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The modifying effect of selenium and vitamins A, C, and E on the genotoxicity induced by sunset yellow in male mice

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ABSTRACT

The use of food additives in various products is growing up. It has attracted the attention towards the possible correlation between the mutagenic potential of food additives and various human diseases. This work evaluated the protective role of selenium and vitamins A, C and E (selenium ACE)[1] against the genotoxic effects induced by a synthetic food additive, sunset yellow, in mice. Six groups were studied including two control groups (negative and positive control), two groups are given single dose of sunset yellow (either 0.325, 0.65 or 1.3 mg/kg body weight[2] alone or with selenium ACE) and two groups are given sunset yellow daily for 1, 2 or 3 weeks (0.325 mg/kg b.wt./day alone or with selenium ACE), respectively. The study examined the induction of sister chromatid exchanges (SCE's)[3] in bone-marrow cells, chromosomal aberration in somatic (bone-marrow) and germ cells (spermatocytes) after single and repeated oral treatment, and the induction of morphological sperm abnormalities. The results showed that sunset yellow had genotoxic effects as indicated by increased frequency of SCE's, by chromosomal aberrations in both somatic and germ cells, and by increased morphological sperm abnormalities and DNA fragmentation. The results also indicated that the oral administration of selenium ACE significantly reduced the genotoxic effects of sunset yellow, a result that may support the use of antioxidants as chemopreventive agents in many applications.

1. Introduction

Natural or synthetic food additives have been used as coloring, curing, and/or sweetening agents in many food stuffs. Recently, food additives have attracted the attention as potential causes of various human diseases; food additives might be among the factors responsible for the outbreak of cancer, hepatic and nephritic failures, and have mutagenic potential [1–3].

Sunset yellow (FD & C Yellow No. 6) is a synthetic coloring dye belonging to azo compounds. It is formed from arenediazonium ions reacting with highly reactive aromatic hydrocarbon compounds. The dye is isolated as dried sodium salt [4], and may be added to orange squash, orange jelly, marzipan, Swiss roll, apricot jam, citrus marmalade, lemon curd, sweets, hot chocolate mix, packet soups, snack chips, trifle mix, breadcrumbs, cheese sauce mix and soft drinks. The acceptable daily intake (ADI)[4] of sunset yellow for man is 0–1 mg/kg b.wt. [5].

Several studies suggested that certain types of azo dyes, including sunset yellow, exhibit mutagenic effect [6–9]. The toxicity and carcinogenicity of sunset yellow in mammalian system may result either via interactions of intact molecules with cytosolic receptors [10–12] or via the formation of free radicals and arylamines azoreduction [13,14]. Reactive oxygen species (ROS) such as hydroxyl radicals ($^{\bullet}OH$), superoxide anion radicals ($O_2^{\bullet -}$), and hydrogen peroxide (H_2O_2) are produced during normal metabolism or as a consequence of response to abnormal stress. They are implicated in the pathogenesis of aging and diseases, including cancer. Mammalian cells are equipped with both enzymatic and non-enzymatic

Abbreviations: Selenium ACE, selenium and vitamins A, C and E; mg/kg b.wt., mg/kg body weight; ADI, acceptable daily intake; SCE's, sister chromatid exchanges; CP, cyclophosphamide; M.A., more than one aberration; R.T., Robertsonian translocation.

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[1] Selenium and vitamins A, C and E, selenium ACE.
[2] mg/kg body weight (mg/kg b.wt.).
[3] Sister chromatid exchanges (SCE's).
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[4] Acceptable daily intake, ADI.

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antioxidant mechanisms to minimize cellular damage resulting from ROS [15]. It has been reported that a combination of the supplemental antioxidants selenium ACE can protect cells against genetic damage, cancer development, mycotoxin and radiation induced oxidative damage [16–18].

Selenium, an essential trace element, has been suggested to protect against a number of diseases including cancer [17,19,20]. An important metabolic role of selenium in mammalian species is its presence in the active site of the selenoenzyme glutathione peroxidase. This enzyme, together with superoxide dismutase and catalase, can protect cells against damage caused by free radicals and hydroperoxides or lipoperoxides [21–23].

The identification of vitamins A, C, and E as antioxidants has shed more light onto importance of antioxidants in the biochemistry of living organisms [24,25]. These vitamins may scavenge harmful radicals or electrophiles, which damage DNA and other cell targets. They also play an important role in regulating the immune system [26–29]. Vitamin C or ascorbic acid [30], vitamin A (beta-carotene and retinol) [15], and vitamin E (mostly alpha tocopherols, lipid-soluble antioxidant) [31] can all participate in the redox mechanism of the cell, and thereby neutralize ROS.

The present work evaluated the ability of sunset yellow to damage DNA, and the protective role of selenium ACE in somatic and germ cells of male mice.

2. Materials and methods

2.1. Animals and treatments

Male white Swiss mice of similar age (8–12 weeks) and weight (20–25 g) were obtained from the animal house of the National Research Centre, Cairo, Egypt. Animals were maintained under controlled conditions of temperature and humidity and received food and water *ad libitum*. The selenium ACE drug was purchased from Wassen International Ltd., England (100 mg selenium, 1.500 IU vitamin A, 90 mg vitamin C and 30 mg vitamin E). Sunset yellow, an azo dye with the formula $C_{16}H_{10}N_2Na_2O_7S_2$, a molecular weight of 452.37 g/mol and CAS Registry Number 2783-94-0, was purchased from the local market (96% product of Kamena Industries, Canada).

The mutagenic effect of sunset yellow was estimated in mice receiving sunset yellow alone (either the single or repeated doses) and the possible protective role of selenium ACE was studied in mice treated as previously mentioned. Mice were divided into control, single and repeated dose groups. Control group is divided into negative control (did not receive any treatment and was maintained under the same living conditions of temperature and humidity) and positive control (intraperitoneally injected with 20 mg/kg b.wt. of cyclophosphamide (CP)[5] dissolved in distilled water), samples were taken 24 h after treatment in all experiments or after 5 consecutive days of treatment for sperm abnormalities experiment. Single dose treated groups received sunset yellow corresponding to either 1×, 2×, or 4×-ADI, (0.325, 0.65 and 1.3 mg/kg b.wt., respectively) or the same treatment with the therapeutic dose of selenium ACE (50 mg/kg b.wt.). The sunset yellow dose was dissolved in distilled water and was orally administered to the mice. Repeated dose treated groups are used to study the impact of repeated oral administration of sunset yellow. Mice received successive daily dose (0.325 mg/kg b.wt.) of sunset yellow for 1, 2 or 3 weeks or the same treatment with selenium ACE (50 mg/kg b.wt.), respectively.

In all cases, samples were collected 24 h following the last treatment. Samples were tested for the induction of SCE's, chromosomal aberrations in bone-marrow cells and in spermatocytes, sperm-shape abnormalities and DNA fragmentation.

2.2. Cytogenetic experiments

2.2.1. SCE's in bone-marrow (somatic cells)

SCE'S in bone-marrow cells was tested according to the method of Allen [32], with some modifications [33]. 5-Bromodeoxyuridine powder (55 ± 5 mg, Sigma) was pressed to form BrdU tablet, and these were stored frozen in the dark. Tablets were implanted inside a subcutaneous pocket; the wound was then closed with 2–3 sutures taking care not to break the tablet. All mice were orally administered the prescribed dose 30 min after the implantation of BrdU tablet. All animals were injected i.p. with 0.1 ml of 40 mg% of colchicine for 20–22 h following BrdU treatment. Bone marrow cells were harvested 2 h later. Animals were sacrificed by cervical dislocation and both femurs were dissected; bone marrow was obtained by injecting 0.072 M KCl solution into one end of the femur. Bone marrow cells were routinely processed by the standard procedure and slides were stained according to Goto et al. [34]. At least 25 well spread metaphases were analyzed for each mouse.

2.2.2. Chromosomal aberrations analysis in both bone-marrow (somatic cells) and spermatocytes (germ cells)

In order to arrest cell division, cholchicine (2 mg/kg b.wt.) was injected intraperitoneally 2 h before the animals were sacrificed by cervical dislocation. Preparation of mitotic and meiotic chromosomes were done according to the method of Yosida and Amano [35] and Evans et al. [36], respectively. The chromosomes were stained by Giemsa stain and 75 well spread metaphases were analyzed per mouse. Metaphases with both structural and numerical chromosomal aberrations were recorded and 100 well spread diakinesis-metaphase 1, were examined for each animal.

2.2.3. Sperm-shape abnormalities

The protocol recommended by Wyrobek and Bruce [37] was followed. Each group of mice was treated orally with a daily dose of the tested materials for 5 consecutive days. Animals were sacrificed 35 days after the 1st treatment. Sperms were sampled from the caudae epididymis. Sperm preparations were examined by light microscopy at 1000× magnification. For each dose, about 5000 sperms were assessed for morphological abnormalities of the sperm shape.

2.2.4. DNA fragmentation assay

Agarose gel electrophoresis was used for separating and analyzing DNA. DNA extracts were separated on agarose gel according to Leonard et al. [38] as modified by Chen et al. [39]. Nucleic acids were extracted from liver tissue using SS-phenol/chloroform. Concentration was determined spectrophotometerically.

2.3. Statistical analysis

Student's *t*-test was applied for the statistical analysis in all experiments according to Ronald et al. [40]. Significant means that $P < 0.05$ and highly significant means $P < 0.01$. The resulting data between groups were compared using Fisher's least significance differences and Duncan's multiple range test [41].

3. Results

Table 1 shows the effect of a single-dose of sunset yellow on the induction of SCE's in bone-marrow cells. The effect of CP indicated that the frequency of SCE's/cell had reached 34.55 ± 1.46 compared to 2.4 ± 1.05 for the non-treated animals. The difference was highly significant ($P < 0.01$). The sunset yellow induced statistically significant increases in the frequency of SCE's over that of the control. The increased frequency reached 5.44 ± 1.62, 7.65 ± 0.58 and 8.44 ± 0.7 after oral administration of 0.325, 0.65 and 1.3 mg/kg b.wt., respectively. Single, double and triple SCE's were observed in all cases of treated doses (Table 1).

Table 2 shows the chromosomal aberrations induced by sunset yellow with and/or without selenium ACE on bone-marrow cells. Structural chromosomal aberrations included chromatid and/or chromosome gap, fragment and/or break, chromatid deletion and Robertsonian translocation. CP induced chromosomal aberrations in mouse bone marrow (35.2 ± 0.7 compared to 2.4 ± 0.2 for the control). The difference was highly significant ($P < 0.01$). The results also demonstrated that the difference remained highly significant ($P < 0.01$) even after excluding the number of metaphases with gaps (28.5 ± 0.31 versus 1.3 ± 0.2). Sunset yellow, at all the tested concentrations, induced dose-dependent increases in the percentage of chromosomal aberrations. The highest percentage increase (24.26 ± 0.2) was observed after oral administration of the highest tested dose. The difference in induced aberrations was statistically significant ($P < 0.01$) at all tested doses even after excluding gaps (Table 2). The results also showed that selenium ACE alone did not induce significant increases in the percentage of chromosomal aberrations (2.66 ± 0.31 versus 2.4 ± 0.2 for the control). The administration of selenium ACE with sunset yellow significantly decreased the percentage of chromosomal aberration ($P < 0.01$) in all the tested doses comparing with the sunset yellow treated mice. Table 2 also shows that both structural (chromatid and/or chromosome gap, fragment and/or break, deletions) and numerical aberrations (aneuploidy, polyploidy) were recorded.

[5] Cyclophosphamide (CP).