Structure and Functions of Biopolymers

The study on membrane-stabilizing and anti-inflammatory actions of yeast RNA *in vivo* and *in vitro*

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It has been shown that nucleic acids reveal membrane-stabilizing and anti-inflammatory actions depending on the origin and the purity level in in vitro and in vivo experiments. The purified yeast RNA has been found to be the most effective.

Keywords: nucleic acids, yeast RNA, cell membranes, anti-inflammatory action

The inflammation is a localised reaction of tissues to injury, caused by different factors of exogenous and endogenous origin. Physical, chemical and biological factors are distinguished among exogenous factors. Inflammatory mediators, antigens, antibodies are the representatives of the endogenous factors. The inflammatory reaction is necessarily followed by the disorders of structure and the cell membrane penetration.

The functions of cell membranes as well as their relation to inflammation are well-studied. The inflammation is always connected with the changes in arachidonic acid, nitric oxide exchanges, and free radicals formation. Due to the inflammatory process starting a great amount of metabolic cascades, the use of inhibitors, or the analogues of arachidonic acid metabolites, does not allow balancing all reactions and thus regulating the inflammatory process in a complex way. Non-steroidal anti-inflammatory drugs (NSAID), *e.g.* aspirin, may block certain stages of inflammatory process, but they can not stabilize cell membranes, thus it limits their influence on the inflammatory process.

Nucleic acids have attracted many scientists' attention for a long time as pharmacological preparations. Besides, the RNA, the products of its partial hydrolysis and synthetic polynucleotides, are known for their wide biological activity spectre [1] as they stimulate protein synthesis in cells [2] and have anti-tumour activity [3].

The RNA preparations may increase antibodies formation and decrease inductive phase of their genesis [4-6]. Yeast RNA was shown to be capable of normalizing some immunological indices, namely, T-lymphocytes, B-lymphocytes, and macrophages. Exogenous RNA was shown to be included into RNA of lymphocytes and macrophages in 2 hours after insertion [7]. The data obtained allow supposing that yeast tRNA may penetrate into the cells in the form of intact molecules [8].

The goal of the present work was to study anti-inflammatory action of nucleic acids on the models of erythrocyte membranes stabilisation *in vitro* and *in vivo* at local inflammation caused by carragheenin.

Materials and methods

The following preparations of nucleic acids have been investigated: DNA-T (thymus of cattle), DNA-CE (chicken erythrocytes) (*Reanal*, Hungary), tRNA (*Escherichia coli*) (*Serva*, Germany) and total RNA-D (yeasts) in final concentration of 0.01 %. As the standard of anti-inflammatory preparation, aspirin 0.06 mg per vial with enriched plasma was accepted. RNA-D was obtained from *Saccharomyces cerevisiae* yeast and RNA-P, RNA-PN, and RNA-F which were different in degree of purification from protein, DNA, and polysaccharides were obtained from *Candida utilis*. RNA was extracted by 10-12% NaCl solution at 100-110?C with subsequent cooling to 0?C and acidizing by HCl to pH 1-2. After precipitation, RNA was washed by ethyl alcohol, dried and dissolved in water. pH of the solution was made 8.0-8.2 by sodium hydroxide and then pancreatine was added. Solution was sustained for 1 hour at 37-40?C. The enzyme was inactivated by boiling and then solution was filtered with subsequent precipitation by cold ethyl alcohol, acidized by HCl to pH 1-2, and dried. RNA-F was obtained. Then the procedure was repeated in such a way in order to obtain more purified RNA preparations.

To estimate the effectiveness of membrane-stabilizing action of the nucleic acids preparations in case of damage by free radicals, acid resistance of washed erythrocytes of normal mice blood was determined. Acid haemolysis of washed erythrocytes was induced by sodium nitrite in the concentration of 250 ?g/ml for the initiation of oxide damage of erythrocytes.

Acid haemolysis of erythrocytes is presented in the form of integral parameter of this process: summary acid resistance of erythrocytes which was defined, multiplying the obtained erythrocytes cells which were haemolysed in the certain period of time (a_i) by t_i. The summary resistance is $I = a_i \cdot t_i$

Results and Discussion

We showed that biological activity of RNA depends on its origin kind. The preparations of purified yeast RNA turned out to be twice more active than the preparation of *E.coli* tRNA. Yeast RNA has membrane stabilizing properties in the wide concentration range. The detailed study showed that membrane protective action of yeast RNA depends on its purity and the presence of protein.

The anti-inflammatory action of nucleic acids *in vivo* was also studied on the model of local inflammation of mice paws, caused by caragheenin, which we compared to the influence of aspirin. The preparation of yeast RNA was found to show significant concentration dependency of anti-inflammatory activity.

In the concentration of 5, 10, and 15 mg per mouse the preparation inhibits oedema formation in 36.74 %, 47.17%, and 53.13% respectively. The preparations DNA-T and DNA-EK also showed anti-inflammatory activity, though in rather high concentrations (15 mg per mouse), and the indices of anti-inflammatory action were 26.58 % and 33.27 % respectively.

The next stage of the work was the study of yeast RNA influence on the NO-synthetase (NOS) activity in blood plasma and erythrocytes of mice. It is known that oxide damage of protein and lipid components of plasmatic erythrocytes membrane takes place at inflammatory processes, the result of which is the activation of nitric oxide of biosynthesis, an active oxidizing agent especially for haemoglobin of erythrocytes. It was shown that yeast RNA causes substantial inhibitor action on the activation of oxidizing way of L-arginin metabolism at the caragheenin injection which results in the inhibition of NOS activity in blood plasma.

On the basis of obtained results it is possible to make the conclusion that nucleic acids have rather expressed anti-inflammatory properties compared to aspirin. In particular, yeast RNA showed the most significant anti-inflammatory activity.