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ROLE OF DNA REPAIR PROCESSES IN SISTER CHROMATID EXCHANGE FREQUENCY CHANGES IN PERIPHERAL BLOOD LYMPHOCYTES DURING INFLAMMATORY DISEASES

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KEY WORDS: sister chromatid exchanges; DNA repair; inflammatory diseases of the lungs.

Since the discovery of sister chromatid exchanges (SCE) in cells [12] and the beginning of their active study with bromodeoxyuridine [7] many aspects of this phenomenon have been studied and, in particular, variability of the SCE level depending on different factors. It has been observed that the final yield of SCE is largely dependent on the characteristics of the stage of the cell cycle during exposure, the clinical nature of the induced lesion, the rate of its removal and the degree of preservation of lesions of different types, leading to the appearance of SCE [13].

DNA injuries arising spontaneously in the cell during its biological activity (apurine and apyrimidine regions, etc.) activates a number of mechanisms leading either to chromosomal aberrations or to SCE. The main role in these mechanisms is played by repair processes, aimed at removing the lesions or converting them into a less dangerous form for the cell. Changes in the intensity of these processes and, in particular, in human lymphocytes, lead to changes in the SCE level [3].

Recent data showing the higher frequency of reduced ability of lymphocytes to carry out repair in patients with nonspecific lung diseases (NSLD) and a further weakening of this ability after a course of antibiotic therapy [1] suggested that changes may also occur in parameters such as SCE.

The aim of this investigation was to study the frequency of SCE in human peripheral blood lymphocytes in patients with nonspecific inflammatory diseases of the lungs.

EXPERIMENTAL METHOD

Patients hospitalized for NSLD (acute pneumonia, chronic bronchitis, bronchial asthma during an exacerbation) were investigated before the beginning of treatment, i.e., before administration of any therapeutic substances, and immediately after the end of antibacterial therapy (5-7 days after the beginning of administration of drugs). Healthy persons of the corresponding age and sex served as the control group.

Whole blood (0.5 ml) was cultured for 72 h in medium RPMI 1640 with the addition of L-glutamine and inactivated calf serum, in the presence of bromodeoxyuridine (10 μg/ml). Colchicine (0.5 μg/ml) was added 2 h before fixation. Hypotonic treatment was carried out in KCl solution, followed by fixation with a mixture of ethanol and acetic acid (3:1). Films were stained by the method in [2].

Ability of peripheral blood lymphocytes to repair DNA was expressed in conventional units, and was assessed as the ratio between reparative synthesis, stimulated by a standard dose of UV irradiation (100 J/m^2) and spontaneous reparative synthesis. An ability to repair of under 2 conventional units (c.u.) was considered to be low [1].

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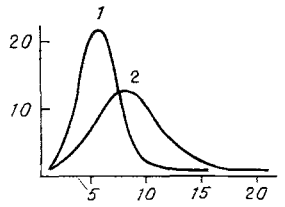

Fig. 1

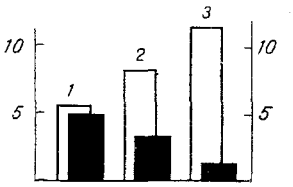

Fig. 2

Fig. 1. Distribution of number of SCE per cell in peripheral blood lymphocytes from normal subjects (1) and patients with NSLD (2). Abscissa, number of SCE per cell; ordinate, number of cells with the given number of SCE (in per cent).

Fig. 2. Frequency of SCE and DNA-repair ability of peripheral blood lymphocytes of normal subjects (1) and patients with NSLD before (2) and after (3) antibiotic therapy. Ordinates: left (white columns) frequency of SCE per cell, right (black columns), ability to repair DNA (in c.u.).

EXPERIMENTAL RESULTS

A marked increase in the frequency of SCE was observed in lymphocytes from patients with NSLD compared with healthy subjects ($P < 0.01$). The frequency of SCE in healthy human lymphocytes varied between 1 and 17 SCE per cell, compared with between 1 and 22 SCE per cell in the patients' lymphocytes, and it increased after antibiotic therapy. No change was found in the proliferative ability of the cells as shown by the relative numbers of cells in the first, second, and third mitoses during inflammatory diseases compared with the control (in all cases $t < 1$ for paired comparison of means). The increase in the frequency of SCE in the patients took place on account of an increase in the number of cells carrying an increased number of exchanges (Fig. 1).

Data characterizing the ability of lymphocytes from normal subjects and patients before and immediately after the end of antibiotic therapy to repair DNA, compared with the SCE level in lymphocytes are given in Fig. 2. It will be clear that in patients with NSLD the average ability of the peripheral blood lymphocytes to repair DNA was rather lower (3.8 ± 0.4) than normally (4.9 ± 0.4, $P > 0.05$). A low level of DNA-repairing ability of the lymphocytes was observed in 35-40% of patients (but only in 2.5% of normal subjects). Immediately after the end of the course of treatment with antibiotics of the penicillin or tetracycline series, the ability of the lymphocytes to repair DNA was profoundly depressed. Correlation was thus found between the increase in the number of SCE and the decrease in the ability of peripheral blood lymphocytes from patients with NSLD to repair DNA.

The phenomenon of SCE formation is linked with the appearance of breaks in the parental DNA chains in the replicating chromosome. Integrity of the DNA molecule in the cell may be disturbed through influences directed toward DNA or toward the mechanisms maintaining its structure.

Incidentally, repair processes play a passive role in the realization of DNA injuries in the form of SCE, but they facilitate changes in the number of these injuries in the cell. For instance, differences have been demonstrated in the frequency of SCE appearing in cases when the cells were held up in the presynthetic phase [5], and also during induction of injuries at different stages of the G_1 phase [8, 10]. Participation of repair processes in the final manifestation of DNA injuries in the form of SCE is definitely confirmed by observations [6] showing differences in the frequency of SCE in human lymphocytes during induction of injuries that are recognized differently by the repair enzymes and removed at different rates.

Correlation between DNA repair processes and SCE formation can also be studied on the basis of the results of an investigation [4] which revealed different levels of SCE in cells of the second reduction division in mice of different strains [BALB/c > Dub: (IKR) > C57BL/6j], fertilized with spermatozoa from IKR mice, irradiated beforehand with UV light. Before this, a similar order of arrangement was obtained for the level of extraordinary DNA synthesis in mouse spermatogonia after treatment with methyl methanesulfonate [9, 11], which suggests that differences in the level of UV-induced SCE may be attributed to differences in the number of unrepaired DNA injuries.

There is no doubt that direct correlation does not exist between the ability of cells to repair DNA and the SCE level in all cases, for many factors can influence the course of these processes in cells under normal conditions and during changes in physiological status. In the writers' view, toxic products (endotoxins, free-radical products) produced in NSLD may cause injury to DNA that is manifested as an increase in the frequency of SCE. Antibiotic treatment may lead to an increase in bacteriolysis, with massive release of bacterial toxins, and may increase the degree of injury to the cell DNA. The sharply reduced ability of the patients' lymphocytes to repair DNA may be due to two causes: inhibition of activity of the DNA excision repair enzymes by toxic products, and deficiency of repair enzymes for the "healing" of both types (endogenous and UV-induced) DNA injuries.

Injury to the chromosomal material, manifested as an increase in the frequency of SCE, observed during an inflammatory process, and the simultaneous decrease in the ability of lymphocytes to repair DNA may thus be evidence of disturbance of the intracellular mechanisms aimed at maintaining the structural integrity of DNA.

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INCREASED NUMBER OF REPEATED NUCLEOTIDE SEQUENCES IN TRANSCRIPTIONALLY ACTIVE DNA AND POLY A^+-mRNA FROM RAT LIVER AND INDUCTION OF ITS INCREASED TRANSLATION ACTIVITY

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KEY WORDS: DNA, liver, induction

It was shown previously that the number of repeated sequences (RS) [4-8] in the composition of transcriptionally active DNA (taDNA) [4, 5] is increased in rat liver cells under the influence of various factors inducing gene expression (cortisol, phenobarbital, regeneration). These RS are actively transcribed and are found among giant nuclear RNA (gnRNA) [4]. No appreciable changes take place in the composition of the unique sequences of liver taDNA on induction of transcription by cortisol [4]. We know that cortisol induces enzymes of gluconeogenesis in the liver of animals [15], and that certain xenobiotics, including the aminoazo

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