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Some Biochemical Effects of Sub-Acute Oral Administration of L-Arginine on Monosodium Glutamate-Fed Wistar Albino Rats 2: Serum Alkaline Phosphatase, Total Acid Phosphatase and Aspartate Aminotransferase Activities

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Abstract: The present study is aimed at substantiating whether monosodium glutamate, MSG, could induce toxic effects at an appreciably lower dose and to examine the possible role of arginine, ARG, on such MSG-induced effects. Thus, MSG at a dose of 5 mg kg^{-1} body weight was administered to adult male Wistar rats by oral intubation. Treatment was daily and lasted for 28 days. The MSG treatment significantly ($p < 0.05$) decreased the serum alkaline phosphatase (ALP) activity (71.97%) but increased the activities of the serum total acid phosphatase (TAP) (6222.80%) and the serum Aspartate Amino Transferase (AST) (66.86%) and the serum aspartate aminotransferase-to-alanine aminotransferase (AST-to-ALT) ratio (56.59%). Arginine (ARG) (20 mg kg^{-1} b.wt.) co-administered with MSG significantly ($p < 0.05$) decreased the serum Alkaline Phosphatase (ALP) activity (90.47%) representing a decrease of only 18.50% relative to the MSG-treatment alone, but increased the serum Total Acid Phosphatase (TAP) activity (11119.27%), the serum Aspartate Aminotransferase (AST) activity (133.35%) and the serum aspartate aminotransferase-to-alanine aminotransferase (AST-to-ALT) ratio (147.25%). The results showed that MSG at a dose of 5 mg kg^{-1} b.wt. probably affected the synthesis of the above enzymes and that ARG at 20 mg kg^{-1} b.wt. potentiated the MSG-induced effects. Thus, ARG at 20 mg kg^{-1} b.wt. may significantly exacerbate the possible MSG-induced adverse effect on the prostate and major organs with high metabolic activities especially the liver.

Key words: Monosodium glutamate, L-arginine, alkaline phosphatase, total acid phosphatase, aspartate aminotransferase

INTRODUCTION

Amino acids and their derivatives constitute an important class of nutritional supplements used the world over (Field *et al.*, 2000). Monosdium glutamate, MSG, a derivative of the acidic amino acid glutamate, is a food additive generally used to enhance

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food flavor. The MSG is involved in the excitatory central nervous system, CNS, transmission (Rang *et al.*, 2003) and could alter the mitochondria lipid peroxidation as well as the antioxidant status in the different regions of the brain (Singh and Ahluwalia, 2003). Several MSG-induced side effects have been reported by Allen *et al.* (1987), Asnes (1980), Frieder and Grimm (1984) and Ohguro *et al.* (2002) hence, its continued use as a flavor enhancer is controversial.

Although, the above information on MSG-induced toxicity exist, the studies of the possible effects of amino acids, especially those that are consumed in food or used as medicine, on MSG-induced toxicity are lacking. Furthermore, very high doses (40 mg kg^{-1} b.wt. and above) have been used in most of the studies on MSG.

Arginine, ARG, together with MSG may be present in human diet. ARG, a basic amino acid, is essential for the normal immune system activity by enhancing the activities of the natural killer and lymphocyte activated killer cells (Harlod, 2004). Further to this, ARG is the principal precursor in the synthesis of Nitric Oxide (NO) (Vallance, 2000). In particular, NO, is a free radical that, apart from playing diverse roles in the pathogenesis of many ailments (Dawson and Dawson, 1996), has many promising possible therapeutic benefits reported by various groups of researchers (Trousoulis *et al.*, 1997; Cooke *et al.*, 1992; Creager *et al.*, 1992). Since, ARG may have therapeutic effect through its major metabolite Nitric Oxide (NO) it is necessary to study the possible effects of the simultaneous use of ARG and MSG so as to establish weather ARG would exacerbate, attenuate or fail to alter possible effects induced by MSG intake. These, therefore, warranted the present study aimed at substantiating whether MSG could induce biochemical effects at an appreciably lower dose and to examine the possible effects of ARG, a widely used and therapeutically promising amino acid, on such possible MSG-induced effects.

We, therefore, investigated the effect of the oral administration of MSG (5 mg kg^{-1} b.wt.) either alone or simultaneously with ARG (20 mg kg^{-1} b.wt.) on the serum Alkaline Phosphatase (ALP), Total Acid Phosphatase (TAP) and Aspartate Aminotransferase (AST) activities and the serum Aspartate aminotransferase-to-alanine aminotransferase (AST- to-ALT) ratio.

MATERIALS AND METHODS

Chemicals

A brand of MSG (Ajinomoto) was purchased from a regular foodstuff market at Nsukka. ARG, a product of Sigma Chemical Co. St. Louis, Mo. USA, was obtained at the chemical store of Biochemistry Department, University of Nigeria, Nsukka. Other chemicals were of certified analytical grade unless otherwise stated.

Animals and Treatments

The animals used in this study were adult male Wistar rats. They were procured from the animal house of the Faculty of Biological Sciences, University of Nigeria, Nsukka. All the animals received humane care in accordance with the guidelines of the National Institute of Health, USA for ethical treatment of laboratory animals as adapted by the University of Nigeria, Nsukka Ethical Committee.

Twelve adult male Wistar rats with mean body weight (b.wt.) of 93±0.5 g were acclimatized for one week before they were randomly assigned to three groups of four rats each. Group II were fed MSG (5 mg kg^{-1} b.wt.) alone whereas group III were fed ARG with MSG (20:5 mg kg^{-1} b.wt.). Group I rats were given distilled water (1 mL kg^{-1} b.wt.). Treatment

was by daily oral incubations for 28 days. The rats were housed in stainless steel cages at room temperature ($28\pm2°C$) and exposed to a normal daylight/dark cycle under humid tropical condition. They were supplied with enough rat feed and drinking (portable) water *ad libitum* throughout the duration of the experiment.

Blood Collection and Preparation

Blood samples of the rats were collected individually with sterile capillary tubes into properly labeled polystyrene centrifuge tubes as previously described by Egbuonu *et al.* (2009a). The blood samples thus collected were allowed to clot and then centrifuged at 3000 rpm for 10 min. The resultant sera were collected individually in stoppered polystyrene tubes and stored in deep freezer for the determination of the serum Alkaline Phosphatase (ALP), Total Acid Phosphatase (TAP) and Aspartate Aminotransferase (AST) activities and the serum aspartate aminotransferase-to-alanine aminotransferase (AST-to-ALT) ratio.

The Assay of Alkaline Phosphatase (ALP) Activity

The assay of Alkaline Phosphatase (ALP) activity was by the method of Walter and Schutt (1974a). The buffer/substrate solution and serum sample were independently equilibrated to room temperature ($28\pm2°C$) before the start of the assay. To 1.00 mL of alkaline phosphatase buffer/substrate solution in test tubes were pipetted 0.01 mL of serum samples, thoroughly mixed and allowed to stand for 1minute before measuring their absorbencies at 1 min intervals for three times at 405 nm, using Unico-UV-2102 PC Spectrometer, Unico USA.

The Determination of Total Acid Phosphatase (TAP) Activity

Total Acid Phosphatase (TAP) activity was determined by the method of Walter and Schutt (1974b). Into separate test tubes labeled samples and respective blanks were pipetted 0.5 mL of acid phosphatase buffer/substrate solution. The 0.025 mL of tartarate solution was added to the blanks only before adding 0.1 mL of serum to both sample and blank tubes. The content of the tubes were mixed and incubated for 30 min at room temperature before the addition of 0.1 N sodium hydroxide to all the tubes. The absorbance was read at 405 nm as in ALP measurement.

The Assay of Aspartate Amino Transaminase (AST) Activity

The assay of Aspartate Aminotransaminase (AST) activity was carried out by the method of Reitman and Frankel (1957). To 0.05 mL of each serum sample in a test tube was added 0.25 mL of buffer/substrate solution. The content was incubated at $37°C$ for 60 min in a water bath followed by the addition of 0.25 mL of chromogen solution. The content was mixed and allowed to stand for 20 min at room temperature after which 2.5 mL of sodium hydroxide (0.4 N) was added and mixed. The absorbance was read after 5 min against blank at 540 nm as in ALP measurement. The blanks were treated as the samples but without the addition of chromogen solution used to stop all enzymatic reactions. The AST activity in IU L^{-1} was read off from the standard curve.

Calculation of the AST-to-ALT Ratio

The AST-to-ALT ratio was calculated from the results of the serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) activities obtained from the same experiment. However, the AST results are not presented in this study.

Statistical Analysis

The data obtained from the present study were analyzed by one-way Analysis of Variance (ANOVA) as described by Egbuonu *et al.* (2009a). Differences were considered significant at $p<0.05$ level of significance.

RESULTS

Serum Alkaline Phosphatase (ALP) Activity

The assay of serum ALP activity show a significant ($p<0.05$) decrease in MSG-treated rats (group II) (71.97%) and in the rats co-treated with ARG and MSG (group III) (90.47%). It was observed that the serum ALP activity decreased in MSG +ARG-fed rats by 18.5% more than in MSG-treated rats (Table 1).

Serum Total Acid Phosphatase (TAP) Activity

The results of the determination of serum TAP activity as presented in Table 2 show a significant ($p<0.05$) increase in group II (MSG) (6222.80%) and in group III (MSG+ARG) (11119.29%). The increase in the serum TAP activity was further and markedly potentiated by more than three-folds (4896.49%) in the ARG + MSG-treated rats.

Serum Aspartate Aminotransferase (AST) Activity

The results of the determination of assay of serum AST activity presented in Table 3 shows a significant ($p<0.05$) increase in the MSG group (66.86%) and in ARG + MSG group (133.35%). Further, the serum AST activity in group of rats fed ARG with MSG increased by 66.49% relative to the MSG-treated rats.

Serum Aspartate Aminotransferase (AST)-to-Alanine Aminotransferase (ALT) Ratio

The serum AST-to-ALT ratio as calculated from the results of the serum AST and alanine aminotransferase activities was shown to increase in rats given 5 mg kg^{-1} body of

Table 1: The effect of distilled water, MSG and MSG + ARG on the serum Alkaline Phosphatase (ALP) activity[§]

Groups	Serum ALP activity (IU L^{-1})	Relative value (%)	Difference from control (%)	Differencefrom MSG (%)
Distilled water	87.37±0.15	100.00		
MSG alone	24.49±0.14**	28.03*	-71.97*	
MSG plus ARG	8.33±0.11*	9.53*	-90.47*	-18.50**

[§]The results are Mean±SEM for four rats in each group. *Significantly different from control ($p<0.05$). **Significantly different from MSG-treated rats ($p<0.05$). #From Egbuonu *et al.* (2009b)

Table 2: The effect of distilled water, MSG and MSG + ARG on the serum Total Acid Phosphatase (TAP) activity[§]

Groups	Serum TAP activity (IU L^{-1})	Relative value (%)	Difference from control (%)	Differencefrom MSG (%)
Distilled water	0.57±0.01	100.00		
MSG alone	36.04±0.06*	6322.80*	+6222.80*	
MSG plus ARG	63.95±0.03*	11219.29*	+11119.29*	+4896.49**

[§]The results are Mean±SEM for four rats in each group. *Significantly different from control ($p<0.05$). **Significantly different from MSG-treated rats ($p<0.05$)

Table 3: The effect of distilled water, MSG and MSG + ARG on the serum Aspartate Aminotransferase (AST) activity[§]

Groups	Serum AST activity (IU L^{-1})	Relative value (%)	Difference from control (%)	Differencefrom MSG (%)
Distilled water	13.40±0.20	100.00		
MSG alone	22.36±0.15**	166.86*	+66.86*	
MSG plus ARG	31.27±0.23*	233.35*	+133.35*	+66.49**

[§]The results are Mean±SEM for four rats in each group. *Significantly different from control ($p<0.05$). **Significantly different from MSG-treated rats ($p<0.05$). #From Egbuonu *et al.* (2009b)

Table 4: The effect of distilled water, MSG and MSG + ARG on the serum Aspartate Aminotransferase (AST)-to-Alanine Aminotransferase (ALT) ratio

Groups	Serum AST-to-ALT activity (IU L^{-1})	Relative value (%)	Difference from control (%)	Difference from MSG (%)
Distilled water	0.182±0.00	100.00		
MSG alone	0.28±0.00*#	156.59*	+56.59*	
MSG plus ARG	0.45±0.00*	247.25*	+147.25*	+90.66**

§The results are Mean±SEM for four rats in each group. *Significantly different from control ($p < 0.05$). **Significantly different from MSG-treated rats ($p < 0.05$). #From Egbuonu *et al.* (2009b)

MSG by 56.59% compared to control ($p < 0.05$). In addition, rats co-treated with MSG and ARG markedly potentiated the MSG-induced increase in the serum AST-to-ALT ratio by 90.66% (Table 4).

DISCUSSION

The serum ALP activity was observed to decrease in the rats that were fed MSG and in those that were fed ARG with MSG. The observed significant reduction of the serum ALP activity by MSG may imply the absence of obstructive liver diseases at the tested dose with ARG enhancing the MSG-induced effect. This is because an increased serum ALP activity has been associated with obstructive liver diseases (Rodwell and Kennelly, 2003). Thus, it may be advisable to use MSG or ARG together with MSG when treating obstructive liver diseases. The observation in the present study that co-treating ARG with MSG enhanced the MSG-induced effect on the serum ALP activity by 18.5% may be an indication of possible synergistic interactive effect of ARG with MSG on serum ALP activity or synthesis and possible benefit.

The effects of the various treatments on the serum TAP activity of the male albino rats were compared. The result revealed an increased serum TAP activity in rats that were fed MSG alone and in those that were fed MSG and ARG together. These results seem to indicate that the use of MSG either alone or in combination with ARG may have adverse effect on the prostate, since the serum TAP activity has been used to assess metastatic carcinoma of the prostate (Stuart, 1996; Rodwell and Kennelly, 2003). Co-treatment with ARG enhanced the MSG-induced effect by 4896.49% (more than three-folds), which could be a pointer to a significant enhancement by ARG of the possible MSG-induced harm to the prostate. Thus, the intake of MSG especially together with ARG should be avoided in the cases of prostate disorders.

An increase in the serum AST activity was observed in rats that were fed MSG alone (group II) and also in rats that were fed a combination of ARG with MSG (group III). This could be predictive of damage to the organs with high metabolic activity (such as the liver, brain, heart and lungs) as previously reported by Bain (2003) or of myocardial infarction (Rodwell and Kennelly, 2003). The increased serum AST activity observed in MSG-fed rats agrees with the previous reports of Farmobi and Onyema (2006) and Onyema *et al.* (2006) that the activity of serum AST increased in male rats that were fed MSG due to MSG-induced oxidative stress. These results may, therefore, imply that the oral intake of MSG alone or in combination with ARG may be destructive to the organs with high metabolic activity, or may cause myocardial infarction, hence has to be used with caution. Co-treating MSG with ARG enhanced the effect of MSG by 66.49% suggesting that ARG possibly potentiated the MSG-induced increase in serum AST activity and the consequent harm.

Unlike the Alanine Aminotransferase (ALT) enzyme that is found mainly in the liver, aspartate aminotransferase enzyme is found in the liver as well as in the other major organs (including the heart and brain). For this reason, it is not easy to associate any observed increase in the serum AST activity with possible damage to any of these organs where AST

enzyme could be found. Hence, the calculation of AST-to-ALT ratio may be of biochemical /diagnostic importance in that the result could help to solve the above problem. This is because value of AST-to-ALT ratio greater than one could be suggestive of possible damage to other major organs (and not the liver) being responsible for the increase in the serum AST activity whereas value of AST-to-ALT ratio equal to or less than one could suggest that an increase in the serum AST activity may be as a result of damage to the liver (and not the other major organs).

In the present study, an increased serum AST-to-ALT ratio was observed in rats that were fed MSG alone and in those that were fed MSG with ARG. However, MSG with ARG treatment in the albino rats increased the serum AST-to-ALT ratio relative to MSG treatment by over one fold (90.66%). The observations indicate possible cirrhosis (hardening) of the major organs with ARG co-treatment possibly potentiating the MSG-induced effect. Furthermore, since the serum AST-to-ALT ratio (as calculated in the present study) was less than one, it is more likely that the increase in the serum AST activity observed in the present study was as a result of possible damage to the liver (and not the other major organs). The possible liver damage may not include obstructive liver diseases as the possible absence of obstructive liver diseases was implied (Rodwell and Kennelly, 2003) from the decreased serum ALP activity observed in the present study. Thus, the implication of the liver as the damaged organ leading to the increased serum AST activity observed in the present study, as adduced from the calculated AST-to-ALT value of less than one, appears irreconcilable with the possible absence of obstructive liver diseases as indicated by the decreased serum ALP activity, also, observed in the present study. We interpreted this to be proof of the onset of liver damage without significant ailments due to obstructed liver. Nevertheless, these findings could be indicating that the use of MSG, especially in combination with ARG, may be harmful to the liver, hence should be used with caution. This is so because the continued use of MSG either alone or together with ARG may damage the liver and increase the possible risk of developing metabolic syndrome (including Non Insulin Dependent Diabetes Mellitus (NIDDM)) since Hanley *et al.* (2005) had associated an increased liver marker (and consequent liver damage) with the development of metabolic syndrome.

The results showed that MSG at a dose of 5 mg kg^{-1} b.wt. probably affected the synthesis of the above enzymes and that ARG at 20 mg kg^{-1} b.wt. potentiated the MSG-induced effects. Thus, we concluded that ARG at 20 mg kg^{-1} b.wt. may significantly exacerbate the possible MSG-induced adverse effect on the prostate and major organs with high metabolic activities, especially the liver. Therefore, caution should be exercised in the use of MSG together with ARG at the tested dose in the treatment of the diseases of either the prostate or the liver.

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