DISTURBANCES OF REPAIR PROCESSES IN LYMPHOCYTES FROM SCHIZOPHRENIC PATIENTS CULTURED IN VITRO

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Disturbances of various stages of repair of injuries to cellular DNA, induced by mutagens, are found in certain hereditary human diseases. Some diseases are characterized by disturbance of the "long" repair path, which is normally responsible for repair of DNA lesions after UV-irradiation or exposure to chemical UV-mimetics (xeroderma, Cockayne's syndrome, etc.). In other diseases, one stage of the "short" repair path, normally responsible for repair of lesions induced by gamma-irradiation of DNA or corresponding chemical gamma-mimetics (ataxia-telangiectasia, Down's syndrome, etc.), is involved. In diseases of this sort changes of immunoreactivity are found. In some cases these diseases are accompanied by retardation of mental development which may amount to feeblemindedness (Cockayne's and Down's syndromes, ataxia-telangiectasia, etc.).

Schizophrenia is a disease with a marked hereditary predisposition, in which certain changes of the immunologic status are found.

The aim of this investigation was to study the ability of lymphocytes obtained from schizophrenic patients and cultured in vitro, to repair DNA injuries induced by UV-irradiation and by 4-nitroquinoline-1-oxide (4NQO, a UV-mimetic, producing injuries whose repair requires activity of enzymes of the "long" repair path) and by gamma-irradiation. Formation of mutagen-induced sister chromatid exchanges (SCE) — structural transformations of chromosomes reflecting recombination events, the number of which is sharply increased as a result of exposure to mutagens in cells with a disturbed repair system [2] — also was investigated.

EXPERIMENTAL METHOD

To assess disturbances of repair processes in the patients' cells in culture 13 criteria reflecting the activity or the result of activity of an enzyme or combination of functions of enzymes were used: the spontaneous SCE level and generation time, the mitogen-induced SCE level and the corresponding generation time, reparative synthesis (RS) of DNA and the level of reactivation of mutagen-treated virus and its mutability.

The investigations were carried out on lymphocytes from patients with the diagnosis of schizophrenia, with a progressive type of course. The lymphocytes were cultured by the usual method. Phytohemagglutinin (PHA) was obtained from Wellcome (England).

The cells were irradiated with gamma-quanta on a ^{60}Co gamma-ray apparatus at the Institute of General Genetics, Academy of Sciences of the USSR. The doses of gamma-irradiation, depending on the aims of the experiment, varied from 2 to 100 Gy, and the dose rate was 7 Gy/min. The source of UV rays was a pair of BUF-15 lamps (254 nm). The dose of UV-irradiation was 20 J/m^2 and the intensity of radiation 1.6 J/sec/m^2. To induce RS of DNA the chemical mutagen NQO was used in a concentration of $2.5 \cdot 10^{-6}$ M with an exposure of 30 min, and in some experiments in concentrations of $2.5 \cdot 10^{-5}$ and $2.5 \cdot 10^{-7}$ M (exposures of 30 and 60 min, respectively).

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TABLE 1. Criteria of Evaluation of Repair Activity and SCE Induction in Lymphocytes from Schizophrenic Patients Treated with Mutagens

Patient No.	Spontaneous level of SCE	Spontaneous generation time	4NQO						Gamma-irradiation					
			induced SCE level	induced generation time	RS	repair of DNA breaks	reactivation of virus	induced mutagenesis of virus	induced SCE level	induced generation time	repair of DNA breaks	reactivation of virus	induced mutagenesis of virus	duration of disease (years)
1	+	N	+	+		def.	N	+	+	+	def.	N	+	17
2	++	N	++	+		def.	N	++	+	N	def.	N	N	15
3	+++	N	++	N		inh.	N	+++	+	N	N	+	N	30
4	++	N	+	N	inh.	def.	N	++	++	N	def.	+	N	8
5*	+	N	+	N			+	++	+	N		+	N	13
6	++	+	++	+			N	++	+	+		+	N	10
7					def.	N	N	++			def.	N	+	
8					def.	inh.	N	++			inh.	N	N	20
9					inh.									10
10*					def.		+	++				+	N	3
11					def.	inh.					inh.			40
12					UV-inh.	N	+	++		d	def.	N	N	10
13				UV-def	N						def.			5
14				UV-N	N						def.			3
15*				UV-def		+	+					N	+	20

Legend. Spontaneous level of SCE (number of cell): N (normal) = 11, +) from 12 to 15, ++) from 16 to 20 or higher; induced SCE level: N) from 16 to 18, +) more than or equal to 20, ++) more than or equal to 30. Generation time: N = 2, +) delay (under 1.6), ++) marked delay (under 1.2). Reparative DNA synthesis in SI values: N) SI below or equal to 2, defect) SI under or equal to 1, inhibition) SI from 1.3 to 1.9. Repair of breaks: N) restoration of original DNA structure, inh. (inhibition) — incomplete restoration of original DNA structure (up to 80% of the control), def. (defect) — absence of restoration or further degradation of the original DNA structure. Reactivation of virus: N) equal survival rate of virus treated with mutagens in cells from patient and healthy blood donors, +) differences in survival rate of virus (log PFU/ml greater than or equal to 1); induced mutagenesis: N) percentage of induced small-plaque variants the same in cells from patient and healthy blood donors; +) percentage of mutations increased twofold, ++) percentage of mutations increased sevenfold or more. *) Untreated patients.

All the experiments to assess and analyze SCE were conducted in accordance with the scheme described in detail by the writers previously [3]. During analysis of SCE 900-1200 chromosomes were investigated.

The level of RS of DNA induced by NQO was determined by scintillation radiometry, based on incorporation of ^{3}H-thymidine into the total lymphocyte pool which, at the beginning of the experiment, was $5 \cdot 10^5$-10^6 cells/ml. To inhibit residual replicative DNA synthesis, before exposure to the carcinogen the cells were incubated for 30 min in medium containing 10 mM hydroxyurea. Experiments to study the intensity of UV-induced RS of DNA were conducted in the radiomodification, using a technique of microculture of whole blood lymphocytes. The intensity of RS of DNA was judged by the level of the stimulation index (SI), the ratio of radioactivity (in cpm) in cells treated with the mutagens to radioactivity counted in untreated (control) cells.

To determine single-stranded breaks in DNA and their repair, the method of chromatography of cell lysates on columns with hydroxyapatite [1] was used.

Reactivation of mutagen-treated vaccinia virus was estimated from the survival rate and the percentage yield of induced mutations. The survival rate was calculated as the difference in titers of virus, expressed in log of plaque-forming units (PFU) in 1 ml, in the presence

or absence of treatment with mutagens. The mutability of the virus was estimated as the percentage of induction of small-plaque variants.

The results were subjected to statistical analysis by the chi-square test.

EXPERIMENTAL RESULTS

The results of investigation of the patients' lymphocytes by different methods, characterizing activity of the "long" (UV-irradiaton, 4NQO) and "short" (gamma-irradiation) repair pathways, are given in Table 1. It follows from Table 1 that one criterion, namely mutability of the virus, i.e., the sharply increased induction of small-plaque mutations under the influence of 4NQO, was positive in the cells of all 11 patients tested. These findings indicate that premutation injuries of DNA induced by a mutagen of "long" type are not repaired by the cell, but are realized as mutations. In the cells of four patients, moreover, reactivation of the virus was depressed.

The complete absence of repair of DNA breaks or partial inhibition of this process was observed in six of the 10 patients investigated with respect to this criterion and in nine of 10 patients investigated in respect of the criterion of reparative synthesis recorded after UV-irradiation or treatment with 4NQO. In three of seven cases, incidentally, inhibition (or profound inhibition) of activity of both stages of repair was found: both reparative synthesis and repair of DNA breaks. As regards spontaneous SCE, a marked increase in the level of SCE was observed in cells of four of the six patients tested, whereas in the remaining two patients the SCE level also was significantly raised compared with that in healthy human cells. In the experiments with 4NQO the induced SCE level also was sharply raised (in three patients) and moderately raised (in three patients) compared with the induced SCE level in cells of healthy blood donors.

According to the data in the literature treatment of schizophrenic patients with chlorpromazine together with other neuroleptics did not lead to an increase in the number of SCE in the cells [4]. Analysis of the "short" path of repair revealed deep inhibition of the stage of repair of DNA breaks in seven of 10 patients tested and partial inhibition in two patients. A raised gamma-induced SCE level was observed in the cells of all six patients (in two cases it was sharply increased). In the cells of five of the 11 patients tested, reduced reactivation of the virus was observed, whereas a small increase in the number of gamma-induced mutations was recorded only in cells of two patients (by contrast with the experiments with 4NQO). We obtained similar data on disturbances in repair processes in other hereditary diseases: Marfan's syndrome [2] and homocystinuria.

However, no changes in the levels of mutability of the virus induced by these same mutagens were observed in the cells of any of the 20 patients (with Marfan's syndrome) studied. A small rise in the level only of gamma-induced mutations was found in cells from patients with homocystinuria (characterized by retardation of mental development). It must be emphasized that the group of patients chosen for testing was heterogeneous. No correlation was found between the duration of the disease and changes in the various criteria.

Thus disturbances of repair processes were found in cell cultures from 15 schizophrenic patients, and four of the 13 criteria tested distinguished the group of patients as a whole quite distinctly from the healthy group: raised levels of SCE, both spontaneous and induced by the two mutagens, and increased mutability of the virus. Disturbances of individual stages of repair were recorded in cells from the overwhelming majority of patients. Although only three of the 15 patients were untreated, no differences in principle were observed between the results of their investigation compared with the treated patients. Nevertheless, a definite influence of pharmacotherapy cannot be completely ruled out. If the results reflect the etiopathogenic mechanisms of the disease, they do not contradict the hypothesis of the hereditary nature of schizophrenia, for the number of hereditary diseases in which disturbances of repair processes are found is steadily increasing as new syndromes become investigated.

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CHOLESTEROL EXTRACTION FROM RABBIT BLOOD INTO MULTIPLE EMULSIONS

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Components of biological fluids can be extracted from blood with the aid of liquid membranes, in the multiple emulsions version [5, 6]. For instance, experiments with solutions of lipoproteins, isolated by preparative ultracentrifugation, have shown that emulsions containing glycosides in their internal phase selectively extract nonesterified cholesterol (ChS) of atherogenic β- and pre-β-lipoproteins (LP).

The aim of this investigation was to study the possibility of extracting ChS directly from the blood of a rabbit with experimental atherosclerosis, followed by reinjection of the decholesterolized blood into the animal.

EXPERIMENTAL METHOD

To extract the ChS an emulsion of the following composition was prepared: the liquid membrane — mineral oil (39 ml) and sorbitan oleate (surfactant, 1 ml), internal phase — buffered isotonic solution, pH 7.4 (90 ml), and digitonin (500 mg, 4.06 mM in the internal phase).

The freshly prepared emulsion obtained by dispersion of the internal phase in the membrane by mixing on a propeller mixer, was exposed for 30 min in a graduated funnel with the isotonic buffer solution. This last operation prevents hemolysis (Fig. 1) due to the fact that during preparation of the emulsion a certain amount of digitonin (1-3%) may be adsorbed on the outer surface [3, 6]. No digitonin is subsequently lost into the extracted phase (blood) from an emulsion prepared in this way, and the high stability of the emulsion thus prepared (the half-separation time into layers exceeds 24 h) makes it possible for ChS to be extracted from blood without preliminary separation of the erythrocytes.

Under intraperitoneal pentobarbital anesthesia (40 mg/kg) the rabbit's femoral vein was exposed and catheterized, for removal and reinjection of the blood. To prevent the blood from clotting heparin was injected intravenously in a dose of 500 U/kg. Portions of rabbit blood (20 ml) were exposed with the digitonin emulsion in the separating funnel at room temperature and with moderate mixing. The ratio of the phases of blood and emulsion was 1:1 by volume. Under these circumstances the emulsion was distributed among the volume of blood in the form of globules measuring 2-3 mm. After extraction for 40 min the phases were separated and the blood returned into the rabbit's femoral vein. For subsequent exposure blood was again taken from the femoral vein. The process of blood extraction was repeated five times, and 24 h later it was repeated a further four times.

In the course of 1 day of the experiment, i.e. from the 1st through the 5th extraction on the 1st day and from the 1st through the 4th extraction on the 2nd day, the concentration of ChS in blood samples taken from the femoral vein did not change significantly. Accordingly, total initial concentrations were used for the calculations: 11.90 mM (1st day) and 12.10 mM (2nd day). The ChS concentration in the blood stream after the end of each day of the experiment was determined 3.5-4 h after the blood sample had been returned into the vein.

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