Genotoxic Evaluation of Furfuryl Alcohol and 2-Furyl Methyl Ketone by Sister Chromatid Exchange (SCE) Analysis

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Genotoxicity of furfuryl alcohol and 2-furyl methyl ketone is evaluated by sister chromatid exchange analysis (SCEs) in mouse bone marrow system using standard technique. Three doses of the compounds ranging from 1000–4000 ppm were given by oral gauvage to experimental animals for one day in single dose series and for five consecutive days in multiple dose series to simulate a human situation. 5-Bromodeoxyuridine (5-Brdu) was intraperitoneally injected at hourly intervals for 6 hr at a concentration of 0.04 mg/g body weight. In both single and multiple dose series the animals were exposed to 5-Brdu for 26 hr and were sacrificed after 12, 24 and 48 hr following last feeding. Control animals received distilled water and same concentration of Brdu. Bone marrow slides were prepared and stained as per standard procedure. 100 metaphase spreads in second mitotic division were scored from each dose and period and subjected to statistical analysis. There was significant induction of SCEs in both single and multiple dose series with both the compounds. It was found to be dose dependant and the period dependancy showed a decline after 24 hr.

Key words —— genotoxicity, furfuryl alcohol, 2-furyl methyl ketone, 5-Bromodeoxyuridine, sister chromatid exchange analysis

INTRODUCTION

Processed and unprocessed food consumed by human beings contain many mutagenic and carcinogenic substances. It is estimated that 1–2 g of potentially mutagenic substances are consumed by us every day from foods and beverages alone.[1,2] Furans are volatile chemicals with a number of derivatives known to be present in cooked or thermally processed foods. They are formed by the oxidation of polyunsaturated fatty acids at high temperature and by the decomposition of ascorbic acid derivatives. They are also widely used in the manufacture of plastics, agrochemical dyes,[3] corrosion resistant polymers, high temperature laminates, in the formation of foundry moulds and as solvents in textile industry.[4,5] Furfuryl alcohol is also used in the manufacture of abrasive wheels and as a liquid propellant.[6]

Furfuryl alcohol is commercially obtained by the catalytic hydrogenation of furfural while acetylation of furan gives 2-furyl methyl ketone. In both these furans, electrophilic substitution takes place at fifth position of the furan ring. Furan is proved to be a rodent carcinogen.[7] An increased clastogenic activity is reported with furan, furfural, 5-methyl furfural and furfuryl alcohol in Chinese Hamster Ovary (CHO) cells in presence of *S9* mix.[2] Furfuryl alcohol was found to be a negative mutagen in germ cells of Drosophila melanogaster.[8] Earlier studies with furfuryl alcohol and 2-furyl methyl ketone in mouse system in our laboratory have shown that they are mild clastogens.[9–11] Cytotoxic studies have shown that they cause liver damage and induce stress proteins. Since Sister chromatid exchange (SCE) analysis is considered to be more elegant and sensitive indicator to detect the action of many mutagens at molecular level, the present study is carried out from the bone marrow system of mouse after exposure to furfuryl alcohol and 2-furyl methyl ketone.

MATERIALS AND METHODS

Animals —— Eight to ten weeks old pure bred Swiss Albino female mice (average body weight 25 g) were obtained from M/S Biological Evans, Hyderabad, India. They were maintained in an animal house attached to our laboratory under the animal maintenance guidelines from the Ministry of Social Justice and Empowerment, Government of

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India. The temperature of the room was controlled to 24 ± 1°C and the relative humidity to 45–50%. They were fed on standard pelleted diet (Gold mohur, manufactured and marketed by M/S Lipton Ltd. Mumbai, (India)) and germ free water *ad libitum* in clean bottles. After their acclimatisation, they were treated with the test compounds for different durations.

Dose Selection —— Three doses selected for the present study ranged from 1000 to 4000 ppm *i.e.*, 1000, 2000, and 4000 ppm for furfuryl alcohol (99% pure, Oxoid Ltd, Basingstoke, Hampshire, U.K.) and 1000, 2000, and 3000 ppm for 2-furyl methyl ketone (99% pure, Fluke, AG). The highest dose selected for 2-furyl methyl ketone was 3000 ppm since 4000 ppm was found to be toxic to animals. This dose selection was based on the clastogenic doses reported for furans in food which ranged from 100 to 3900 ppm[12] and three minimum doses are recommended for such clastogenic analysis.[13] The desired concentrations of the above compounds were prepared by mixing them in sterile double distilled water. As furans are found in a wide variety of food products, these compounds were given to the animals by oral guavage in 0.5 ml volume with the help of an improvised oral tube. In single dose series, the animals received the test compound only once, whereas in multiple dose series, they received the same concentration for five consecutive days at 24 hr intervals to simulate a human situation.

SCE Analysis —— 5-Bromodeoxyuridine (5-Brdu, Sigma Chemical Co., St. Louis, MO, U.S.A.) was given by six intraperitonial injections at hourly intervals at a concentration of 0.04 mg/g body weight.[14] In both single and multiple dose series the animals were exposed to 5-Brdu for 26 hr and sacrificed after 12, 24 and 48 hr of last treatment. The design of Brdu treatment for 26 hr was to treat the cells for approximately two cell cycles.[15, 16] Control animals received the same concentrations of Brdu and double distilled water under identical experimental conditions. Two animals per dose per period were used.

Slide Preparation —— Bone marrow slides were prepared as per the standard procedure and kept in dark for three to five days for reliable differentiation of chromatids and processed for differential staining.[17] This procedure make use of the flourescent dye, Hoechst 33258 and exposure of slides to UV light followed by Giemsa staining. One hundred well differentiated second metaphases were scored per dose per period from control and treated animals.

Statistical Analysis —— Data were subjected to statistical analysis by following the Student's "t" test. Significance was calculated at 1% and 5% level and given at respective places.

RESULTS AND DISCUSSION

Table 1 depicts the occurance of SCEs after single and multiple dose exposure to furfuryl alcohol. In single dose series there was a significant induction of SCEs with 2000 ppm and 4000 ppm at all periods and with 1000 ppm at 24 hr. In multiple dose series, the significant induction of SCEs could be attributed even to 1000 ppm after 12 and 24 hr. There was a dose dependant increase in both series and the period response showed a decline after 24 hr.

Similarly when the animals were treated with 2-furyl methyl ketone for one day the induction of SCEs was significant with the two higher concentrations after 12 and 48 hr exposure and with all the doses after 24 hr. In multiple dose exposure significance could be attributed to all the concentrations at all periods tested (Table 2). There was a gradual increase in the frequency of SCEs from lower to higher concentrations and the period response showed an increase from 12 to 24 hr followed by a decline.

Inspite of their wide occurrence and industrial applications, informations available on the toxic potentials of these furans are scanty. Furans in general are found to be effective after metabolic activation. They produce reactive electrophilic intermediates which react with proteins, nucleic acids or other macromolecules.[18, 19] Earlier investigations with the same test system have shown that these compounds were mild clastogenic and also hepatotoxic.[10, 11] Studies with furfuryl alcohol had shown a negative response to SCEs in human lymphocytes *in vitro*.[20]

Though these furans failed to produce a significant increase in chromosome aberrations except with the highest doses,[10, 11] the present investigations showed an increased incidence of SCEs after single and multiple exposures. They were also found to be dose dependant as in the case of many chemicals tested. It is well known that the mechanism of formation of SCEs and chromosome aberrations are different[21] yet the efficiency of former protocol to detect changes at DNA level is highly

Table 1. Sister Chromatid Exchanges Induced by Furfuryl Alcohol

			Single dose series			Multiple dose series		
Period (in hr)	Dose (in 1000 ppm)	No. of meta phases scored	No. of SCEs Scored	SCEs/cell (Mean ± SE)	t-values	No. of SCEs Scored	SCEs/cell (Mean ± SE)	t-values
12	C	100	173	1.73 ± 0.21	—	180	1.80 ± 0.21	—
	1	100	247	2.47 ± 0.31	2.68	240	2.40 ± 0.20	3.70[a)]
	2	100	263	2.63 ± 0.69	4.10[a)]	313	3.13 ± 0.31	6.33[b)]
	4	100	369	3.69 ± 0.19	12.02[b)]	359	3.59 ± 0.22	12.43[b)]
24	C	100	180	1.80 ± 0.20	—	175	1.75 ± 0.24	—
	1	100	253	2.53 ± 0.25	3.96[a)]	247	2.47 ± 0.31	3.77[a)]
	2	100	303	3.03 ± 0.15	8.48[b)]	336	3.36 ± 0.05	10.39[b)]
	4	100	363	3.63 ± 0.15	12.62[b)]	363	3.63 ± 0.21	16.07[b)]
48	C	100	177	1.77 ± 0.25	—	182	1.82 ± 0.18	—
	1	100	227	2.27 ± 0.25	2.50	218	2.18 ± 0.16	2.62
	2	100	243	2.43 ± 0.13	4.09[a)]	245	2.45 ± 0.09	5.48[b)]
	4	100	291	2.91 ± 0.09	7.55[b)]	307	3.07 ± 0.15	9.39[b)]

C-Control. *a*) Significant at 5% level (Expected value is 2.78). *b*) Significant at 1% level (Expected value is 4.60).

Table 2. Sister Chromatid Exchanges Induced by 2-furyl methyl ketone

			Single dose series			Multiple dose series		
Period (in hr)	Dose (in 1000 ppm)	No. of meta phases scored	No. of SCEs Scored	SCEs/cell (Mean ± SE)	t-values	No. of SCEs Scored	SCEs/cell (Mean ± SE)	t-values
12	C	100	173	1.73 ± 0.21	—	180	1.80 ± 0.21	—
	1	100	230	2.30 ± 0.30	2.71	266	2.66 ± 0.20	5.28[b)]
	2	100	287	2.87 ± 0.31	5.43[b)]	303	3.03 ± 0.15	8.48[b)]
	3	100	340	3.40 ± 0.10	12.46[b)]	388	3.88 ± 0.03	17.93[b)]
24	C	100	180	1.80 ± 0.20	—	175	1.75 ± 0.14	—
	1	100	270	2.70 ± 0.26	4.71[b)]	283	2.83 ± 0.25	3.80[a)]
	2	100	337	3.37 ± 0.15	10.82[b)]	317	3.17 ± 0.21	7.59[b)]
	3	100	383	3.83 ± 0.11	15.49[b)]	407	4.07 ± 0.15	19.66[b)]
48	C	100	177	1.77 ± 0.25	—	182	1.82 ± 0.18	—
	1	100	217	2.17 ± 0.15	2.39	257	2.57 ± 0.20	4.87[b)]
	2	100	277	2.77 ± 0.15	5.95[b)]	277	2.77 ± 0.15	7.14[b)]
	3	100	312	3.12 ± 0.10	8.71[b)]	330	3.30 ± 0.26	8.13[b)]

C-control. *a*) Significant at 5% level (Expected value is 2.78). *b*) Significant at 1% level (Expected value is 4.60).

recommended.[22–24)] There are many chemicals like mitomycin C and nitrogen mustard which produce SCEs at very low concetrations which are not necessarily associated with an increased level of chromosome aberrations.[25–27)] They occur during the DNA synthesis phase of the cell cycle but the actual DNA damage that results in SCEs could be produced by chemicals at any stage of the cell cycle.[25)] Usually they are produced either by DNA damage or by the inhibition of DNA synthesis.[28)]

On the basis of the above, the potential of these two furans to induce SCEs should be reckoned. The decline of SCEs during later periods might be due to the repair of lesions or due to the death of lesion bearing cells or due to the lack of persistence of chemicals and/or their metabolites at the target site. This study gains significance since people get exposed to these furans either through food and beverages or their workfront. This also gains importance, as these DNA lesions, if not eventually repaired, can lead to neoplasia.[25)]

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