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The Effect of Aspartame Administration on Oncogene and Suppressor Gene Expressions

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Abstract. *Background: Aspartame (L-phenylalanine N-L-α-aspartyl-1-methyl ester) is an artificial sweetener with widespread applications. Previously published results have shown that among rats receiving aspartame a significant increase of lymphoreticular neoplasms, brain tumours and transitional cell tumours occurred. The aim of our short-term experiment was to investigate the biological effect of aspartame consumption by determining the expressions of key oncogenes and a tumour suppressor gene. Materials and Methods: After one week per os administration of various doses of aspartame to CBA/CA female mice, p53, c-myc, Ha-ras gene expression alterations were determined in individual organs. Results: The results showed an increase in gene expressions concerning all the investigated genes especially in organs with a high proliferation rate: lymphoreticular organs, bone-marrow and kidney. Conclusion: Aspartame has a biological effect even at the recommended daily maximum dose.*

Aspartame (L-phenylalanine N-L-α-aspartyl-1-methyl ester) an artificial sweetener widely used all over the world since 1981, has already exceeded its original diabetological application fields. Aspartame is classified as a general purpose sweetener by the FDA and is approved for use in all foods and beverages; it is approved for use in more than 100 countries and is the sweetening ingredient in 6,000 food and beverage products (1). Two thousand tons of aspartame per year is consumed by 375 million people in Europe (2). A study carried out on the American population between 1984 and 1992, showed that the average daily intake of aspartame is 2-3 mg/bodyweight kg in adults and 2.5-5 mg/kg bodyweight in children because of the lower bodyweight (3, 4). Although these amounts are far from the recommended maximum daily intake, the rapidly widening arsenal of foods containing aspartame means that the recommended maximum daily intake [40 mg/kg bodyweight in Europe and 50 mg/kg bodyweight in the United States (4)] is approachable by simultaneous consumption of food products containing aspartame.

Aspartame is a dipeptide methyl ester, built up of a phenylalanine molecule, an aspartate molecule and a methyl group esterified to the carbonic acid group of the phenylalanine. In the upper part of the small intestine, methanol is released by hydrolysis of the methyl ester, by pancreatic chymotrypsin. This is immediately absorbed in the small intestine (5). In the tissues, methanol is oxidised by alcohol dehydrogenase to formaldehyde and through further oxidation catalysed by formaldehyde dehydrogenase to formate. In this transformation, formaldehyde is a highly reactive intermediate product with 1.5 minute half-life (6). Trocho *et al.* came to the conclusion that methanol developing from aspartame plays a role in adduct formation caused by formaldehyde, resulting in DNA-protein crosslink formation (7).

Sofritti *et al.* have published the results from an experiment on the effects of aspartame consumption, that involved the largest number of animals in long-term experiments on this subject so far. Among rats receiving aspartame, the occurrence of neoplasms of the nervous system, transitional cells of the renal pelvis and ureters and the lymphoreticular system was significantly more frequent compared to the controls (8).

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Table I. *Gene expression alterations in individual organs after aspartame consumption in treated and control groups (as percentage of β-actin).*

	c-myc gene expression (%)				*Ha-ras* gene expression (%)				*p53* gene expression (%)			
	Control	Aspartame (mg/kg bodyweight)			Control	Aspartame (mg/kg bodyweight)			Control	Aspartame (mg/kg bodyweight)		
Organ		40	200	2500		40	200	2500		40	200	2500
Lymph nodes	16.8	65.6	76.1	69.2	8.4	73.2	85.7	88.5	20	82.2	100	87.2
Bone-marrow	13	42.3	16.4	53.4	22.1	42.5	27	68.3	45.4	61.8	48.1	92.9
Thymus	40.4	61.4	82.8	54.1	29.7	82.8	87.8	43.9	66.8	82.2	100	76.1
Kidney	52.7	63.3	74.5	60.6	45.3	82.4	92.2	52.9	76.4	100	100	70.4
Liver	51.6	58.2	59.3	35.8	59.9	72.1	73.5	58.2	63.0	72.5	82.9	74.9
Spleen	64.7	34.1	81.9	35.9	76.6	41.5	100	58.6	93	84.7	100	79.3
Lung	68.2	8.6	90.3	49.3	79.1	21.4	99.2	36	93.1	35.6	100	64

The determination of the alterations in oncogene and tumour suppressor gene expressions has been suggested to be usefull for the detection of carcinogen exposure and identification of early stages of carcinogenesis (9).

The aim of our experiment was to answer the question whether aspartame consumption results in biological effects on several organs. A detectable biological effect would suggest the possibility of a potential carcinogenic effect. In this short-term experiment we determined gene expression alterations concerning key oncogenes and a suppressor gene expression in the investigated organs. This is the first investigation on the biological effects of aspartame *via* measurements of gene expression.

Materials and Methods

Five-week-old female inbred CBA/CA mice were given oral doses of water solutions of 99% pure technical aspartame powder (at doses of 40, 200 and 2500 mg/kg bodyweight, Asp-Phe-OMe Fluka BioChemika, Sigma-Aldrich, Buchs, France). For one week aspartame solutions were administered *per os* periodically for 12 hours; mice were given tap water *ad libitum* for the following 12 hours. Controls received only tap water with the same drinking conditions as the treated groups. Overall we had four groups, six animals in each group. Mice were separated in standard cages and maintained under conventional conditions.

All the groups were autopsied after one week, then bone-marrow, liver, spleen, thymus, lymph nodes, lung and kidneys were removed and 100 mg samples of each tissue were pooled separately according to groups, so we had one pooled sample per group. After homogenization of the organs, total cellular RNA was isolated using TRIZOL reagent (Invitrogen, Paisley, Scotland, UK). The concentration and quality of the RNA was checked by absorption measurement at 260/280 nm. RNA of each sample (10 μg) was dot-blotted onto Hybond N+ nitrocellulose membranes and hybridized with chemiluminescently-labelled (ECL kit, Amersham, Little Chalfont, England) *c-myc, p53* and *Ha-ras* (Prof. J. Szeberényi, University of Pécs, Hungary) gene-specific probes. The RNA isolation, hybridization and detection were performed according to the manufacturer's instructions. The signals were detected on X-ray films. The dots were evaluated by Quantiscan software (Biosoft, Cambridge, UK). We used beta-actin activity as an endogenous control. This gene shows permanent expression and its activity is considered as 1 unit or 100%. We compared the investigated genes to beta-actin and their expressions were expressed as percentages of beta-actin activity.

Results

Our results showed considerable differences in the key oncogene and tumour suppressor gene expressions between treated and control groups (Table I). A considerable increase was detected in the expressions of *Ha-ras* and *c-myc* oncogenes and *p53* tumour suppressor gene in all treated groups (all doses) in lymphoreticular, bone-marrow and kidney tissues (Figure 1). In the liver, spleen and lung the increases in gene expression were remarkable only at 200 mg/kg bodyweight dose due to the high gene expression levels of the control group.

We were not able to demonstrate a dose-dependent pattern. In the 2500 mg/kg bodyweight treated group an osmotic diarrhoea occurred due to the high osmotic concentration of the aspartame solution. It is likely that the inadequate absorption of aspartame resulted in lower gene expressions at the high dose level.

Discussion

The carcinogenic effect of aspartame is currently under debate. Trocho *et al.* (7) have labelled the carbon atom of aspartame involved in the methanol formation with ^{14}C isotope. The labelled aspartame was given orally to rats at 200 mg/kg bodyweight dose. They came to the conclusion that methanol developing from aspartame plays a role in the adduct formation of formaldehyde, and thus results in DNA-protein crosslink formation. The carbon atom of the aspartame methyl group does not partake in the biosynthesis of nucleotide bases mediated by the S-adenosyl-methionine transport system. In addition, the

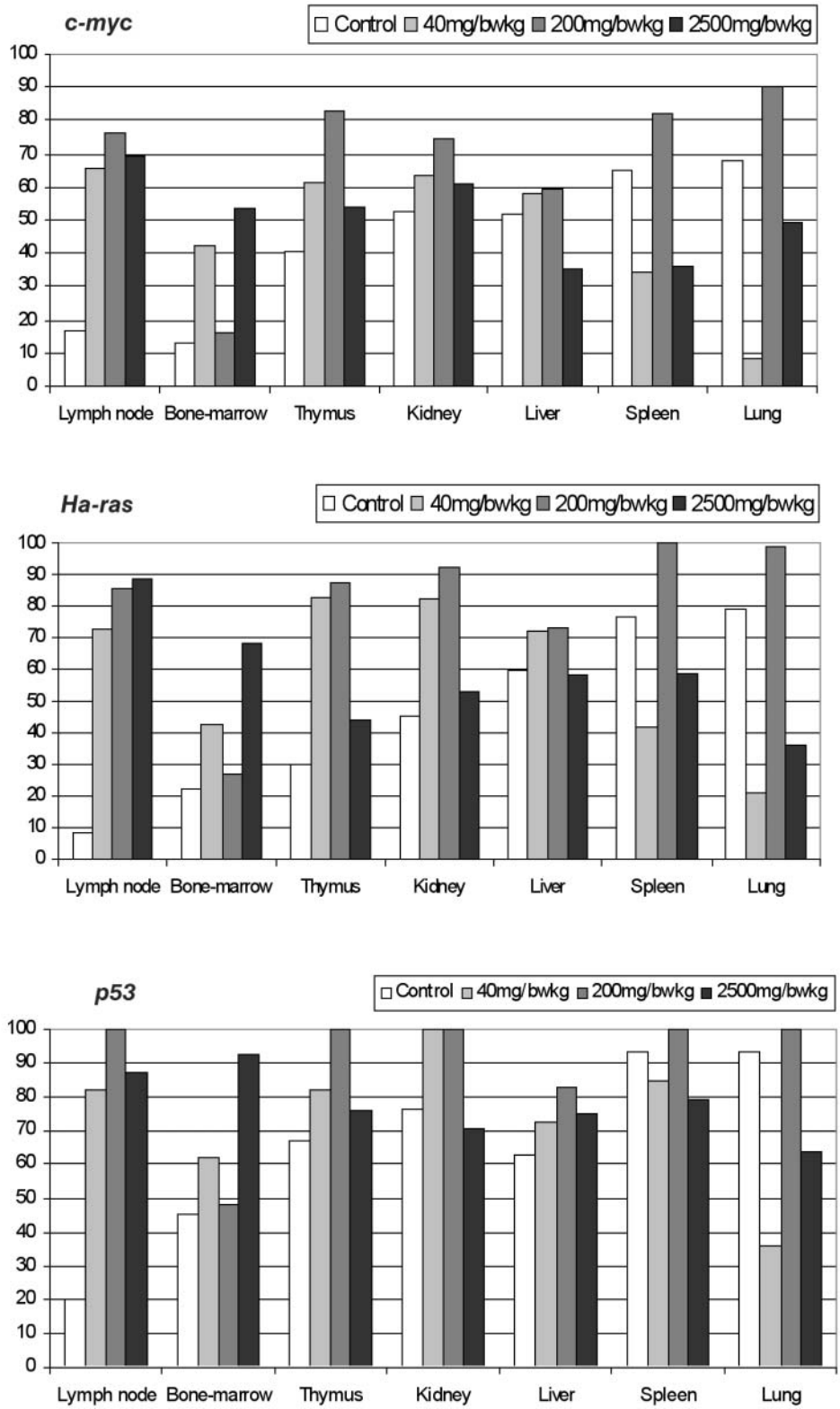

Figure 1. *Comparative figure of c-myc, Ha-ras and p53 gene expression alterations in all ivestigated organs after aspartame treatment (the arbitrary unit is gene expression % of β-actin).*

higher radioactivity detected in rats receiving aspartame for longer periods suggests the possibility of progressive accumulation of intermediate products developing during regular aspartame consumption (7).

Aspartame has not been found to be genotoxic in the following tests: dominant lethal mutation assay in rats, host-mediated assay in rats and mice, *in vivo* cytogenetic assay in rats, and the Ames test (10). Results of an assay to measure induction of unscheduled DNA synthesis in rat hepatocytes treated with aspartame *in vitro* were negative indicating the absence of aspartame-induced DNA damage (11). Mukhopadhyay *et al.* reported that a mixture of aspartame and acesulfame potassium (up to 150 mg/kg bodyweight) was negative in a test for the induction of chromosomal aberrations in bone-marrow cells of male Swiss mice (12). However, a dose related increase in the percentage of cells with chromosomal aberrations was noted with increasing doses of the two sweeteners, even though the increase was not statistically significant. Results published by Soffritti *et al.* also called attention to the possible carcinogenic effect of aspartame (8). Using methods of molecular epidemiology, we aimed to answer the question whether aspartame consumption results in a detectable biological effect. In our short-term animal experiment, we detected remarkable alterations in the expression of key oncogenes and a tumour suppressor gene – a proven method adapted for the recognition of carcinogene exposure developed in our Institute – in tissues with a high proliferation rate (lymphoreticular organs, bone-marrow and kidney). In our investigations, we detected a biological effect of aspartame, supporting the possibility of carcinogenic impact. We observed considerable gene expression alterations in similar organs which had shown significantly increased occurrence of malignancies in the long-term feeding carcinogenicity bioassay on aspartame consumption (8). Soffritti *et al.* noticed significant increase of tumour occurrence concerning principally the central nerve system, haemo- and lymphoproliferative system, renal pelvis and ureter (8).

In our investigation, it was demonstrated that aspartame has a detectable dose-dependent biological effect.

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