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Influence of caffeine on arecoline-induced SCE in mouse bone-marrow cells in vivo

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Summary

The effect of exposure of mice for 5, 10 and 15 days to arecoline or/and caffeine on the frequency of sister-chromatid exchanges (SCEs) in bone-marrow cells was evaluated by using the fluorescence plus Giemsa technique. There was a significant increase in the frequency of SCEs after exposure to either arecoline or caffeine. When these two alkaloids were given in combination, the SCE frequency-enhancing effect was additive. The implications of coffee/tea drinking and betel chewing on oral cancer are discussed.

Betel-quid chewing is a common habit in oriental countries. About one-tenth of the world population is habituated to chewing betel quid (see Encyclopedia Britannica). Extensive epidemiological studies show that the high incidence of oral and oropharyngeal cancers in these countries is associated with betel-quid chewing (Jusawalla and Deshpande, 1971; Wahi, 1976; Hirayama, 1979; Sanghvi, 1981). While the betel quid is being chewed, many carcinogens and mutagens that are suspected to be released into the buccal pouch may play an important role in the process of carcinogenesis (Stich et al., 1983). Betel nut, a major ingredient of betel quid, contains different types of chemical like tannins, polysaccharides, fats and alkaloids. Arecoline, a major alkaloid of betel nut, is released into the buccal cavity in the process of chewing of the quid. Alkaloids from various plants that are consumed either as food or local medicines are reported to be carcinogenic (see Hirono, 1981) and mutagenic (Gladwin and Michael, 1977; Hirumichi et al., 1979; Hitoshi et al., 1980) in different test systems.

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Many betel chewers are exposed to another important alkaloid, caffeine, which occurs in a number of plants used in the preparation of widely consumed drinks, i.e., coffee and tea. Moreover, caffeine is used clinically in combination with other drugs. Caffeine affects DNA in many ways, but the most important one is its ability to potentiate the lethal mutagenic and chromosome-breaking effects of mutagens and carcinogens (Kihlman, 1977; Timson, 1977). Caffeine affects post-replication repair in mammalian cells (Trosko et al., 1973; Lehman and Kirk-Bell, 1974). Because the coffee/tea-drinking habit goes hand in hand with the betel-chewing habit in many oriental countries, and because these two habits are associated epidemiologically with various types of cancer, the study of the genotoxicity of arecoline in combination with caffeine is of considerable importance. Therefore, the present study was undertaken to find out the genotoxic effects of arecoline (by taking changes in the base-line frequency of sister-chromatid exchange as a parameter) and its modulation, if any, by caffeine.

Materials and methods

Inbred Swiss albino mice (Cancer Research Institute, Bombay) of both sexes and 4–6 months of age were used. The mice were maintained in an air-conditioned animal facility with standard feed (Hindustan Lever Limited, India) and water (see below) ad libitum.

Experimental design. The mice were sorted into 24 groups as depicted in Table 1. Arecoline dissolved in normal saline (5 mg/ml) was injected daily by the intraperitoneal route at the dose levels of 0.5, 1 and 2 mg for 5, 10 or 15 days. Caffeine was added to the drinking water (0.5%) given to the animals of relevant groups.

One day before the animals were killed, they were given 9 (0.4 ml) one-hourly injections of BrdU (10^{-2} M) (Sigma Chemical Co., U.S.A.). Deoxycytidine (5×10^{-3} M) (Sigma Chemical Co., U.S.A.) was added into the injections to reduce the toxicity of the BrdU. At 19 h after the BrdU injections, the animals were killed by cervival dislocation. 2 h before the killing, colchicine (Sigma Chemical Co., U.S.A.) dissolved (4 mg/ml) in distilled water was injected i.p. at the dose level of 4 mg/100 g body weight.

Long bones were excised and their epicondyle tips were removed. Marrow was expelled by aspiration with Hank's balanced salt solution through a 24-gauge hypodermic needle into a centrifuge tube. Additional balanced salt solution was added to make it 7 ml and the mixture was agitated vigorously for proper cell dissociation. By using standard procedures, slides of metaphases were prepared, aged for some days and then stained for 15 min with 100 μg of 33258 Hoechst (Germany). Slides were rinsed with distilled water and put into petri dishes having cotton soaked with citrate buffer (pH 7.0). All the petri dishes were placed under the sun for 2.5 h for excitation of fluorescent dyes. Slides were removed from the petri dishes, washed

TABLE I

FREQUENCIES OF SCEs INDUCED BY ARECOLINE AND CAFFEINE AT VARIOUS EXPOSURE INTERVALS AND DOSE LEVELS

Groups	Treatment(s)	Number of mice	Exposure time (days)	Number of metaphases scored	SCE/cell (mean ± S.E.)
1	Control	3	5	75	3.01 ± 0.177
2	Caffeine	3	5	75	3.88 ± 0.143[a]
3	0.5 mg arecoline	3	5	75	4.97 ± 0.234[a]
4	0.5 mg arecoline + caffeine	3	5	75	6.18 ± 0.296[a]
5	1 mg arecoline	2	5	45	4.66 ± 0.259[a]
6	1 mg arecoline + caffeine	2	5	50	6.72 ± 0.463[a]
7	2 mg arecoline	3	5	75	5.10 ± 0.275[a]
8	2 mg arecoline + caffeine	3	5	75	8.89 ± 0.289[a]
9	Control	3	10	75	3.21 ± 0.176
10	Caffeine	3	10	75	5.34 ± 0.148[a]
11	0.5 mg arecoline	3	10	75	5.05 ± 0.178[a]
12	0.5 mg arecoline + caffeine	3	10	75	5.50 ± 0.155[ab]
13	1 mg arecoline	3	10	75	4.95 ± 0.141[a]
14	1 mg arecoline + caffeine	3	10	74	7.31 ± 0.345[a]
15	2 mg arecoline	3	10	75	5.76 ± 0.239[a]
16	2 mg arecoline + caffeine	3	10	75	9.66 ± 0.307[a]
17	Control	3	15	75	4.10 ± 0.324
18	Caffeine	3	15	75	7.30 ± 0.214[a]
19	0.5 mg arecoline	3	15	75	4.13 ± 0.122[c]
20	0.5 mg arecoline + caffeine	3	15	75	6.28 ± 0.220[a]
21	1 mg arecoline	2	15	51	4.80 ± 0.184[c]
22	1 mg arecoline + caffeine	2	15	50	8.4 ± 0.190[a]
23	2 mg arecoline	3	15	74	5.97 ± 0.202[a]
24	2 mg arecoline + caffeine	3	15	75	10.70 ± 0.396[a]

[a] $P < 0.001$ on comparison with control values of the respective intervals.
[b] $P < 0.05$ comparison between arecoline and arecoline + caffeine.
[c] Not significant.

with distilled water and stained with Giemsa stain (5%), prepared in phosphate buffer (pH 6.8), for 20 min. Slides were air-dried, dehydrated in acetone, acetone:xylol and xylol, then mounted in DPX. About 25 differentiated metaphase plates were analysed per animal. Student's *t*-test was used for statistical analysis of the data.

Results

Table 1 depicts the effect of arecoline (Groups 3, 5, 7, 11, 13, 15, 19, 21 and 23), caffeine (Groups 2, 10 and 18) and the combination of these two (Groups 4, 6, 8,

12, 14, 16, 20, 22 and 24) on the frequency of SCEs in marrow cells of mice. The spontaneous rate of SCE (Groups 1, 9 and 17) was very low and comparable to that recorded in an earlier study in vivo (Nakanishi, 1979).

Mice killed 5 days after treatment with daily doses of 0.5, 1 and 2 mg of arecoline (Groups 3, 5 and 7, respectively) displayed significantly ($P < 0.001$) higher frequencies of SCEs in their marrow cells. Likewise, administration of caffeine (Group 2) for 5 days significantly ($P < 0.001$) increased the frequency of SCEs. Furthermore, when the caffeine was given in addition to various doses of arecoline (Groups 4, 6 and 8) for 5 days, the frequencies of SCEs increased significantly ($P < 0.001$) over the control value (Group 1) as well as the values recorded in corresponding mice treated with arecoline only (Groups 3, 5 and 7).

When the mice were put on arecoline treatment for 10 days (Groups 11, 13 and 15), the frequencies of SCEs in their marrow cells were also significantly ($P < 0.001$) higher than that seen in the corresponding control mice (Group 9). Mice exposed concomitantly to caffeine and arecoline for 10 days showed significantly higher frequencies of SCEs in comparison with the control value. However, the levels of significance differed when the damage was compared with that in the corresponding mice, treated with arecoline only. Thus, the SCEs found in Groups 14 and 16 differed at the $P < 0.001$ level from those seen, respectively, in Groups 13 and 15. But the significance level at which the SCE frequency in Group 12 differed from that in Group 11 was comparatively lower ($P < 0.05$).

The mice of Groups 19, 21 and 23 were exposed only to arecoline for 15 days.

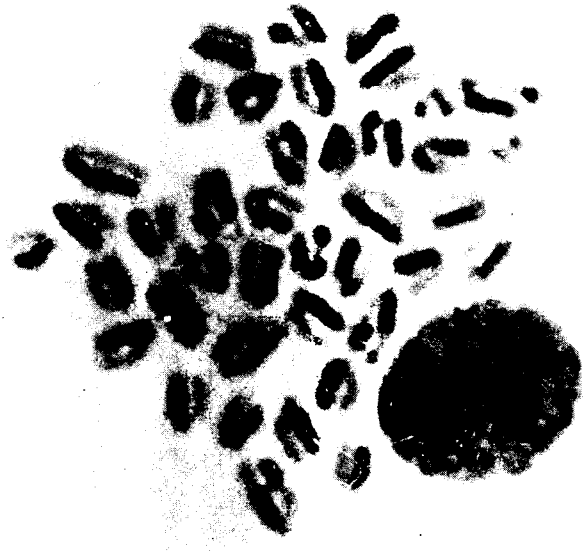

Fig. 1. Differentially stained sister chromatids showing exchanges, after intraperitoneal injections of 2 mg arecoline and 0.5% caffeine in drinking water for 15 days.

The frequency of SCEs in the mice of Groups 19 and 21 did not differ significantly from the control value (Group 17). On the other hand, the frequency of SCEs in the mice of Group 23 was significantly ($P < 0.001$) higher than that in Group 17. Concomitant treatment of animals with caffeine and arecoline for 15 days resulted in a highly significant increase ($P < 0.001$) of SCE frequencies (Fig. 1) in comparison with the values of control as well as arecoline-only treated groups of this interval.

Discussion

Our findings show conclusively that the chronic exposure of mice to arecoline results in the enhancement of SCEs over the control value. The exchanges were pronounced at the highest dose level used. Caffeine alone, at the concentration used, also induced SCEs. The frequency of SCEs increased as the days of exposure of caffeine increased. Caffeine increased rather additively the SCE frequencies induced by various doses of arecoline given for different intervals of time.

The clastogenic property of arecoline has been reported in mouse bone-marrow cells in vivo (Panigrahi and Rao, 1982) and in Chinese hamster cells in vitro (Stich et al., 1981). Arecoline gives positive results in the Ames test (Shirname and Bhide, 1982). Caffeine causes chromosomal aberrations in different test systems (Ostertag, 1966; Ostertag and Greif, 1967; Kuhlman et al., 1968; Kao and Puck, 1969). In the present study, caffeine increased the frequency of SCE which is in conformity with the findings of earlier workers (Basler et al., 1979; Vogel and Bauknecht, 1978). However, caffeine is also known not to elicit chromosomal aberrations when given alone (Adler and Rohrborn, 1969) or in combination with other mutagenic agents (Simons et al., 1977).

The carcinogenic properties of arecoline have been reported by some workers (Boyland and Nery, 1969; Nery, 1971). The cell-transformation ability of arecoline was reported by Ashby et al. (1979). Papilloma in the upper third of the oesophagus, basal cell proliferation and atypia in the oesophagus were reported by Dunham et al. (1974) after arecoline feeding together with calcium hydroxide.

In a number of epidemiological studies, coffee drinking was associated with cancer (Kihlman, 1977; McMohan et al., 1981; Trichopoulous et al., 1981). But the experiments on caffeine show that it decreased the yield of tumours (see Roberts, 1978). The present study shows that both arecoline and caffeine independently, but not synergistically, enhance the SCE frequency in the bone-marrow cells of mice. If enhanced frequency of SCEs is taken as an indication of mutagenicity/carcinogenicity of chemical substances under test, then the present study suggests that coffee/tea drinking and betel-quid chewing together might increase the risk of oral cancer in the human population. Because betel-nut chewers also drink coffee/tea in certain geographical areas of the world having higher incidences of oral cancer, it

would be worth while conducting further long-term and short-term carcinogenicity tests for arecoline and caffeine.

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