ACTION OF N-ALKYL-N-NITROSOUREA ON THE PROTEIN-SYNTHESIZING SYSTEM IN CELLS OF THE LIVER AND HEPATOMA 22a

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A single injection of N-methyl-N-nitrosourea (80 mg/kg) into mice with a hepatoma causes injury to the principal components of the protein-synthesizing system (the polyribosomal complex and soluble factors of the cell juice) in the liver cells (reversible changes) and in cells of hepatoma 22a (irreversible changes). The results are confirmed by experiments in vitro on a cell-free protein-synthesizing system.

Despite the successful clinical use of N-alkyl-N-nitrosourea and the results of investigations into the mechanism of their antitumor action, the causes of the selectivity of action of these compounds on dividing cells have not yet been discovered [5, 10].

The effect of N-methyl-N-nitrosourea (MNU), which has mutagenic, carcinogenic, and antitumor activity [2, 4, 7, 8], on the protein-synthesizing system was studied in liver cells of adult mice and on cells of hepatoma 22a.

EXPERIMENTAL METHOD

Male C3HA mice of three groups were used in the investigation: healthy newborn (1 week) and adult animals (weighing 20-22 g) and animals with hepatoma 22a on the 12th-13th day after inoculation [3]. MNU in 0.9% NaCl solution was injected intraperitoneally in doses of 20-80 mg/kg. These doses are close to the therapeutic level (LD_{50} = 138 mg/kg, maximal permissible dose 100 mg/kg). Incorporation of leucine-C^{14} (8-10 μCi) into total proteins of the liver and hepatoma tissue homogenate and into complete polypeptide chains of polysomes (mice decapitated 15 min after intraperitoneal injection of the isotope) and in growing polypeptides (decapitation 5 min after injection) was determined [1]. Sedimentation analysis of the polysomal-ribosomal material was carried out in a sucrose density gradient with low and high ionic strength.

Experiments in vitro. MNU was injected into the animals in a dose of 80 mg/kg, and they were killed 1, 19, and 48 h later. Samples of a cell-free system (0.1 ml) contained the following components [6, 9]: 1 mmole ATP, 0.1 mmole GTP, 0.6 mmole CTP, 7 mmole mercaptoethanol, 10 mmole creatine phosphate, 0.16 mg/ml creatine phosphokinase, 60 mmole tris-HCl, pH 7.6, 55 mmole KCl, 55 mmole NH_4Cl, 5 mmole $MgCl_2$, a mixture of unlabeled L-amino acid (without valine, leucine, lysine, and phenylalanine) in concentrations of 0.04 mmole each, and of C^{14} amino acids (valine, lysine, leucine, and phenylalanine) with activities of 0.3 μCi each, and 30 μl of the 15S or 30S fraction. The content of polysomes and 150S protein in the experiments with the reconstituted cell-free system varied. Samples were incubated for 30-45 min at 37°C.

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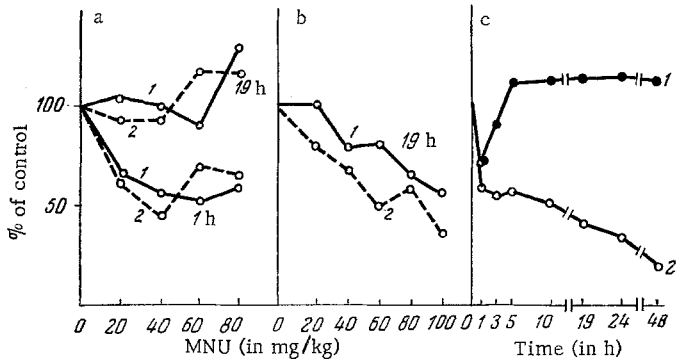

Fig. 1. Inhibition of protein synthesis (in vivo) in liver and hepatoma cells depending on dose and time of action of MNU. MNU injected intraperitoneally into animals (5-7 in a group) followed by leucine-C^{14} after specified time intervals (growing polypeptides labeled in 5 min, total proteins in 15 min): a, b) effect of different does of MNU given as a single injection 1 and 19 h before injection of leucine-C^{14} on incorporation into total protein (1) and incorporation into growing polypeptides (2) of liver (a) and hepatoma (b); c) degree of inhibition of protein synthesis in liver (1) and hepatoma cells (2) as a function of time of action of MNU (80 mg/kg). Mean values of five determinations given.

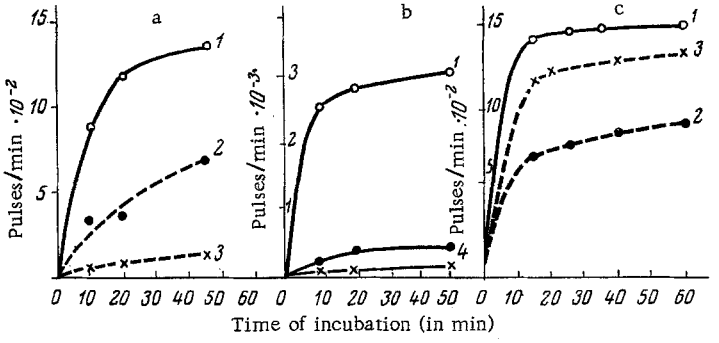

Fig. 2. Activity of unfractionated protein-synthesizing cell-free system of hepatoma (a, b) and liver (c) of mice treated with MNU: a, c) 15S supernatant (10 min at 15,000 g); b) 30S supernatant (1 min at 30,000 g). Kinetics of incorporation of C^{14} amino acids expressed in pulses [min] A_{260} unit: 1) control; 2) 1 h, 3) 19 h, 4) 48 h after injection of MNU (80 mg/kg).

The reaction was stopped by the addition of 0.1 N KOH solution and the samples incubated for 20 min at 37°C and 20 h at 5°C. Acid-soluble label was removed by washing on millipore filters. Samples (0.1 ml) for determination of the acceptor activity of tRNA contained 3 mmole ATP, 50 mmole tris-HCl, pH 7.6, 55 mmole KCl, 50 mmole NH_4Cl, 5 mmole $MgCl_2$, and 0.375 μ Ci C^{14} amino acids. After incubation for 15 min at 37°C, 2 ml of ice-cold 5% TCA containing 0.1% of unlabeled amino acid was added, and 30 min later the samples were washed in millipore filters with 5% TCA and ethanol.

The concentrations of polysomes and tRNA were determined spectrophotometrically [6, 9]. The concentration of 15S protein was expressed in optical units per ml and calculated by the formula: $(A_{260} - A_{280}) \times$ dilution. The concentration of 150S protein was expressed in mg protein/ml and calculated by the formula: $(A_{280} - 0.5 \times A_{260}) \times 1.5 \times$ dilution. Radioactivity was determined by a scintillation counter (Mark II, Nuclear Chicago, USA), using millipore filters of AUFS, RUFS, and HUFS (Chemopol, Czechoslovakia) types and a PPO–POPOP toluene system. All the samples for comparison contained identical quantities of protein.

EXPERIMENTAL RESULTS AND DISCUSSION

A single injection of various doses of MNU into healthy animals and animals with tumors caused the absolute rate of protein synthesis in the experiments in vivo in the liver and hepatoma cells to be reduced by 50-60% after 1 h, but in the liver cells the rate of protein synthesis returned to its initial level after 15-24 h while in the hepatoma cells it still remained low (Fig. 1).

Analysis of the polysomal-ribosomal material in a sucrose density gradient showed that by the action of MNU the relative number of 80S- and 105S-particles and of ribosomes capable of dissociating into 60S- and 40S-subunits in a medium of high ionic strength was increased. This effect was found in both types of cells, but in the liver after 19 h it was completely reversible while in the hepatoma its effect increased with time.

In the experiments in vitro on cell-free systems of protein synthesis the functional activity of the "coarse" system (postmitochondrial or postmicrosomal supernatant + necessary additives) was reduced after a single injection of MNU, but after 19 h the activity of the liver system was practically completely restored although the hepatoma activity continued to fall, and by 48 h it was only 5-10% of the control (Fig. 2).

Special experiments with the reconstituted system showed that during the action of MNU both polysomes and components of the cell juice, but not tRNA, are damaged in the hepatoma cells.

After a single injection of MNU into mice with hepatoma the principal components of the protein-synthesizing system in the cells of the liver (reversible changes) and in the cells of hepatoma 22a (irreversible changes) are thus damaged.

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