EFFECT OF ALKYLATION AND CARBAMOYLATION ON THE CHARACTER OF GENETIC INJURIES IN MAMMALIAN SOMATIC CELLS CULTURED IN VITRO

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The genetic consequences of the combined action of alkylation of carbamoylation on the character of genetic injuries in mammalian somatic cells and also the concrete contribution of each of these factors to mutagenesis are virtually unstudied.

The aim of this investigation was to study the time course of appearance of different types of genetic injuries and, in particular, of gene mutations, micronuclei, and major chromosomal disturbances, culminating in fragmentation of the cell nucleus, as a result of alkylation or the combined action of alkylation and carbamoylation during the action of N-nitroso-N-methylurea (NMU).

EXPERIMENTAL METHOD

Chinese hamster cell line CHO-AT3-2, obtained from Dr. G. M. Adair (Texas Medical Center, USA) was used. To record alkylating activity the cell culture was treated with NMU for 7 min, and to evaluate the combined action of alkylation and carbamoylation, it was treated with NMU for 60 min [1, 3]. Gene mutations affecting the HGPRT and Na^+/K^+-ATPase loci were taken into account, with demonstration of clones of mutant cells on the corresponding selective media with 8-azaguanine and ouabain [5]. The cytogenetic activity of NMU was determined by counting the number of micronuclei in the cells at the interphase stage by the method [2] in our modification. When cells with micronuceli were identified, criteria suggested for human leukocyte cultures [6] were used. Cells with fragmented nuclei were counted separately. The data were subjected to statistical analysis by the method used to analyze induced mutations in cultured CHO cells [7].

EXPERIMENTAL RESULTS

With an increase in the duration of exposure to NMU from 7 to 60 min, the mortality among the cells was greatly increased and the difference in the lethal effect of the two alternative treatments was particularly marked with the higher doses.

With an increase in the duration of exposure of the cells to NMU the number of mutations induced at the HGPRT locus increased considerably. The absolute number of mutations affecting the Na^+/K^+-ATPase locus also increased, but when high concentrations of NMU were used it was lower than after short exposure. Thus the Na^+/K^+-ATPase locus was more sensitive to alkylation and the HGPRT locus was more sensitive to alkylation accompanied by carbamoylation.

After treatment with NMU for 7 and 60 min a concentration-dependent increase in the number of micronuclei was induced; the number of cells with micronuclei after treatment with NMU for 60 min, moreover, was greater. After exposure to high NMU concentrations for 60 min a fall of the level of induction of micronuclei and an increase in the number of cells with fragmented nuclei, evidently connected with a considerable lethal effect, was observed (Table 1). With the transition from alkylation to a combination of alkylation and carbamoylation, the number of cells with fragmented nuclei increased by an order of magnitude.

The different mutagenic effects of NUM depending on the duration of exposure can be explained by different types of induced lesions.

The HGPRT locus records a wide spectrum of mutations: point mutations, small and large (more than 28 kbp) deletions, chromosomal aberrations [4, 8, 10]. The Na^+/K^+-ATPase locus records only point mutations of the "mis-sense" type [4].

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TABLE 1. Effect of Treatment of Cells with NMU for 7 and 60 min on Induction of Micronuclei and Fragmented Nuclei in CHO-ATŽ-2 Cells

Concentration of NMU, μg/ml	Duration of exposure of cells, min	Number of cells tested, thousands	Number of cells with micronuclei (per thousand cells)	Number of cells with fragmented nuclei (per thousand cells)
Control (solvent)	—	6	7,4±1,03	6,6±0,97
10	7	4	19,0±2,52*	8,6±1,7
10	60	4	44,7±3,33*	42,7±3,27*
25	7	6	38,0±4,36*	23,0±3,39*
25	60	4	61,3±4,52*	135,7±6,73*
50	7	7	59,3±4,45*	27,0±3,0*
50	60	3	38,0±4,36*	338,0±13,0*

Legend. *$p \leq 0.001$.

The increase in the sensitivity of the HGPRT locus to a 60-min exposure to NMU and the absence of response of the Na^+/K^+-ATPase locus can be explained by broadening of the spectrum of induced lesions. After exposure to NMU for 7 min mainly point mutations arose, but after an exposure of 60 min, besides continuing induction of mutation of the base pair substitution type, there was an increase also in the proportion of more severe injuries. Broadening of the spectrum of mutagenic action may be connected with various factors, including the appearance of unrepaired single-strand breaks in DNA under the conditions of the inhibitory action of carbamoylation on repair of DNA injuries induced by alkylation [3]. A certain proportion of the number of mutations affecting the HGPRT locus may also be accounted for by chromosomal aberrations [9], induced by double-stranded breaks in DNA, which also are recorded after the modifying effect of carbamoylation on effects induced by alkylation by NMU [1].

A comparative investigation of the two mechanisms of action of NMU on chromosomes and chromatids from the point of view of induction of more severe injuries revealed the different character of the effects. In the case of alkylation, a predominantly dose-dependent increase in the number of induced micronuclei, the result of nonincorporation of acentric fragments, remaining behind from chromosomes and chromatids, in the nuclei of the daughter cells, was recorded. In response to the combined action of alkylation and carbamoylation the general character of the genetic effects changed in favor of induction of more serious injuries, namely fragmentation of the nuclei. At the same time the number of cells with micronuclei increased considerably.

The increase in the number and the change in the character of predominant genetic lesions as a result of combined exposure to alkylation and carbamoylation are evidently not only connected with continuing alkylation, but also reflect the result of strengthening and widening of the spectrum of mutagenic action of alkylation, when followed by carbamoylation. Under these conditions the agreement between the increase in number of NMU-induced gene mutations affecting the HGPRT locus and the number of micronuclei may be partially dependent on realization of the same primary changes, which are based on single-stranded breaks in DNA.

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