ACTION OF THE PESTICIDE COTORAN (FLUOMETURON) ON RNA SYNTHESIS AND TRANSPORT IN THE RAT LIVER

D. Kh. Khamidov, A. K. Mirakhmedov, G. A. Sagatova, and Sh. S. Azimova

UDC 574.2.24 + 576.315.42

KEY WORDS: RNA transport and synthesis; pesticide Cotoran

To study the mechanism of the toxic action of pesticides on mammals the study of the effect of these compounds on function of the nuclear apparatus of the cell is very important. Pesticides have been shown to penetrate inside cells, to accumulate in them, and to exert their effect through modification of certain systems controlling the genetic response of the cell. Under these circumstances the frequency of chromosomal aberrations and sister chromatid exchanges is increased [9], biotransformation of embryonic cells takes place [13]; unforeseen synthesis of DNA is observed in human lymphocyte cultures [12], and processes of DNA, RNA, and protein synthesis are disturbed [10]. It has been shown [3] that induction of cytochromes of the P-450 group is accompanied by increased transcription of genetic loci coding for particular forms of hemoprotein. One result of this is the accumulation of corresponding mRNAs of cytochrome P-450 in animal cells, potentiation of their translation by ribosomes and, ultimately, the more rapid biosynthesis of forms of cytochrome P-450 specific for the particular inducer promoting these processes [6].

In this connection the study of the toxic action of the pesticide Cotoran (a urea derivative widely used in agriculture as a herbicide) is interesting, for discovery of the principles governing disturbance of cell metabolism will go some way to elucidating the mechanisms of the pathology.

The aim of this investigation was to study the effect of the pesticide Cotoran on RNA synthesis and to study RNA transport from nuclei into cytoplasm.

EXPERIMENTAL METHOD

Wistar rats weighing 120-150 g were used in the experiments. The animals were poisoned with Cotoran by its administration through a special gastric tube. The dose of Cotoran was $0.05LD_{50}$. The animals were killed by decapitation. The nuclei were isolated [4], and the cytosol obtained by centrifugation of the postmitochondrial supernatant at 105,000*g* for 1.5 h on a "Beckman" ultracentrifuge. RNA synthesis was judged by incorporation of ^{3}H-uridine, injected intraperitoneally into the animals in physiological saline, in a dose of 100 μCi/100 g, into the nuclei and cytoplasm of the rat liver cells. The animals were killed 1, 3, and 5 days after injection of the labeled precursor of RNA synthesis and Cotoran. The nucleocytoplasmic RNA transport [15] and Mg-ATPase activity were studied, protein was determined by Lowry's method [11], and the DNA concentration was measured on an SF-26 spectrophotometer at a wavelength of 260 and 300 nm in lysate in a solution of 1% SDS, using the empirical formula C_{DNA} (μg/ml) = $(A_{260} - A_{300}) \cdot 32$. The isolated nuclei and cytosol, each in a volume of 100 μl, were applied to millipore filters, which were washed twice with cold 5% TCA and ethanol; the dried filters were counted on a "RackBeta-1217" Radioactivity Counter (LKB, Sweden).

Laboratory of Structural Organization of Biological Membranes, Institute of Biochemistry, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 1[illegible] No. 1, pp. 40-42, January, 1992. Original article submitted April 2, 1991.

 0007-4888/92/0001-0056$12.50

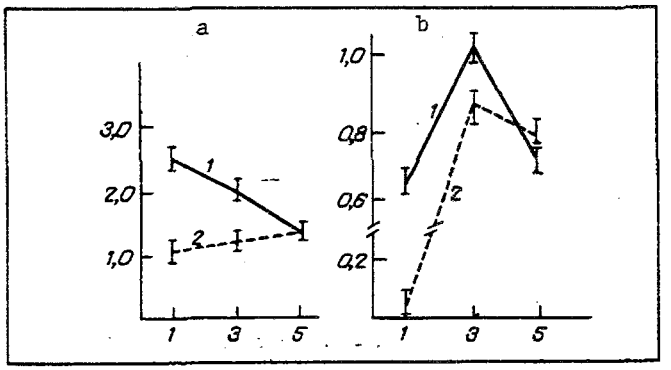

Fig. 1. Incorporation of ^{3}H-uridine into RNA of rat liver cell nuclei and cytoplasm under normal conditions (1) and after administration of Cotoran in a dose of $0.05LD_{50}$ (2). a) incorporation into RNA of rat liver nuclei, b) incorporation into RNA of rat liver cytoplasm. Abscissa, time (in days); ordinate, radioactivity (in cpm/μg DNA for a, μg protein for b).

EXPERIMENTAL RESULTS

Experiments to study synthesis of nuclear RNA, in which a single injection of Cotoran and of ^{3}H-uridine was given showed that one day after poisoning with Cotoran incorporation of the labeled precursor of RNA synthesis in the rat liver cell nuclei was reduced by almost 60% compared with the control; on the 3rd day this difference was reduced somewhat (to 50%) but not until the 5th day did the level of RNA synthesis return to control values (Fig. 1a).

The time course of incorporation of ^{3}H-uridine into cytoplasmic RNA in the liver of the normal and Cotoran-poisoned rats is shown in Fig. 1b. Clearly Cotoran considerably inhibits incorporation of the label into cytoplasmic RNA of rat liver (by almost 90%); on the 3rd day after poisoning some increase was observed in the rate of incorporation of the labeled precursor of RNA synthesis into the liver cytoplasm of the poisoned rats, although a significant difference still remained compared with the control values (up to 30%), and not until the 5th day had incorporation of ^{3}H-uridine into cytoplasmic RNA in the liver of rats treated with Cotoran returned to normal.

Thus the quantity of newly synthesized RNA in the liver nuclei and cytoplasm of the poisoned rats was considerably less than the control values, but subsequently incorporation of the labeled precursor of RNA synthesis into the nuclei and cytoplasm (on the 5th day) was restored virtually completely.

The pesticides Dieldrin and DDT [5] also have a significant effect on RNA synthesis and transport. An increase was observed in incorporation of the labeled precursor of RNA synthesis into 4S RNA, whereas incorporation of ^{14}C-uridine in 18S and 28S RNA was reduced. The decrease in synthesis of 28S RNA in the nuclear fraction is explained by the authors cited by the reaction of the pesticide-treated cells to replacement of part of the degraded cytoplasmic 28S RNA. The inequality of incorporation of labeled uridine into the different forms of RNA in the presence of pesticides is evidence of the multiphase effect of pesticides on RNA biosynthesis, and also perhaps of the more rapid degradation of cytoplasmic forms of RNA.

One of the most important features of eukaryote cells is the uncoupling of transcription, which takes place mainly in the nucleus, and translation, taking place in the cytoplasm. Consequently, in eukaryote cells a special stage of metabolism known as RNA transport appears. RNA transport is not simply the transfer of RNA from its point of synthesis to its point of function, but it also embraces a whole range of metabolic conversions of RNA. There is evidence in the literature that much of the heterogeneous nuclear RNA formed in eukaryote cells is not transport into the cytoplasm. We know [14] that 70% of primary transcripts of heterogeneous nuclear RNA do not contribute to cytoplasmic RNA. The next stage in the study was to examine the effect of Cotoran on RNA transport from nuclei into cytoplasm of rat liver cells.

It follows from the data in Fig. 2 that under normal circumstances RNA transport from nuclei into cytoplasm takes place very rapidly in the first 5-10 min, during the next 20 min of incubation the curve of the velocity of RNA transport from nuclei into cytoplasm flattens out on a plateau, and after incubation for 30 min RNA transport from nuclei into cytoplasm of rat liver cells decreases. A similar time course of RNA transport from nuclei into cytoplasm also is observed

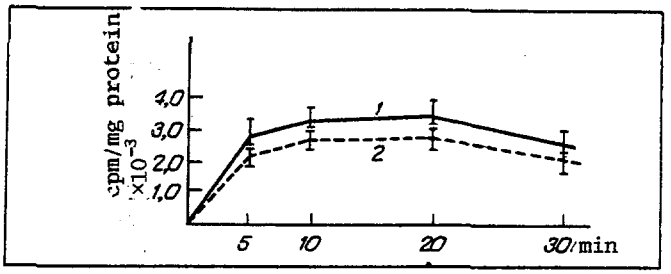

Fig. 2. RNA transport from nuclei into cytolasm of rat liver cells under normal conditions (1) and under the influence of Cotoran in a dose of $0.05LD_{50}$ (2).

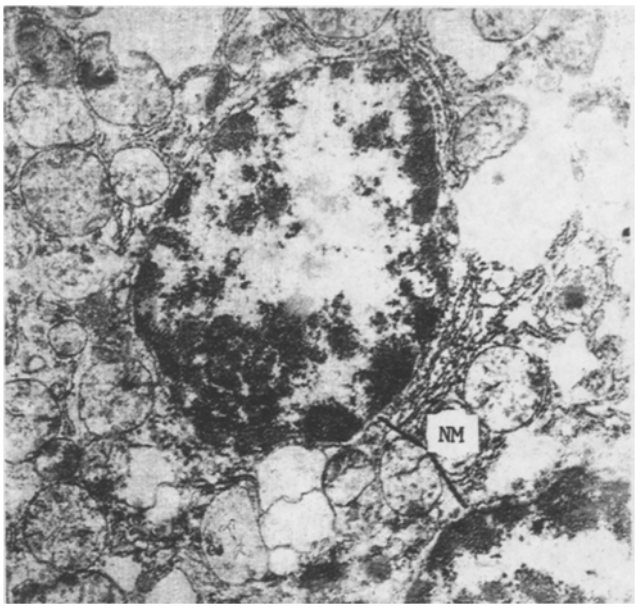

Fig. 3. Fragment of hepatocyte of a rat poisoned with Cotoran in dose of $0.05LD_{50}$. Magnification 8000. Embedding in Epon-Araldite. Stained with uranyl acetate. NM) nuclear membrane.

under the influence of Cotoran, but under these circumstances inhibition of RNA transport by 20-25% is observed at all times of incubation.

Cotoran thus inhibits RNA transport from nuclei into cytoplasm.

Let us examine why this should happen. We know that Mg-ATPase, which is essential for the provision of energy for mRNA transport [2], is a specific marker enzyme of the nuclear membrane [8]. Particularly high ATPase activity has been discovered in pores of the nuclear membrane, which are sites of permeability for RNA. Moreover, RNA transport is connected with the central granule of the pore complexes [1], and it is also possible that the process of maturation of the nuclear precursors of RNA and of cytoplasmic forms of RNA and its completion may also be connected with the pore complex.

We accordingly used the value of Mg-ATPase activity as a marked of functional activity of the nuclear membranes and of their integrity. We found that the time course of Mg-ATPase activity in the liver nuclei of rats poisoned with Cotoran is almost indistinguishable from the time course of Mg-ATPase activity in the liver nuclei of normal rats.

A photomicrograph of a section through the liver of a rat poisoned with Cotoran is given in Fig. 3. The nuclear membrane is clearly defined, although certain structural changes can be observed in the form of widening of the perinuclear space and condensation and compaction of chromatin.

The results described above show that Cotoran has no marked effect on the structural and functional state of the nuclear membrane.

LITERATURE CITED

1. G. P. Georgiev and O. P. Samarina, Usp. Biol. Khim., (Moskva), **10**, 5 (1969).
2. Ya. M. Koen and I. B. Zbarskii, Biokhimiya, **43**, No. 12, 2115 (1978).
3. V. V. Lyakhovich and I. B. Tsyrlov, Induction of Enzymes of Xenobiotic Metabolism [in Russian], Novosibirsk (1981).
4. G. Blobel and V. R. Potter, Science, **1**, 1662 (1966).
5. R. A. Chung and C. S. Williams, Arch. Environ. Contam. Toxicol., **15**, 581 (1986).
6. J. Fagan, J. Pastewka, F. Guengerich, and H. Gelboin, Biochemistry, **22**, No. 8, 1927 (1983).
7. R. M. Hays and P. Barland, J. Cell Biol., **31**, 209 (1966).
8. H. Ishikawa, S. Sato-Odani, and K. Ogata, Biochim. Biophys. Acta, **521**, 650 (1978).
9. H. Konietzko, W. Haberlandt, H. Hellbronner, et al., Arch. Toxicol., **40**, 201 (1978).
10. J. M. Lockard, B. P. Schuette, and P. S. Sabharwal, Experientia, **38**, 586 (1982).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., **193**, 265 (1951).
12. P. Perrocco and G. Prodi, Cancer Lett., **13**, 213 (1981).
13. P. Price, C. M. Hassett, and J. I. Mansfield, In Vitro, **14**, 290 (1978).
14. M. Salditt-Georgieff and J. E. Darnell, Molec. Cell Biol., **2**, No. 6, 701 (1982).
15. D. B. Schumm and T. E. Webb, Biochem. Biophys. Res. Commun., **58**, 354 (1974).