Scientific Research and Essay Vol. 2 (1), pp. 010-012, January, 2007
Available online at http://www.academicjournals.org/SRE
ISSN 1992-2248 © 2007 Academic Journals

Short Communication

In vivo effects of sodium benzoate on plasma aspartate amino transferase and alkaline phosphatase of wistar albino rats

Ibekwe, Sixtus E, Uwakwe, Augustine A.* and Monanu, Michael O.

Department of Biochemistry,University of Port Harcourt, P. M. B. 5323 Port Harcourt.

Accepted 12 December, 2006

The *in vivo* effects of varying concentrations of sodium benzoate (30, 60 and 120 mg/kg body weight) administered by gavage to male albino wistar rats (100 g average weight) over a 14-day period on the plasma levels of aspartate amino transferase and alkaline phosphatase were investigated. Thirty-six (36) male wistar albino rats (average weight of 100 g) were divided into four groups and housed in well-kept cages at the animal house. The rats were fed ad libitum on normal diets while the sodium benzoate solutions in water were given orally at 2-day intervals for 14 days. Blood samples were obtained from sacrificed animals at zero, 2-, 6- and 14-day and levels of plasma enzymes determined by standard methods. The results revealed that for aspartate amino transferase (AST), at 30 mg/kg, enzyme activity (IU/l) increased progressively from 27.33 ± 1.04 on the zero-day to 36.83 ± 1.25 on the 14th day; at 60 mg/kg, the increase was to 38.50 ± 0.50 on the 14th day, while the enzyme level increased to 36.40 ± 0.96 on the 14th day at 120 mg/kg of sodium benzoate. The increases were significant ($p<0.05$) relative to the control. For alkaline phosphatase (ALP), enzyme activity (IU/l) at 30 mg/kg increased from 114.83 ± 0.76 on the zero-day to 130.33 ± 1.04 on the 14th day; at 60 mg/kg, the increase was to 130.16 ± 0.76 on the 14th day, with an increase to 130.00 ± 0.40 noted on the 14th day at 120 mg/kg sodium benzoate. The increase observed for ALP were significant ($p<0.05$). The findings suggest that short-term administration of sodium benzoate produce notable increases in serum levels of the two enzymes investigated.

Key words: Sodium benzoate, aspartate amino transferase, alkaline phosphatase.

INTRODUCTION

Benzoic acid and sodium benzoate (C_6H_5COONa) remain widely applicable as preservatives in a number of products consumed by humans (Chipley, 1983; Baldwin et al., 1995; Ishida, 1996; Villanueva et al., 1994). While several studies had been done aimed at ascertaining what short- and long-term effects of consuming products preserved with these compounds, most of the studies investigated organ disposition as well as clinical parameters of experimental animals and human subject. Some reports suggest adverse effects due to both chronic and sub-chronic intake of sodium benzoate (Fujitani, 1993; Vogt, 1999). Other reports suggest absence of negative effects of sodium benzoate intake (Sodemoto and Enomoto, 1980; Toth, 1984).

The pharmacokinetic studies in human subjects reported by several workers indicated that after oral and dermal, benzoate is metabolized in the liver by conjugation with glycine, resulting in the formation of hippuric acid (Feldman and Maibach, 1970; Fillet and Leonard, 1998). Furthermore, the transformation of orally administered benzoate to hippuric acid was reported to be a saturable process in humans (Kubota et al., 1988; Kubota and Ishizaki, 1991).

Sodium benzoate at a general optimum concentration of 0.1% could be used for preservation of such products as soft drinks, fruit drinks, margarine, and certain fish products (Villanueva et al., 1994; Baldwin, 1995; Srour, 1998). The upper limits of benzoate allowable in foods is about 0.1% for United States of America, while a range of

*Corresponding Author's E-mail: uwadik2006@yahoo.co.uk .

Table 1. Plasma levels of AST (IU/L) following administration of sodium benzoate.

Conc. (mg/kg)	0-day	2nd Day	6th Day	14th Day
Control	27.74±0.95	27.79±1.25	27.77±1.04	27.78±1.02
30	27.75±1.06	29.75±1.96	30.80±1.13 [a]	37.50±0.70 [a]
60	27.70±0.98	30.50±0.70	33.25±0.35 [a]	38.75±0.53 [a]
120	27.80±0.98	27.26±0.35	30.25±0.45 [a]	36.75±1.06 [a]

Values are means ± SD of triplicates and those with superscript 'a' are considered to be significantly ($p<0.05$) different from each other and the control.

0.15 to 0.25% had been reported for other countries of the world (Chipley, 1983). For European countries, the limit reported range is from 0.015 to 0.5% (European Commission, 1995). It therefore follows that sodium benzoate could be assimilated widely by consuming a wide range of food products consumed by man.

The determinations of blood levels of several enzymes have served as a useful tool in clinical diagnosis, especially those enzymes know to be preferentially associated with certain cells and tissues of an organism. Aspartate amino transferase (AST) is a marker enzyme for liver function. The blood levels of alkaline phosphatase (ALP) could give indications of liver and bone malfunctions (Sturgill and Lambert, 1997). The present report addresses the effects of short-term intake of sodium benzoate at varying concentrations of the plasma levels of AST and ALT. The findings would aid our understanding of the effect of sodium benzoate in the status of the experimental animals as relates to these key enzymes. It was envisaged that some indications would be obtained on the possible interference of intake of sodium benzoate-containing food products before blood-draw for blood chemistry determinations.

MATERIALS AND METHODS

Enzyme kits for AST and ALP were obtained from Randox Laboratory Ltd., San Francisco, U. S. A. Sodium benzoate was from May & Baker Ltd., England while all other reagents were of analytical grades.

Animals

A total of thirty-six (36) male wistar albino rats, average weight of 83.3 g, were obtained from the animal house of the Department of Biochemistry, University of Port Harcourt. They were grouped into four (4), housed in stainless steel cages in a well-ventilated room under 12 h light/dark cycle, and maintained on normal diet ad libitum, for the duration of the experiment (14 days). The groups were: G1 (control), G2 [30 mg/kg sodium benzoate (SB)], G3 (60 mg/kg SB) and G4 (120 mg/kg SB). The varying concentrations of sodium benzoate were administered orally in 0.5 ml portion at 48 h intervals for 14 days. One rat per group was sacrificed at 0, 2, 6 and 14 days in the course of the experiment.

Sample collection

The rats were anesthetized with chloroform and dissected for blood collection. Blood was collected into lithium heparin bottles and analysis performed within one (1) h of collection. Before enzyme assays, the blood samples were centrifuged for 5 min using a bench-top centrifuge and the supernatant plasma was used for enzyme assay.

Enzyme assays

Aspartate amino transferase activity of the plasma was determined at 37°C using the Randox kit by monitoring the amount of oxaloacetate hydrazone formed in the presence of L-aspartate, α-oxoglutarate and 2,4-dinitro phenyl hydrazine. Absorbance measurement was at 520 nm and the calculation of enzyme activity followed the directive of the kit manufacturer. Alkaline phosphatase activity was measured at 37°C using the Randox kit as reported by Haussament (1977). The absorbance of p-nitrophenol formed from p-nitrophenyl phosphate was determined at 405 nm and calculation of enzyme unit followed as described by the kit manufacturer.

Statistical analysis

All data were expressed as mean ± SEM and statistically analyzed with the student's t-test at 95% confidence limit.

RESULTS AND DISCUSSION

The results for AST level determinations are shown in Table 1. The levels of this enzyme increased progressively at the three concentrations of sodium benzoate. The increases from the 6th to 14th day were significantly different from the control. For ALP, the results are shown in Table 2. Significantly different levels in comparison with the control were seen from the 6th day for the three sodium benzoate concentrations.

Earlier reports on studied to investigate short-term effects of both benzoic acid and sodium benzoate showed varied results. While no adverse effect was seen for cats fed with benzoic acid in blood samples, histopathology of the liver, kidneys and lung indicated degenerative changes. Increases were reported for alanine amino transferase activity, suggesting liver and kidney damage (Bedford and Clarke, 1972).

Fujitani (1993) reported changes in serum levels of cholesterol, albumin, and total protein with enlargement of hepatocytes with glassy cytoplasm in peripheral area of the liver of rats and mouse fed sodium benzoate of varying concentrations. Also, Sodemoto and Enomoto (1980) noted atrophy of the spleen and lymph following short-term feeding of rats with sodium benzoate. The fin-

Table 2. Plasma levels of alkaline phosphatase (IU/l) following administration of sodium benzoate.

Conc. (mg/kg)	0-day	2nd Day	6th Day	14th Day
Control	114.80± 0.40	114.85± 0.38	114.77±0.44	114.79±0.42
30	114.75±0.35	115.25±0.36	118.75±0.55 [b]	129.75±0.45[b]
60	114.80±0.56	115.25±0.35	119.01±0.70 [b]	129.75±0.45[b]
120	114.80±0.70	113.80±0.28	120.70±0.98 [b]	129.80±0.28

Values are means ± SD of triplicates and those with superscript 'b' are considered to be significantly ($p<0.05$) different from each other and the control.

findings in our report of increased level of the two enzymes agree with possible damages to the liver of the experimental animals. It is notable that the concentrations of sodium benzoate used in the present study were much lower than that by the previous works reported and well below the 'safe' limits in foods of 0.1% (Chipley, 1983).

It is remarkable that determination of plasma/serum levels of AST and ALP are routinely performed in clinical diagnosis. The finding that intake of sodium benzoate at concentrations below that 'safe' limits for the use of this compound as a preservative suggests that caution be taken when interpreting blood chemistry data, especially for samples drawn from individual who may have consumed sodium benzoate-containing foods before sample collection.

REFERENCES

Baldwin EA, Nisperos-Carriedo MO, Baker RA (1995). Use of edible coatings to preserve quality of lightly (and slightly) processed products. Critical reviews in food science and nutrition 35(6): 509-524.

Bedford PGC, Clarke EGC (1972). Experimental benzoic acid poisoning in the cat. Veterinary record, 90: 53-58.

Chipley JR (1983). Sodium benzoate and benzoic acid. In: Branen AL, Davidson PM, eds. Antimicrobials in foods. New York, NY, M. Decker. pp. 11-35.

EC (1995). European Union Directive 95/2/CE from 20.02.1995 on food additives, colourants and sweeteners. European Commission.

Feldmann RJ, Maibach HI (1970). Absorption of some organic compounds through the skin in man. J. Invest. Dermatol. 54: 399-404.

Fujitani T (1993). Short-term effect of sodium benzoate in F344 rats and $B6C3F_1$ mice. Toxicol. Lett. 69: 171-179.

Haussament, TU (1977). Quantitative determination of serum alkaline phosphatase. Clin. Chem. Acta 35: 271-273.

Ishida H (1996). Levels of preservatives in toothpastes and possibility of their intake during brushing of teeth. Shokuhin Eiseigaku Zassh.i J. Food Hygiene Society Japan 37: 234-239.

Kubota K, Horai Y, Kushida K, Ishizaki T (1988). Determination of benzoic acid and hippuric acid in human plasma and urine by high performance liquid chromatography. J. chromatogr., 425(1): 67-75.

Kubota K, Ishizaki T (1991). Dose-dependent pharmacokinetics of benzoic acid following oral administration of sodium benzoate to humans. Eur. J. Clin. Pharmacol. 41(4): 363-368.

Srour R (1998). Benzoic acid and derivatives. In: Srour R, ed. Aromatic intermediates and derivatives. Paris, pp. A.IV.1- A.IV.17 (unpublished report).

Sturgill, MG, Lambart GH (1997). Xenobiotic-induced hepatotoxicity: Mechanisms of liver injury and methods of monitoring hepatic function. Clin. Chem. 43: 1512-1526.

Toth B (1984). Lack of tumorigenicity of sodium benzoate in mice. Fundam. Appl. Toxicol. 4: 494-496.

Vogt T, Landthaler M, Stolz W (1999). Sodium benzoate-induced acute leukocytoclastic vasculitis with unusual clinical appearance. Arch. Dermatol. 135: 726-727.