

STIMULATION OF ILEAL EPITHELIUM GROWTH AND REGENERATION BY DIETARY NUCLEOTIDE EXTRACTS

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Summary: The gastrointestinal tract epithelium plays an important role not only in digestion and absorption of nutrients, but also in antigen and pathogen signal translocation toward the gut associated lymphoid tissue. Malnutrition in various degrees is recognized as the most common cause of the immune system dysfunction. Research done in the past several years has revealed that dietary nucleotides (dNT) represent an essential compound of nutrition because of their importance in metabolic pathways, energetic processes and nucleic acid synthesis during tissue renewal. Much evidence accumulated suggests that dNT are essential for the growth and maturation of the gut epithelia. In previous experiments we have documented immunoregulative properties of dNT-containing extracts. In this study Balb/c female mice were fed (1) standard diet, (2) dNT-supplemented diet, and (3) dNT-supplemented water for 4 weeks. The supplement in dose of 100 mg/kg/l comprised original extract (Imuregen, Uniregen Ltd., Náchod, Czech Republic). Samples of terminal ileum in each dietary group were removed for histological examination. The length of villi was evaluated by computer morphometry. The highest growth of intestinal villi was observed in group administered dNT-supplemented water. We have found no pathological changes of intestinal epithelium in any experimental group.

Key words: *Dietary nucleotides; Terminal ileum; Tissue renewal; Nutrition*

Introduction

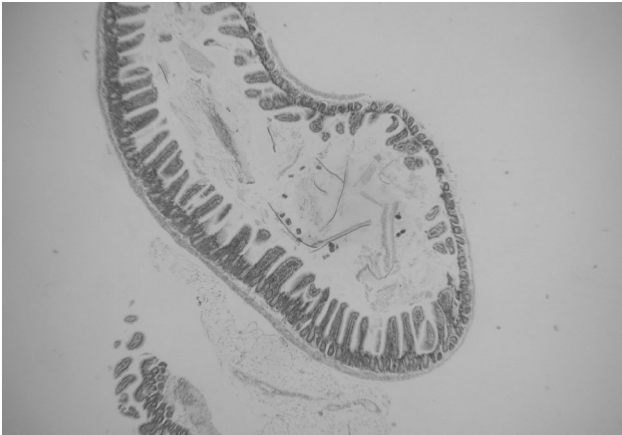
Epithelium of the gastrointestinal tract besides its important role in digestion and absorption of nutrients is involved in spreading of antigenic and pathogenic signals to gut associated lymphatic tissue. Various forms and degrees of malnutrition are considered to be the most frequent causes of immune system dysfunctions.

Recent papers solving the relationship of immune system and dietary nucleotides (dNT) conclude that food oligo- and polynucleotides represent one of the most important component of nutrition regarding their meaning for a lot of metabolic and energetic processes in human body (14).

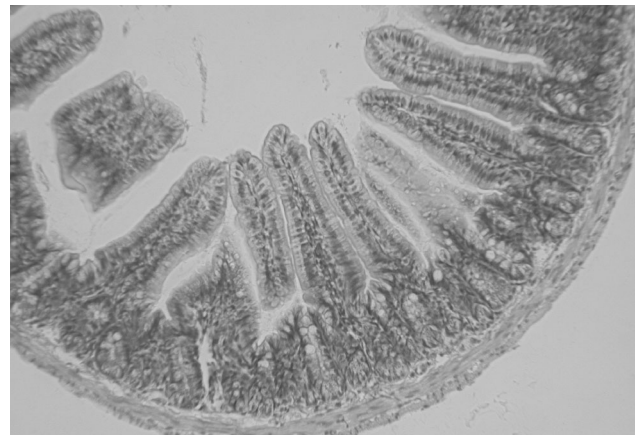
The immune system, in order to develop properly and to overcome infections without complications, demands increased supply of some substances which are present in current diet, however not in sufficient quantities. These are preferably dietary nucleotides, their consumption reaches such levels that they become essential in above mentioned situations. Based on recently obtained evidence, dNT com-

bined with some other compounds, e.g., glutamin, arginin, are considered to be the basic component of immunonutrition. In nineties, there were published sound proofs that dNT are important and from many points of view are an inevitable nutrition component. The body is able to use them directly from food without energetically demanding synthesis *de novo*. Their insufficient supply by food may result in dysbalance of immune functions, moreover it may be reflected in dysfunction of liver, heart, and gut. In childhood, it may manifest as developmental retardation of central nervous system, immune system and disorders of general growth (4,8).

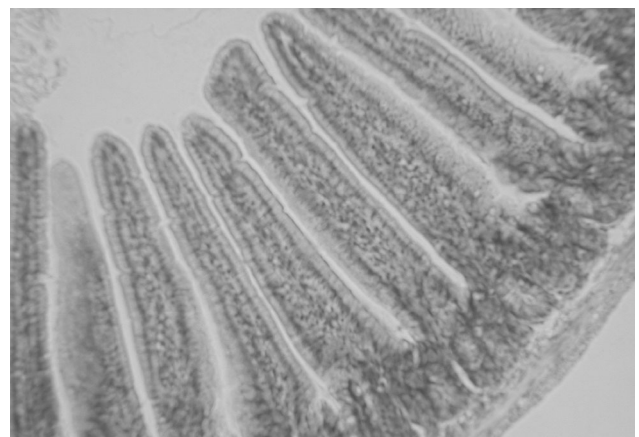
Hitherto available results suggest that dietary nucleotides are essential also for growth and maturation of gut epithelium. In previous experiments in animal models, we have documented immunoregulatory capabilities of animal extracts containing dNT, above all their influence on the cellular component of immune response (11). In this work we studied changes of terminal ileal epithelium in animal model after administration of dNT mixture in food or water lasting several weeks.



Figs. 1A, B: Control group. A. Short and thin villi of terminal ileum. 40x. B. Detailed view. Enterocytes and goblet cells of villi. 100x.



Figs. 2A, B: Experimental group fed by dNT-supplemented diet. A. Higher and thicker villi of terminal ileum. 40x. B. Detailed view of epithelium. 100x.



Figs. 3A, B: Experimental group fed by standard diet and drinking dNT-supplemented water. A. The highest ileal villi. 40x. B. Detailed view of epithelium. 100x.

Figs. 1-3: Histological samples of small intestine (terminal ileum). Female mice Balb/c, 3 months old. Staining: hematoxylin-eosin.

Material and Methods

Experimental animals: 15 female mice Balb/c, 3 months old with an average weight 22 g.

Experimental groups: (5 mice each)

1. Control group fed standard diet ad libitum,
2. Group fed dNT- supplemented diet,
3. Group fed standard diet and drinking dNT-supplemented water for 4 weeks.

Imuregen - dNT supplemented substance (Uniregen Ltd., Náchod, Czech Republic) - was administered in dose 100 mg/kg of food or 100 mg/l of drinking water. (Composition of Imuregen: free amino acids 8,56 mg, minerals 68 mg, and nucleotides 0,07 mg/100 mg.)

Experimental design:

Animals were subdivided into 3 groups and fed (1) standard diet ad libitum, (2) dNT-supplemented diet, (3) standard diet and drinking dNT-supplemented water for 4 weeks. At the end of experiment, the animals were killed by cervical dislocation. Samples of digestive organs, thymus, lymphatic nodes, and spleen were taken. In this study we present findings on terminal ileal specimens. Tissues for histological examination were fixed, embedded in paraffin, stained by hematoxylin-eosin and by stain for acid mucopolysaccharides according to Hale-Müller. During light microscopic examination the height of villi was evaluated by computer morphometry.

Methods of statistic evaluation

In each group there were measured heights of villi. Variances of measured heights of villi in control and both experimental groups were compared by Snedecor's F-test. Student's t-test was used for checking the validity of null hypothesis on difference of measured average height values. Differences in average height values were considered statistically significant if the value of the test characteristics was higher than corresponding quantile of Student's distribution at 5 % level of significance ($p < 0.05$).

Results

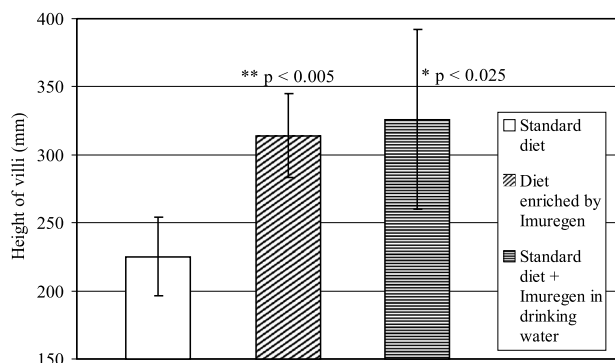
We found neither pathological changes in morphology of intestinal villi in any experimental group nor significant differences in percentual ratio of enterocytes and goblet cells in histological sections stained by hematoxylin-eosin or according to Halle-Müller.

The height of intestinal villi was significantly different in experimental groups. The lowest villi were found in control group fed standard diet only (Figs. 1A, 1B). Increase in height of villi was observed in both experimental groups administered by dNT-supplement (Figs. 2A, 2B, 3A, 3B).

Evaluation of morphometric data proved statistically significant difference in height of intestinal villi in both experimental groups when compared with a control group (Graph 1). More conspicuous increase was found in case of

supplementing drinking water ($p < 0.005$) than after consumption of supplemented diet ($p < 0.025$).

In given experimental design, we proved positive effect of nucleotide mixture of dietetic immunostimulating preparation Imuregen on regeneration and growth of terminal ileal epithelium.



Graph 1: Statistic evaluation of the length of terminal ileum.

Discussion

Complex role of dNT in human body is now in the focus of theoretical and clinical experts. Above all, there is studied the impact of dNT on postnatal development, processes of regeneration, support of immune functions during recovery after surgery, in chronic infections, and in old age.

Nucleotides are structural elements of nucleic acids and as mono-, di-, and triphosphates are included in almost all metabolic functions (13). In form of nucleosides they are inevitable components of some metabolic active substances, especially the coenzymes. They act in energetic metabolism as donors of the phosphates, in form of mediators they are indispensable, e.g., for aggregation of thrombocytes or during vasodilatation. They are also necessary in organism for cell division in regeneration of tissues, desquamation of skin, renewal of mucous epithelia, and for hemopoiesis (11).

It is assessed that during 24 hours there is renewed about 300 grams of enterocytes (9). The need of nucleotides is increased during developing immune response due to clonal expansion of antigen activated-lymphoid cells, and moreover during differentiation of immunocompetent cells (12).

Neither endogenous synthesis nor reutilization are fully sufficient to meet increased demands of metabolically high active cells and tissues (immunocytes, enterocytes, liver parenchyma, processes of regeneration, epithelization, etc.). Food containing utilizable dNT may contribute to equilibrated balance of endogeneous nucleotides, and as it was said they are inevitable for growing and renewing tissues, and during stress conditions, too (4).

It was revealed in animal experiments that nucleosides are absorbed by enterocytes of the small intestine (5,6,7),

are taken up by hepatic cells (1) and skeletal musculature (10).

Accelerated repair of enterocytes and increased contents of mucoproteins, engaged in indirect augmentation of secretion immunity, was observed during administration of dNT after chronic inflammatory conditions (2).

Conclusions

The results of our experiment proved that 4 weeks lasting administration of oligo- and polynucleotide mixture of dietetic preparation Imuregen caused statistically significant increase in average height of terminal ileal villi in female mice Balb/c of both experimental groups when compared with control group. More remarkable changes in group drinking supplemented water may be dose-related. We suppose that dNT induce morpho-functional changes of villi resulting in both higher resorptive capability and positive impact on immune functions.

Research of dNT effects and production of supplemented foodstuffs meet proposed objectives of the World Health Organization focused on suppression of infectious, cardiovascular, tumorous, and neurodegenerative diseases.

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