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Sulphadoxine-pyrimethamine alters the antioxidant defense system in blood of rabbit

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Summary: Sulphadoxine-pyrimethamine (SP) despite reported resistance remains an important drug of choice for the treatment and control of malaria in most endemic areas. Exacerbation of intra-erythrocytic oxidative stress might contribute to the process of elimination of malaria parasites in the body. The effect of treatment with SP on the antioxidant defense system was investigated using rabbit as a model. Ten male rabbits were divided into two groups of five animals each. The first group was administered with normal saline and served as control. The second group received a single dose of SP (26.25mg/kg body weight). Blood samples were collected before and at 6, 12 and 24 h after drug administration. Activity of cellular enzymatic antioxidants, superoxide dismutase (SOD) and catalase (CAT), and level of reduced glutathione (GSH) were assayed using standard spectrophotometric methods. Serum lipid peroxidation was assessed by the formation of thiobarbituric acid reactive species (TBARS) while protein content was assayed by the method of Lowry *et al*., 1951. SOD activity was observed to increase progressively by 4.9, 63.4 and 120.8% at 6, 12 and 24 h respectively, after drug administration. Similarly, CAT activity increased by 44.5, 82.6 and 116.3% at 6, 12 and 24 h, respectively. TBARS level also increased significantly by 45.5, 118.2 and 186.4%, respectively. However, the level of GSH decreased by 41.9% at 6 h and remained so up till the 12 h, but by 24 h after drug administration, the level of the thiol substance has increased considerably up to 48.4% above the baseline level. SP treatment altered the antioxidant defense system in blood and may therefore induce oxidative stress by generating reactive oxygen species. This might play significant role in the therapeutic and adverse effects associated with the drug.

Keywords*:* Sulphadoxineipyrimethamine, Lipid peroxidation, oxidative stress, Blood, Rabbit

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INTRODUCTION

More than two billion people around the world are at risk for malaria infection. The estimated annual global incidence of malaria is about 250 million clinical cases and about one million deaths occur worldwide, each year (WHO, 2009).

Chloroquine used to be the most favoured drug for the treatment of malaria in Africa. However, the growing problem of resistance to this drug has rendered it almost useless for the treatment of malaria, therefore, sulphadoxine-pyrimethamine (SP) became the alternative drug for the treatment of malaria in endemic areas (Kamya *et al.*, 2001). SP has been used extensively against chloroquine-resistant malaria, but increasing resistance to this drug which has also developed in most parts of Africa has led to a decline in its effectiveness (Alloueche *et al.*, 2004). Consequently, artemisinin-based combination therapy (ACT) has been adopted as choice drug for the treatment of malaria in most African countries (Hastings *et al.*, 2002; Ajayi *et al*, 2008). The main problem with ACT is its relative high cost, which may be beyond the reach of many Africans.

SP comprises of 500mg, N-(5,6–dimethoxy–4–pyrimidinyl) sulphanilamide (sulphadoxine) and 25mg, 2,4–diamino-5-(p-chlorophenyl)-6 ethylpyrimidine (pyrimethamine). It acts by reciprocal potentiation of its two components, achieved by a sequential blockade of two enzymes involved in the biosynthesis of folic acid within the parasite. Thus, sulphadoxine and pyrimethamine are folate antagonists. Pyrimethamine inhibits the utilization of folate by inhibiting dihydrofolate reductase while the sulphonamide inhibits the synthesis of folate by competing with P-aminobenzoic acid (PABA) (Sibley *et al.*, 2001). SP causes serious skin reactions such as

blood dyscrasias and allergic alveolitis. In high doses, pyrimethamine may inhibit mammalian dihydrofolate reductase, resulting in megaloblastic anaemia (Scott and Weir, 1980).

Both sulfadoxine and pyrimethamine are excreted mainly by the kidney and appears in the breast milk of nursing mothers. The combination of pyrimethamine and sulphadoxine is generally well tolerated but severe and fatal reactions including erythema multiform, Stevens – Johnson syndrome and toxic epidermal necrosis could be observed (Koch-Weser, 1982). Hepatic toxicity due to sulphadoxine has been reported (Lazar *et al.,* 1985). Pyrimethamine reportedly caused foetal abnormalities in humans and animals, consequently, the use of folic acid supplements has been recommended (Peter *et al*., 2007).

Cellular antioxidants include radical scavenger enzymes such as superoxide dismutase (SOD), glutathione peroxidase and catalase (CAT); fat soluble vitamins A and E; as well as water soluble, low molecular weight substances such as vitamin C, glutathione (GSH) and carotenoids (Akkus *et al.*, 1996). SOD promotes the dismutation of superoxide anion (O_2^-) into hydrogen peroxide, which can be detoxified by both CAT and GSH peroxidase (Guemorri *et al*., 1991). Lipid peroxides are indicators of membrane peroxidation resulting from radical attacks on membranes by ROS. In view of the observed adverse effects of SP and the evidence that some antimalarials induce oxidative stress by generating large quantities of reactive oxygen species, we evaluated the effects of SP on cellular antioxidant defense system and extent of generation of lipid peroxides in the blood, which is the main medium of drug distribution in the present study.

MATERIALS AND METHODS

Chemicals

Sulphadoxine- Pyrimethamine (Fansidar®) was obtained from Evans Pharmaceutical company, Lagos, Nigeria. 5, 5' – dithio-bis- 2– nitrobenzoic acid (DTNB), glutathione, thiobarbituric acid, epinephrine and hydrogen peroxide were purchased from Sigma Chemical Company. MO, USA. All other reagents were of analytical grade and were obtained from the British Drug House, BDH, Poole, UK.

Animals and treatment

Male rabbits weighing between 720 and 800g were obtained from the animal house of the College of Medicine, University of Ibadan, Nigeria. They were kept in wire-meshed cages and fed with commercial rabbit feed (Ladokun Feeds, Nigeria, Limited) and liberally supplied with water. Ten rabbits were divided into two groups of 5 animals each. The first group, used as controls received physiological saline, while the second group was treated orally with a single therapeutic dose of SP (26.25mg/kg body weight).

Blood collection

Two millilitres of blood was collected from the marginal veins of the outer ear of rabbits into heparinised tubes and plain sterile tubes at 6, 12 and 24 h after drug administration. One milliliters of blood from each animal were also put into EDTA anticoagulated and plain bottles. Serum was subsequently collected from the samples in plain bottles. The whole blood was used for CAT and SOD assays while serum was used for protein and lipid peroxidation determinations.

Antioxidant enzyme assays

SOD activity was determined by measuring the inhibition of auto-oxidation of epinephrine at pH 10.2 at 30^0C by the method described by Tapiwanashe *et al.*, (1997). Activity of CAT was determined according to the procedure of Claiborne, (1995) by following the absorbance of hydrogen peroxide at 240nm, pH 7.0 and 25^0C. GSH was determined according to the procedure described by Ellman, (1959).

Measurement of serum lipid peroxides

Lipid peroxidation in serum was determined by the quantification of thiobarbituric acid reactive species as described by the procedure of Hunter *et al.,* (1963) and modified by Gutteridge and Wilkins (1982).
Protein content of the samples was estimated by the standard method of Lowry *et al.,* (1951) using bovine serum albumin as standard.

Statistical analysis

The data generated were analyzed using student's t-test. P values less than 0.05 were considered statistically significant between controls and SP treated rabbits.

RESULTS

Figure 1 shows the effect of oral administration of SP on SOD activity in the erythrocytes of rabbits. The data obtained showed that there was a significant increase ($p<0.05$) in the activity of erythrocytic SOD with time. SOD activity increased by 4.9, 63.4 and 120.8% at 6, 12 and 24 h, respectively after drug administration. Similarly, CAT activity increased by 44.5, 82.6 and 116.3% at 6, 12 and 24h respectively, compared with control values (Figure 2).

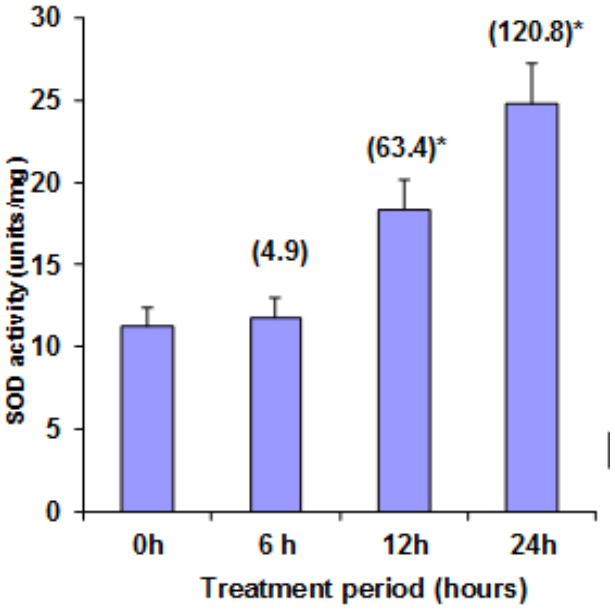

Figure1:
Effect of oral administration of a single dose of SP (26.25mg/kg) on superoxide dismutase activity in rabbits 1 Unit of superoxide dismutase (SOD) is the amount that inhibits autoxidation of epinephrine by 50% at pH 10.2 and 30^{O}C. Values are expressed as mean ± SD for 5 rabbits in each group. Figures in parenthesis are percentage differences relative to control *Significantly different from control ($p<0.05$).

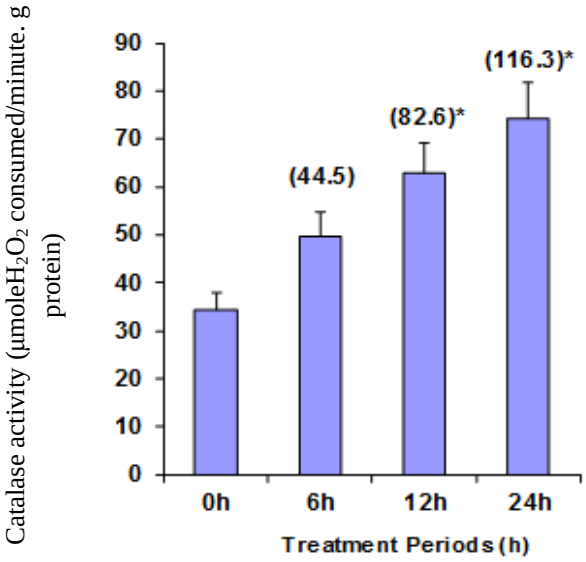

Figure 2:
Effect of oral administration of a single dose of SP (26.25mg/kg) on superoxide dismutase activity in rabbits.1 Unit of catalase (CAT) decomposes 1µmol H_2O_2 /min at pH 7.0 and 25^{O}C. Values are expressed as mean ± SD for 5 rabbits in each group.Figures in parenthesis are percentage differences relative to control *Significantly different from control ($p<0.05$)

Table 1:
Effect of a single dose SP (26.25mg/kg) treatment on serum lipid peroxidation and reduced glutathione.

Variables	Control	Post treatment		
	0	6h	12h	24h
TBARS (nmole /mg protein)	110±24.0	160±11.0* (45.5%)	240±39.0* (118.2%)	315±63.7* (186.4%)
GSH (nmole /mg protein)	3.1+0.3	1.8 ± 0.1* (41.9%)	1.8 ± 0.2* (41.9%)	4.6±0.2* (48.4%)

Values are given as mean ± SD for 5 rabbits in each group.
* Significantly different from control at $p< 0.05$
Figures in parenthesis are percentage difference relative to control

Thiobarbituric acid reactive species (TBARS) levels increased significantly ($p<0.05$) by 45.5%, 118.2% and 186.4% at 6, 12 and 24h respectively, when compared with the baseline values. While GSH level significantly decreased ($p<0.05$) by 41.9% at 6 hours and was constant up to 12 hours after drug administration. It increased significantly ($p<0.05$) by 48.4% over the baseline value at 24h as shown in table 1.

DISCUSSION

In spite of resistance to SP, the drug still plays a significant role in the control of malaria in endemic areas. It is very popular as an alternative to chloroquine as it is cheap and readily available. It is also being indicated in intermittent preventive treatment (IPT) of malaria in infants and pregnant women (Staalose *et al.*, 2004; Schellengberg *et al*, 2005). The current investigation demonstrates that treatment with SP alters the enzymatic antioxidant defense profile and induces oxidative stress in the body. Single dose treatment of the drug resulted in an increase in the level TBARS and activities of SOD and CAT, while there was an initial marked decrease in GSH level which increased afterwards at 24h after drug administration.

The increased TBARS level in the blood following SP administration is an indication of lipid peroxidation enhanced by the drug. The suitability of the thiobarbituric acid (TBA) assay as a reliable means of measuring malondialdehyde levels has been shown to be reliable in assessing lipid peroxidation in most biological samples (Draper and Hadley, 1990). The apparent increase in SOD activity suggests that

the blood in rabbit responded to an increased production of reactive oxygen species generated by SP. This is similar to reported observations with chloroquine treatment (Magwere *et al*., 1997; Farombi, 2000; Ogunbayo *et al*., 2006).

CAT is a tetrameric haemoprotein that undergoes alternate divalent oxidation and reduction at its active site in the presence of hydrogen peroxide. Studies have shown that it can be inhibited by superoxide anion, which converts it to ferroxyl and ferryl states i.e. the inactive forms of the enzymes (Kono and Fridovich, 1982). Induction of CAT is expected if increased SOD activity leads to an increased production of hydrogen peroxide in the blood. It is known that hydrogen peroxide can be destroyed by CAT which removes it when present in high concentration (Casado *et al.*, 1995). The observed increased CAT activity is therefore an indication that the blood may not be readily susceptible to the effects of hydrogen peroxide-induced oxidative stress following SP administration as it will be broken down by the enzyme.

Studies have shown that moderate oxidative stress may result in an induction of GSH-S-transferase while severe oxidative stress may result in its decrease with concomitant depletion of GSH (Aniya and Naito, 1993) which is a measure of the cellular redox status (Chance *et al.*, 1979). Our result shows that SP appears to affect the overall redox status of the cells in the blood, as indicated by the alteration in the levels of GSH. This result is in agreement with the report of Bhattacharyya *et al.,* (1983) who reported a decrease in the GSH levels of rat retina up to about four hours after a single dose of chloroquine. The increased serum level of GSH at 24 hours after treatment to pretreatment level is an indication that the body has restored the earlier depleted stock of the thiol substance after an initial response which induced oxidative stress in the blood.

In conclusion, it has been demonstrated that SP induces oxidative stress and alters the antioxidant defense system in the blood. The body however, seems able to cope with the stress through the activities of enzymatic and non- enzymatic antioxidants. The mechanism of the alteration and the significance of these changes on therapeutics and adverse events of SP need to be further investigated.

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