IN VITRO ANALYSIS OF TARTRAZINE GENOTOXICITY AND CYTOTOXICITY

Anja Haverić[1*], Damira Inajetović[2], Aneta Vareškić[2], Maida Hadžić[1], Sanin Haverić[1]

[1]*University of Sarajevo, Institute for Genetic Engineering and Biotechnology (INGEB), Sarajevo, B&H*
[2]*University of Sarajevo, Faculty of Science, Sarajevo, Bosnia and Herzegovina*

***Correspondence**
E-mail: anja.haveric@ingeb.unsa.ba

Received
January, 2017
Accepted
April, 2017
Published
June, 2017

Copyright: ©2017 Genetics & Applications, The Official Publication of the Institute for Genetic Engineering and Biotechnology, University of Sarajevo

Research article

Abstract

Tartrazine (E 102) is widely used yellow food colorant. It is used in nonalcoholic and sports drinks, spicy chips, jams, jelly and chewing gum and also found in many non-food products like soaps, cosmetics, shampoo, vitamins and some drugs. Tartrazine belongs to the most important and diverse group of synthetic dyes – azo dyes. Their use often creates controversies in the public since some of them are toxic, carcinogenic, mutagenic and cause different disorders or allergic reactions. In this study we aimed to evaluate genotoxic potential of tartrazine in human lymphocytes culture and its cytotoxic potential in human lymphocytes and melanoma GR-M cell line. For testing of its genotoxic and cytotoxic potential in human lymphocyte culture, we used chromosome aberration analysis and cytokinesis-block micronucleus cytome assay. For the analysis of its cytotoxic potential in human melanoma cell culture, we applied trypan blue exclusion assay.

Key words: *chromosome aberrations, micronuclei, trypan blue, human cell culture*

Introduction

Synthetic food dyes are extensively used in many aspects of human life. The beginning of synthetic dyes production dates back to the middle of the 19th century and the use of natural dyes is completely overshadowed. Implementation of new analyses in the 20th century revealed significant negative and harmful impacts of certain food dyes on human health thus inducing restrictions on their use. Aside from their negative, mutagenic and carcinogenic, impact on health, synthetic dyes from household or industry also have negative impact on the environment even at very low concentrations (Forgacs et al, 2004; Hosseini et al., 2011). Establishing of appropriate mechanisms for the detection and monitoring of food dyes negative effects on human health is essential to define adequate protection and prevention measures.

Although exacerbating effects of tartrazin in chronic urticaria and asthma sufferers are long known (Lockey, 1977), tartrazine (E 102) is widely used yellow food colorant (Mittal et al., 2007, Saxena and Sharma, 2015). Tartrazine is used in nonalcoholic and sports drinks, spicy chips, jams, jelly and chewing gum, bakery goods, cereals, candies, gelatin and numerous other commodities (Saxena and Sharma, 2015). It is also found in non-food products like soaps,

cosmetics, shampoos, vitamin supplements and some drugs (Amin et al., 2010). In many countries tartrazine is used in catering as an alternative to saffron (Mehedi et al., 2009). Tartrazine belongs to the most important and diverse group of synthetic dyes – azo dyes, which includes about 3000 different compounds. They are widely used and distributed in the environment due to their convenient and inexpensive synthesis, stability and wide range of shades in comparison to natural dyes (Saratale et al., 2011). Use of azo dyes often causes controversies in the public since some of them are toxic, carcinogenic, mutagenic and cause different disorders (Saxena and Sharma, 2015) or allergic reactions in the organism (Bhatia, 2000; Ardren and Ram, 2001; Bourrier, 2006). Studies have also linked the ingestion of dyes (mostly azo dyes) in candies and drinks with hyperactivity and other disruptive behavior in children (McCann et al., 2007).

In this study we aimed to evaluate genotoxic potential of tartrazine in human lymphocytes culture and its cytotoxic potential in human lymphocytes and melanoma GR-M cell line.

Materials and methods

Lymphocyte culture

Peripheral blood was collected into heparinized vacutainers from a healthy female volunteer who signed informed consent form. Lymphocyte cultures were induced in 15-mL sterile, plastic tubes with conical base (Isolab GmbH, Wertheim Germany), which contained 5 mL of PB-MAX Karyotyping Medium (GIBCO-Life Technologies, Grand Island, NY, USA) and 400 µl of peripheral blood. Cultures were harvested after 72 hours of lymphocytes cultivation at 37ºC, using standard procedure (hypotonic treatment with 0,075M KCl, triple ethanol-acetic acid fixation, followed by microscope slide preparations and staining in 5% Giemsa).

Tested substance

Tartrazine (E-102) was dissolved in dH2O and added to the cultures in the 25th hour of cultivation to the final concentrations of 2.5, 5 and 10 mM. The concentrations were determined according to literature (Mpountoukas et al., 2010). Negative controls with equivalent volume of dH2O and positive controls with 0.25 µg/mL of mytomicine C (Sigma-Aldrich Co, St Louis, MO, USA) were set up as well.

Cytokinesis-block micronucleus cytome (CBMN Cyt) assay

The analysis of genotoxic, cytotoxic and cytostatic potential of tartrazine was evaluated in human lymphocyte cultures by applying cytokinesis-block micronucleus cytome assay in vitro. In order to block cytokinesis, 4.5 µg/ mL of cytochalasin B (Sigma-Aldrich Co., St Louis, MO, USA) was added to the cultures in the 45th hour of cultivation. Microscopic analysis at 400x magnification using Olympus BX51 microscope (Tokyo, Japan) included observation of 2000 binuclear cells per each treatment and controls, equally divided among two replicates (OECD, 2014). The frequencies of genotoxicity markers: micronuclei, nucleoplasmic bridges and nuclear buds, were determined according to the defined criteria (Fenech, 2007; Fenech et al., 2003). Cytotoxic and cytostatic effects were assessed by calculating nuclear division index (NDI) and nuclear division cytoxicity index (NDCI) (Fenech, 2000). The frequencies of mononuclear, binuclear, trinuclear, and quadrinuclear cells, as well as apoptotic and necrotic cells, were registered in the total number of at least 1000 cells, counted per each treatment and controls, equally divided among two replicates.

Chromosome aberration assay

For the chromosome aberration analysis, lymphocytes were arrested in metaphase by addition of colcemid (0.18 μg/ml), 90 min before cell harvesting. 300 metaphases (46±1 chromosome) were analyzed per treatment and controls, equally divided among three replicates (OECD, 1997) at 1000× magnification. Structural chromosome aberrations were scored and registered according to the International System for Human Cytogenetic Nomenclature - ISCN (Mitelman, 1995).

Melanoma cell line

Human GR-M melanoma cell line (Culture Collections, Public Health England, London, UK, Cat. No. 95032301) was cultured in RPMI 1640 (Gibco-Invitrogen, Grand Island, NY) supplemented with L-glutamine, 10% of fetal bovine serum (FBS) and penicillin/streptomycin (Sigma-Aldrich, St. Louis, MO) in T-25 flasks (NUNC, Rochester, NY) at 37˚C, 5%CO2 atmosphere with 95% humidity. At the beginning of experiment cells were seeded at a density of 1×105 cells, pre-incubated for 24 h and finally incubated with the selected concentrations of tartrazine for another 48 h.

Trypan blue exclusion assay

For the cytotoxicity analysis trypan blue exclusion assay was performed after 48h of incubation of melanoma cell cultures with tartrazine. Each treatment was carried out in triplicate. Cells were harvested by trypsinization and cell viability (%) was determined as [number of viable cells / (number of viable + non-viable cells)] x 100.

Statistical analysis

Differences in observed frequencies of genotoxic and cytotoxic parameters were calculated using proportion comparison Z-test in WINKS 4.5 Professional Software (TexaSoft, Cedar Hill, TX). Statistical significance threshold was set at 0.05.

Results and discussion

Results of tartrazin genotoxicity analysis, obtained in human lymphocytes cultures and GR-M melanoma cell line appling CBMN Cyt assay, chromosome aberration assay and trypan blue exclusion assay are presented in tables 1-3. Frequencies of genotoxicity biomarkers in CBMN Cyt assay incrased with the increase of tartrazin concentration (Table 1, Figure 1).

Table 1. Results of CBMN Cyt assay in human lymphocyte culture

Treatment	Genotoxicity biomarkers			Cytostasis/cytotoxicity	
	MN	NB	NPB	NDI	NDCI
Negative control	2	0.5	0	1.768	1.752
2.5 mM	1[b]	0[b]	1.5[b]	1.816	1.303
5 mM	2[b]	0.5[b]	6.5[a]	1.584	1.573
10 mM	8[b]	1	8[a]	1.586	1.574
Positive control	65.5	6	8.5	1.308	1.303

Note: MN – micronuclei; **NB** – nuclear buds; **NPB** – nucleoplasmic bridges. Genotoxicity analized on 1000 BN cells per culture, 2 replicate cultures per treatment; Cytostasis/cytotoxicity analized on 500 cells per culture, 2 replicates per treatment. (a) Significantly different against negative control; (b) Significantly different against positive control.

However, comparison with negative controls revealed no significant differences in MN and NB frequencies. In concentrations of 5 mM and 10 mM of tartrazine, significant increase in

frequency of NPB against negative control was detected (p<0.5 for 5 mM; p=0.005 for 10 mM).

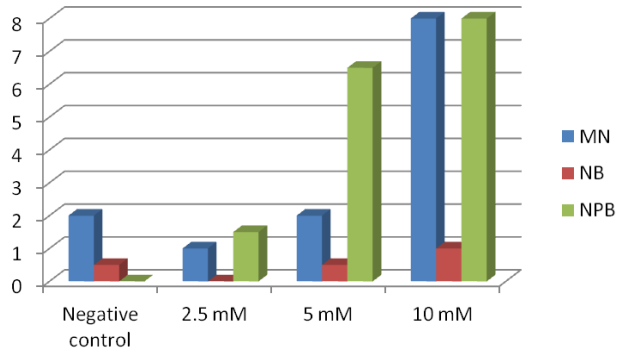

Figure 1. Average frequencies of genotoxicity markers in CBMN Cyt assay expressed per 1000 BN cells in human lymphocyte cultures (negative controls + treatments)

Comparison against positive control were significant for MN frequencies in all three tested concentrations (p=0.0), for NB frequencies in 2.5 mM (p=0.01) and 5 mM (p=0.03) concentrations and for NPB in the lowest tested concentration of 2.5 mM (p=0.026). Neither tartrazine nor mytomicin C induced significant differences in nuclear division indexes (NDI and NDCI) against negative control although nuclear division indexes were decreased in the highest tested concentrations and positive control (Table 1, Figure 2). However, cytotoxic effects of mytomicin C, an antitumor antibiotic, are confirmed in several oral squamous cell lines (HSC-2, HSC-3, HSC-4, Ca9-22 and NA) and human promyelocytic leukemic cell line HL-60 (Sasaki et al., 2006).

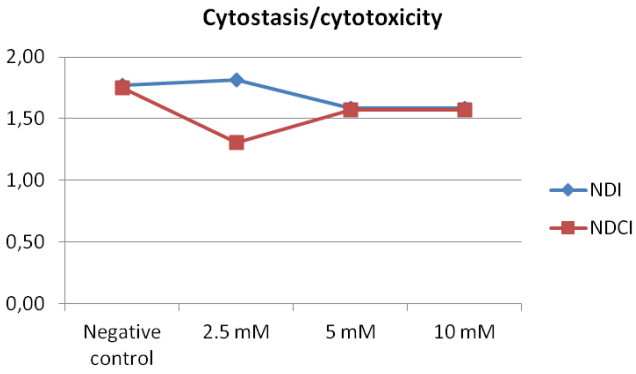

Figure 2. NDI and NDCI values calculated using average frequencies of analyzed cells in cytokinesis blocked human lymphocyte cultures, negative controls and treatments

Proportion comparisson of observed chromosome aberrations revealed the lack of significant differences between each of three tested concentrations and negative control while positive control significantly differed for all tested concentration in chromatid- (breaks and minute fragments) and chromosome-type aberrations (breaks, double minutes) and chromosome rearrengements that were calculated separately (p=0.0). Incidence of pulverzations (pvz) and premature centromere separations (pcs) did not differ between each of three treatments or when compared with positive or negative control (p>0.05).

Previously published data regarding tartrazine genotoxicity are contradictory. Bastaki et al. (2017) dismissed tartrazine genotoxicity in vivo, while Khayyat et al. (2017) reported tartrazine

Table 2. Results of chromosome aberration analysis in human lymphocyte culture

Treatment	chtb, min	chrb, ace, dmin	chre	pvz	pcs
Negative control	0	0	0	0	0
2,5 mM	1	1	0	0	0
5 mM	1	1	0	0	0
10 mM	0.666	1.333	0	0	0.666
Positive control	21	74	24	1	0.333

potencial to induce structural and functional aberrations and genotoxic effects in vivo. Atli-Sekeroglu et al. (2017) also found that tartrazine and its metabolites have genotoxic potential on human lymphocyte cultures both with and without metabolic activation (S9) and can induce cytotoxic effects without S9 and in the highest of concentrations tested (2500 µg/ml). Tartrazin is shown to induce DNA-damage in the gastrointestinal organs at low doses, even at doses approaching the acceptable daily intake (Sasaki et al., 2002) of 7.5 mg/kg/bw/day, which is significant since many products, such as ice creams, desserts, cakes are often marketed without labeling (Elhkim et al., 2007). Study of cytotoxic and genotoxic effects of tartrazine on DNA repair in human lymphocytes demonstrated no cytotoxic effects but revealed significant genotoxic effects in all tested concentrations ranging from 0.25-64.0 mM (Soares et al., 2015), that are far below the concentrations tested in our study (2.5; 5 and 10 mM).

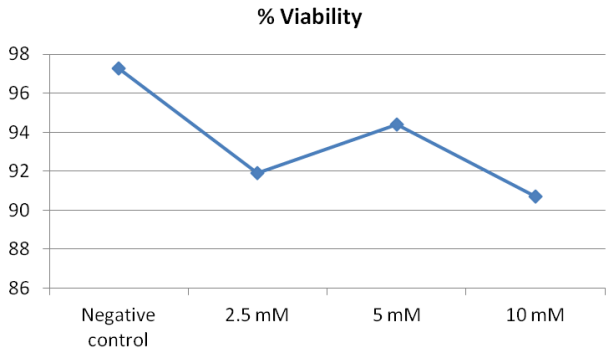

Figure 3. Cytotoxicity analysis using Trypan blue assay in human melanoma GR-M culture

Trypan blue exclusion assay was performed in GR-M human melanoma cell culture in order to estimate tartrazine cytotoxicity. The decrease in cell viability (%) was obvious and significant against negative control (p=0.0), contrasting negligible cytotoxic effects recorded in human lymphocytes culture by CBMN Cyt assay.

Table 3. Results of CBMN Cyt assay in human lymphocyte culture

	Viable	Nonviable	Viability %
Negative control	277	7.75	97.28
2.5 mM	122	10.75	91.90[a]
5 mM	130.5	7.75	94.394[a]
10 mM	124.25	12.75	90.693[a]

Note: (a) Significantly different against negative control.

Overall results suggest that concerns regarding tartrazine use are not unfounded, especially regarding cytotoxicity of tartrazine that should be further evaluated.

Conclusions

Obtained results and conducted statistical analysis show that tartrazine is not genotoxic in human lymphocytes in tested concentrations. Its cytotoxic effects are negligible in human lymphocyte culture but significant in GR-M melanoma cell culture. This finding suggests that future studies of tartrazine should be focused on evaluation of cytotoxic effects using human lymphocytes but also additional cell models, cytotoxicity assays in comparison against cytotoxic effects of other cytotoxicity inducers.

References

Amin KA, Abdel Hameid II H, Abd Elsttar AH (2010) Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. Food Chem Toxicol 48:2994-2999.

Ardern KD, Ram FS (2001) Tartrazine exclusion for allergic asthma. Cochrane Database Syst Rev 4: CD000460.

Atli-Sekeroglu Z, Gunes B, Kontas Yedier S, Sekeroglu V, Aydin B (2017) Effects of tartrazine

on proliferation and genetic damage in human lymphocytes. Toxicol Mech Methods 27(5): 370-375.

Bastaki M, Farrell T, Bhusari S, Pant K, Kulkarni R (2017) Lack of genotoxicity in vivo for food color additive Tartrazine. Food Chem Toxicol 105:278-284.

Bhatia M.S (2000) Allergy to tartrazine in psychotropic drugs. J Clin Psychiatry 61:473-476.

Bourrier T (2006) Intolerance and allergy to colorants and additives. Revue Française D'allergologie et D'immunologie Clinique, 46: 68-79. DOI: 10.1016/j.allerg.2005.12.002.

Elhkim M, Heraud F, Bemrah N, Gauchard F, Lorino T, Lambre C, Fremy JM., Poul JM (2007) New considerations regarding the risk assessment on tartrazine An updated toxicological assessment, intolerance reactions and maximum theoretical daily intake in France. Regul Toxicol Pharmacol 47:308-316.

Fenech M (2000) The in vitro micronucleus technique. Mutat Res 455:81-95.

Fenech M, Chang WP, Kirsch-Volders M, Holland N, Bonassi S, Zeiger E (2003) HUMN project: detailed description of the scoring criteria for the cytokinesis–block micronucleus assay using isolated human lymphocyte cultures. Mutat Res 534:65-75.

Fenech M (2007) Cytokinesis-block micronucleus cytome assay. Nat Protoc 2:1084- 1104.

Forgacs E, Cserhati T, Oros G (2004) Removal of synthetic dyes from wastewaters: a review. Environ Int 30(7):953–971.

Hosseini KE, Alavi Moghaddam MR, Hashemi SH (2011) Post-treatment of anaerobically degraded azo dye Acid Red 18 using aerobic moving bed biofilm process: Enhanced removal of aromatic amines. J Hazar Mater 195:147–154.

Khayyat L, Essawy A, Sorour J, Soffar A (2017) Tartrazine induces structural and functional aberrations and genotoxic effects in vivo . PeerJ 5:e3041; DOI 10.7717/peerj.3041.

Lockey SD Sr. (1977) Hypersensitivity to tartrazine (FD&C Yellow No. 5) and other dyes and additives present in foods and pharmaceutical products. Ann Allergy 38(3):206-10.

McCann D, Barrett A, Cooper A, Crumpler D, Dalen L, Grimshaw K, Kitchin E, Lok K, Porteous L, Prince E, Sonuga-Barke E, Warner JO, Stevenson J (2007) Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomised, double-blinded, placebo-controlled trial. The Lancet 370:1560-1567.

Mehedi N, Ainad-Tabet S, Mokrane N, Addou S, Zaoui C, Kheroua O, Saidi D (2009) Reproductive toxicology of tartrazine (FD and C Yellow No. 5) in Swiss albino mice. Am J Pharmacol Toxicol 4(4):128–133.

Mitelman F (1995) ISCN - An International System for Human Cytogenetic Nomenclature. Cytogenetics and Cell genetics, S. Karger, Basel.

Mittal A, Kurup L, Mittal J (2007) Freundlich and langmuir adsorption isotherms and kinetics for the removal of tartrazine from aqueous solutions using hen feathers. J Hazar Mater 146(1–2):243–248.

Mpountoukas P, Pantazaki A, Kostareli E, Christodoulou P, Kareli D, Poliliou S, et al. (2010) Cytogenetic evaluation and DNA interaction studies of the food colorants amaranth, erythrosine and tartrazine. Food Chem Toxicol 48(10):2934-2944.

OECD Guideline for the testing of chemicals (1997) In Vitro Mammalian Chromosome Aberration Test. Test guideline 473.

OECD Guideline for the testing of chemicals (2014) Mammalian Erythrocyte Micronucleus Test. Test guideline 474.

Saratale RG, Saratale GD, Chang JS, Govindwar SP (2011) Bacterial decolorization and degradation of azo dyes: A review. J Taiwan Inst Chem Eng 42:138–157.

Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, Taniguchi K, Tsuda S (2002) The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutat Res 519:103-119.

Sasaki M, Okamura M, Ideo A, Shimada J, Suzuki F, Ishihara M, Kikuchi H, Kanda Y, Kunii S, Sakagami H (2006) Re-evaluation of Tumor-specific Cytotoxicity of Mitomycin C, Bleomycin and Peplomycin. Anticancer Res 26:3373-3380.

Saxena B, Sharma S (2015) Food Color Induced Hepatotoxicity in Swiss Albino Rats, Rattus norvegicus. Toxicol Int 22(1):152-157.

Soares BM, Araujo TM, Ramos JA, Pinto LC, Khayat BM, De Olivieira Bahia M, Montenegro RC, Burbano RM, Khayat AS (2015) Effect on DNA repair in human lymphocytes exposed to the food dye tartrazine yellow. Anticancer Res 35(3):1465-1474.