Saudi Journal of Biological Sciences (2016) **23**, 503–506

King Saud University

Saudi Journal of Biological Sciences

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ORIGINAL ARTICLE

Investigation of role of aspartame on apoptosis process in HeLa cells

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Received 25 December 2014; revised 26 May 2015; accepted 3 June 2015
Available online 15 June 2015

KEYWORDS

Aspartame;
HeLa;
SRB;
mRNA;
p53

Abstract Aspartame is an artificial sweetener used as an alternate for sugar in several foods and beverages. The study reports that consumption of aspartame containing product could lead to cancer. However, the effect of aspartame on apoptosis process in cancer is not yet understood clearly. HeLa cells were exposed to different concentrations (0.01–0.05 mg/ml) of aspartame for 48 h. Cytotoxicity of aspartame on cancer cells was determined by SRB assay. The result indicates no significant changes on cell viability. Aspartame suppresses apoptosis process in cancer cells by down-regulation of mRNA expression of tumor suppressor gene p53, and pro-apoptotic gene bax. It up-regulates anti-apoptotic gene bcl-2 mRNA expression. In addition, Ki 67 and PCNA mRNA, and protein expressions were determined. Taking all these together, we conclude that aspartame may be a potent substance to slow-down the apoptosis process in HeLa cells. Further works are ongoing to understand the biochemical and molecular mechanism of aspartame in cancer cells.

1. Introduction

Aspartame is widely used as an artificial sweetener in several foods (Magnuson et al., 2007). It is commonly found in low calorie beverages and desserts (Oyama et al., 2002). Chemically, it is a dipeptide of aspartyl-phenylalanine methyl ester which facilitates its intestinal hydrolysis and absorption of amino acids (aspartic acid and phenylalanine) together with free methanol (Lipton et al., 1991). Aspartic acid and phenylalanine are commonly found in natural proteins, which are beneficial under normal circumstances (Woodrow, 1984). Consumption of aspartame has been linked with several neurological and behavioral disturbances (Humphries et al., 2008). However, most of the results yielded inconclusive correlations.

Aspartame is distributed under several trade names and approved by the FDA. However, the safety of aspartame was renewed by a report suggesting that an increase in the number of people with brain tumors might be associated with the introduction and use of this sweetener in the USA. A study reports that lymphomas and leukemias were induced in rats

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2015.06.001
1319-562X

fed with very high doses of aspartame (Soffritti et al., 2005). Saccharin is another artificial sweetener which increases incidence of urinary bladder cancer at high doses in male rats. The aim of the present study was to investigate the short-term effect of aspartame on apoptosis in cancer cells.

2. Materials and methods

2.1. Materials

HeLa cells were obtained from American Type Culture Collection. DMEM and MTT were provided by Sigma–Aldrich Chemical (St Louis, Mo). Aspartame was provided by Supelco. Fetal bovine serum (FBS), penicillin–streptomycin and trypsin–EDTA were purchased from Gibco. Primers of p53, bax, bcl-2, Ki 67 and PCNA were purchased from Macro Gen (South Korea). Primary antibodies were purchased from Bioscience Technology (Seoul, South Korea).

2.2. Methods

2.2.1. SRB assay for cell viability

The effect of aspartame on HeLa cells was measured by the SRB assay (Vichai and Kirtikara, 2006). HeLa cells were seeded at a density of 2.2×10^4 cells/well into 96-well plates and allowed to adhere for 24 h at 37 °C. Then the cells were treated with aspartame at different concentrations (0.01–0.05 mg/ml) for 48 h. At the end of the treatments, cells were fixed with acetone and air dried. After fully dried, each well was added with 100% of SRB solution (0.4% w/v) and incubated for 3 h at room temperature. Microplate was washed with 1% of acetic acid and then dried under drying oven. Stained HeLa cells were photographed. Finally, 10 mm of Tris-base was added and kept overnight. Following complete dissolution of SRB in Tris-base, the absorbance was measured at 540 nm.

2.2.2. qPCR

HeLa cells were cultured in T75 flask and treated with aspartame (0.01–0.03 mg/ml) for 48 h. Total RNA was isolated from the control and aspartame treated samples (Chomczynski and Mackey, 1995). The first-strand c-DNA was synthesized from 1 μg of the total RNA using the M-MLV reverse transcriptase with the anchored oligo $d(T)_{12–18}$ primer. qPCR was performed using a cDNA equivalent of 10 ng of total RNA from each sample with primers specific for p53, bax, bcl-2, Ki 67, PCNA and a housekeeping gene GAPDH. The reaction was carried out in 10 μl using SYBR Green Master Mix (Bioneer) according to the manufacturers' instructions. Relative ratios were calculated based on the $2^{-\Delta\Delta}_{CT}$ method (Pfaffl, 2001). PCR was monitored using the CFX96™ Real-Time System (Bio-Rad).

2.2.3. Western blot analysis

HeLa cells were cultured in T75 flask and treated with aspartame (0.03 mg/ml) for 48 h. Control and aspartame treated HeLa cells were washed three times with ice-cold PBS and lysed with 10 mM Tris–HCl (pH 7.5), 100 mM NaCl, 1% NP-40, 50 mM NaF, 2 mM EDTA (pH 8.0), 1 mM PMSF, 10 μg/ml leupeptin, 10 μg/ml aprotinin. Equal amounts of lysate protein samples were run on SDS polyacrylamide gel, and then transferred onto a PVDF membrane. Non-specific binding was blocked by soaking the membrane in Tris buffered saline-Tween (TBST) buffer that contained 5% nonfat dry milk for 1 h. The membrane was probed overnight with an antibody against Ki 67 and PCNA. Following washing with TBST buffer, the membrane was incubated for 1 h with horseradish peroxidase conjugated goat anti-rabbit IgG. The

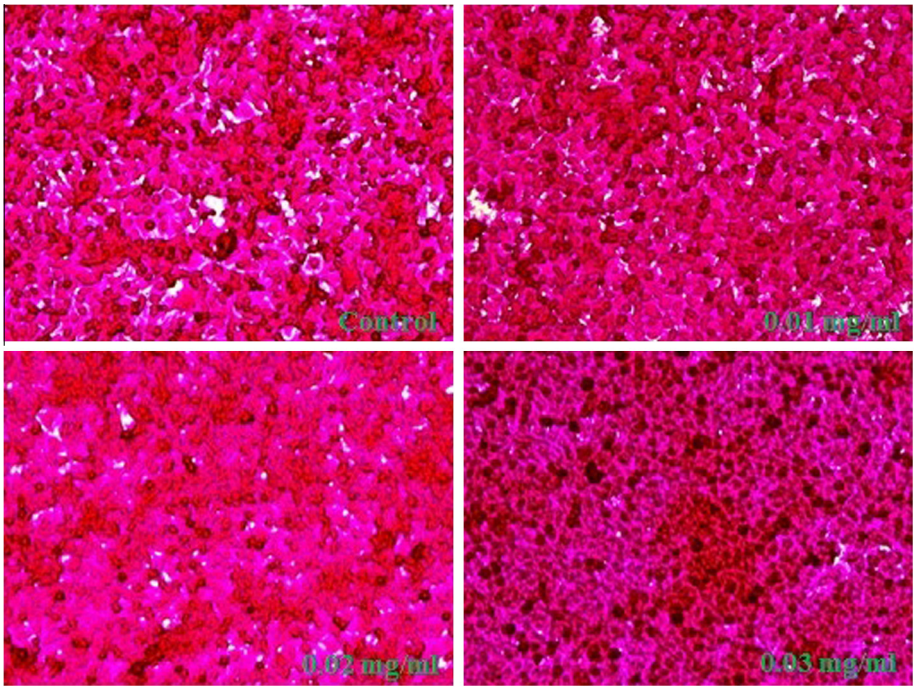

Figure 1 Effects of various concentrations of aspartame on cell viability. HeLa cells were seeded at seeding densities of 2×10^4 cells/ml into 96 well micro plates and allowed to adhere for 24 h and treated with 0.01–0.05 mg/ml of aspartame for 48 h.