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Full Length Research Paper

Effect of administration of acetylsalicylic acid on phosphatase enzymes in liver of metabisulphite treated rats

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Membrane stabilization is an established attribute of most anti-inflammatory drugs. In this work the membrane stabilization capability of acetylsalicylic acid (ASA) was investigated in the liver of metabisulphite insulted experimental rats. Previous reports have shown that sodium metabisulphite administered to rats resulted in labilization of plasma membrane of some rat tissues while acetylsalicylic acid, an anti-inflammatory drug, like certain others, has been reported as membrane stabilizer. The organ of interest (liver) was collected into a 0.25 M sucrose and the homogenate was prepared for enzyme analyses. The activities of the phosphatases were measured using standard methods. Initial administration of metabisulphite alone showed an immediate significant decrease with (P<0.05) in alkaline phosphatase activities. Loss in activities was recorded throughout the experimental period but became insignificant after day 3. The activities of the phosphatases in acetylsalicylic acid treated rats were lower when compared with the control group but not as obvious as with the metabisulphite treated rats. The combination treatment (metabisulphite and acetyl salicyclic acid) gave a trend in activities that was in between the administration of individual compounds. This study therefore has shown that acetylsalicyclic acid prevented disruption of liver cellular membrane caused by the administration of sodium metabisulphite.

Key words: Acetylsalicylic acid, metabisulphite, liver, phosphatases, administration.

INTRODUCTION

Acetylsalicylic acid (ASA) is the chemical name for a member of the salicylate drugs commonly known as Aspirin. It is produced by the substitution of the phenolic group of salicylic acid with acetic anhydride (Roger et al., 1981). Acetylsalicylic acid is the prototype for the non-steroidal anti-inflammatory drugs (NSAIDS) which inhibits the production of thromboxane (Wu, 2000). Ibuprofen and related drugs are other members of NSAIDS. Most NSAIDS have been described as potent membrane stabilizers with aspirin being shown (Ngaha and Akanji, 1982) to stabilize kidney lysosomal membrane after its labilization by chloroquine. Though discovered more than 100 years ago, little is known about how aspirin actually works to control inflammation. What is known is that it acts in two pathways, one involving prostaglandins (Abramson and Weissmann, 1989) and the other a more recent discovery, involving nuclear factor-K (Kappa) B (NF-KB) (Holger and Angus, 2001). Acetylsalicylic acid inhibits the activation of NF-KB molecules that activate chemicals to trigger inflammation (Holger and Angus, 2001). However, it was shown in recent works that these anti-inflammatory drugs act by inhibiting prostanoid synthesis through acetylation of fatty acid cyclooxygenase thus irreversibly blocking cyclooxygenase system (Friedrich et al., 2001; Durand et al., 2002a). This effect depends on the type of cells involved due to resynthesis (Dutrand et al., 2002b). The dependant of blockade by acetylsalicylic acid on cell type is the rationale for the recommended dose of (50 - 1500) mg/day with long inter dose intervals (Hla and Bailey, 1989; Patrono et al., 1998; Abouelenin et al., 2002).

Membrane of cells is vulnerable to be attacked by both endogenously produced toxic substances and those con-

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sumed exogenously. Sodium metabisulphite is a food preservative that produces sulphur dioxide (Wedzicha, 1984). Its interaction with dietary components (Bhagat and Lockett, 1964) resulting in toxic products have rescinded its generally regarded as safe (GRAS) status through some toxicological findings (Taylor et al., 1986).

Akanji et al. (1993; 1996) and Akanji and Yakubu (2000) have shown the potentiality of metabisulphite in tissue damage in rats. Metabisulphites generate sulphite and sulphites are known to have inhibitory action on some enzymes such as the lactate dehydrogenases of the heart and malate dehydrogenases (Akanji and Yakubu, 2000). Bisulphites (0.5 mM) have earlier been shown to cause induced oxidation in corn-oil emulsified in 1.5% polysorbate solution (Kaplan et al., 1975) just as unsaturated membrane lipids incubated with a large excess of bisulphite was reported to have different chromatographic pattern indicative of addition of bisulphite across double bonds and hence alteration of membrane structure (Akogyeram and Southerland, 1980). With its high oxygen content metabisulphite generates oxygen radical $\dot{O}_2$; a good nucleophile that could react readily with electrophilic sites on biological molecules such as in the membrane of cells (Halliwell, 1974; Akanji et al., 1993; 1996). The potentiality of acetylsalicylic acid in preventing the cellular damage caused by metabisulphite is investigated in this study using changes in pattern of activity of alkaline and acid phosphatases both marker enzymes for specific regions of the cell as index of its effect.

MATERIALS AND METHODS

Experimental animals

Male albino rats (Wister Strain) weighing between 150 – 200 g were acclimatized for four weeks and fed with rat cubes made from animal feed obtained from ECWA feed mills Ltd, Jos, Plateau State, Nigeria, and water ad labium. The experiment was performed according to current guidelines for the care of laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals (Zimmerman, 1983). The design/protocol for the study is as approved by the Animal Experiments Ethics Review Board of the Department of Biochemistry, Kogi State University, Anyigba, Nigeria

Acetylsalicylic acid used for the work is a product of Tega Laboratories Chelsea London, while sodium metabisulphite used is a product of May and Baker Ltd, Dagenham England.

Design

The experiment consisted of thirty three rats divided randomly into three groups of 10 rats each, numbered group 1, 2, and 3. The fourth group consisted of 3 rats as the control group. Each group of animals was kept in separate metabolic cages and fed with rat cubes and water *ad libitum*. Each set was replicated trice. The rats in group 1 were administered daily with solution of sodium metabisulphite (10 mg/kg body weight) while rats in group 2 were administered daily with solution of acetylsalicylic acid (10 mg/kg body weight). Group 3 rats were administered daily with solution of the two chemical compounds concurrently while rats in the control Groups were administered with distilled water alone. The route of administration was intraperitoneal.

Statistical analysis

Results were analyzed using Analysis of Variance (ANOVA). A value of $P < 0.05$ was considered as statistically significant.

Drug and metabisulphite administration

Solution of 2 mg/ml Sodium metabisulphite and 2 mg/ml acetylsalicylic acid were prepared in distilled water. The solution of sodium metabisulphite was administered daily to rats in group 1 while the solution acetylsalicylic acid was administered daily to rats in group 2. The rats in group 3 were administered solution of sodium metabisulphite and acetylsalicylic acid concurrently. All administeration lasted for fifteen days. Day 1 represents rats that were given one daily dose of appropriate chemical compound or the combination of the compound and were sacrifice 24 h after the administration while other days (3, 5, 10 and 15) represent rats that were given those numbers of daily doses and sacrifice thereafter (Akanji and Nlumanze, 1987). The rats in the control group were administered with only distilled water daily for 15 days and sacrifice 24 h after the 15^{th} dose.

Preparation of homogenate

The rats were anaesthetized in a glass jar containing cotton wool soaked in chloroform until they go unconscious. They were immediately removed for dissection to avoid damage to the organ of interest. The liver was removed immediately. One gram of the liver was weighed, cut into pieces and homogenized in an ice- cooled 0.25 M sucrose solution (1:5 w/v) using a pre-cooled enamel mortar and pestle. Triton X-100 was added to the homogenate to a final concentration of 1% (Ngaha et al., 1979; Yakubu et al., 2001). The homogenate was frozen over night to allow unbroken cells to lyse before being used for enzyme assay (Akanji and Ngaha, 1989; Adesokan and Akanji, 2003).

Tissue dilution and enzyme measurement

The stock homogenate of the tissue (1:5 w/v; tissue: 0.25 M sucrose) was diluted using 0.25 M as diluents. The dilution factors of the homogenate were 30 and 600 for the assays for alkaline phosphatase and acid phosphotase. Protein determination was carried out with the method of Gromal et al. (1949) and the phosphatase activities were measured as described by Wright et al. (1972). The methods depends on the determination of the amount of phosphate ester that is being hydrolyse within a given period of time in an alkaline pH and acidic pH for alkaline phosphatase and acid phosphatase respectively. In this work, the hydrolysis of para-nitrophenylphosphate (colourless) into para-nitrophenol was followed spectrophotometrically at 400 nm. Para-nitrophenol gives yellow colour in alkaline medium. The intensity of which can be measured maximally at 400 nm. All measurements were carried out using Agilent 8453 UV- visible spectrophotometer. The experiment was perform in triplicate.

RESULTS

The results of administration of sodium metabisulphite (10 mg/kg body wt), acetylsalicylic acid (10 mg/kg) and combination treatment on the activity of alkaline phospha-

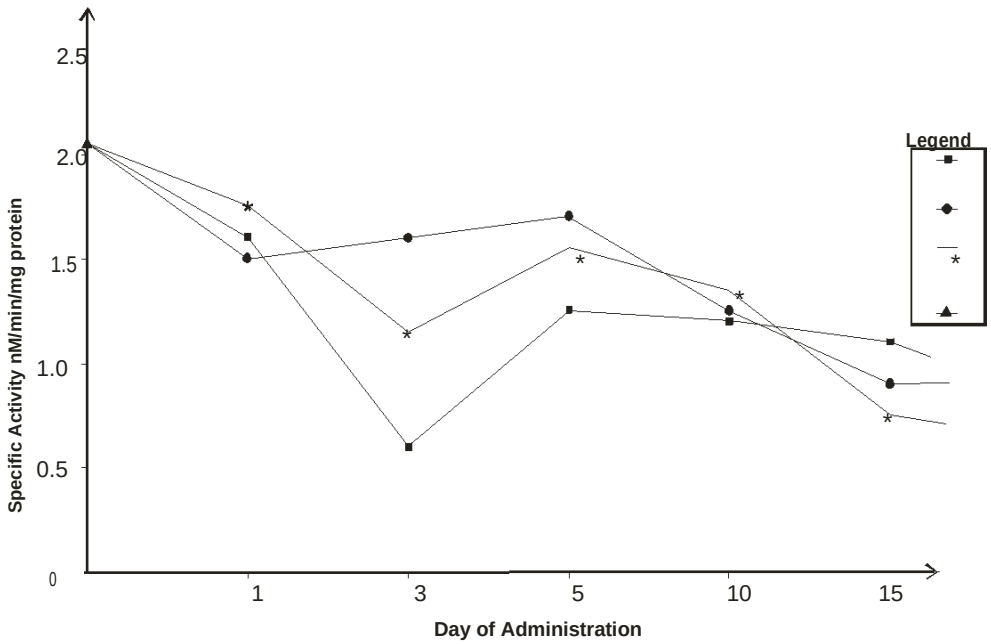

Figure 1. Effect of daily administration of sodium metabisulphate (10 mg/kg b.wt), acetyl salicyclic acid (10 mg/kg b.wt), and combination of the two compounds on the activities of acid phosphatase of rat liver.

tase in the liver of rats are shown in Figure 1. Upon initial administration of sodium metabisulphite alone, there was an immediate decrease ($P<0.05$) in the activity of alkaline phosphatase. The decrease in values of enzyme activity was observed throughout the experimental period when compared with the control value. After the 3rd day, a 70.70% decrease in alkaline phosphatase activity was obtained when compared with the control value. The activities of the enzyme under the influence of administration of acetylsalicylic acid was reduced but was insignificant ($P> 0.05$) as was observed with the administration of metabisulphite.

Administration of both sodium metabisulphite and acetylsalicylic acid gave a trend of activity that was in between the administration of the individual chemical compound. There was a significant: ($P< 0.05$) recovery from loss of enzyme activity under the combination treatment when the results were compared with when metabisulphite alone was administered.

The effect of the individual chemical compound and the combination treatments on the activity of acid phosphatase in the liver of the rats is shown in Figure 2. The three treatments produced same pattern of enzyme activity but with the combination treatment being between the two single treatments. The initial loss of enzyme acti-vity following the first dose of metabisulphite was insignificant ($P>0.05$) with the activity stabilized by the fifth day. On the whole, all experimental values of acid phosphatase activity obtained from the various test administrations showed insignificant ($P>0.05$) decreases when compared with the control value. Summarily, the activities of acid phosphatase in the liver of rats under treatments were not significantly different ($P>0.05$). Results were analyzed using Analysis of Variance (ANOVA). A value of $P < 0.05$ was considered as statistically significant.

DISCUSSION

This work has revealed the membrane stabilization ability of acetylsalicylic acid. This finding confirms further the earlier works of Ignarro (1971), Ngaha and Akanji (1982); Olajide et al., (2005) on the membrane stabilizing role of most non-steroidal anti-inflammatory agents, the class of drugs to which acetylsalicylic acid belongs. The metabolism of xenobiotics is achieved largely in the liver because the drug metabolizing enzyme system is located therein. In this work the potentiality of sodium metabisulphite to disrupt cellular membrane as reported in some earlier works (Akanji et al., 1993; Akanji et al., 1996; Akanji and Yakubu, 2000) was affirmed. In this study, administration of sodium metabisulphite resulted in a significant decrease ($P< 0.05$) in alkaline phosphatase activities in the liver (Figure 1). But the activity of acid phosphatase was not affected to any appreciable extent in the tissue (Figure 2).

Adesokan et al. (2006) showed that the activity pattern of rat liver and kidney enzymes change following administration of xenobiotics. The values of activity of acid phosphatase in the tissue when metabisulphite was administered showed no significant difference from the control value ($P>0.05$). This may imply that the integrity of the lysosomes where acid phosphatase is located was maintained despite the chemical insult.

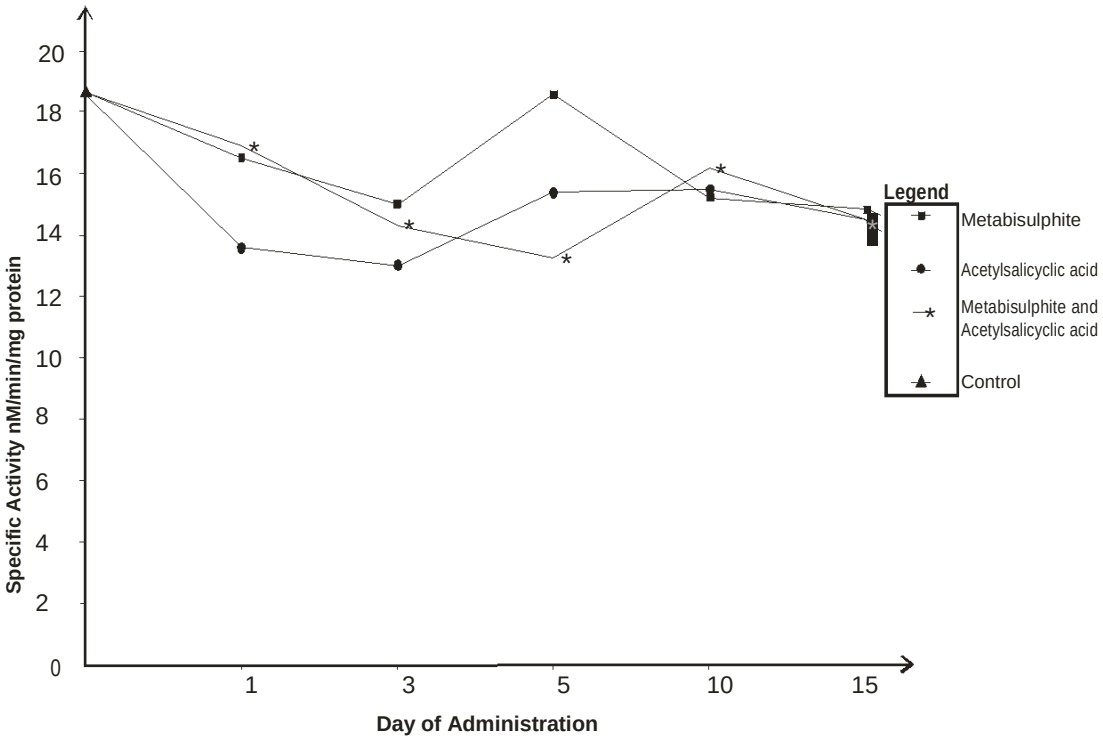

Figure 2. Effect of daily administration of Sodium metabisulphate (10 mg/kg b. wt), acetylsalicyclic acid (10 mg/kg b. wt), and combination of the two compounds on the activities of acid phosphatase of rat liver.

When acetylsalicylic acid alone was administered to the rats, it was observed that the activities of the phosphatases in the liver tissue were not appreciably affected when compared with the control (Figures 1 and 2). This finding supports the work of Ngaha and Akanji (1982) on the stabilizing role of acetylsalicylic acid on rat kidney lysosomal membrane after its labilization by chloroquine.

Despite that the liver tissues contain the enzymes involved in drug metabolism, its cellular membrane damaged by the administration of sodium metabisulphite was repaired by acetylsalicylic acid like drugs by preventing the disruption of kidney cellular membrane (Olajide et al., 2005). From the result, the loss of alkaline phosphatase activities in the liver of rats was prevented following the concurrent administration of sodium metabisulphite and acetylsalicylic acid to the experimental animals (Figure 1). Therefore in this study, acetylsalicylic acid further demonstrated its stabilizing role by preventing the disruption of liver cellular membrane caused by the administration of sodium metabisulphite. This role could be due to the ability of molecules of acetylsalicylic acid to lodge itself in gaps created by sodium metabisulphite between the ordered membrane molecules on the membrane wall

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REFERENCES

Abou-elenin K, Xydakis A, Hamndy O, Horton S (2002). The effect of aspirin and various iontophoresis solution vehicles on skin microvascular reactivity. Microvasc. Res. 63: 91-95.

Abraham SB, Weissmann G (1989). The mechanism of action of nonsteroidal anti inflammatory drugs. Arthritis Rheum. 32: 1-9.

Adesokan AA, Akanji M.A (2003). Effect of administration of aqueous extract of Enantia chloronta on the activities of some enzymes in the small intestine of rats. Nigerian. J. Biochem. Mol. Bio. 18:103-105.

Adesokan AA, Akanji MA, Balogun EA, Aderibigbe A (2006). Effect of oral administration of Aloe Barbadensis-Miller juice on selected biochemical parameters of rat liver and kidney. In : Recent Progress in Medicinal Plants, Volume 13: In Govil et al. (eds) Search for Natural Drugs, Studium Press LLC, Houston, Texas USA. Chapter 3: pp 73-80.

Akanji MA, Nlumanze SE (1987). "Alkaline phosphatase activities following repeated suramin administration in some rats tissues in relation to their functions". Pharmacol. Toxicol. 61: 182-183.

Akanji MA, Ngaha EO (1989). "Effect of repeated administration of berenil on urinary excretion with corresponding tissue pattern in rats". Pharmacol. Toxicol. 64: 272-275.

Akanji MA, Olagoke OA, Oloyede OB (1993). Effect of chronic consumption of metabisulphite on the integrity of the rat kidney cellular

system. Toxicology 81: 173-179.
Akanji MA, Olagoke OA, Ipinmoye IT, Oloyede OB (1996). Effect of chronic consumption of metabisulphite on the activities of some enzymes in selected parts of gastro-intestinal tract of rats. Nig .J. Biochem. Mol. Bio. 11: 11-14.
Akanji MA, Yakubu MT (2000). - Tocopherol protects against metabisulphite induced tissue damage in rats. Nig.J. Biochem. Mol. Bio. 15: 179-183.

Akogyeram C, Southerland WM (1980). "The interaction of bisulphite with membrane lipids". Fed. Proc. Am. Soc. Exp. Biol. Abstract 39: 1836.
Bhaggat B, Lockett M.F (1964). "The effects of sulphites in solid diets on the growth of rats ". Fed. Cosmets Toxicol. 2: 1-13.
Durand S, Fromy B, Koital A, Abraham P, Saumet JL (2002 a). Oral single high-dose aspirin results in a long-lived inhibition of anodal current – induced vasodilation. British .J. Pharmacol. 137: 384-390.
Durand S, Fromy B, Koital A, Abraham P, Saumet JL (2002 b). Vasodilation in response to repeated anodal current application in the human skin relies on aspirin sensitive mechanism. J. Physiol. 540: 261-269.
Friedrich CL, Dominic NM, Ralf Dechend (2001). Aspirin inhibits NF-KB and protects from angiotensin II – induced organ damage. The FASEB .J. Express Article 10. 1096/fj.00-0843 fje.
Gromal AG, Bardwill C. J, David MM (1949) "Determination of serum protein by means of biuret reaction". J. Biol. Chem. 177: 571-766.
Halliwel B (1974). "Super-oxide dismutase, catalase and glutathione peroxidase-solutions to problem of living with oxygen". New Phytol. 73: 1075-1086.
Hla TT, Bailey JM (1989). Differential recovery of prostacyanin synthesis in cultural vascular endothelial versus smooth muscle cells after inactivation of cyclooxygenase with aspirin. Essen. Fatty Acids, 36:175-184.
Holger H, Angus T (2001). Aspirin targets key cells that trigger organ rejection and other immune responses. Science Daily (June 25, 2001). Pittsburgh.
Ignarro LS (1971). "Effects of anti-inflammatory drugs on the stability of rat liver lysosomes in-vitro". Biochem. Pharmacol. 20: 2847-2853.
Kaplan D, Mejilton C, Lutchel D (1975). "Bisulphite induced lipid oxidation". Arch. Environ. Health 30:507-509.
Ngaha EO, Fry M, Plummer DT (1979). "The effect of cephaloridine on the stability of rat kidney lysosomes". Chem. Biol. Interacts. 24: 199-208.
Ngaha EO, Akanji MA (1982). "Effect of chloroquine in the stability of rat kidney lysosomes *in vivo* and *vitro* comp". Biochem. Physiol. 73c: 109-113.
Olajide JE, Akanji MA, Sanni M, Omale J (2005). Acetylsalicylic acid and cellular damage in kidney of metabisulphite treated rats. AnI. Res. Int. 2(3): 388-392.
Patrono C, Collar B, Dalen JE, Roth G (1998). Platelet activating Drugs; The relationship among dose, effectiveness and side effects. Chest. 114: 470s-488s.
Roger HJ, Spectre RG, Trounce JR (1981). "Salicylates. In a textbook of clinical pharmacology". Acad. Press. New York, Pp 281-286.
Taylor SL, Higley NA, Bush RK (1986). Sulfites in food: uses, analytical methods, residue, fate exposure assessment, metabolism, toxicity and hypersensitivity". Adv. Food Res. 30: 1-8.
Wedzicha BL (1984). "In: chemistry of sulphur dioxides in foods". Elsevier Applied Sci. Publisher Borking Essex. Pp 214-215.
Wright PJ, Leathwood FD, Plummer DT (1972). "Enzymes in rat urine: Acid Phosphatase". Enzymologia 42: 317-327.
Wu KK (2000). Aspirin and Salicylate: An old remedy with a new twist. Circulation 102: 2022-2023.
Yakubu MT, Akanji MA, Saliu IO (2001). Protective effect of ascorbic acid on some selected tissues of rantidine-treated rats. Nig. J. Biochem. Mol. Bio. 16: 177-182.
Zimmerman M (1983): Ethical guidelines for investigation of experimental pain in conscious animal. Pain 16: 109- 110