EFFECTIVENESS OF RIBONUCLEOTIDES FOR REPLACEMENT THERAPY IN CERTAIN DISEASES

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Yeast RNA and products of its enzymic degradation have a replacement therapeutic effect in degenerative lesions of the retina, in spinal amyotrophies, and in chronic trophic ulcers. It is postulated that congenital or acquired disturbances of nucleotide metabolism (dysnucleotidoses) are an element in the pathogenesis of certain diseases.

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In the last decade reports have been published indicating that some cell and body functions can be stimulated by interference with metabolism of nucleic acids and their precursors. It has been shown that oligoribonucleotides and oligodesoxyribonucleotides stimulate the immune response in normal and irradiated animals and activate proliferation of antibody-forming and other cells [4-6, 11, 12, 15, 18]. Polymers of cytidylic and guanylic acids activated desoxycytidylate- and desoxyguanylatekinases and stimulated proliferation of bacteria [10].

Ribonuclease, in low concentrations, stimulated biosynthesis of antibodies and other proteins in fragment cultures of the spleen [2, 14]. A mixture of oligoribonucleotides accelerated hypertrophy of the nucleolus and muscle fibers of the heart in experimental myocardial hypertrophy. RNA stimulated wound healing [8]. Data of this type served as the basis of new research in this field.

In the present investigation the effect of ribonucleotides was studied in certain diseases.

EXPERIMENTAL METHOD

A preparation of yeast RNA was given by mouth in courses of 0.1-0.2 g three times daily for 7-21 days. A sterilized mixture of oligonucleotides – products of enzymic hydrolysis of yeast RNA (RNA in a solution containing 5-10 mg/ml was treated at 45° for 1 h with "Reanal" pancreatic ribonuclease, 200 μg/ml) – was applied locally.

EXPERIMENTAL RESULTS

The preparation was given to 15 patients (studied by S. F. Shershevskaya). These included 14 patients with central and peripheral tapeto-retinal degenerations and one patient with secondary degenerative changes of the macula lutea associated with progressive myopia. Before administration of RNA, all the patients had been treated unsuccessfully by repeated and prolonged courses of vitamins and vasodilator drugs.

Subjectively, 14 patients described a marked improvement in vision and clarity of orientation in darkness. Control perimetry, campimetry, and investigation of dark adaptation, carried out before the start and after the finish of treatment, showed considerable improvement in function in 13 of the 15 patients. The maximal effect was obtained in relation to dark adaptation, which was considerably improved in 13 patients. The twofold improvement in dark adaptation observed in 2 patients with typical pigmental degeneration was particularly demonstrative in this respect. An increase in visual acuity by 0.3-0.5 took place in 5 patients, by 0.2 in 4 patients, and by not more than 0.1 in the rest.

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The greatest effect was observed in patients with typical pigmental degeneration, where in one case the visual acuity was increased from 0.2 to 0.7-0.8, and in the other from 0.2 to 0.5-0.6. Widening of the visual field by 20-35° was observed in the two patients with pigmental degeneration, and by not more than 12-15° in the rest. As a rule widening of the visual field occurred mainly in the temporal portion. Treatment was ineffective in the patient with changes in the macula lutea associated with progressive myopia, and also in secondary optic atrophy. In central tapetoretinal degenerations the results were not as good as in typical pigmental degeneration. In all cases the effect was transient and disappeared toward the end of the first month of observation. Repeated administration of RNA was accompanied by an early (after 2-3 days) and more lasting (1.5 months) effect.

RNA was given to 5 patients with spinal amyotrophies (studied by L. M. Popova). All these patients had extensive flaccid pareses and paralyses of the muscles of the trunk and limbs. One patient had bulbar manifestations (dysarthria, dysphagia, fibrillary twitching of the facial muscles and tongue). Two patients had been on artificial respiration for 1 and 7 years, respectively. Respiratory failure or the first degree was present in three other patients.

The most marked effect after administration of RNA was observed in patient G (with bulbar disturbances), who had been on artificial respiration for 7 years. The progression of the bulbar disorders ceased in this patient. He was able to manage for 10-15 min without the respirator. The strength of his hands and of the trunk muscles increased, so that he was able to write and to do mental work for 6-8 h daily. The effect was temporary in character, and four further courses of treatment were required during a period of 7 months. The disease did not progress further. In a second patient, the strength of the paretic muscles of the limbs increased. However, after stopping treatment, the pathological process continued to progress. In two patients a slight increase in strength was observed without neurological changes. In one patient no effect was found.

Enzymic RNA hydrolysate was applied as external dressings on 10 patients with chronic indolent trophic ulcers in various situations (studied by A. L. Shnaper); this group included 5 patients with trophic ulcers of the leg, 1 with an ulcer in the region of an irreducible subcutaneous hernia, 1 with an ulcer of the buttock due to syringomyelia, and 2 with posttraumatic ulcers of the leg. The leg ulcers reached 10 × 17.5 cm in size. The ulcers in other situations measured up to 5.5 × 11 cm. The duration of the disease ranged from 9 months to 30 years. All patients had been treated repeatedly. In two patients the ulcer had never closed completely. Before treatment with nucleotides, the ulcers had never remained healed for 9-18 months despite persistent treatment. In 7 of the 10 patients treated with RNA hydrolysate, the ulcers healed in the course of 7-45 days. In three cases, in elderly patients with severe concomitant diseases (cardiovascular failure, diabetes mellitus, chronic osteomyelitis) the ulcers did not heal.

Before discussing these results it should be mentioned that the most marked effect was observed in patients with retinal degenerations. In experimental degeneration of the retina, a rapid decrease in the RNA content in the cytoplasm of the nerve cells has been observed [3], direct justification for the clinical administration of nucleotides. According to some observations [1], functions of the retinal nerve cells are connected with the state of their RNA metabolism. An appreciable effect was observed also in one patient and a moderate effect in two patients with spinal amyotrophies, but these effects were temporary. The improvement in subjective and objective indices several days after the beginning of each course of treatment and the gradual disappearance of the effect after stopping treatment are presumptive evidence of the effectiveness of treatment. Objective evaluation in the group of patients with trophic ulcers is more difficult. These ulcers healed in the course of 7-45 days. Evidence of the effectiveness of administration of RNA hydrolysate was given by the fact that prolonged preliminary treatment (1-1.5 years) by other methods was unsuccessful.

When RNA is given by mouth, it undergoes enzymic degradation initially to oligonucleotides. The possibility that some of these products may pass from the intestine via the liver into the blood stream (when high doses of RNA are given) has never apparently been investigated. The problem of the effectiveness of products of more intensive RNA degradation taking place in the intestine and liver thus remains.

The daily dose of RNA given to the patients in this investigation corresponded to its content in about 300-400 g muscle tissue. The qualitative composition of the yeast RNA preparation is specific: it consists mainly of transfer RNAs with a large quantity of so-called minor bases. Ultimately, therefore, it is possible for large quantities of oligoribonucleotides (or their decomposition products) of specific structure

to enter the bodies. It has been shown [9] that RNA-C^{14}, when given parenterally, disappears rapidly from the blood stream. In the blood and tissues,RNA undergoes degradation to nucleotides, riboses, and free bases. Nucleotides help to form the nucleotide pool of the cells. Ribose is oxidized to CO_2 or used for synthesis of carbohydrates. Nitrogenous bases are used for resynthesis of DNA or RNA or are excreted as allantoin and urea. Autoradiographically, this worker found labeled bases in the DNA and RNA of various tissues. The paper cited includes a survey of the extensive literature on nucleic acid reutilization. Its author states that RNA which has not been broken down is not utilized in the cell. However, a number of tissue culture investigations have demonstrated that this can in fact take place [7, 16, 19]. At the same time, it has been shown [7] that RNA assimilated by cells (lymphoblasts) undergoes degradation within the cell.

There are two points of view regarding the possible action of its degradation products. One is widely accepted: degradation products of RNA are utilized for synthesis of cell RNA and DNA.

The second point of view is based on the assumption that RNA degradation products in some way regulate certain cell functions. It has been shown that products of hydrolysis of RNA and DNA activate cell growth and multiplication [17]. A mixture of bases (adenine, guanine, cytosine, uracil) or of the corresponding ribonucleotides was ineffective. These workers conclude that oligonucleotides are the active principle. It is known [10] that synthetic polymers of cytidylic and guanylic acids activate desoxycytidylate- and desoxyguanylatekinases.

Some pathological processes of uncertain etiology (especially in the nervous system) are accompanied by a decrease in the content and a relocation of the RNA in the cell. Very probably this is due not only to disturbance of DNA-dependent RNA synthesis, but also to disturbances of synthesis of precursors and of utilization of degradation products of nucleic acids. It may thus be a matter of an insufficiency of nucleotides or disturbances of their metabolism, i.e., of dysnucleotidoses of a specific type. A direct argument in support of the validity of this assumption in the present series of observations is the demonstrable replacement therapeutic effect of degradation products of RNA in the disease mentioned above, characterized by disturbances of cell nutrition.

The writers consider that the replacement effect of hydrolysis products of yeast RNA is based on collection of the deficiency of products of this type in the cell. The assumption that local disturbances of nucleotide metabolism in the cells and tissues may be present is perfectly justified, for differentiated cells evidently do not obtain substances of this type from outside sources, but synthesize them themselves.

LITERATURE CITED

1. V. Ya. Brodskii, Cell Nutrition [in Russian], Moscow (1966).
2. I. V. Konstantinova, L. V. Van'ko, B. B. Fuks, et al., Dokl. Akad. Nauk SSSR, Ser. Biol., 176, No. 4, 942 (1967).
3. S. F. Shershevskaya and Yu. E. Morozov, Byull. Éksperim. Biol. i Med., No. 11, 37 (1968).
4. W. Braun, in: Molecular and Cellular Basis of Antibody Formation, Prague (1965), p. 525.
5. W. Braun, M. Nakano, M. Hechtet, et al., Fed. Proc., 24, 286 (1965).
6. W. Braun and M. Nakano, Science, 157, 819 (1967).
7. P. Chin and M. Silverman, J. Immunol., 99, 476 (1967).
8. A. F. Dumorot, Ann. Surg., 150, 799 (1959).
9. H. Enesco, Exp. Cell. Res., 42, 640 (1966).
10. W. Firshein, R. Benson, and M. Sease, Science, 157, 821 (1967).
11. H. Friedman, Ann. N. Y. Acad. Sci. 114, 444 (1964).
12. B. N. Jaroslow and W. H. Taliaferro, J. Infect. Dis., 98, 75 (1956).
13. H. Keir, Biochem. J., 85, 265 (1962).
14. K. Merritt and A. G. Johnson, J. Immunol., 94, 416 (1965).
15. G. Shanmugam and P. M. Bhargava, Biochem. J., 99, 297 (1966).
16. K. Shepley, E. Ambrose, and K. Kirby, Nature, 208, 1072 (1965).
17. M. Simić, P. Petkovic, and D. Mancić, Bull. Inst. Nucl. Sci. "Boris Kidrich" (Beograd), 9, 155 (1959).
18. C. Yoon, Exp. Cell Res., 38,386 (1965).