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Enhancing effect of tetrandrine on sister-chromatid exchanges induced by mitomycin C and cigarette-smoke condensate in mammalian cells

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Summary

The enhancing effect of tetrandrine, an antisilicosis, antitumor and antiinflammatory drug, on the genotoxic activity of two known mutagens, mitomycin C (MMC) and cigarette-smoke condensate (CSC), has been studied using cultured Chinese hamster lung (V79) cells. The sister-chromatid exchange (SCE) was used as genetic endpoint to measure genotoxicity. One-day cultured cells were exposed to the test chemicals for 3 h with or without metabolic activation. The results show that the frequencies of SCE induced by MMC or CSC were enhanced by tetrandrine. The percent of enhancement was dependent on the concentration of tetrandrine.

Some chemicals, such as nitrofurans (Shirai and Wang, 1980), ethinyl estradiol (Purdy and Marshall, 1984), caffeine (Faed and Mourelatos, 1978), 12-*O*-tetradecanoylphorbol 13-acetate (Dewdney and Soper, 1984) zinc acetate (Sakai et al., 1985) are known to enhance the activity of mutagens and/or carcinogens. Recently, tetrandrine, an antisilicosis, antitumor, and antiinflammatory drug (Berezhinskaya et al., 1971; Deconti et al., 1975; Kuroda et al., 1976; Li et al., 1981; Lu et al., 1983), has also been reported as possessing the genotoxic enhancing effect in *Salmonella typhimurium* (Whong et al., 1988). This drug is also a weak genotoxic agent to *S. typhimurium* and mammalian cells in vivo and in vitro with metabolic activation (Dong et al., 1982; Whong et al., 1988; Xing et al., 1988).

Because silicosis and tumors are chronic diseases, patients may concurrently be treated or exposed to different drugs or chemicals. It is important, therefore, to examine the effects of combining tetrandrine with other substances. To our knowledge, no data on the genotoxic enhancing effect of tetrandine in mammalian cells has been reported. Mitomycin C (MMC) has been used for treatment of cancer, and has been shown to be a mutagen and carcinogen in animals (Tomatis et al., 1978; Jenssen and Ramel, 1980). Cigarette-smoke condensate (CSC) could induce somatic cell and germ cell mutations that may lead to cancer, cor-

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onary artery disease and heritable defects (DeMarini, 1983). The sister-chromatid exchange (SCE) assay, which is considered to be a sensitive and less time consuming method for monitoring and screening of mutagenic and potential carcinogenic chemicals (Carrano et al., 1978; Latt et al., 1981; Abe and Sasaki 1982), was employed for the genotoxicity studies to determine the effect of tetrandrine on the genotoxicity of MMC and CSC in V79 Chinese hamster lung cells.

From two burning cigarettes, cigarette-smoke condensate was prepared by trapping smoke in cold acetone. The acetone was evaporated and the residue redissolved in 2 ml dimethyl sulfoxide (Ong et al., 1986). For SCE analysis, one-day V79 cultures were exposed to the chemicals for 3 h with (for CSC) or without (for MMC) S9 in vitro activation. After being washed two times with PBS, the cells were cultured in Eagle's minimum essential medium containing 12.5 μM BrdU for 42 h. Colcemid (0.1 μg/ml) was added 3 h before cells were harvested by mitotic shake-off (Galloway, 1985). Cells were expanded with 0.075 M KCl, fixed with methanol-acetic acid (3:1), and stained by the fluorescence-Giemsa method (Perry and Wolff, 1974).

50 cells each having 22 ± 2 well spread chromosomes were scored for SCE. The cell-cycle kinetics were determined by scoring the number of first, second and third generation metaphases in 100 consecutive metaphase cells. Two sets of experimental data were obtained. One was from the combined treatment (tetrandrine + CSC or tetrandrine + MMC), and the other from the treatment with CSC, MMC, or tetrandrine, individually. Statistical analysis was performed using the Student's *t* test to compare the net increase in the SCE frequencies induced by the combined treatment with those induced by compounds alone.

The results of the studies are shown in Tables 1 and 2. Both chemicals, MMC and CSC, induced more than a 2-fold increase in SCE frequencies. Tetrandrine alone without in vitro metabolic activation did not cause a significant increase in SCE. In the presence of in vitro metabolic activation, however, tetrandrine per se at or above the concentration of 80 μg/ml caused a significant increase in SCE (Table 1) which occurs with results reported

TABLE 1

ENHANCING EFFECT OF TETRANDRINE ON SISTER-CHROMATID EXCHANGE FREQUENCIES INDUCED BY CIGARETTE-SMOKE CONDENSATE IN CHINESE HAMSTER V79 LUNG CELLS WITH S9 ACTIVATION

Treatment	Concentration (μg/ml)	SCEs/cell ± SE	Induced SCE[a]/cell (% enhancement)	Replicative index
Cigarette-smoke condensate	20 μl[b]	13.92 ± 0.84[c]	7.34	2.25
Tetrandrine	40	7.04 ± 0.62	0.46	2.33
	80	10.68 ± 0.74[c]	4.1	1.88
Tetrandrine	20 + 20 μl	15.34 ± 0.95[c]	8.76(19)	2.09
+	40 + 20 μl	18.48 ± 1.16[c]	11.9 (53)[d]	1.91
Cigarette-smoke condensate	80 + 20 μl	23.92 ± 1.55[c]	17.34(52)[d]	1.25
Dimethyl sulfoxide (solvent control)	20 μl	6.58 ± 0.36		2.33

[a] Background SCE (solvent control value = 6.58) was subtracted.
[b] Equivalent to the amount of smoke from 1/50 cigarette.
[c] Significant ($p < 0.001$) as compared to solvent control.
[d] Significant ($p < 0.005$) as compared to the sum of SCEs induced individually by CSC and tetrandrine.

TABLE 2

ENHANCING EFFECT OF TETRANDRINE ON SCE FREQUENCIES INDUCED BY MITOMYCIN C IN CHINESE HAMSTER V79 LUNG CELLS WITHOUT S9 ACTIVATION

Treatment	Concentration (μg/ml)	SCEs/cell ± SE	Induced SCE[a]/ cell (% enhancement)	Replicative index
Mitomycin C	0.01	14.48 ± 0.76[b]	8.56	2.01
Tetrandrine	40	6.78 ± 0.62	0.86	1.96
	80	7.24 ± 1.04	1.32	1.85
Tetrandrine	20 + 0.01	15.02 ± 0.68[b]	9.1 (6)	1.88
+	40 + 0.01	17.46 ± 0.83[b]	11.54(23)	1.85
mitomycin C	80 + 0.01	21.48 ± 1.08[b]	15.56(57)[c]	1.58
Phosphate buffer saline (solvent control)		5.92 ± 0.45		2.05

[a] Background SCE (solvent control value = 5.92) was subtracted.
[b] Significant ($p<0.001$) as compared to solvent control.
[c] Significant ($p<0.001$) as compared to the sum of SCEs induced individually by MMC and tetrandrine.

previously (Xing et al., 1988). In the combined treatment, over 50% increase in SCE was observed when cells were treated with CSC (20 μl/ml) plus tetrandrine (40 and 80 μg/ml) as compared to the sum of SCEs induced individually by CSC and tetrandrine at the same concentrations (Table 1). A similar phenomenon was also found for the combined treatment of MMC (0.01 μg/ml) plus tetrandrine (80 μg/ml) (Table 2). The increase was dose-dependent and statistically significant. These results indicate that tetrandrine is an effective enhancer of the genotoxic activity of MMC and CSC. The mechanism of mutagenic enhancement by tetrandrine is not known. It may be due to the increase of DNA damage, because the induction of SCE is an indicator of the production of lesions in DNA (Wolff, 1983). The enhancing effect of tetrandrine may also be attributed to interference with excision repair by tetrandrine leading to an accumulation of a large number of unrepaired lesions which may subsequently cause SCE (Faed and Mourelatos 1978). Whong et al. (1988) have suggested that the mutagenic enhancement by tetrandrine in *S. typhimurium* TA98 results from the potentiation of error-prone repairs. It seems that the influence on DNA repairs (excision and/or error prone) by tetrandrine might be the possible mechanism by which tetrandrine enhanced genotoxic effects.

While cultures treated with CSC or MMC alone showed similar cell-cycle kinetics in comparison with the control values, the combined treatment (tetrandrine plus CSC or MMC) induced a dose-related cell-cycle delay. Tetrandrine, therefore, is not only a mutagenic enhancer but also a cytotoxic enhancer. The changes in cell-cycle kinetics after treatment with tetrandrine may be attributed to inhibition of DNA synthesis (Kaina, 1982).

The enhancing effect of tetrandrine on the genotoxicity of chemicals has also been reported in the gene mutation and DNA-repair assay systems (Whong et al., 1988). However, the health impact of this drug, regarding genotoxic enhancement, is unknown. Nevertheless, because CSC-induced SCE can be markedly potentiated by tetrandrine, the possibility of an increase in genotoxic risk in tetrandrine-treated silicotic patients who are smokers needs to be further investigated.

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