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Genotoxicity testing of four food preservatives and their combinations in the *Drosophila* wing spot test

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Abstract

In this study, four food preservatives (sodium nitrate, sodium nitrite, potassium nitrate and potassium nitrite) and there five combinations at a concentration of 25 mM have been evaluated for genotoxicity in the somatic mutation and recombination test (SMART) of *Drosophila melanogaster*. Three-day-old larvae trans-heterozygous including two linked recessive wing hair mutations (multiple wing hairs and flare) were fed at different concentrations of the test compounds (25, 50, 75 and 100 mM) in standard Drosophila Instant Medium. Wings of the emerging adult flies were scored for the presence of spots of mutant cells, which can result from either somatic mutation or mitotic recombination. Also lethal doses of food preservatives used were determined in the experiments. A positive correlation was observed between total mutations and the number of wings having mutation. In addition, the observed mutations in each wing were classified according to the size and type of the mutation. For the evaluation of genotoxic effects, the frequencies of spots per wing in the treated series were compared to the control group, which is distilled water. Chemicals used were ranked as sodium nitrite, potassium nitrite, sodium nitrate and potassium nitrate according to their genotoxic and toxic effects. Moreover, the genotoxic and toxic effects produced by the combined treatments were considerably increased, especially when the four chemicals were mixed. The present study shows that correct administration of food preservatives/additives may have a significant effect on human health.

Keywords: *Drosophila melanogaster*; Somatic mutation and recombination test; Food preservatives; Genetic toxicology

1. Introduction

Food additives have been increasingly used in the food industry as a result of production technologies. These chemicals are mainly used for diverse preferential taste of consumers and also for their protective effects from food contaminants. In fact, when the food additives are given to organisms in excessive amounts, they may cause toxic reactions. For example, nitrite–nitrate containing foods may react with endogen amines, forming carcinogenic and mutagenic (Ertuğrul, 1998).

It is well documented that certain types of foods and beverages for human consumption may pose toxic, genotoxic or carcinogenic hazards (Aeschbacher, 1990; Wakabayashi, 1990). The sources of these hazards can be divided into four categories. First, certain food additives may have harmful effects (IARC, 1983). Secondly, food staffs may be contaminated either by environmental pollutants or by microbial toxins (Williams, 1986). Thirdly, the processing of food (e.g. cooking, broiling, smoking, pickling, etc.) may produce carcinogenic compounds (Sugimura et al., 1986). Fourthly, certain natural constituents foods are also known to possess mutagenic and/or carcinogenic potential (Ames, 1986).

There are thousands of additives used by the food industry for a variety of purposes. However, only a small number have been implicated in causing adverse reactions in humans. Although there are reported cases of individuals who have reactions to single additives, most of the medical literature involves patients with asthma or chronic idio-

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pathic urticaria/angioedema whose conditions are exacerbated after ingestion of food additives. Many of these reports are characterized by poorly controlled challenge procedures. Recent studies performed under properly controlled conditions imply that sensitivity to food additives in patients with chronic urticaria/angioedema is very uncommon (Simon, 2003).

Since nitrite has various disadvantages and technological difficulties when added directly, it is more suitable add as a curing salt. The maximum amount of nitrite, which can be added in meat product, is defined as 150 ppm in the Food Additives Regulation, but the maximum amount of nitrite present in the final products must be 50 ppm. In case the amount of nitrite used is more than the above figures, it causes excess amounts of free nitrite and results in the formation of nitrosamines. On the other hand, the usage of nitrite in small amounts results insufficient reactions and various disadvantages, such as inadequate coloring or insufficient antimicrobial, effects in the final product. For example, nitrite prevents the growth of *Clostridium botulinum* and the formation of toxins (Simon, 2003). Nitrosocompounds formed by the interaction of nitrites and secondary amines are neurotoxic in human and different rodent species. Human exposures of nitrosocompounds are widespread affected by different modes like nitrite–nitrate preserved foods, beverages like beer, formed in the stomach following uptake of the precursors nitrates, nitrites and secondary amines. The productions of alkylating metabolites during the breakdown of nitrosocompounds are the causative agents for the neurotoxic changes of the neural cells (Mukherjee et al., 2004).

Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) may cause tumor in lungs and liver. Sulphate and its salts may cause sulphite allergy and saccarin may cause bladder cancer, though not fully proved yet. The diseases that occur due to canthaxanthin is accumulation of chemicals in retina in the form of crystals, erythrosine causes thyroid disorders, amaranth causes the damage by urticaria and chromosome, tartrazine causes hypersensitive reactions like exema, mygren and asthma and beta carotene causes hypercarotinemia (Ertuğrul, 1998).

There are several advantages that make the fruit fly *Drosophila melanogaster* the species of choice for developing fast and reliable assay systems for detection of chemicals with genotoxic activity. The main points are: it is a eukaryotic organism with a short generation time (approximately 10 days at 25 °C); it has easily detectable genetically controlled morphological characters; large numbers of mutants and genetically characterized strains are available; culture media are inexpensive and allow the breeding of large numbers of animals using simple facilities and it is capable of activating enzymatically promutagens and procarcinogens in vivo.

The extensive knowledge of the genetics of *D. melanogaster* and the long experimental experience with this organism has made it of unique usefulness in mutation research and genetic toxicology. The development of somatic mutation and recombination tests (SMART) has provided sensitive, rapid and cheap assays for investigations of mutagenic and recombinogenic properties of chemicals. It is a versatile short-term in vivo assay that not only detects different kinds of mutational events but also allows the detection of mitotic recombination; being the quantitation of the recombinagenic activity of a compound of primary importance for genotoxicity screening. The use of two genetic markers, multiple wing hair (*mwh*) and flare (*flr*) in the third chromosome, makes it possible to discern localized recombinogenic effects on the two intervals the major, euchromatic, part of the chromosome, and the mostly heterochromatic centromere region. The distribution of induced mitotic recombination varied between test chemicals. Many of the enzyme activities found in mammals can be demonstrated in *Drosophila* adults especially the larvae, which also contain cytochrome P-450 and cytochrome oxidase systems (Ramel and Magnusson, 1992).

As cell genetics show, wing spot test assays detect several genetic endpoints. Genetic changes induced in somatic cells of the wing's imaginal discs leads to the formation of mutant clones on the wing blade. Single spots are produced by somatic point mutation, deletion, etc. and mitotic recombination occurring between the two markers. Twin spots are produced exclusively by mitotic recombination occurring between the proximal marker *flr* and the centromere of chromosome 3. To determine the recombinagenic activity, the frequency of *mwh* clones on the marker-heterozygous wings (*mwh* single spots plus *mwh* clones of twin spots) is compared with the frequency of *mwh* clones on the balancer-heterozygous wings. The difference in *mwh* clone frequency is a direct measure of the proportion of recombination (Frei et al., 1992).

The SMART assays are not only useful to analyze single pure compounds for genotoxic activity, but also to investigate genotoxicity of complex mixtures of various origins. The aim of this study was to assess the possible genotoxicity of four food preservatives (sodium nitrate, sodium nitrite, potassium nitrate and potassium nitrite) and their five combinations in *Drosophila* wing spot test. Since these preservatives are widely used, there is a need for more data on mutagenity in order to assess their potential hazards to human health.

2. Materials and methods

2.1. Chemicals

Four food preservatives and their five combinations were tested. Sodium nitrate (CAS No: 7631-99-4, 99% purity), sodium nitrite (CAS No. 7632-00-0, 99.5% purity), potassium nitrate (CAS No. 7757-79-1, 99% purity) and potassium nitrite (CAS No. 7758-09-0, 96% purity) were obtained from Sigma (St. Louis, MO, USA). The all chemicals, which are used in the experiment, were dissolved in distilled water to obtain the required concentrations.