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EXPERIMENTAL / LABORATORY STUDIES

In Vitro Testing for Genotoxicity of 4-CPA by Sister Chromatid Exchange in Human Lymphocyte Culture

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Abstract: Certain phenoxyacetate derivatives used as plant growth regulators act as plant hormones. 2,4-D dichlorophenoxyacetic acid and 4-chlorophenoxyacetic acid are widely used in vegetable growing, especially tomatoes, in our region. These phenoxyacetic acid compounds are a potential hazard for the environment. There are conflicting results and inconsistent data about these herbicides in the literature. This is the first report of genotoxicity of 4-CPA evaluated by sister chromatid exchange in human lymphocyte culture. No significant change was found in sister chromatid exchange frequency at the tested 4-CPA concentration.

Key Words: 4-CPA, Phenoxy herbicides, genotoxicity, sister chromatid exchange, tomato.

Indroduction

Humans may be affected by a large number of environmental contaminants via respiration, ingestion and absorption. The contamination of the environment by chemicals, drugs, pesticides and solvents is a major concern (1,2). Certain phenoxyacetate derivatives are used as plant growth regulators that act as plant hormones. For this reason they are used commonly as herbicides in agriculture to enhance and accelerate the growth of commercial foods such as tomato, cucumber, pepper, eggplant and strawberry. Since excess amounts of such chemicals are released continuously into the environment they are a potential hazard, physiologically and genetically, to humans as well as to other species. Therefore, it is necessary to continue and to extend the evaluation of the genotoxicity of these chemicals by using different sensitive assays. The possible genotoxic effects are of special concern due to the generally irreversible nature of the process and the long latency associated with their manifestations. Serious exposure to genotoxic agents may result in mutations, metabolic disorders and reduced fertility. In addition, epidemiological evidence suggests that 60%-80% of all human cancers may be the result of lifestyle and environmental factors (1-3).

With reference to the possible genotoxicity of phenoxyacetic acid compounds, different reports indicate inconsistent data since their genotoxicity depends on the chemical(s) selected and/or the genetic end-point(s) analysed (4-8). Various authors have been working on the genotoxic evaluation of 2,4-D dichlorophenoxyacetic acid (2,4-D) (4-6, 8-12) using different genotoxicity assays, and report both positive and negative results. However, there are no data on the possible genotoxicity of 4-chlorophenoxyacetic acid (4-CPA) in the literature. One of the widely used tests, sister chromatid exchange (SCE), is a sensitive cytogenetic assay for detecting genotoxic effects of chemical mutagens and carcinogens (13-19).

4-CPA is widely used in vegetable growing, especially tomatoes, in the Mediterranean region. Considering the scarcity of the genotoxicity data in the literature, we report the genotoxic evaluation of 4-CPA using the SCE test in human lymphocyte culture.

Materials and Methods

Subjects

Fifty subjects (19-25 years old) were enrolled . All volunteers answered a detailed questionnaire in which health conditions and past and present exposure to possible genotoxic agents such as drug and alcohol use, smoking, pesticides and herbicides were considered, and written informed consent was obtained from all volunteers.

Human Lymphocyte Cultures

Using a heparinised syringe, 5 ml of healthy donor blood was extracted by venipuncture. Eight drops of blood were added to 3 ml of McCoy's 5A medium with L-glutamine (Seromed) plus 0.2 ml of phytohaemagglutinin (Seromed). BrdU (Sigma) was added to the culture medium at a final concentration of 10 mg/ml and incubated at 37 °C for 72 h in darkness. Direct treatment of human lymphocytes in culture at 0, 10, 15 and 20 µg/ml with 4-CPA (Sigma) was carried out (prepared just before use, 4-CPA was dissolved in Basal Medium (Sigma)); l0 µg/ml colchicines (Seromed) was added 2 h prior to the harvest. Metaphase cells were harvested by centrifugation, treated with 0.075 M KCl and fixed in methanol:acetic acid (3:1). Slides were stained by the Fluorescence-plus-Giemsa technique (20). SCE analysis was performed on second division metaphases in which all 46 chromosome modal numbers and in which 25 well spread metaphases for each 4-CPA concentration per donor were scored, were possible.

Data Analysis

SCEs were examined for each experimental concentration using ANOVA (SPSS 9.0) to determine any possible significant differences among the groups.

Results

We examined the genotoxicity of 4-CPA using SCE in human lymphocyte culture. Fifty donors were studied, although 6 donors were excluded from statistical analysis due to culture failure in certain 4-CPA concentrations (Figure 1).

No significant differences were found between control and test concentrations of 4-CPA (P = 0.355) (Table 1).

In addition, we tested possible effects of age and gender on SCE frequency, but determined no correlation between SCE, gender (P = 0.549 for male subjects, P = 0.698 for female subjects) and age (P = 0.099) (Tables 2, 3).

Discussion

No genotoxicity was observed by treatment with the 3 different 4-CPA concentrations. We prepared a detailed questionnaire to eliminate any possible factors which might affect the SCE frequency of the volunteers, especially unwitting exposure to any herbicide, pesticide or other chemicals. Our subject age range was relatively low, 17-25 years. In some studies it is reported that SCE

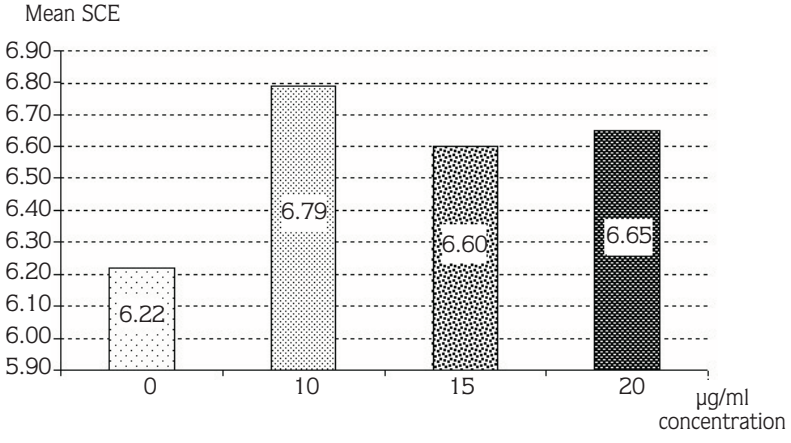

Figure 1. Mean SCE frequencies in the three 4-CPA concentrations of 44 donors.

Table 1. Comparison of mean SCE frequencies of control and three 4-CPA concentrations

Groups	n	Mean SCE	Standard Error	Standard Deviation
C	44	6.218	0.24	1.597
I	44	6.793	0.251	1.664
II	44	6.598	0.214	1.423
III	44	6.553	0.234	1.555

C: 0 µg/ml control group; I, II, III: 10, 15 and 20 µg/ml 4-CPA test groups, respectively; n: subject number.

Table 2. Comparison of mean SCE frequencies in male subjects.

Groups	n	Mean SCE	Standard Error	Standard Deviation
C	26	6.216	0.284	1.451
I	26	6.715	0.3	1.532
II	26	6.649	0.241	1.231
III	26	6.555	0.217	1.109

C: 0 µg/ml control group; I, II, III: 10, 15 and 20 µg/ml 4-CPA test groups, respectively; n: subject number.

Table 3. Comparison of mean SCE frequencies in female subjects.

Groups	n	Mean SCE	Standard Error	Standard Deviation
C	18	6.221	0.431	1.831
I	18	6.907	0.443	1.879
II	18	6.527	0.4	1.698
III	18	6.795	0.487	2.067

C: 0 µg/ml control group; I, II, III: 10, 15 and 20 µg/ml 4-CPA test groups, respectively; n: subject number.

frequency does not change up to 60 years of age (21,22). Our SCE frequencies volunteer's did not exhibit any changes by age. Additionally, no significant differences were found when we compared the SCE frequencies to our laboratory values in different age groups. Although gender does not affect SCE frequency, certain studies have reported that the SCE frequency in the female population may be higher than that in the male population, depending on hormonal differences and use of contraceptives (23-25). In our study the sex distribution was 26 males / 18 females and no significant difference was determined between the genders. Herbicides are used widely in cultivation vegetable, especially tomatoes, and the tested concentrations were within the range of formal regulations. However, in the field they could be over used due to concern over profits. Therefore, they could be tested in different concentrations or via metabolites extracted from tomato or from in vitro experimental metabolising systems. Additionally, different genotoxicity tests should be examined to determined potential hazards of 4-CPA. There are several reports on the genotoxic evaluation of 2,4 dichlorophenoxyacetic acid, although with reference to the possible genotoxicity of 4-CPA no data were found in the literature. Recently, Kaya et al. (19) reported genotoxicity of 2,4-D and 4-CPA using the wing spot test in *Drosophila melanogaster*. In the study, 4-CPA had no genotoxic effect in the wing spot test. Although Atabey et al. (26) reported a significant increase in SCE frequencies of some exposed workers who lacked protective equipment, and they did not find any significant effect of SCE frequencies on sprayers chronically exposed to organophosphorus compounds and pyrethrin. In addition, as an alternative subject-effect test system, herbicide sprayers exposed to these chemicals should be examined for genotoxicity and other biohazards. In conclusion, we report that the tested 4-CPA concentrations had no genotoxic effect on cytogenetic levels.

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