1999

Reversal of Sodium Arsenite Inhibition of Rat Liver Microsomal Ca2+ Pumping ATPase by Vitamin C

Odutayo O. Odunuge
Stephen F Austin State University, odunugaoo@sfasu.edu

Gbolahan W. Okunade

Olufunso O. Olorunsogo

Bioscience Reports, Vol. 19, No. 1, 1999

Reversal of Sodium Arsenite Inhibition of Rat Liver Microsomal Ca^{2+} Pumping ATPase by Vitamin C

Odutayo O. Odunuga,[1,2] Gbolahan W. Okunade,[1] and Olufunso O. Olorunsogo[1]

Received October 5, 1998

Sodium arsenite ($NaAsO_2$), at 10% of its median lethal dose, was administered to rats with and without vitamin C pretreatment. Liver microsomal fraction was isolated and the activity of Ca^{2+}-ATPase was assayed. Sodium arsenite was found to inhibit the activity of the liver microsomal Ca^{2+}-ATPase to 50% to that of control rats. The specific activity of the enzyme in rats administered sodium arsenite with vitamin C pretreatment was not significantly different from that of control rats.

KEY WORDS: Sodium arsenite; inhibition; microsomes; Ca^{2+}-ATPase; reversal; vitamin C.

INTRODUCTION

Sodium arsenite ($NaAsO_2$) is used widely as a component of insecticides, herbicides, fungicides, algicides and for killing vegetation. The chemical is highly toxic and has a high degree of retention in tissues. It has been found that arsenite markedly decreases total hepatic cytochrome P_{450} content and dependent monooxygenase activities [1]. Rumos *et al.* (1995) [2] have shown that sodium arsenite decreases glutathione (GSH) levels and increased lipid peroxidation in liver, kidney and heart, [3] have also shown that arsenite mimics okadaic acid, a potent tumor promoter and selective inhibitor of phosphatase. Brown and Kitchen (1996) [4] suggested that sodium arsenite, like phorbol esters, might be a promoter rather than an initiator of carcinogenesis.

Adenuga *et al.* (1972) [5] observed that depression of ER Ca^{2+}-ATPase was a manifestation of the toxicological effect of tumor promoters. Lowry *et al.* (1981) [6] have also observed that decreased microsomal calcium pumping is one of the earliest signs of chemical hepatotoxicity using haloalkanes. Studies on thapsigargin have shown that it induces Ca^{2+} mobilization by ER Ca^{2+} pump inhibition [7]. It has long been recognized that gap junctional intercellular communication is a function of cytoplasmic calcium concentration [8]. The endoplasmic reticulum is a major organelle in the fine regulation of intracellular calcium ion concentration. Therefore,

[1]Laboratory for Biomembrane Research, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Nigeria.
[2]To whom correspondence should be addressed.

0144-8463/99/0200-0011$16.00/0

the endo (sarco) plasmic reticulum Ca^{2+}-ATPase may play a vital role in the mechanism of action of tumor promoters.

Vitamin C is known generally as a detoxifying agent. Antimutagenic effect of ascorbic acid has been investigated against mutagenic agents such as dimethylnitrosomine, benzo(a)pyrene, benzidine and aflatoxin B_1 [9]. The anticancer effect of vitamin C is a consequence of the removal of free radicals from the cells.

We report here the effect of sodium arsenite alone and in combination with vitamin C on the liver microsomal Ca^{2+} pump.

METHODS

Chemicals

Sodium arsenite and vitamin C were obtained from Sigma Chemical Company, U.S.A. and BDH Chemicals Ltd., England, respectively. Adenosine triphosphate (ATP) was also obtained from Sigma Chemical Company. All other chemicals and reagents were of analytical grade.

ANIMALS

Male Wistar rats were obtained from the Department of Biochemistry, University of Ibadan, Nigeria. The rats were kept in well-ventilated cages and allowed food and water freely throughout the period of the experiments. The weight of the rats just before sacrificing were between 130 and 150 g. The control animals (Group A) were fed normal rat pellets before and during the experiments. Group B rats were given a mega dose of vitamin C daily for seven (7) days before administration of sodium arsenite. Animals in Group C received sodium arsenite alone. The sodium arsenite dose given in each case was 2.4 mg/kg body weight, which is a tenth part of the LD_{50} for rats. All drugs were administered by oral intubation. Twenty four (24) hours after sodium arsenite administration, the rats were starved overnight and sacrificed by cervical dislocation. Livers were quickly removed, washed in ice-cold 0.2 M sucrose/20 mM Tris, pH 7.2 buffer and weighed.

PREPARATION OF MICROSOMAL FRACTION

10% (w/v) liver homogenates were prepared using the homogenizing buffer, 0.25 M sucrose/20 mM Tris, pH 7.2, by five strokes in a Poter–Elverjem homogenizer. The homogenates were centrifuged at 12,000*g* for 20 minutes in a Hermle 2-323K centrifuge at 4°C. The microsomal vesicles were sedimented at 105,000*g* for 1 hr at 4°C in a Beckman L5-50B ultracentrifuge. The microsomal pellets were resuspended in 130 mM KCl/120 mM Tris, pH 7.2 and stored frozen.

PROTEIN DETERMINATION

Protein content was determined by the method of Lowry *et al.* (1951) [10] using bovine serum albumin as standard.

Table 1. Liver Microsomal Ca^{2+}-ATPase Activities of Rats Exposed to Sodium Arsenite (2.4 mg/kg body weight) Alone and After Pretreatment with Vitamin C. ATPase Activity was Determined as Described in Materials and Methods

Group	Number of experiments	Microsomal protein (μg/ml)	Microsomal Ca^{2+}-ATPase activity (μmole Pi/mg protein/hr)
$NaAsO_2$	6	7.50 ± 2.80	21.30 ± 1.29*
$NaAsO_2$ + Vit. C	6	7.90 ± 2.44	44.10 ± 2.25
Control	6	7.16 ± 2.94	45.21 ± 1.75

Values are means ± SD.
*Significantly different from control at $p < 0.05$.

ER Ca^{2+}-ATPase Assay

The Ca^{2+}-dependent ATPase activity was assayed in a final reaction mixture of 1 ml containing 100 mM KCl, 20 mM Tris, pH 7.2, 2 mM NaN_3, 1 mM $MgCl_2$, with or without 2 mM $CaCl_2$. When $CaCl_2$ was not present, 4 mM EGTA was used instead. The reaction was initiated by adding 5 mM ATP and terminated after 30 minutes of incubation at 37°C, with the addition of 50 μl of 50% trichloroacetic acid. Liberated inorganic phosphate (Pi) was measured by the conventional method of [11]. The difference in activity in the presence and absence of 2 mM $CaCl_2$ was used to calculate the Ca^{2+}-ATPase activity. The procedure was repeated using different concentrations or $CaCl_2$. One unit of enzyme was taken to be the amount of enzyme that released 1 nmole of Pi per minute.

RESULTS

Table 1 shows the liver microsomal Ca^{2+}-ATPase activities of rats after exposure to sodium arsenite (2.4 mg/kg body weight) alone and in combination with vitamin C. There was a marked decrease in the microsomal Ca^{2+}-ATPase activities of rats given $NaAsO_2$ alone to about 50% in comparison with that of the control rats. Similarly, the activity of the enzyme in rats pretreated with vitamin C and later administered $NaAsO_2$ was found to be twice that of rats given $NaAsO_2$ alone. However, no appreciable difference was observed between the microsomal Ca^{2+}-ATPase activities of rats given the combined regimen and that of the control group.

Figure 1 presents the Ca^{2+}-dependence profiles of liver microsomal Ca^{2+}-ATPase of rats treated with sodium arsenite alone and after vitamin C pretreatment. The result showed that the affinity of liver ER Ca^{2+}-ATPase for Ca^{2+} was drastically reduced after *in vivo* administration of sodium arsenite. Treatment with vitamin C prior to the administration of the carcinogen significantly lowered the extent of inhibition of the enzyme.

DISCUSSION

The endoplasmic reticulum Ca^{2+}-ATPase is involved in the fine regulation of calcium ion level in the cell [12]. Investigations have revealed that tumor promoters

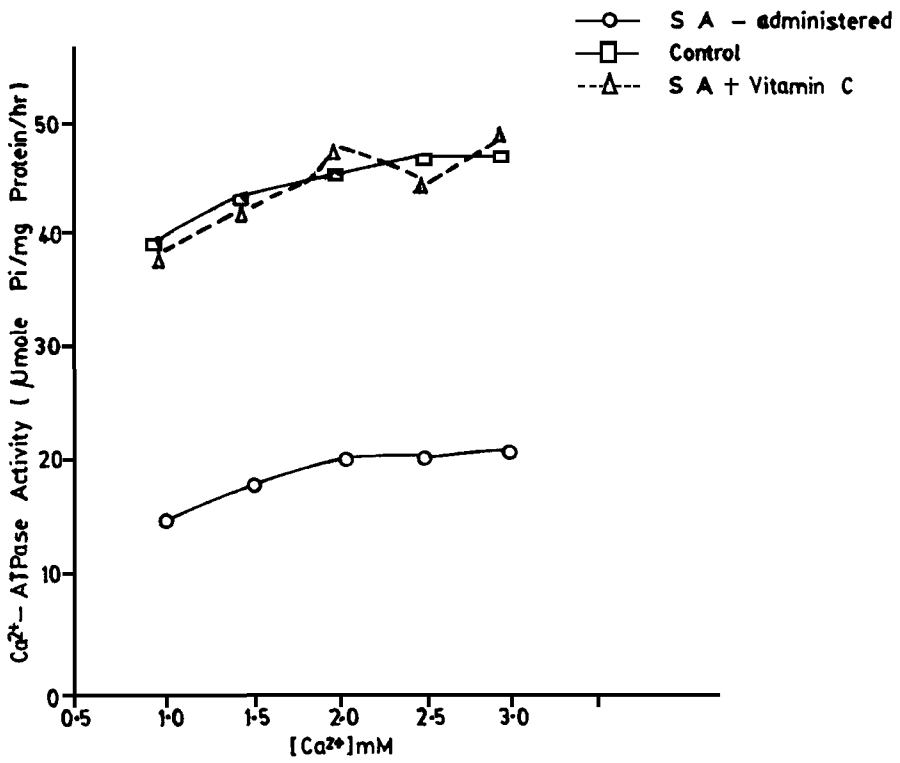

Fig. 1. Activity of liver microsomal Ca^{2+}-ATPase of rats administered sodium arsenite (SA) alone and in combination with vitamin C, at different Ca^{2+} concentrations. Ca^{2+}-ATPase activity was determined as described in Materials and Methods.

depress the ER Ca^{2+}-ATPase [5–7, 13]. In this experiment sodium arsenite was found to significantly decrease the specific activity of the hepatic endoplasmic reticulum Ca^{2+}-ATPase in rats (Table 1). This observation supports previous findings [5–7, 13]. The specific activity of the enzyme in sodium arsenite—rats previously treated with vitamin C did not show significant difference when compared with control rats. When compared with that of rats exposed to $NaAsO_2$ alone a significant decrease to about 50% was observed.

The drastic depression of the activity of the ER Ca^{2+} pump will result in an increase in cytosolic free Ca^{2+}, a condition which favors positive response of cells to mitogen including growth factors and tumor promoters. For instance, [14] reported that administration of triiodo-L-thyronine to rats lowered the activity of the liver microsomal Ca^{2+}-dependent ATPase and altered Ca^{2+} sequestration in the rat liver microsomes.

The mechanism by which this inhibition occurs is not known. It may be due to non-competitive inhibition of the enzyme by $NaAsO_2$. Sodium arsenite may bind covalently to groups on the enzyme molecule. On the other hand, the inhibition could be brought about by indirect interaction microsomal membrane. If this is the

case, the topology of the Ca^{2+}-ATPase, an enzyme known to be sensitive to its immediate lipid environment will be adversely affected. This may result in drastic reduction in the activity of the enzymes. This suggestion is further supported by the fact that many carcinogens act by generating free radicals. Generation of free radicals will ultimately result in membrane damage via peroxidation of membrane phospholipids. More investigations are however required to unravel and establish the mechanism of action of the compound.

Vitamin C has been shown to possess antimutagenic property against a number of carcinogens [9]. However, ascorbic acid seems not to be effective when toxicological manifestations are already established. This is in conformity with the role of vitamin C as an extracellular free radical scavenger molecule [15].

In conclusion, sodium arsenite at one tenth of its median lethal dose was found to depress rat liver endoplasmic reticulum (ER) Ca^{2+}-ATPase by 50% in comparison with control. This depression however was significantly reversed by vitamin C pretreatment.

REFERENCES

1. Webb, J. L. (1966) *Enzyme and Metabolic Inhibitors.* Academic Press. New York **3**:595–793.
2. Ramos, O. *et al.* (1995) *Environ. Health Perspect.* **103**: Suppl **1**:85–88.
3. Huang, R. N., Ho, I. C., Yin, L. H., and Lee, T. C. (1995) *Environ. Mol. Mutag.* **25**:188–196.
4. Brown, J. L. and Kitchen, H. T. (1996) *Cancer* **98**(2):227–231.
5. Adenuga, G. A., Bababunmi, E. A., and Hendrickse, R. G. (1972) *Toxicol.* **71**:1–6.
6. Lowrey, K., Glende, E. A. Jr., and Recknagel, R. O. (1981) *Biochem. Pharmacol.* **30**:135.
7. Thastrup, O., Orobak, B. K., Hemley, M. N., and Dawson, A. P. (1990) *Proc. Nat. Acad. Sci. U.S.A.* **87**:2466–2470.
8. Loewenstein, W. R. (1979) *Biochem. Biophys. Acta* **560**:1–59.
9. Lim-Sylianco, C. N. (1993) *Molecular Nutrition.* The Academy, Metro Manila, Philipines.
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randal, N. J. (1951) *J. Biol. Chem.* **193**:265–275.
11. Fiske, G. H. and Subbarow, Y. (1925) *J. Biol. Chem.* **66**:375–400.
12. Carafoli, E. (1987) *Ann. Rev. Biochem.* **56**:395–433.
13. Odunuga, O. O. and Adenuga, G. A. (1997) *Biosci. Rep.* **17**(6):543–546.
14. Balestrero, F., Morelli, A., Damonte, G., Yoci, A., and Orunesu, M. (1990) *Ital. J. Biochem.* **39**(4):250–257.
15. Halliwell, B. and Gutteridge, J. M. C. (1990) *Arch. Biochem. Biophys.* **286**:1–8.