

ACETYLSALICYLIC ACID AND CELLULAR DAMAGE IN KIDNEY OF METABISULPHITE TREATED RATS

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ABSTRACT

The effect of acetylsalicylic acid (ASA) as membrane stabilizers was investigated on the kidney of experimental rats treated with sodium metabisulphite. Administration of sodium metabisulphite has been shown to labilize the plasma membrane of some rat tissues. Sodium metabisulphite (10 mg/kg b.wt) acetylsalicylic while both chemical substances of same dose were both chemicals were concurrently administered to three group of rats for two weeks (14 days) while the fourth (4th) group of rats served as control and were given physiological saline alone. Two 'marker' enzymes, alkaline phosphatase (ALP) and acid phosphatase (ACP) activities were spectrophotometrically determined to monitor the efficacy of acetylsalicylic acid in membrane stabilization. Following the initial administration of metabisulphite alone, immediate significant decreases ($p < 0.05$) in ALP activities were observed. The activity latter recovered towards control value by the tenth day. For ACP, the loss in activities was sustained throughout the experimental period. However, the difference showed no significant difference ($p > 0.05$). In acetylsalicylic acid administered rats the activities of ALP were higher than for the control group while the activities of ACP were not appreciably affected. The combined treatment gave values that were not significantly different from the control values ($p < 0.05$).

Keywords: Acetylsalicylic acid, Kidney, Metabisulphite, Cellular damage

INTRODUCTION

Acetylsalicylic acid (ASA) is a member of the salicylate drugs earlier known in use to the Greeks and Romans (Roger *et al.*, 1981). Substitution of the phenolic group of salicylic acid with acetic anhydride produced acetyl salicylic acid.

Acetylsalicylic acid is a non-steroidal anti-inflammatory drug (Roger *et al.*; 1981). It has earlier been reported that certain anti-inflammatory drugs are potent membrane stabilizers (Ignarro, 1971). These anti-inflammatory drugs probably act by inhibiting prastanoid synthesis by acetylation of fatty acid cyclooxygenase (EC. 1.14.99.1) (Durand *et al.*, 2002a). Acetylsalicylic acid thus irreversibly blocks cyclooxygenase (COX) (Durand *et al.* 2002), an effect short-lived in endothelial or smooth muscle cells due to resynthesis (Durand *et al.*, 2002b). the duration of cyclooxygenase blockade by ASA however depends on the type of cell studied (Hla and Bailey, 1989) and this difference in the duration of the effect of ASA is the rationale for the use of 50 – 1500 mg/day (Abou-Elenin *et al.*, 2002) of this drug with long inter – dose intervals in an attempt to inhibit thromboxane production in platelets (Patrono *et al.*, 1998). Baghat *et al.*, (1995) reported the possibility of ASA having longer effects *in vivo* than invitro depending on the type of cells studied. The capacity of the anti – inflammatory drugs to stabilize lysosomal membrane of rat liver under invitro conditions have been demonstrated (Ignarro 1971). The stabilization of the Kidney lysosomal membrane by ASA after its labilization by

chloroquine has been reported (Ngaha and Akanji, 1982).

Through sodium metabisulphite and other sulphiting agents employed in food preservation have been generally regarded as safe, some recent toxicological findings tend to underscore their safety (Taylor *et al.*; 1986).

The possibilities of toxic products resulting from interaction between sulphur dioxide, a compound readily generated by sodium metabisulphite (Wedzicha, 1984) and dietary components had earlier been studied (Baghat and Lockett, 1964).

Sulphites are known to have inhibitory action on some enzymes such as Lactate dehydrogenase and Malate dehydrogenase (Pfleiderer *et al.*, 1956). Sulphonation at the N⁶ atom of flavin – active site of Flavo proteins, occurs through formation of chemical adducts with sulphites (Gunnison *et a.*, 1981).

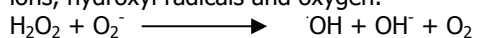
In aqueous solution sodium metabisulphite like other sulphites from sulphurous acid, H₂SO₃ (William and Dennis 1995), the active compound in its preservative property. This acid has two dissociation constants at pH 2 and pH 7. At pH 7 the HSO₃⁻ and SO₃²⁻ are in about equal proportion. At pH 5 most of the compound is in HSO₃⁻. At lower pH value protonation by bisulphate ion HSO₃⁻ results in molecular SO₂ (George, 2002).

Sulphites inactivate certain enzymes systems such as cytoplasmic membrane of cells thereby altering its permeability (George, 2002) as well as react with pyrimidine bases altering their properties.

Kaplan *et al.* (1975) reported induced oxidation in corn- oil emulsified in 1.5% polysorbate

solution by low concentrations (0.5 mM) of bisulphate. Unsaturated membrane lipids incubated with a large excess of bisulphate was reported to have different chromatographic pattern indicative of addition of bisulphate across double bonds. Such changes in membrane lipids could account for the irritant effect of sulphites (Akagyeran and Southerland, 1980).

Sodium metabisulphite is highly rich in oxygen and oxygen radicals. O_2^- is good nucleophile which could react readily with electrophilic sites on biological molecules (Halliwell, 1974). Nevertheless O_2^- does not seem to be especially reactive but dismutation of O_2^- either spontaneously or by action of super oxide dismutase give hydrogen peroxide, (H_2O_2) whose reactivity is enhanced in the presence of transition metal ions. Transition metal ions break H_2O_2 into reactive radical species (Halliwell, 1978). Thus it was proposed that O_2^- react with H_2O_2 to produce hydroxyl ions, hydroxyl radicals and oxygen.



The non – enzymatic dismutation of O_2^- has been reported to generate oxygen, in the singlet state Khan, (1970). Singlet oxygen is reactive enough to attack molecules such as alkenes and the polyunsaturated fatty acids found in membrane lipids (Halliwell, 1974). The toxicity of sodium metabisulphite through induction of oxidation of lipids of cell membrane because of its high content of polyunsaturated fatty acids chain have been reported (Halliwell, 1978).

Alkaline phosphatase, ALP (EC.3.1.3.1) is a 'marker' enzyme for plasma membrane and endoplasmic reticulum (Wright and Plummer, 1974). It is found mainly in the liver and Kidney (Folley and kay, 1935; Kaplan, 1972). Acid phosphatase, ACP, (EC.3.1.3.2) is a 'marker' enzyme for lysosomal membrane (de Duve *et al*, 1962). Found mainly in many animals tissues such as the prostate the seminal plasma (Wilkinson, 1963), showed intense activity in the convoluted tubules of rat Kidney.

These mono ester phosphohydrolases are active at pH 10.1 and pH 4.5 respectively. Their activities in the Kidney are monitored after being insulted with sodium metabisulphite to establish cell damage and the potency of acetylsalicylic acid in repairing such damaged cell membrane.

MATERIALS AND METHODS

Male white albino rats (150 – 200 g) were obtained from the Research Laboratory, Biochemistry Department, University of Ilorin, Nigeria. Sodium metabisulphite was purchased from May and Baker Ltd, Dagenham, England. Acetylsalicylic acid was obtained from Tega Laboratories, Chelsea, London. 4 – Nitrophenyl orthophosphate (disodium salt) was obtained from British Drug Houses (Chemicals) Ltd, Prole, England.

Animal Grouping: Forty rats weighing between (150 – 200 g) were divided randomly into four group of 10 rats each. The first three groups were experimental groups and the fourth the control group. Each group of animals was kept in separate metabolic cages and fed

with rat cubes and water *ad libitum*. Each set up was replicated thrice.

Group 1 rats were administered daily with sodium metabisulphite (10 mg/kg body weight).

Group 2 rats were administered daily with solution of acetylsalicylic acid (10 mg/kg b.wt).

Group 3 rats were administered daily with solutions of the two chemical compounds concurrently while rats in the control group were administered with physiological saline alone.

Drug Administration: Solution of sodium metabisulphite (2 mg/ml) and acetylsalicylic acid (2 mg/ml) were prepared in distilled water. They were administered intraperitoneally to rats daily (24 hourly) as enumerated above for 15 days.

Animal Sacrifice: Rats from each group were sacrificed on alternate days (1, 3, 5, 10, 15) starting from the day when administration commenced. Day 1 represents rats that were given one daily dose of appropriate chemical compound or its combination and thereafter sacrificed 24 hours, while day 15 represents rats that were given 15 daily doses of the appropriate chemical compound or its combination and left thereafter for 24 hours before sacrifice (Akanji and Nlumanze, 1987). Rats in the control group administered with physiological saline were sacrificed 24 hours after the 15th dose.

Preparation of Tissue Homogenates: A desiccator containing cotton wool soaked in chloroform was used to anaesthetize the rats until they go unconscious. The rats were taken out and immediately dissected. The Kidney was removed decapsulated, washed, weighed (1 g) and cut into pieces for homogenization in ice – cooled 0.25 M sucrose solution (1.5 w/v) (as buffer to maintain the integrity of the organ) using a pre – cooled enamel mortar and pestle. Triton X-100 was added to a final concentration of 1% (Ngaha *et al*; 1979). The kidney homogenates was frozen over night. This allows unbroken cells to lyse being used for enzyme assay (Akanji and Ngaha, 1989).

Tissue Dilution: The tissue homogenates were diluted using 0.25 M sucrose solution as diluent, before being assayed for protein and enzyme activities. The dilution factors are presented on table 1.

Table 1: Dilution factors for kidney tissue

	Protein	ALP	ACP
Tissue (Kidney)	30	600	600

Enzyme and protein Measurements: The Biuret method of Gromal *et al* (1949) was used to determine protein concentration. The absorbance was read at 540 nm and extrapolated in standard protein curve. The absorbance obtained for each sample was used to obtain the corresponding protein concentration from the standard protein curve. Protein concentration (mg/ml) = C x F where

C = Protein concentration from standard curve, and F = Dilution factor.

The activities of the phosphatase were followed using the assay method described by (Wright *et al*; 1972). All measurements were carried out using spectronic 20, Bauch and Lamb Rochet.

Data Analysis: The data obtained were subjected to statistical analysis (ANOVA) to determine the level of significance.

RESULT AND DISCUSSION

Figures 1 and 2 illustrate the results obtained following the administration of the chemical compounds on the activities of the phosphatases (ALP and ACP) on rat Kidney respectively.

Figure 1 reflects the effect of daily administration of the chemical compounds on the activities of alkaline phosphatase of rat kidney. Following administration of metabisulphite, there was an immediate decrease of enzyme activities which persisted until after the fifth dose. Thereafter a recovery towards control value was obtained. By the 10th day, values were not significantly different from control value ($P > 0.05$).

Contrarily, administration of acetylsalicylic acid resulted in increased enzyme activity throughout the experimental period. Combination of the two chemical compounds gave values that are not significantly different ($P > 0.05$) from those obtained when acetylsalicylic acid alone was administered.

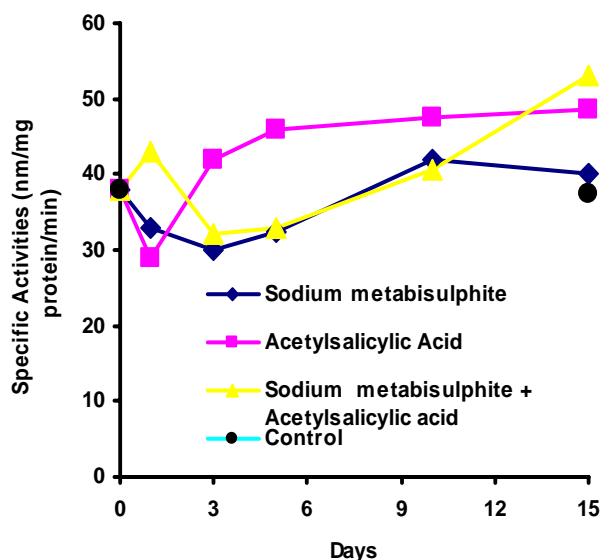


Figure 1: Effect of administration of acetylsalicylic acid on ALP of the kidney of metabisulphite treated rat

Figure 2 illustrated the effects of the individual chemical compounds and the combination treatment on the activities of acid phosphatase. Administration of metabisulphite resulted in loss of enzyme activity up to the fifth day. Thereafter there was recovery of activity and this lasted to the end of the experimental period. A continuous reduction in enzyme activity throughout the duration of experiment attended administration of acetylsalicylic acid. The same pattern was obtained

when the two compounds were administered together although the magnitude of loss of activity was smaller.

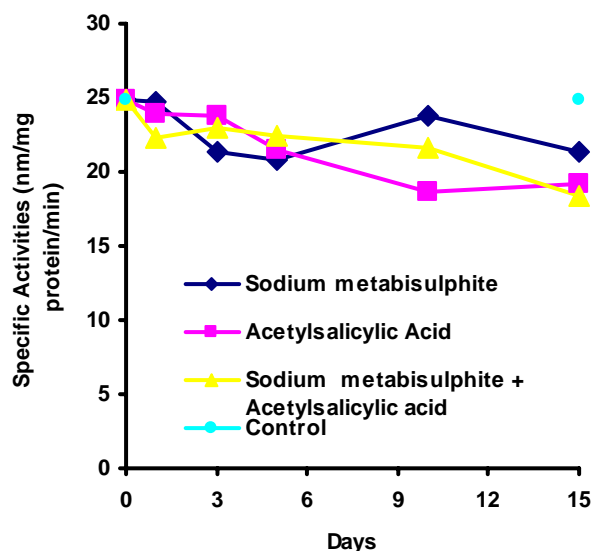


Figure 2: Effect of administration of acetylsalicylic acid on ACP of the kidney of metabisulphite treated rat

It has been shown that biochemical parameters like enzyme assay indicate tissue/cellular damage long before structural damage that can be picked up by conventional biological techniques (Ngaha, 1979 and Akanji 1986). Two 'marker' enzymes were assayed in the present study. These enzymes are found in specific regions of the cell. Alkaline phosphatase is a plasma membrane enzyme (Wright and Plummer, 1974), while acid phosphatase is a lysosomal enzyme (Shibko and Tappel, 1965). The activities of these enzymes before and after the tissue is insulted with chemical agents, can be monitored to deduce pattern and sequence of cell damage.

The organ studied was the Kidney, an organ involved with active absorption of substances (Schanker *et al*, 1957; Clegg and Clegg, 1975). In this work, administration of sodium metabisulphite resulted in a significant decrease ($P < 0.05$) in alkaline phosphatase activities in the kidney (figure 1). The loss on the activities of alkaline phosphatase on the tissue could be as a result of either or combination of the following reasons:

- i. Damage to the cell plasma membrane resulting from the administration of metabisulphite.
- ii. Inactivation of the enzyme in situ (Ngaha, 1982).
- iii. Increase in synthesis of other cellular protein elicited by the compounds (Harkness and Roth, 1969).

The result of this study showed that the level of reduction in enzyme activities may not likely be due to inhibition of alkaline phosphatase, but may be as a result of damage to plasma membrane. The kidney plays vital primary functions in all organisms. It is involved in active transportation of molecules and ions across cell membrane (Akanji and Nlumanze, 1987).

The activities of acid phosphatase were not affected to any appreciable extent in the kidney following the administration of the compound. The values obtained showed no significant difference from the control value ($P > 0.05$). It may imply that the integrity of the lysosome where acid phosphatase is located was maintained despite the chemical result. A possible explanation for this might be that sodium metabisulphite has been completely eliminated before coming in contact with the organelles since they have their individual organelle membrane (Wright *et al.*, 1979).

When acetylsalicylic acid alone was administered to rats and the kidney enzymes assayed, it was observed that the activities of the enzymes in the kidney were not appreciably affected (fig. 1 and 2). There were high fluctuations in enzyme activities around the control levels.

Acetylsalicylic acid seems to play a stabilizing role, and thus maintained the integrity of the cell membranes. This accounted for the stable activities of the enzymes throughout the period of the experiment.

The concurrent administration of sodium metabisulphite and acetylsalicylic acid to the animals produced distinct pathways from what was observed when metabisulphite alone was injected. The attendant decrease in alkaline phosphatase activities in the kidney when metabisulphite was administered were no longer observed.

Enzyme activities were not higher, but brought towards control level on administration of both metabisulphite and acetylsalicylic acid. Miller and Smith (1966) reported that acetylsalicylic acid stabilizes rat liver lysosomes *in vitro* while Ngaha and Akanji, (1982) have shown that acetylsalicylic acid stabilizes rat kidney lysosomal membrane after its labilization by chloroquine.

Acetylsalicylic acid has been demonstrated in plasma at level of 0.2 – 1.4 mg/100 ml, 30 minutes after oral administration of 1.2 g of acetylsalicylic acid. It was also been recovered in hydrolysed form in urine indicating that complete hydrolysis of acetylsalicylic acid *in vivo* does not occur (Miller and Smith, 1966). Thus, this concentration which has stabilizing effect on lysosomes *in vitro* can be achieved *in vivo* following administration of a single dose of 1.2 g of acetylsalicylic acid orally (Miller and Smith, 1966).

Proposed Mechanism: Olagoke (1991) summarized the probable mechanisms to explain the mode of toxicity of metabisulphite in tissues in the following ways,

- i. Oxidation of lipids of the cell membrane arising from high oxygen content of metabisulphite.
- ii. Production of very reactive free radicals ($\cdot\text{SO}_3$) by the compound, which disrupt the ordered lipid bilayer of the cell membrane.
- iii. Production of sulphate radical anion $\cdot\text{SO}_3$ in (ii) above can lead to further production of oxidizing radical $\cdot\text{O}_2$ or $\cdot\text{OH}$, which can eventually lead to peroxide formation.

The design of this study is such that it will use a block membrane stabilizer (acetylsalicylic acid) to show whether metabisulphite, disrupts affected membrane by creating gaps along the membrane walls.

Acetylsalicylic acid as a membrane stabilizer (Miller and Smith, 1966; Ngaha and Akanji; 1982) is expected to prevent the disruption of the membrane when it is in concurrent administration with sodium metabisulphite. The results (Figure 1) showed that the loss of alkaline phosphatase activities was prevented when acetylsalicylic acid was concurrently administered with metabisulphite to the experimental animals.

The stabilizing effect of acetylsalicylic acid in preventing the disruption of cell membrane induced by metabisulphite was demonstrated. This may be attributable to the ability of acetylsalicylic acid to lodge itself in spaces created between molecules on the membrane structure.

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