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Effects of Amodiaquine Hydrochloride and Artemisinin on Indomethacin-Induced Lipid Peroxidation in Rats

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Abstract: In this study, the effects of two antimalarials-amodiaquine hydrochloride and artemisinin were investigated in ulcerated albino rats of Wistar strain. Rats were treated with amodiaquine (30 mg kg^{-1}) and Artemisinin (2.86 mg kg^{-1}) for 24 h after formation of ulcers induced by indomethacin. Treatments with Amodiaquine Hydrochloride led to significantly increased gastric lesions while artemisinin led to significantly decreased gastric lesions. Also, amodiaquine hydrochloride seemed to elaborate the indomethacin induced effects on gastric juice volume, pH and acid output, while artemisinin attenuated these changes. The data indicates that the use of amodiaquine hydrochloride may be dangerous to the integrity of the stomach, especially in existing gastric ulcers, while artemisinin is mild and ameliorating, may result from their lipid peroxidation/apoptosis activity interference.

Key words: Amodiaquine hydrochloride, artemisinin, indomethacin, lipid peroxidation, apoptosis

INTRODUCTION

Malaria has been expressed as the most prevalent, pernicious, parasitic disease of humans which is estimated to kill between one and two million people, mainly children, each year (White, 2004). Other high risk groups include pregnant women, non-immune travellers/HIV patients, refugees, displaced persons, or labour forces entering into endemic areas (Phillips, 2000).

Although it is a global disease, most of the burden is in the sub-Saharan Africa, (Olliaro and Taylor, 2003). The safety and effectiveness of the available antimalarials still remain priority to health givers in Africa.

Amodiaquine hydrochloride belongs to the class of antimalarials that are easily affordable by many malaria-endemic countries (Olliaro and Taylor, 2003).

Its combination with artemisinin in the treatment of malaria is significantly more effective than amodiaquine alone (Adjuik *et al*., 2002).

Artemisinin is currently the most rapidly acting and potent antimalarial (White, 1997; Nosten *et al*., 2000). The pharmacodynamic effects are due to their rapid absorption and activity against many stages of the malaria life cycle (Targett *et al*., 2001). The artemisinin derivatives in combination with other standard antimalarials are now been promoted as the best therapeutic option for treating drug-resistant malaria and retarding the development of resistance (White, 1999; WHO, 2001).

Though artemisinin derivatives , at therapeutic doses, lack adverse effects (White, 1997), amodiaquine is associated with pruritus (Ajayi *et al*., 1998), gastro-intestinal irritation and ocular damage, low white blood cell count, hepatitis, weakness (Westley, 2003, University of Florida, personal communication).

Misuse of antimalarials is widespread especially in the tropics (White, 2004).

Peptic ulcer disease, also, is not uncommon in malaria endemic areas.

Gastric ulcers occurrence is known to be associated with oxidative stress increases by pro-ulcerative factors in the gut like *Helicobacter pylori* (Yamaguchi and Kakizoe, 2001) use of Non steroidal anti-inflammatory drugs, NSAIDs (Rostom *et al*., 2000), smoking (Ma *et al*., 2000) psychological stress (Mawdsley and Rampton 2006), lead exposure (Olaleye *et al*., 2007) and dietary intake of potential ulcerogens (Ibironke *et al*., 1997).

From available literature, there is a dearth of documented information on possible role of antimalarials in the aetiology of inflammatory disorders of the gastrointestinal system. Hence, the effects of Amodiaquine hydrochloride and Artemisinin on experimental gastric ulceration were investigated in rats.

The objective of the study is to investigate the effect of two antimalarials amodiaquine hydrochloride and artemisinin in ulcerated albino rats of Wistar strain.

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MATERIALS AND METHODS

The study was carried out between September and October, 2006 in the Department of Physiology, College of Medicine, University of Ibadan, Nigeria.

Drugs: Amodiaquine hydrochloride and Artemisinin used were obtained from a local pharmacy duly registered by the Pharmacists' Council of Nigeria (PCN). Indomethacin was obtained from Strides, Belgium. All other reagents were of analytical grade and obtained from the British Drug Houses, Poole, UK.

Animals: Thirty two healthy adult albino rats of Wistar strain weighing between 180-220 g each were used in the study. The animals were housed under standard conditions of temperature (23±2°C), humidity (55±15%) and 12 h light (7.00 am-7.00 pm).

They were kept in wire meshed cages and fed with commercial rat pellets (Ladokun Feeds Ltd., Ibadan, Nigeria) and allowed water *ad libitum*.

Treatment: The animals were divided into four groups with eight rats each. Group 1 was treated with normal saline after 24 h fasting. Group 2 was treated with indomethacin (40 mg kg^{-1}) orally after 24 h fasting. This served as the treated control group. Group 3 and 4 received intramuscularly amodiaquine hydrochloride (30 mg kg^{-1}) and artemisinin (2.86 mg kg^{-1}), respectively 4 h after oral indomethacin administration.

Ulcer induction and index determination: Indomethacin (Strides, Belgium) dissolved in sodium bicarbonate was administered orally (40 mg kg^{-1}) to 24 h fasted rats.

Four hours later (for the treated control) and 24 h later (for the experimental group), the animals were killed with ether anaesthesia. The stomachs were opened along the greater curvature, washed in normal saline to remove debris and pinned on a cork mat for ulcer scoring. This was done by locating the wounds in the glandular regions under a simple microscope. The lengths (mm) of all the elongated black-red lines parallel to the long axis of the stomachs in the mucosa was measured. The ulcer index was calculated by adding the lengths of all the lesions in the glandular region of the stomach (Rifat-uz-Zaman *et al*., 2002; Tanaka *et al*., 1993). The wounds were assessed independently by two observers.

Gastric juice volume, pH and acid output: According to the method of Tanaka *et al*. (1993), the volumes and pH of centrifuged gastric secretion were measured by pipette and pH meter respectively, the acid output were calculated using the following equation (Ishizuka *et al*., 1996):

$$EqH^{+}/100\ g/4\ h = 1/\text{antilog pH} \times 1000 \times \text{volume of gastric juice (mL)} \times 100/\text{body weight of animals (g)}$$

Determination of gastric mucous: Adherent gastric glandular mucous was measured by the method of Corne *et al*. (1974). The excised stomachs were soaked for 2 h in 0.1% Alcian blue dissolved in buffer solution containing 0.1 M sucrose and 0.05 M sodium acetate (pH adjusted to 5.8 with hydrochloric acid). After washing the stomach twice in 0.25 M sucrose (15 and 45 min), the dye complexed with mucous was eluted by immersion in 10 mL aliquots of 0.5 M $MgCl_2$ for 2 h. The resulting blue solution was shaken with equal volumes of diethyl ether and the optical density of the aqueous phase measured at 605 nM using a spectrophotometer.

Using a standard curve, the absorbance of each solution was then used to calculate the various concentration of the dye and the weight of dye (expressed in mg). The weight of the dye was then expressed over the weight of the stomach.

Statistical analysis: The data obtained were expressed as Means±SEM. (Standard Error of Means of eight experiments) and analysed statistically by application of the Statistical Package for Social Sciences (SPSS).

The student's t-test was applied and p-values were determined. Differences were considered significant at $p<0.05$ and highly significant at $p<0.001$.

RESULTS AND DISCUSSION

Indomethacin causes increased gastric secretion volume and acid output, with significant decreased gastric pH (Table 2). Similarly, gastric ulcer lesions were formed by indomethacin in the experimental rats (Table 1) ($p<0.001$). This is in agreement with the report of several authors on the role of indomethacin on gastric ulcer and erosion formation (Reeves and Stable 1985; Christopher *et al*., 1998).

Amodiaquine administration on existing ulcer in the rat led to an increase in the ulcer index; 12.92±1.49 mm (when compared with 8.58±1.44 mm index of the indomethacin treated group) as shown in Table 1, ($p<0.001$). However, the result obtained with Artemisinin showed a significant decrease in the existing ulcers, the index being 7.33±0.92 mm when compared with the indomethacin treated group ($p<0.05$).

Table 1: Effect of amodiaquine hydrochloride and artemisinin on ulcer index in rats

Groups	Treatments	Weight (g)	Ulcer index (mm)
1	Normal saline	193.00±4.35	0.00
2	Indomethacin (oral) (40 mg kg^{-1})	201.70±6.54	8.58±1.44**
3	AQ (i.m) (30 mg kg^{-1}) + Indo (oral) (40 mg kg^{-1})	203.30±8.03	12.92±1.49**
4	AS (i.m) (2.86 mg kg^{-1}) + Indo (oral) (40 mg kg^{-1})	205.00±2.93	7.33±0.92*

Indomethacin: Highly significant from normal saline treated, **p<0.001 Test drugs: Highly significant from indomethacin treated, **p<0.001 and significant *p<0.05

Table 2: Gastric juice profile of normal saline, indomethacin, amodiaquine and artemisinin-treated animals

Groups	Treatments	Volume (mL)	pH	Acid output (uEq/100 g/4 h)
1	Normal saline	3.50±1.20	2.51±0.57	2.60±0.10
2	Indo (oral) (40 mg kg^{-1})	6.20±0.59	1.65±0.20*	103.25±12.41**
3	AQ (i.m) (30 mg kg^{-1}) + Indo (oral) (40 mg kg^{-1})	8.20±0.10	1.10±0.22	130.65±8.22**
4	AS (i.m) (2.86 mg kg^{-1}) + Indo (oral) (40 mg kg^{-1})	5.25±0.06	2.61±0.05*	89.53±6.45*

Indomethacin: Highly significant from saline treated **p<0.001, Test drugs: Significant from indomethacin treated *p<0.05 and highly significant **p<0.001

Table 3: Barrier mucous concentration of normal saline, indomethacin, amodiaquine and artemisinin treated rats

Groups	Treatments	Gastric mucous (mg kg^{-1})
1	Normal Saline	7.50± 0.95
2	Indo (oral) (40 mg kg^{-1})	5.25± 0.70*
3	AQ (i.m) (30 mg kg^{-1}) + Indo(oral)(40 mg kg^{-1})	3.45± 0.55*
4	AS(i.m) (2.86 mg kg^{-1}) + Indo (oral) (40 mg kg^{-1})	5.00±0.90#

Indomethacin: Significant from saline treated *p<0.05, Test drugs: Significant from indomethacin treated *p<0.05 and Non Significant #p>0.05

In addition, amodiaquine seemed to elaborate the gastric changes induced by indomethacin while Artemisinin attenuated these changes. This is depicted in Table 2.

Figure 1 shows the status of the existing ulcer in the rats treated with amodiaquine and artemisinin, expressed as percentage change in ulcer index of indomethacin.

Table 3 shows the effect of amodiaquine and artemisinin treatment on gastric barrier mucous. The mean value of gastric barrier mucous in saline treated animals was 7.50±0.85 mg kg^{-1}, while indomethacin treatment led to a significantly decreased gastric barrier

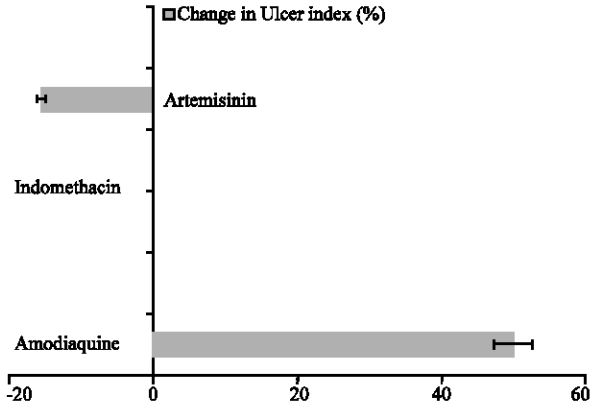

Fig. 1: Percentage change in ulcer index in rats treated with Amodiaquine and Artemisinin

mucous; 5.25±0.90 mg kg^{-1}. Gastric barrier mucous is further decreased significantly with Amodiaquine and no significant change in the animals treated with Artemisinin .

The results of this study show a kind of relationship between the use of antimalarials and gastric ulceration. Amodiaquine hydrochloride, one of the first defense synthetic drugs in Nigeria and many African countries because of its commonality and cheap status is discovered to aggravate the existing ulcer in the stomach of the rat.

Previous studies have shown that chloroquine phosphate, another drug of choice in most part of malaria endemic areas (Gustafsson *et al*., 1983), cause significant increase in gastric acid secretion (Etimita *et al*., 2005), one of the risk factors in the development of ulcer.

This, therefore, suggests that indiscriminate and unguided use of Amodiaquine to ulcer patients, may be dangerous.

Artemisinin ameliorates the existing ulcer in the stomach of the rat. Gastric changes induced by indomethacin were also attenuated. This could interpret to reduction in inflammation and pain associated with it.

Indomethacin-induced ulceration is precipitated by inhibition of prostaglandin synthesis (Desai *et al*., 1997). Progressive decrease in the mucosal contents of prostaglandins e.g., PG_2, PGE_2 and TXA_2 and increase in leukotrienes, (Lts) due to selective inhibition of cyclo-oxygenase enzyme leads to inflammation and pain (Kapui *et al*., 1993; Levine, 2001). This eventually results to lipid peroxidation. Principally, mucosal blood flow, secretion of mucus and bicarbonate ion (HCO_3) and maintenance of a hydrophobic surface are compromised (Hawkey, 1996).

The findings of the present study suggest that there is an interference in the lipid peroxidation/apoptosis activity of indomethacin by the antimalarials, as observed in the significant reduction of mucous barrier by Amodiaquine. Further studies to investigate the role of anti-oxidant enzymes in the aggravation and attenuation of gastric lesions by amodiaquine hydrochloride and artemisinin are on-going in our laboratory, the result of the present study show that there is a potentiation of ulceration in the stomach of rats treated with amodiaquine hydrochloride and attenuation in ones treated with artemisinin.

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