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Sister chromatid exchange and DNA damage in human lymphocytes induced by air-dust in Lanzhou City: involvement in free radicals*

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Abstract—The air-dust samples collected from petro-chemical industrial region in the suburb of Lanzhou and from a certain rural region 64 km away from the city were extracted, with a mixed solvent (benzene: hexane: isopropanol=7:2:1) for 8 hours. A strong free radical signal at g= 2.00 of air-dust itself and a hyperfine splitting EPR signal of extract from air-dust have been detected. The sister chromatid exchange frequency(SCE) was increased by extracts of both dusts from the industrial region and from the rural region. If a chemical is able to increase SCE up to twice as high as the control, this chemical is considered to be mutagenic and/or carcinogenic. The double SCE frequency concentration is 23 μg/ml for the dust extract obtained from the industrial region and 47μg/ml for that from the rural region. Extracts were able to damage to DNA template. Results indicated that the mutagenicity and/or carcinogenicity of the extracts obtained from the petro-chemical industrial region were stronger than that of the extracts from the rural region.

Keywords: air-dust; sister chromatid exchange; DNA damage; free radical.

INTRODUCTION

A majority of tumors have been shown to be related to environmental factors (Doll, 1981), and much effort has been spent on the identification of their mechanism of effect. Most of the carcinogens are found either to be free radicals involved in free radical reactions or ones to generate free radical intermediates(Zheng, 1987; 1988). The total concentration of free radicals in urban air may reach 1 to 10 percent of the molecular contaminants (Johnson, 1956). Suspected sources of general atmospheric free radicals in urban have been examined including domestic chimney smoke, vehicular exhausts and cigarette smoke (Lyons, 1960). The photochemical smog is formed by free radical reaction, too (Hendry, 1979).

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Lanzhou is the capital of Gansu Province and one of chemical industrial centers of China. It is surrounded on the north and south by mountains and crossed by the Yellow River. It has a long duration of sunshine in a year. All these meteorological and geographical conditions are particularly favorable for the formation of photochemical smog and temperature inversion. The rules for free radical concentrations of air-dust in Lanzhou have been found (Zheng, 1985). The free radical concentrations in air of the industrial region and in downtown are much higher than that in air of the rural region. This paper will focus on the risk of carcinogenesis or mutagenesis induced by air-dust, that is revealed in human neonatal umbilical blood lymphocytes.

MATERIALS AND METHODS

Collection and extraction of air-dust

The samples of air-dust 1.5m above the ground were collected from an industrial region and from a rural region 64 km away from Lanzhou by acetate fiber filters and then extracted by a mixed solvent(benzene: hexane: isopropanol=7:2:1) for 8 hours. After drying, samples were redissolved.in dimethyl sulfoxide.

Electron paramagnetic resonance (EPR) signal detection

Both the air-dust collected and its extract solution were put into quartz tubes respectively for determination of EPR signal by EPR spectroscopy (Bruker, ER200D-SRC). Instrument settings during the recording of the signals were: X-band, 9 GHz; sensitivity, 10^{-10} mol/L DPPH; time constant, 0.8s; gain, max.; scan velocity, 5 mm/s; scan range, 1000g; resolution, 0.1–0.2g.

Sister chromatid exchange(SCE)

Human neonatal umbilical blood was cultureted in RPMI 1640 medium (Nissui Pharmaceutical Co. Ltd.), supplemented with 5% calf serum, penicillin 100 ug/ml, streptomycin 100 μg/ml and phytohemagglutinin 40 μg/ml. Each 0.3 ml of neonatal umbilical blood was added into 4.5 ml medium, and 0.2 ml of 5-bromodeoxyuridine (Fluka AG) was added to give a final concentration of 8 μg/ml. The cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. After incubation for 24 hours, the cultures were treated with various concentrations of extracts for 48 hours. Two or four hours before the end of the cultivation, colchicine was added to give a final concentration 0.005 μg/ml. After centrifugation, cells were rinsed with distilled water. Chromosome slides were stained with acridine orange and Giemsa. The cytological index is SCE number per cell in the 2nd division cycle metaphase. This index is extremely sensitive , i.e., it can detect SCE at far lower concentrations of compounds than those needed to produce ordinary chromosome aberrations (Latt, 1981).

DNA synthesis and DNA template damage measurement

Umbilical blood was treated with various concentrations of extracts and 0.1ml of ^{3}H-thymidine (^{3}H-TdR, Shanghai Institute of Nuclear Research) was added into the medium to a final radioactivity 1 μCi/ml. After incubation at 37°C for 24 hours, the cells were centrifuged,

rinsed with distilled water, fixed by Carnoy fixative and precipitated by trichloroacetic acid. After drying, $HClO_4$ and H_2O_2 were added in drops to the sample and digested at 90°C for 40 minutes. The sample was transferred into a vial containing 10 ml of scintillation solution (0.4% PPO, Koch-light, and 0.01% POPOP of dimethyl benzene solution: glycol monomethyl ether, V:V=6:4) for liquid scintillation counting (FJ-2100) and for calculation of the percentage of incorporation of ^{3}H-TdR into DNA. The DNA template damage experiments were carried out with Painter's method (Painter, 1977). Umbilical blood sample was treated with the extract 32 μg/ml for 2 hours, while a corresponding volume of dimethyl sulfoxide solvent was added into the control blood sample. The extract was washed out twice with saline solution. To all cultures a fresh medium was added. After incubation at 37°C for 0, 1 and 3 hours, ^{3}H-TdR was added and stood at 37°C for 40 minutes and then was removed. The cells were centrifuged and rinsed to determine radioactivity. It has been reported that when an agent that inhibits metabolism is removed from the medium, the metabolic block is stopped, recovery begins immediately and the rate of DNA synthesis should increase. When a DNA-damaging agent is removed from the medium, however the damage remains, and the rate of DNA synthesis should decrease with time. This simple test is used to detect agents that damage cell DNA template (Painter, 1977).

RESULTS AND DISCUSSION

A strong free radical signal at g= 2.00 of air-dust itself can be detected (Fig.1). The types and widths of EPR spectra detected in all samples collected from different regions in Lanzhou area are the same, while only the amplitudes of the spectra vary with regions, seasons and diurnal variations (Zheng, 1985). The free radical signal of air-dust extract is loaded on the EPR spectrum of Mn^{2+}, since the signal hyperfine splitting 6 lines of air-dust and the 6 lines of magnesium ion overlapped (Fig.2). However, the peak pointed by dotted lines arrow could be contributed by free radical, it decayed with time, but the 6 lines did not.

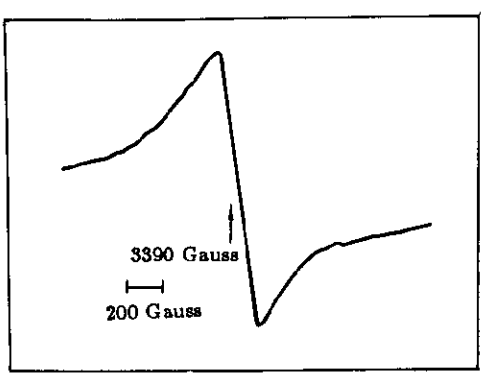

Fig.1 EPR signal of air-dust from Lanzhou

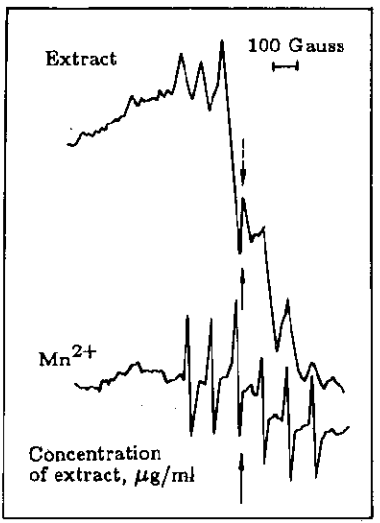

Fig.2 EPR signals of extract from air-dust (industrial region) and of Mn^{2+}

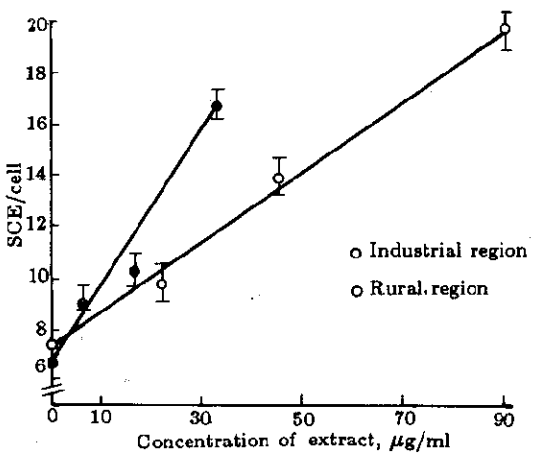

Fig.3 SCE in lymphocytes induced by extracts of air-dust (n=3)

The SCE frequency was induced by extracts either from the industrial region or from the rural region. The regression lines in Fig.3 show a linear relation between SCE frequency and concentration of extract. The SCE frequency induced by the extract obtained from the industrial region is higher than that from the rural region. The concentrations of the extracts to induce 2-fold increase over baseline SCE frequency (the control) are 22 μg/ml (from industrial region) and 47 μg/ml (from rural region) respectively. If a concentration of the agent which

gave a SCE value twice as high as the background, then it is considered to be an effective concentration to induce SCE (Latt, 1981).

The cell DNA synthesis was inhibited by extracts either from the industrial region or from the rural region and this effect was enhanced by the concentration of the extracts (Fig.4). Cell mitosis will be strongly inhibited when the concentration of air-dust extract from the industrial region is above 64 μg/ml and that from the rural region is above 180 μg/ml.

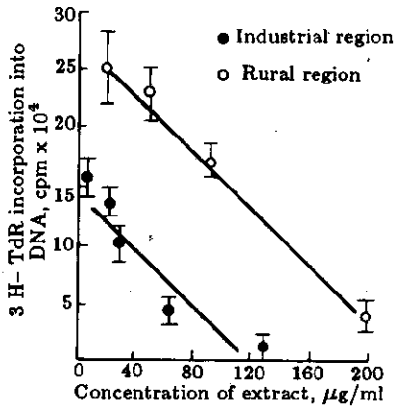

Fig.4 Inhibition of cells DNA synthesis by extracts of air-dust (n=3)

The cell DNA synthetic rate was still inhibited even after the extract was washed out from the medium (Fig.5). Every test has 3 replicates, the tendencies in different tests done under similar experimental conditions are all the same. It indicated DNA template was

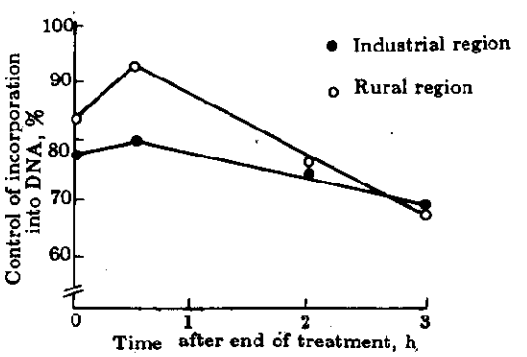

Fig.5 Cell DNA synthetic rate inhibition by extracts of air-dust (n=3)

damaged by air-dust extract. This result is consistent to our previous study (Hu, 1988). DNA damage represents the possibility of mutagenesis or carcinogenesis.

In this paper the mutagenesis and/or carcinogenesis of air-dust were proved by free radicals, SCE and DNA damage. The mutagenicity and/or carcinogenicity of air-dust from the industrial region is stronger than that from rural region.

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