS AND METHODS

The drugs were obtained from Lagos University Teaching Hospital (LUTH) Pharmacy. Artesunate (Gricin ^{R}) was manufactured by Justen Pharm Ltd, Lagos, artesunate - sulfadoxine - pyrimethamine (Farenax^{R}) and sulfadoxine - pyrimethamine (Fansidar^{R}) were manufactured by Swiss Pharma Nigeria Ltd, Lagos, Nigeria. All the drugs were dissolved with distilled water.

Animals

Forty male European rabbits (Oryctolagus cuniculus) weighing 1.00 - 2.10 kg were used in the study. The study was carried out in accordance with the ethical guidelines for animal care and use (Zimmerman, 1983). They were obtained from the Animal house of the Nigerian Institute of Medical Research, Yaba, Lagos. They were housed in the Laboratory Animal Centre of the College of Medicine, University of Lagos in metallic cages kept at temperature of 25–27^{o}C at 70% relative humidity and daylight conditions. They were fed the commercial chow (Pfizer Feeds Plc., Ibadan, Nigeria) and water ad libitum.

Experimental protocol

After 2 weeks of acclimatization, the 40 rabbits were divided into 4 equal groups of 10 animals each. A group served as the control and received 2ml of distilled water while the other groups were orally administered therapeutic doses of 1.25/25mg base/kg body weight of sulfadoxine-pyrimethamine, 3.3mg/kg body weight of artesunate and 1.25/25mg base/kg body weight of sulfadoxine-pyrimethamine plus 3.3mg/kg body weight of artesunate respectively once daily for 3 days per week. Dosing was continued for 4 weeks. At the end of the first week of drug administration and consequently in the 2nd, 3rd and 4th weeks, 3 rabbits from each group were anaesthetized, blood was taken from the jugular veins using sterile needles and serum was extracted and stored at – 80^{o}C. The rabbits were sacrificed by decapitation and the brain tissue excised and homogenized.

The activities of serum alkaline phosphatase (ALP) were determined using the phenolphthalein method (Babson *et al,* 1966), alanine transaminase (ALT) and aspartate transaminase (AST) were determined by the procedure of Rietman and Frankel (1957). Total protein was determined using the method of Lowry *et al* (1951). Serum urea was determined by the urease – Bertthelot method (Weatherbum 1967), total cholesterol with enzymatic endpoint method (Trinder, 1969, Roeschlau *et al.,* 1974), and creatinine levels by alkaline picrate method (Tietz *et al.,* 1986). Albumin level, triglyceride level and creatine kinase (CK) activity were assayed using CX5 Synchron auto analyzer. The brain and liver serotonin levels were determined using high performance liquid chromatography (HPLC).

STATISTICAL ANALYSIS

All the data obtained were expressed as mean ± standard error of mean. The results obtained for both the control and treated groups of rabbits were compared using a one way analysis of variance (ANOVA). Statistical significance was set at $P \leq 0.05$

RESULTS

There were no significant differences ($p \geq 0.05$) in the concentration of serum albumin, urea, creatinine, cholesterol and triglyceride of the rabbits administered sulfadoxine - pyrimethamine (SP), artesunate (A) and sulfadoxine - pyrimethamine - artesunate combination (SPA) for 4 weeks except in serum total protein of the rabbits that were administered SP or SPA. There were no significant differences in the activities of AST, ALT and ALP except in AST of the rabbits administered sulfadoxine - pyrimethamine - artesunate. There were significant ($p \leq 0.05$) differences in the creatine kinase activity of the three groups that were administered SP, A and SPA respectively. These results are summarized in table 1. The brain serotonin levels were observed to be higher in the rabbits that had received 1.25/25mg sulfadoxine – pyrimethamine/kg body weight and 3.3mg Artesunate/kg body weight respectively. The serotonin levels of the animals that received 1.25/25mg sulfadoxine- pyrimethamine plus 3.3mg Artesunate/kg body weight were lower than the values obtained in the control group (table 2).

DISCUSSION

Fansidar containing sulfadoxine and pyrimethamine function by inhibiting folic acid synthetic pathway in the malaria parasite (Wang *et al.*, 1997; Gregson and Plowe, 2005; Hyde, 2005). The rabbit orally administered

Table 1: Serum activities of rabbits orally administered sulfadoxine - pyrimethamine (SP), artesunate (A) and sulfadoxine - pyrimethamine - artesunate (SPA) combination for 4 weeks

Drug	Week 1		Week 2		Week 3		Week 4	
	ALP (iu/l)	CK (mmol/l)	ALP (iu/l)	CK (mmol/l)	ALP(iu/l)	CK (mmol/l)	ALP (iu/l)	CK (mmol/l)
Control	30.03 ± 0.75	158.2 ± 1.41	44.73 ± 0.639	164.1 ± 2.69	37.9 ± 0.23	154.2 ± 0.21	45.2 ± 0.173	151.6 ± 0.38
Artesunate	36.27 ± 1.07[a]	76.13 ± 0.34[a]	54.73 ± 0.348[a]	54.7 ± 0.32[a]	40.63 ± 0.24[a]	0.733 ± 0.15[a]	39.33 ± 0.240[a]	65.83 ± 0.18[a]
Sulfadoxine – Pyrimethamine	49.0 ± 3.91[a]	113.1 ± 4.07[a]	79.13 ± 1.16[a]	80.7 ± 0.70[a]	82.33 ± 1.15[a]	68.4 ± 1.25[a]	52.23 ± 0.809[a]	30.63 ± 0.44[a]
Sulfadoxine – Pyrimethamine Artesunate	46.43 ± 0.35[a]	32.8 ± 0.52[a]	47.2 ± 0.473[b]	67.4 ± 0.38[a]	44.7 ± 0.231[a]	132.4 ± 0.33[a]	56.5 ± 1.31[a]	46.2 ± 0.21[a]

Drug	Week 1		Week 2		Week 3		Week 4	
	AST (iu/l)	ALT (iu/l)	AST (iu/l)	ALT (iu/l)	AST (iu/l)	ALT (iu/l)	AST (iu/l)	ALT (iu/l)
Control	10.03 ± 0.18	8.00 ± 0.252	10.1 ± 0.57	12.2 ± 0.208	16.3 ± 0.35	39.73 ± 0.176	7.13 ± 0.38	21.17 ± 0.601
Artesunate	5.03 ± 0.46[a]	17.07 ± 0.41[a]	16.03 ± 0.18[a]	29.33 ± 0.433[b]	13.67 ± 1.76[a]	21.57 ± 0.353[b]	36.3 ± 0.31[a]	34.03 ± 0.24[b]
Sulfadoxine - Pyrimethamine	16.1 ± 1.83[a]	33.66 ± 1.73[b]	15.97 ± 0.64[a]	24.97 ± 0.384[b]	10.1 ± 0.61[a]	34.16 ± 1.03[b]	36.03 ± 0.90[a]	225.1 ± 0.115[b]
Sulfadoxine – Pyrimethamine Artesunate	15.1 ± 0.115[b]	21.0 ± 0.666[b]	30.3 ± 0.52[a]	84.6 ± 63.6[a]	36.67 ± 0.52[a]	12.5 ± 0.321[b]	41.1 ± 0.3[a]	29.07 ± 0.845[b]

Drug	Week 1		Week 2		Week 3		Week 4	
	Total Protein (mg/dl)	Urea (mg/dl)	Total Protein (mg/dl)	Urea (mg/dl)	Total Protein (mg/dl)	Urea (mg/dl)	Total Protein (mg/dl)	Urea (mg/dl)
Control	45.4 ± 0.321	11.87 ± 0.318	47.63 ± 0.273	19.77 ± 0.240	43.7 ± 0.231	13.67 ± 0.176	41.37 ± 0.3	14.87 ± 0.145
Artesunate	60.23 ± 0.45[a]	8.29 ± 0.140[a]	55.07 ± 0.328[a]	14.87 ± 0.15[a]	62.3 ± 0.473[a]	8.57 ± 0.348[a]	61.4 ± 0.265[a]	14.6 ± 0.208[b]
Sulfadoxine - Pyrimethamine	77.84 ± 0.99[a]	7.07 ± 0.406[a]	73.43 ± 1.20[a]	9.83 ± 0.328[a]	59.87 ± 1.03[a]	7.4 ± 0.361[a]	59.87 ± 1.16[a]	13.73 ± 0.50[a]
Sulfadoxine – Pyrimethamine - Artesunate	62.5 ± 0.36[a]	11.13 ± 0.318[b]	56.23 ± 0.240[a]	16.17 ± 0.33[a]	63.7 ± 0.231[a]	11.6 ± 0.264[a]	66.3 ± 0.231[a]	18.4 ± 0.361[a]

Drug	Week 1		Week 2		Week 3		Week 4	
	Cholesterol (mmol/l)	Creatinine (mmol/l)	Cholesterol (mmol/l)	Creatinine (mmol/l)	Cholesterol (mmol/l)	Creatinine (mmol/l)	Cholesterol (mmol/l)	Creatinine (mmol/l)
Control	2.83 ± 0.291	20.3 ± 0.90	2.27 ± 0.0882	40.27 ± 0.43	3.97 ± 0.176	30.63 ± 0.26	3.63 ± 0.176	29.47 ± 0.56
Artesunate	1.83 ± 0.145[a]	39.17 ± 0.46[a]	3.77 ± 0.260[a]	40.97 ± 0.33[b]	2.87 ± 0.318[a]	34.13 ± 0.31[a]	3.83 ± 0.176[b]	16.87 ± 0.20[a]
Sulfadoxine - Pyrimethamine	2.90 ± 0.173[b]	18.33 ± 1.75[a]	3.10 ± 0.115[b]	21.6 ± 0.44[a]	3.77 ± 0.480[b]	64.77 ± 1.23[a]	3.00 ± 0.208[b]	66.34 ± 0.34[a]
Sulfadoxine – Pyrimethamine - Artesunate	2.07 ± 0.240[b]	14.6 ± 0.36[b]	2.83 ± 0.176[b]	30.83 ± 0.43[a]	4.00 ± 0.208[b]	21.83 ± 0.12[a]	5.27 ± 0.202[a]	34.03 ± 0.09[a]

Drug	Week 1		Week 2		Week 3		Week 4	
	Albumin (mmol/l)	Triglyceride (mmol/l)	Albumin (mmol/l)	Triglyceride (mmol/l)	Albumin (mmol/l)	Triglyceride (mmol/l)	Albumin (mmol/l)	Triglyceride (mmol/l)
Control	44.77 ± 0.524	1.03 ± 0.120	42.2 ± 0.208	0.800 ± 0.153	46.8 ± 0.265	0.400 ± 0.10	42.17 ± 0.233	1.17 ± 0.145
Artesunate	37.87 ± 0.59[a]	1.03 ± 0.233[b]	36.9 ± 0.265[a]	0.70 ± 0.115[b]	45.57 ± 0.32[b]	0.47 ± 0.120[b]	18.4 ± 0.557[a]	0.97 ± 0.145[b]
Sulfadoxine - Pyrimethamine	54.9 ± 1.22[a]	0.84 ± 0.186[b]	39.07 ± 0.851[a]	0.70 ± 0.208[b]	39.47 ± 0.84[a]	0.60 ± 0.231[b]	43.67 ± 0.41[a]	0.84 ± 0.145[b]
Sulfadoxine – Pyrimethamine - Artesunate	36.47 ± 0.32[a]	0.90 ± 0.115[b]	36.73 ± 0.233[a]	0.47 ± 0.120[b]	45.7 ± 0.174[b]	0.73 ± 0.145[b]	39.1 ± 0.115[a]	1.03 ± 0.088[b]

LP = Alkaline phosphatase, ALT = Alanine transaminase, AST = aspartate transaminase, CK = Creatine Kinase.
Data given expressed as Mean ± SEM.
a-indicates a significant difference ($p \leq 0.05$) from control
b-indicates no significant difference ($P > 0.05$) from control

Table 2: Concentration of rabbit brain serotonin orally administered sulfadoxine - pyrimethamine (SP), artesunate, (A) and sulfadoxine - pyrimethamine - artesunate (SPA) combinations for 4 weeks

Drug	Number of weeks after drug Administration			
	1	2	3	4
Control	5.06 ± 0.052	6.67 ± 0.035	4.37 ± 0.103	14.73 ± 0.203
Artesunate	9.34 ± 0.095	22.57 ± 0.433	13.23 ± 0.260	12.70 ± 0.231
Sulfadoxine - Pyrimethamine	17.4 ± 0.265	16.6 ± 0.265	16.23 ± 0.291	13.63 ± 0.240
Sulfadoxine – Pyrimethamine - Artesunate	16.47 ± 0.233	10.2 ± 0.265	13.37 ± 0.240	11.37 ± 0.376

sulfadoxine - pyrimethamine had elevated brain serotonin. This finding agrees with previous reports (Gregson and Plowe, 2005). Addition of artesunate to the regimen did not result in significant reduction in the brain serotonin. The endoperoxide present in the artesunate molecule serves as antioxidant which mops up all the free radicals that could trigger serotonin release (Obianime and Aprioku, 2009; Nwanjo and Oze, 2007). Thus the level in the treated rabbits was lower than either the sulfadoxine - pyrimethamine-artesunate or sulfadoxine - pyrimethamine treated rabbits. The latter is highest since the free radicals which trigger this release of serotonin were available.

The activities of CK, AST and ALT were not significantly altered and suggest that there were no renal or hepatic damage following oral administration of sulfadoxine - pyrimethamine (SP), artesunate, (A) and sulfadoxine - pyrithamine - artesunate (SPA) combinations for 4 weeks. This is in agreement with the previous studies which show that artesunate, sulfadoxine - pyrimethamine and sulfadoxine - pyrimethamine - artesunate combinations are safe regimens.

Alkaline phosphatase activity which is used as indicative of liver function and bone function, maintained a stable level in all the groups throughout the study period. This show the drugs have no effect on the liver. The almost stable protein and albumin levels observed also confirm normal liver functions which were not affected throughout the administration of the drugs. Numerous studies have demonstrated that artesunate has no adverse effects on foetus and is thus ideal in pregnancy (Sowunmi and Fashina 2001). There was significant alteration in the cholesterol and triglyceride levels were also observed.

There were no adverse effects observed on the kidneys as the values for the creatinine, indicative of kidney function was the same for the rabbits who received no drug and those treated with artesunate, sulfadoxine - pyrimethamine or a combination of both.

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