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Relationships Between Molecular Structure and Chromosomal Aberrations in *In Vitro* Human Lymphocytes Induced by Substituted Nitrobenzenes

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Most nitrated aromatics are genotoxic, some structure-activity relationship (SAR) studies have been done about their mutagenicity (Klopman et al 1984a, 1984b), but most studies are concerned with the nitrated polycyclic aromatic hydrocarbons. In this report, we especially studied the substituted nitrobenzenes. Our intention was to study the relationship between molecular structure and genotoxicity of these compounds.

MATERIALS AND METHODS

The genotoxicity of substituted nitrobenzenes were evaluated by the chromosomal aberration (CA) test using in vitro human peripheral lymphocytes. 14 compounds were tested in the present study, the substituent groups include: $-NO_2$, $-NH_2$, $-CH_3$, -OH, -Br and -Cl (Table I).

Peripheral blood was obtained from a healthy male donor, aged 25 years free of any known exposure to genotoxic agents, and to clinical X-ray in recent years. Whole blood cultures were incubated at 37°C. Each culture contained: 4mL of RPMI 1640 medium (Gibco, New York, USA), 1mL fetal bovine serum, penicillin 100Iu/mL, streptomycin 100Iu/mL phytohemagglutinin 25 µL/mL, and human peripheral whole blood 0.2mL. Chemicals dissolved in 10 µL DMSO were added to cultures at 48h after culture initiation. At the same time, 10 µL DMSO was added to the control Five duplicate cultures were made for each chemical. All cultures were incubated for an additional 24h after treatment. 5 µL/ml colchicine was added 2h before the end of incubation. Chromosome preparations were made and stained with Giemsa solution (Jablonicka et al. 1989).

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The number of cells with structural chromosome aberrations among 100 well-spread metaphase cells in one culture was recorded (gaps were not included in making evaluation) (Khalil 1989). The percentage of aberrant cells (PAC) was calculated:

PAC = number of aberrant cells/number of metaphases scored

A two-tailed t-test in Statgraphics software (Ver. 4.0)(STSC. Inc. USA) was used in the statistical analysis. If the PAC was significantly increase ($P<0.01$) above the negative control, the result was considered as positive (+), otherwise the result was negative (-).

RESULTS AND DISCUSSION

Of the 14 tested chemicals, 10 compounds exhibited genotoxic activities while the other 4 compounds were non-genotoxic in the CA test (Table 1).

Only mono- and di-nitro substituted compounds were studied in this report In table 1, all the di-nitro substituted compounds were genotoxic. From the experimental dose listed in table 1, we can see that the di-nitro substituted compounds tend to be far more genotoxic than the mono-nitro substituted compounds.

Earlier studies (Rosenkranz et al. 1983, McCoy et al. 1982, Klopman 1984a) suggested that nitrated aromatics ($ArNO_2$) require metabolic reduction of the nitro group to exhibit genotoxic activity. The mutagenic metabolites are the arylhydroxylamine (ArNHOH) or corresponding hydroxamic esters (ArNHOR). In these metabolites the nitrogen atom produce an electrophilic center, which can react with nucleophilic centers in cellular DNA (eg. the C8 position of guanine residues in DNA) to form adducts (Klopman et al. 1984b).

The substituted di-nitro benzenes are more genotoxic because that these mutagenic compounds may provide two electrophilic centers, which preferentially intercalate between DNA based pairs, and may react with the nucleophilic centers on both strands of DNA simultaneously. The DNA adducts formed in the above manner may induce mutations more effectively by inhibiting DNA repair leading to increased mutagenicity. Similar results have been found in the mutagenicity of polycyclic aromatic hydrocarbons (Qianhuan Dai, 1979).

Table 1. The genotoxicity and physical-chemical parameters of 14 tested compounds

Chemicals	I*		Acti-vity	Dose** (mmol/L)
1,3-dinitrobenzene	2		+	1.0
1,4-dinitrobenzene	2		+	1.0
1,2-dinitrobenzene	2		+	1.0
2,4-dinitro-toluene	2		+	0.5
2,4-dinitro-aniline	2		+	0.2
2,4-dinitrophenol	2		+	0.5
nitrobenzene	1	0	+	50
4-nitro-toluene	1	-0.16	+	5.0
4-nitro-aniline	1	-0.66	+	5.0
4-nitro-phenol	1	-0.37	+	10
3-fluoro-4-chloro-nitrobenzene	1	0.56	-	1000***
4-chloro-nitrobenzene	1	0.23	-	1000***
3,4-dichloro-nitrobenzene	1	0.60	-	1000***
4-bromo-nitrobenzene	1	0.23	-	1000***

* I is the number of nitro groups on benzene ring
** Lowest positive dose tested (the final concentration in the assay medium
*** Highest experiment dose

In the metabolic activation procedure of nitro-aromatics, reduction only occurs on the nitro groups, while the structure of the other parts of the molecule, including the additional substituents on the benzene ring, does not change (Klopman 1984a). Thus, the electronic effects of the additional substituents are anticipated to affect the genotoxicity of the compounds studied due to their influence on the reaction centers in the mutagenic metabolites, because the genotoxic activities of nitro-aromatics are induced by the electrophilic reactions of the mutagenic metabolites with cellular DNA.

The summation of σ ($\Sigma\sigma$) of all the additional substituents to the reaction center were calculated to describe the overall electronic field effects of the substituents (Hansch et al. 1979). Generally, negative $\Sigma\sigma$ values indicate that the substituents present an electron-donating effect on the reaction

center, while positive $\Sigma\sigma$ values indicate an electron-withdrawing effect of the substituents on the reaction center. Since the reaction center of di-nitro substituted benzene can not be determined, only mono-nitro ones in Table I were calculated for $\Sigma\sigma$.

In Table 1, four of the eight mono-nitro substituted compounds studied were genotoxic. The $\Sigma\sigma$ values of all four genotoxic compounds were zero or less than zero, while those of the other four non-genotoxic compounds were positive. It may be concluded that the electron-donating effects of the substituents would increase the genotoxicity of nitro substituted benzenes.

The influence of the electron-donating effects on the genotoxicity of studied chemicals would be explained by the following supposed mechanism of mutagenic reaction between DNA and ultimate mutagen arylhydroxylamines ArNHOH (or arylhydroxamic esters ArNHOR). The reaction is initiated by the departure of hydroxyl group (or alkoxyl group) to form alylnitrenium ions ArN^+as transition state, which immediately react with nucleophilic centers in DNA to form adducts. The electron-donating substituents on the benzene ring would delocalize the positive charge on the nitrogen atom of ArN^+, to reduce the energy of transition state. As a result the mutagenic reaction is accelerated, and the genotoxic responses are increased.

Since the SAR studies were based on an in vitro assay in this report, the general applicability to in vivo genotoxicity observed in present studies needs to be investigated.

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