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Research Article

Effect of Sulfadoxine-Pyrimethamine and Artesunate on Gastric Acid Secretion and Parietal Cell Mass in Rats

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ABSTRACT: In this study, the effects of two antimalarial drugs, sulfadoxine-pyrimethamine and artesunate, on Gastric Acid secretion (GAS), Parietal Cell Mass (PCM) and Gastric Mucous Cell Population (GMP) were investigated in rats randomly assigned into three groups viz: control, Sulfadoxine-pyrimethamine (SP, 1.25/25 mg/kg), artesunate (AS, 2 mg/kg). Basal GAS as well as secretion in response to histamine and carbachol was measured by continuous perfusion of the stomach with normal saline (1ml/minute) under urethane anaesthesia (0.6 mg/100 g). After obtaining a steady basal output response to normal saline in all animals, the antimalaria drugs were administered intramuscularly and the peak responses to each drug obtained. Further assessment of the roles of histaminergic and muscarinic receptors were done using ranitidine (H_2 antagonist) and atropine (M_3 antagonist) in the treated animals. PCM and GMP were determined in the stomach samples by histometry. The basal acid output was 0.70 $\pm$ 0.01 mmol/10 mins. Normal saline and SP produced no statistically different peak output compared with the basal ($p>0.05$). AS produced a significant reduction with a value of 0.45 $\pm$ 0.03 mmol/10 mins ($p<0.05$). Histamine and carbachol elicited 107% and 100% change of acid secretion when compared with the basal output with the value of 1.45 $\pm$ 0.04 mmol/10 mins and 1.40 $\pm$ 0.03 mmol/10 mins respectively ($p<0.001$). SP and AS attenuated histamine-induced acid secretory rate with the peak value of 0.95 $\pm$ 0.01 mmol/10 mins and 0.80 $\pm$ 0.02 mmol/10 mins respectively. Similarly, the carbachol-induced acid secretory response was attenuated by SP and AS to a peak of 0.90 $\pm$ 0.02 mmol/10 mins and 0.90 $\pm$ 0.03 mmol/10 mins ($p<0.05$). SP and AS decreased significantly the parietal cell numbers in the gastric mucosa (13.8$\pm$ 0.3 cells/µm versus 15.2$\pm$0.8 cells/µm control; $p<0.05$) and (13.4$\pm$0.5 cells/µm versus 15.2$\pm$0.8 cells/µm control; $p<0.05$). On the other hand, mucus cell population was significant increased by SP and AS (19$\pm$0.7 cells/µm versus 17.4$\pm$0.5 cells/µm control; $p<0.05$) and (22.2$\pm$0.8 cells/µm versus 17.4$\pm$0.5 cells/µm control; $p<0.05$) respectively. Sulfadoxine-pyrimethamine and artesunate inhibit gastric acid secretion. They inhibited histamine (H_2) and muscarinic (M_3) receptors, and reduced parietal cell mass.

Keywords: Sulfadoxine-pyrimethamine, Artesunate, Gastric acid secretion, Histamine, Parietal cell mass.

INTRODUCTION

Sulfadoxine-pyrimethamine and artesunate are efficacious drugs of choice in the treatment of Chloroquine resistant *Plasmodium falciparum* malaria.

Sulfadoxine-pyrimethamine is a synergistic combination of antifolate drugs that act against parasitic-specific enzymes, dihydropteroate synthase and dihydrofolate reductase (Plowe *et al*, 1997). It has long been proved to be more effective than chloroquine (Randrianasolo *et al*, 2004), and the efficacy is established even as a constituent of either Artemisinin Combination Therapy (ACT) or Non Artemisinin Combination Therapy (NACT) (Menard *et al*, 2007). Artesunate is part of the artemisinin group of drugs that treat malaria. These plant-derived peroxides are unique among anti-malaria drugs in killing the young intra-erythrocytic malaria parasites, thereby preventing their development to more pathological mature stages. This results in rapid clinical and parasitological responses to

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treatment and life-saving benefit in severe malaria (Targett *et al*, 2001; White, 2008).

Sulfadoxine-pyrimethamine and Artesunate are safe with little or no adverse effects when used at the therapeutic dose (Nguoesse *et al*, 2001; White, 2008). These anti-malaria drugs ameliorate the development of gastric ulceration in rats. (Foglio *et al*, 2002; Olaleye and Ajeigbe, 2009).

Hydrochloric acid, produced by the parietal cells in the stomach, is facilitated by the activity of H^+ K^+-ATPase in the apical membrane (Davies, 1951). It is finely regulated by overlapping neural, hormonal, paracrine pathways (Yao and Forte, 2003). When levels of the acid and proteolytic enzymes overwhelm the mucosal defense mechanisms, ulcers occur; and conversely reducing the rate of acid secretion is an important factor in anti-ulcerogenesis (Schmassmann, 1998).

Previous results from our laboratory showed modulation of experimental gastric ulceration by sulfadoxine-pyrimethamine and artesunate (Olaleye *et al*, 2008; Olaleye and Ajeigbe, 2009) probably by reducing oxidative stress induced by ulcerogens.

In view of the fact that gastric acid secretion still plays an important role in the etiology of gastrointestinal ulceration, this study aimed at investigating the effect of sulfadoxine-pyrimethamine and artesunate on acid output, parietal cell mass and mucus cell population.

MATERIALS AND METHODS

Animals.

Healthy male albino rats of Wistar strain weighing between 180-200 g were used in the study. They were housed under standard conditions of temperature (23 $\pm$ 2^{o}C), humidity (55 $\pm$ 15%) and 12 h light (7.00 am-7.00 pm).

They were kept in wire meshed cages and fed with standard commercial rat pellets (Ladokun Feeds Limited, Nigeria) and allowed water *ad libitum* prior to the start of the experiment.

Drugs.

Sulfadoxine-pyrimethamine and Artesunate tablets and atropine injections were purchased from Danax Pharmaceutical Store, Adamasingba, Ibadan. Histamine and carbachol and other analytical grade chemicals used were obtained from Physiology and Pharmacology Departments of the University of Ibadan.

Experimental Design.

*Animal grouping***:** The Eighty (80) rats used for the study were randomly divided between the groups of Sulfadoxine-pyrimethamine (SP) and Artesunate (AS). Each group has forty rats distributed evenly among eight subgroups.

Treatments and drug dosage:

Normal saline (1 ml/kg, control), Sulfadoxine-pyrimethamine (1.25/25 mg/kg), Histamine (1 mg/kg) + Sulfadoxine-pyrimethamine (1.25/25 mg/kg), Ranitidine (4 mg/kg) + Histamine (1 mg/kg) + Sulfadoxine-pyrimethamine (1.25/25 mg/kg), Histamine (1 mg/kg); Carbachol (50 µg/kg) + Sulfadoxine-pyrimethamine (1.25/25 mg/kg), Atropine (1 mg/kg) + Carbachol (50 µg/kg) + Sulfadoxine-pyrimethamine (1.25/25 mg/kg), Carbachol (50 µg/kg).

Artesunate: Normal saline (1 ml/kg, control), Artesunate (2 mg/kg), Histamine (1 mg/kg) + Artesunate (2 mg/kg), Ranitidine (4 mg/kg) + Histamine (1 mg/kg) + Artesunate (2 mg/kg), Histamine (1 mg/kg); Carbachol (50 µg/kg) + Artesunate (2 mg/kg), Atropine (1 mg/kg) + Carbachol (50 µg/kg) + Artesunate (2 mg/kg), Carbachol (50 µg/kg).

Acid secretion studies.

The gastric acid secretion was measured using the continuous perfusion method of Ghosh and Schild (1958), modified by Amure and Ginsburg (1964). The animals were anaesthetized with 25% urethane (ethyl carbamate) at a dose 6 ml/kg body weight. A tracheal cannula was inserted via an incision on the neck to ensure normal breathing throughout the course of the experiment. An abdominal incision through the *linea alba* was made to expose the stomach and a semi-transection made at the junction of the pylorus with the duodenum. A pyloric cannula was inserted and tied to collect gastric contents. An orogastric cannula was inserted for perfusion of pre-warmed (at temperature 37^{0}C) 0.9% normal saline (pH 7.00) at a rate of 1ml/minute using a perfusion pump.

The animals were kept warm by a 100 watts electric lamp to prevent hypothermia. Gastric acid was collected via the pyloric cannula at 10 minutes intervals. In order to determine acidity, 10ml of the stomach perfusate was titrated against 0.01M sodium hydroxide (NaOH) solution with phenolphthalein as indicator. Titrable acidity was expressed in mmol/10mins after calculation in each sample. At the 50th minutes of effluent collection, histamine and carbachol were administered via a femoral cannula vein for the stimulated acid secretory response, and ranitidine and atropine also introduced correspondingly to block the H_2 and muscarinic receptors.

Determination of gastric parietal and mucous cell populations

The animals were sacrificed and the stomach removed as quickly as possible into normal saline. The stomach was opened along the greater curvature, washed and transferred into a beaker containing 10% formalin. Sections were prepared from strips removed from the fundic area of the stomach and stained using the method of Marks and Drysdale (1957) as modified by Oluwole et al (2007), using the Hematoxylin and Eosin stain. The various gastric mucosal secretory cells were clearly differentiated, taking up different colours. The nuclei of the parietal cells were stained deep blue while the mucous cells were clearly vacuolated.

Parietal cell mass index was calculated as described by Perraso *et al* (1991) as the number of cells per mm2 multiplied by the thickness of the glandular layer. Five counts from randomly selected fields were made on each section and the average count per unit area was calculated for each stomach by dividing the number of cells seen by the number of counts made.

Statistical Analysis

Data were expressed as Mean ± SEM (Standard Error of Means of five observations) and analyzed ANOVA and Student's t test at p=0.05.

RESULTS

Effects of SP and AS on basal acid secretion.

The basal gastric acid secretion in the control animals was 0.70 $\pm$ 0.01 mmol/10 mins. In the animals adminstered artesunate, the basal secretion was 0.40 ± 0.02 mmol/10 mins (42% decrease; $p<0.05$). SP administration produced no significant change in the acid output. The time-response relationship is shown in Fig. 1.

Table 1 shows the gastric acid secretory responses of rats to Sulfadoxine-pyrimethamine and artesunate after histamine and carbachol stimulation. Histamine significantly increased the gastric acid secretion from the basal value of 0.70 $\pm$ 0.01 mmol/10 mins to 1.45 $\pm$ 0.04 mmol/10 mins. SP and AS thwarted the histamine-induced secretion to 0.95 $\pm$ 0.05 mmol/10 mins and 0.80 $\pm$ 0.03 mmol/10 mins.

Carbachol significantly increased the gastric acid secretion from the basal value of 0.70 $\pm$ 0.01 mmol/10 mins to 1.40 $\pm$ 0.02 mmol/10 mins. SP and AS attenuated the carbachol-induced secretion to 0.90 $\pm$ 0.03 mmol/10 mins and 0.90 $\pm$ 0.05 mmol/10 mins.

Effect of Ranitidine on peak gastric secretory response to SP and AS

The effect of ranitidine, an H_2 antagonist, on peak gastric secretory response to sulfadoxine-pyrimethamine and artesunate is shown in Fig. 2. Ranitidine further decreased peak acid output in the sulfadoxine-pyrimethamine and artesunate treated.

Effect of Atropine on peak gastric secretory response to SP and AS

Fig. 3 shows the effect of atropine, a muscarinic antagonist, on peak gastric secretory response to sulfadoxine-pyrimethamine and artesunate. Atropine further decreased peak acid output in the sulfadoxine-pyrimethamine and artesunate treated.

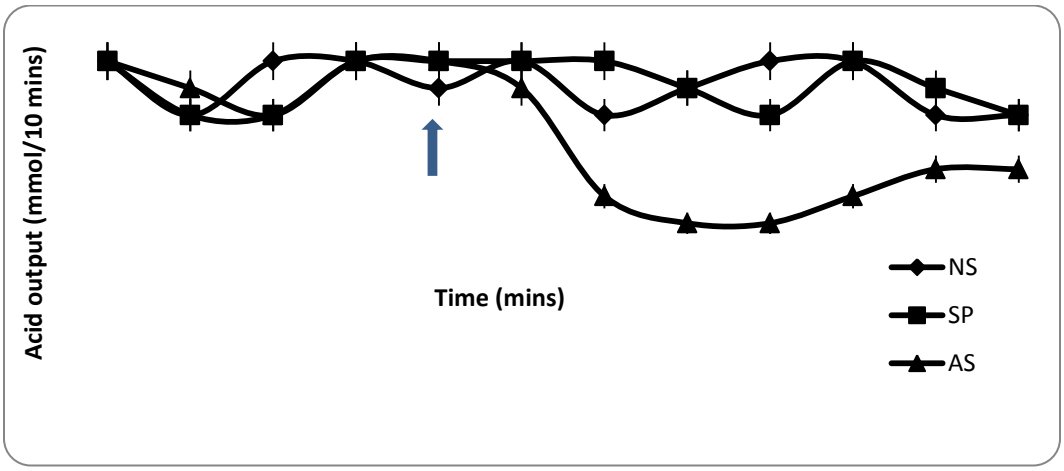

Fig. 1.
Effect of normal saline, SP and AS on acid secretion. Each point value represents Mean$\pm$SEM of five rats ($p<0.05$).
Heavy upward arrow indicates the point of administration

Table 1:
Effect of Sulfadoxine-pyrimethamine and artesunate on gastric acid secretion after histamine and Carbachol stimulation in rats

Drugs	Basal Acid Output	Peak Responses to: Histamine	Carbachol
Normal Saline	0.70 ± 0.01	1.45 ± 0.04 (107%)	1.40 ± 0.02 (100%)
Sulfadoxine-pyrimethamine	0.70 ± 0.03	0.95 ± 0.05 (35%)	0.90±0.03 (28%)
Artesunate	0.40± 0.03	0.80 ± 0.03 (14%)	0.90 ± 0.05 (28%)

Effect of SP and AS on parietal and mucus cell count

SP and AS decreased the parietal cell numbers significantly when compared with the normal gastric mucosa. SP and AS decreased significantly the parietal cell numbers in the gastric mucosa (13.8± 0.3 versus 15.2±0.8 control; $p<0.05$) and (13.4±0.5 versus 15.2±0.8 control; $p<0.05$). On the other hand mucus cell population was significant increased by SP and AS (19±0.7 versus 17.4±0.5 control; $p<0.05$) and (22.2±0.8 versus 17.4±0.5 control; $p<0.05$) respectively. This is shown in Fig. 4.

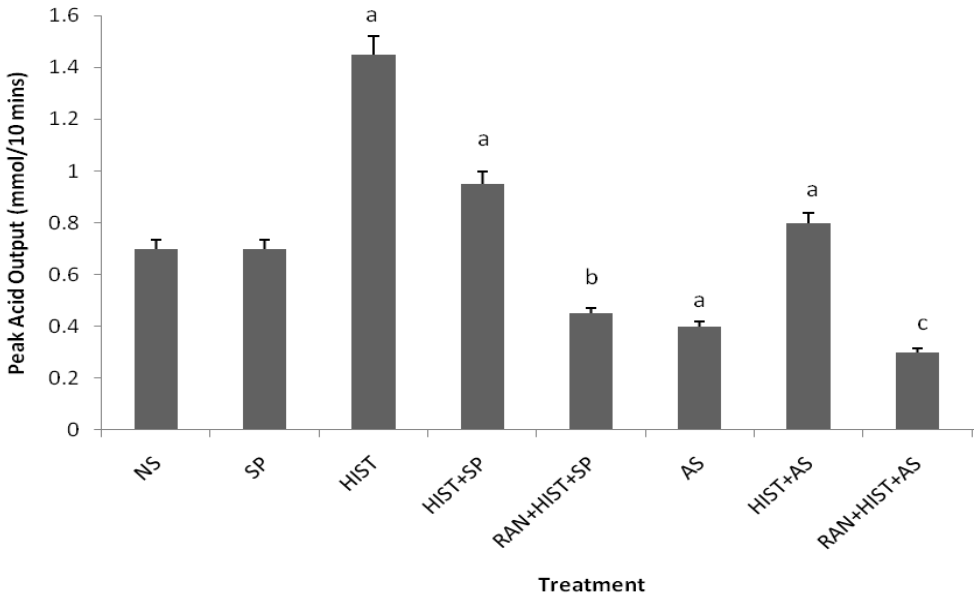

Fig. 2:
Effect of histamine blockade on gastric acid secretory response to sulfadoxine-pyrimethamine. Each vertical bar represents mean ± SEM of five rats per group. [a]P < 0.05 (c.f. NS), [b]P<0.05 (c.f. HIST+SP), [c]P<0.05 (c.f. HIST+AS).

DISCUSSION

In the present study, we investigated the mechanistic events involved in the modulation of gastric acid secretion by Sulfadoxine-pyrimethamine and artesunate.

It was observed that sulfadoxine-pyrimethamine and artesunate inhibit acid output, and the data also suggest that both histamine (H_2) and muscarinic (M_3) receptors play vital roles in this activity.

It is well known that gastric acid secretion is mediated through interplay of neural, hormonal and paracrine pathways (Schubert, 2005). Histamine has been reported to stimulate acid secretion through H_2 receptors (Garrison, 1992) and carbachol M_3 receptors. Though gastrin stimulates acid secretion (via CCK_2 receptors); but indirectly by inducing the release of histamine by ECL cells; its direct effect on parietal cells plays a lesser role.

Sulfadoxine-pyrimethamine seemed not to affect the rate of gastric acid secretion at the basal level while artesunate significantly reduced it. Also, the stimulatory characteristics of both histamine and carbachol were arrested following administration of sulfadoxine-pyrimethamine, and artesunate which clearly suggests the antisecretory potential of the two antimalarials.

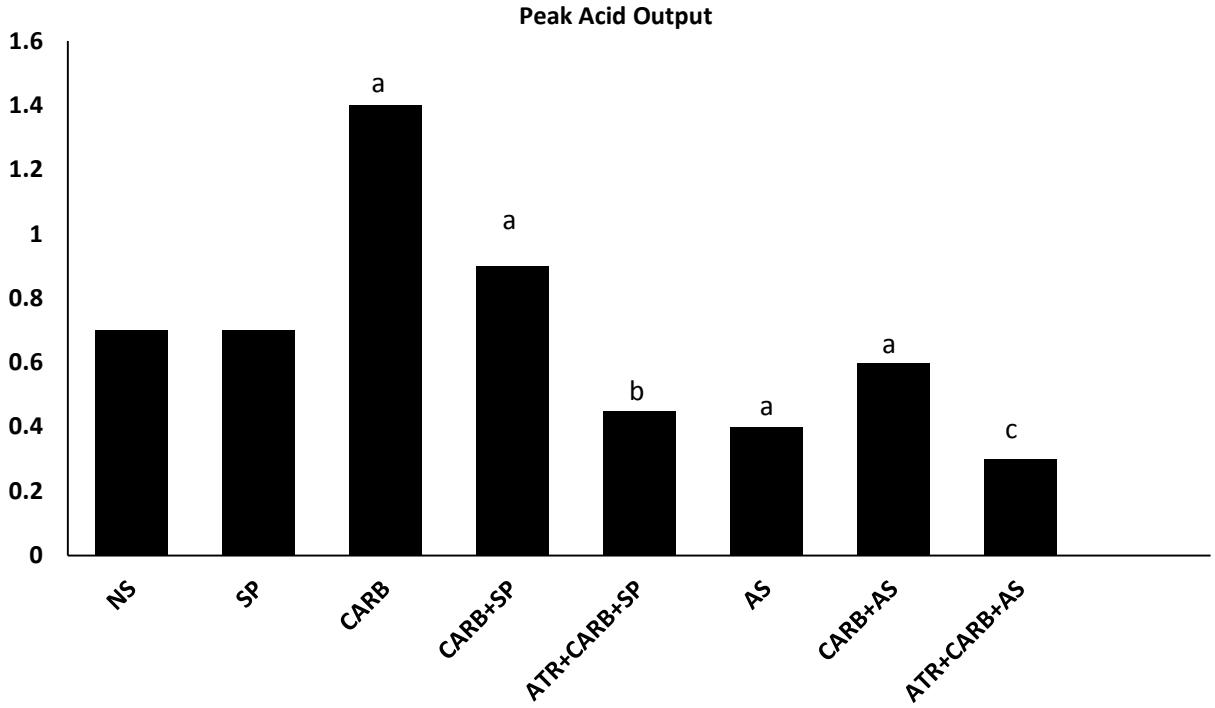

Fig. 3
Effect of muscarinic blockade on gastric acid secretory response to sulfadoxine-pyrimethamine. Each vertical bar represents mean ± SEM of five rats per group. [a]P < 0.05 (c.f. NS), [b]P<0.05 (c.f. CARB+SP), [c]P<0.05 (c.f. CARB+AS).

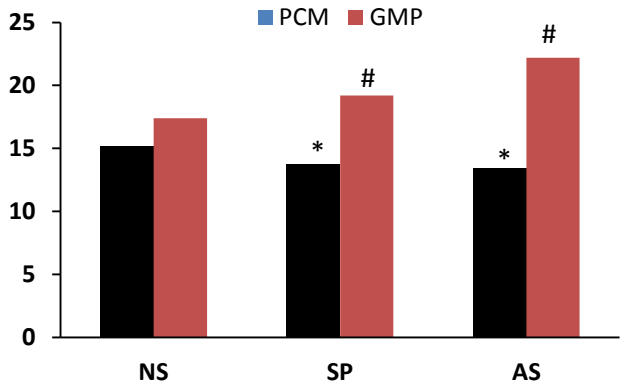

Fig.4:
Effect of Sulfadoxine-pyrimethamine and artesunate on parietal cell and mucus cell population in the rats' gastric mucosa. Each bar represents Mean ± SEM value of five samples. (*,#p<0.05 cf NS). PCM=Parietal Cell Mass, GMP= Gastric Mucous Cell Population.

Another set of experiments performed with ranitidine and atropine prior to administration of sulfadoxine-pyrimethamine, and artesunate even produced lower acid output after a prolonged period of effluent collections. This may indicate a synergistic relationship between the antimalarials and the histaminergic and cholinergic inhibition of the oxynthic cells. Previous studies have also revealed harmoniously that both sulfadoxine-pyrimethamine and artesunate attenuate indomethacin and acidified ethanol effects on gastric juice volume, pH and acid output (Ajeigbe *et al*, 2008a; Olaleye *et al*, 2008; Olaleye and Ajeigbe, 2009). Prior to this, Foglio *et al*, 2002 had demonstrated that the artemisinin extraction by-product exhibited intense anti-ulcerogenic activity in ulcer models induced by indomethacin and ethanol comparable to the standard drug carbenoxolone.

Gastric acid secretion is known to be linearly related with the parietal cell mass, and its attacking effect on the mucosa to be inversely related with the mucus cell population (Goodman and Gilman, 2005). The result of the study revealed that both sulfadoxine-pyrimethamine and artesunate reduce parietal cell number thereby attenuating acid secretion; and increase mucus cell population which depicts the gastroprotective tendencies of the drugs.

Besides oxidative stress (Perry *et al*, 1986) and a number of other factors, gastric acid secretion still remains an important force to reckon with in the pathogenesis of inflammatory disorders of the GIT

especially peptic ulceration. This is evidenced by the action of many anti-ulcerogenic agents which reduce the acid secretion (Schmassmann, 1998; Al Mofleh, 2010). Considering the crucial role gastric acid plays in the development and healing of ulcer, the present study investigated the effect of the antimalarials on basal and secretagogue-induced secretion.

In conclusion, Sulfadoxine-pyrimethamine and Artesunate inhibit gastric acid secretion, both histamine (H_2) and muscarinic (M_3) receptors play vital roles in this activity. If these findings are extrapolated to man, the use of sulfadoxine-pyrimethamine and artesunate may be safe on the integrity of stomach and further underscore mildness and gastroprotective nature of sulfadoxine-pyrimehamine and artesunate (Foglio *et al*, 2002; Marques *et al*, 2006; Olaleye *et al*, 2008). They may even be suggested as part of treatment regimen for gastrointestinal ulceration.

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