CYTOGENETIC ACTION OF 3-HYDROXYKYNURENIN AND 3-HYDROXYANTHRANILIC ACID ON HUMAN CELLS IN EMBRYONIC TISSUE CULTURE

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The carcinogenic tryptophan metabolites 3-hydroxykynurenin and 3-hydroxyanthranilic acid, acting on cells of a primary human embryonic tissue cause chromosomal disturbances, thus demonstrating the mutagenic activity of these substances.

Many tryptophan metabolites are found in high concentrations and with great constancy in the urine of patients with leukemia [11,13] and carcinoma of the urinary bladder [6-9,14-19], and they are capable of inducing tumors in experimental animals [1,2,4,5,10]. In this connection the study of the mutagenic activity of tryptophan derivatives as a possible mechanism of their carcinogenic action is of great interest.

Previous investigations showed that 3-hydroxyanthranilic acid and 3-hydroxykynurenin cause chromosomal aberrations in the hematopoietic cells of mice of lines $CC_{57}Br$ and $C_{52}Bl$ in experiments in vivo, and the mutagenic action of 3-hydroxyanthranilic acid on the mutant strain Escherichia coli K-12 (tre^-, B_1^-) has also been established [3].

In the present investigation the action of 3-hydroxyanthranilic acid and of 3-hydroxykynurenin on chromosomes of human somatic cells was studied in tissue culture.

EXPERIMENTAL METHOD

Human embryos aged 7-9 weeks were repeatedly washed with Earle's solution containing monomycin (1000 units/ml) and minced with curve scissors in a Petri dish. Next, using a pipet with a bulb, the minced tissue was transferred to an Erlenmeyer flask with magnetic mixer where preliminarily warmed 0.25% trypsin solution was added. The first two portions of the cell suspension were discarded because they contained many blood cells. Subsequent separation of the tissue was carried out by alternate treatment with trypsin for 5 min and with medium No. 199 for 10 min. The cell suspension thus obtained was transferred to centrifuge tubes and centrifuged at 1000 rpm for 10 min. Having discarded the supernatant, the cell suspension was transferred by means of a pipet with a bulb to another vessel and resuspended in medium No. 199 containing 10% bovine serum. After filtration through 3 layers of gauze, the cell concentration in the suspension was determined by counting the cells in a Goryaev's chamber. The suspension was then diluted with medium No. 199 with 10% bovine serum until 1 ml contained 1.5 million cells and then poured into Carrel flasks at the rate of 5 ml per flask. The culture was incubated at 37°. The medium was replaced by fresh (medium No. 199 + 10% bovine serum) after 40-41 h, one of the tryptophan metabolites then being added to the experimental flasks in a dose of 40 LD. Preparations of the chromosomes were made after 24 h by a modification of the method of Moorhead and co-workers [12]. The specimens were stained by the Romanovsky method. Only cells with a diploid set were analyzed. Cells with a hyperdiploid set were found equally in the experimental and control series. The mutagenic activity of the compound was judged from the presence of chromosomal aberrations. Experiments were carried out by the methods described above with 3-hydroxykynurenin and 3-hydroxyanthranilic acid (three experiments in each case). The experimental results were subjected to statistical analysis, the degree of significance being calculated from the Student–Fisher table.

EXPERIMENTAL RESULTS

Since the results of each individual experiment for each compound were in agreement, they are presented together in Table 1.

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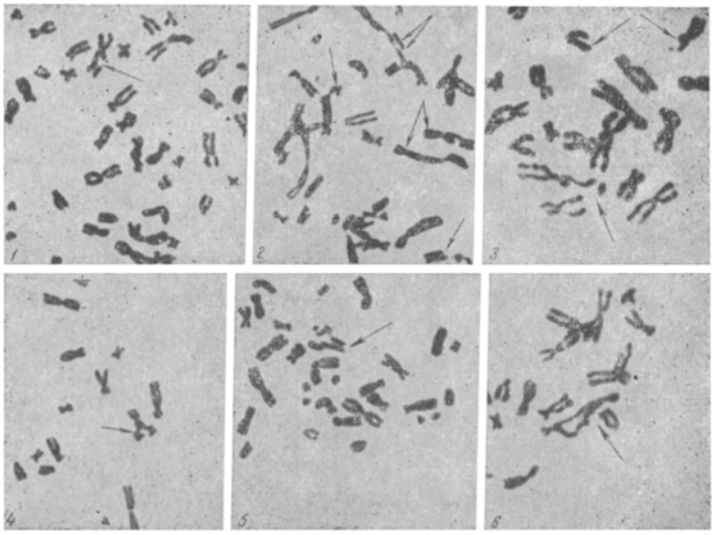

Fig. 1. Chromosomal disturbances caused by the action of 3-hydroxykynurenin and 3-hydroxyanthranilic acid. 1-3) Chromatid breaks; 4-6) chromatid translocations.

TABLE 1. Action of 3-Hydroxykynurenin and 3-Hydroxyanthranilic Acid on Chromosomes of Human Cells

	3-Hydroxykynurenin (50 mg/liter)		3-Hydroxyanthranilic acid (30 mg/liter)	
	experiment	control	experiment	control
Number of metaphases counted	264	237	388	300
Number of cells with aberrations	35 (13.2%)	9(3.8%)	49(12.6%)	8(2.7%)
Level of significance	$P < 0.001$		$P < 0.001$	
Total number of chromatid breaks	83	9	65	8
Mean number of breaks per cell	0.31	0.032	0.16	0.027
Number of translocations	3	–	1	–
Mean number of secondary constrictions and gaps per cell	0.058	0.033	0.08	0.03

As Table 1 shows, the number of cells with aberrations for each compound in the experiments was much higher than their number in the control: 3.5 times higher for 3-hydroxykynurenin and 4.6 times higher for 3-hydroxyanthranilic acid.

Chromosomal analysis showed that 3-hydroxykynurenin and 3-hydroxyanthranilic acid caused mainly chromatid breaks in human somatic cells under tissue culture conditions. Most frequently there were one or two breaks per cell, less commonly 3, 4, or more (Fig. 1, 1-3). In four cases translocations were observed (Fig. 1, 4-6), whose formation was evidently connected also with the formation of chromatid breaks. In a separate line of the table, the number of secondary constrictions and gaps calculated relative to the total number of metaphases counted is given.

The results of these experiments thus confirmed once again the mutagenic properties of 3-hydroxykynurenin and 3-hydroxyanthranilic acid. Comparing them with the previous experiments on mice, in which a correlation between the carcinogenic and mutagenic activities of a series of investigated tryptophan metabolites was demonstrated, it is reasonable to consider that the mutagenic effect is one of the possible mechanisms of the carcinogenic action of tryptophan metabolites.

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