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INFLUENCE of QUERTULIN ON ULTRASTRUCTURE OF MUCOUS MEMBRANE OF COLON OF RATS THAT GOT OVER-OXIDIZED SUNFLOWER OIL (ELECTRONIC-MICROSCOPIC RESEARCH)

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Abstract

Aim: To study the ultrastructure of the crypts of the mucosa of the colon of rats, a long time food content peroxidation sunflower oil and to determine the possible preventive effects on ultrastructure of the cover complex antidysbiosis hepatoprotector Quertulin (quercetin + inulin + calcium citrate)

Methods: Fragments of the mucous membrane of the colon taken from 9 rats of the Wistar, were divided into 3 groups: 1- intact animals, 2 - receiving (per os) 1 ml over-oxidized sunflower oil for 75 days, 3 - receiving over-oxidized sunflowers oil with the addition Quertulin

300 mg/kg from 31 to 75 days. Studied ultrastructural shell colon in electron microscopic TEM-100 - 01.

Results: In rats that received over-oxidized sunflower oil with food for 75 days. Electron microscopy revealed that the apical surface of columnar cells of the crypt of the mucous membrane of the colon has a disturbed plasmolemma, reduced microvilli, the pathological state of individual mitochondria. In its own plate of the mucous membrane there are large electron-transparent patches, indicating the edema of the basic substance. Rats that received the diet over-oxidized sunflower oil and antidysbiosis hepatoprotector Quertulin (quercetin + Inulin + calcium citrate) 300 mg / kg daily, columnar cells without disturbances, tight contact with each other. In the cytoplasm of these cells, there is an increased amount of organelles (ribosomes, mitochondria), the Golgi complex is expressed clearly. And, although in some places there are areas in the mucosal crypts in which columnar cells are destroyed the plasmolemma, but in the cytoplasm contains many mitochondria with electron-dense matrix and clear crypts.

Conclusion: The results of an electron microscopy study showed that after administration of over-oxidized sunflower oil to rats, the structure of the mucous membrane in large areas is significantly damaged. The treatment with Quertulin in rats greatly contributes not only to the restoration of the ultrastructure of the mucous membrane of the large intestine of rats, but also greatly enhances the metabolic processes in them.

Key words: large intestine, over-oxidized sunflower oil, ultrastructure of mucosal of colon, anti-dysbiosis agent, hepatoprotector.

In the process of heat cooking fat of unsaturated fatty acids of dietary fat produces toxic products of peroxidation [1-3].The use of these products leads to the development of pathological processes in the body, primarily in the gastrointestinal tract [4].Unfortunately, the state of the mucosa after consumption peroxidation oil has not been studied. Therefore, the aim of this work was to study the ultrastructure of the crypts of the mucosa of the colon of rats, a long time food content peroxidation sunflower oil and to determine the possible preventive effects on ultrastructure of the cover complex antidysbiosis hepatoprotector Quertulin (quercetin + inulin + calcium citrate) [5].

Materials and methods

Material (fragments of the mucous membrane of the colon) taken from 9 rats of the Wistar, weighing 217– 281g were divided into 3 groups. The first group - intact animals is considered a control. The second group of rats receiving (per os) 1 ml over-oxidized sunflower oil for 75 days. The third group of the rats receiving (per os) 1 ml over-oxidized sunflowers oil with the addition Quertulin 300 mg/kg from 31 to 75 days. Animals were taken from experience in a deep state of anesthesia according to the "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" (Strasbourg, 1986), and the Law of Ukraine № 3447-IV [6].

For electron microscopic examination, pieces of mucous membrane of the large intestine of rats were recorded in 2.5% glutaraldehyde solution in phosphate buffer at pH value of 7.4 with additional fixation of 1% of osmium acid at the same pH of the buffer solution. The samples were then dehydrated in spirits of increasing concentration. Impregnation of the tissues and their polymerization occurred in a mixture of resin epon-araldit. Contrasting ultra-thin sections was carried out using the method of Reynold [7].

Studied ultrastructural shell colon in electron microscopic TEM-100 - 01.

Results and discussion

Ultrastructure of the mucosa of the large bowel of healthy animals. Ultrastructural studies revealed that epithelial cells of the crypts of the colon, located in the crypt is pretty tight, that is, there are tight intercellular contacts. However, these cells are in different functional state. Some of them have several electron-light cytoplasm, which is a small number of organelles: the small number of mitochondria, some of which has destroyed cristae, diffusely located in the cell granules granular endoplasmic reticulum (GER), cisterns and vesicles of smooth endoplasmic reticulum (SER). Ribosomes, on membranes as GER, and free very little.

Microvilli of the brush border on the surface of such cells are uneven, some of them are fragmented. At the same time, there are located epithelial cells with a more electron-dense cytoplasmic matrix with more intracellular organelles than those described above. In these cells the cistern GER have extended profiles (Fig.1). Microvilli on the apical surface of these cells are located sparsely, some of them fragmented. In some cells there are no microvilli. At the heart of the crypt, little differentiated columnar cells lie on the basal membrane, have round or oval nuclei, one or two nucleoli, in which the granular and fibrillar components are well defined. At

the heart of the crypt, little differentiated columnar cells lie on the basal membrane, have round or oval nuclei, one or two nucleoli, in which the granular and fibrillar components are well defined. In the case of chromatin, it is mainly in the condensed state located under the karyolemma, that is, its marginalization, as well as separate lumps lies throughout the karyoplasm, whereas chromatin in the diffuse state is practically absent.

Only the delicate thin-fibrous net is determined. The cytoplasm of cellular cells is electron-transparent, in which diffusely free ribosomes and mitochondria with focal destruction of the cristae are located, which may be due to high energy consumption and intracellular needs (Fig.2).

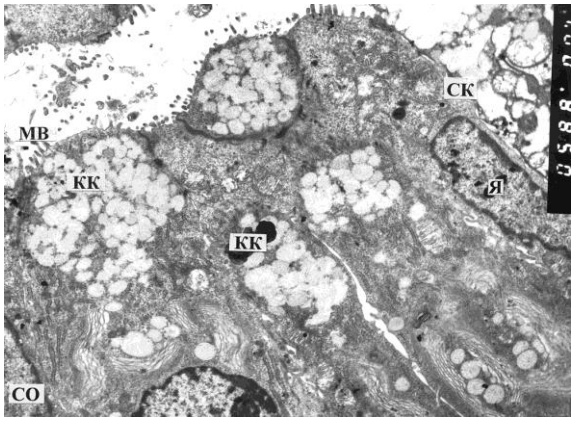

Fig. 1. Ultrastructure of the mucous membrane of the colon of the control animal

A cluster of goblet cells in the epithelial layer of the mucous membrane

Electronic microphotography: X 3,000.

Symbols: N- nucleus, MV- microvilli, GC- a goblet cell, MM- mucous membrane, CC- column cell.

Among the columnar cells are the goblet cells with a high content of secretion in their cytoplasm, especially these cells are many in the basis of crypts. The goblet cells in addition to the mucinous secretion, located on the apical side of the cell, have an elevated electron density of cytoplasm, an oval shape and nucleus with a convoluted karyolemma, in which there is observed there is marginization of chromatin. All the karyoplasm is filled mainly with chromatin in the diffuse state. In karyolemma well defined nuclear pores. It should be noted that in some parts of the crypt on the side surfaces and in their basis microvilli are determined to be greater than on their surface. They are located denser, have a greater height. In the area of the base of the crypt

are cells with acidophilic granules and moderate electric density of cytoplasm, in cells of the Paneth.

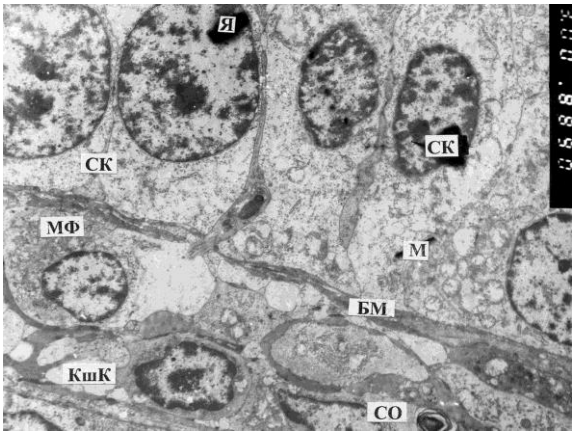

Fig. 2. Ultrastructure of the mucous membrane of the colon of the control animal.

A fragment of columnar cells located on a basal membrane with a normal structure.

Electronic Microphotography: X 4,000.

Symbols: IC - intestinal crypt, N - nucleus, MM - mucous membrane, CC - column cell, MF - macrophage, BM - basement membrane, M - mitochondria.

Its own plate crypt abundantly filled with large number of cells of histological series, which are located among the beams of collagen fibers connective tissue. In this case, in particular, there are plasmatic cells lymphocytes, single macrophages, clusters of lymphocytes, as well as single capillaries.

Ultrastructure of the mucous membrane of the large intestine of rats after feeding them over-oxidized sunflower oil.

In animals, which have received in the ration of the diet over-oxidized sunflower oil apical surface of individual columnar cells of the crypt, or, the hearth, the entire area of the epithelial plast have destroyed plasmalemma. Plots of columnar cells diffuse explode in the cavity. These cells have different degree of damage: the part of these cells remains only the kernel and the narrow rim of the cytoplasm around it, the second part of them with the saved kernel and the cytoplasm around it occupies a larger area, the other destroyed only plasmalemma. (Fig. 3, 4, 5).

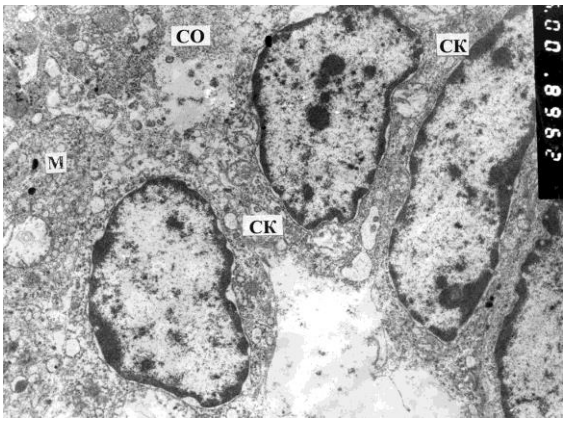

Fig. 3. Ultrastructure of the mucosa of the colon of rats after feeding them with over-oxidized sunflower oil.

Column cells with absent microvilli and focally destroyed cytoplasmic structures. Electronic Microphotography: X 8,000.

Conditional notation: MM- mucous membrane, CC - columnar cell, M - mitochondria.

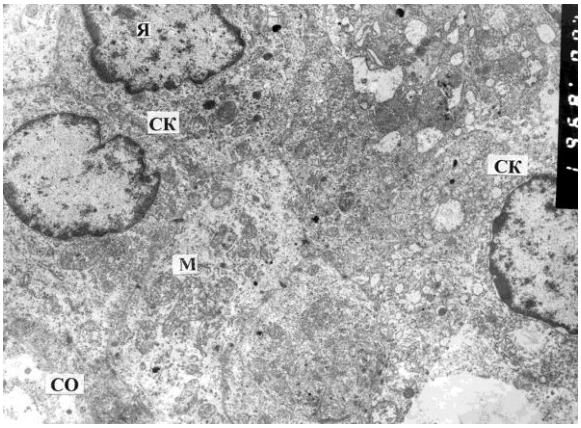

Fig. 4. Ultrastructure of the mucous membrane of the colon of rats after feeding them with peroxidation sunflower oil.

Edema of mitochondria in the columnar cells and absence of microvilli.

Electronic microphotography: X 3,000.

Conditional notation: N- nucleus, MM - mucous membrane, CC - columnar cell, M - mitochondria.

At the same time, nearby are the columns cells in the crypts, which are in a different functional condition. Thus, among the cells that have signs of activation of metabolic activity as evidenced by a slightly higher electron density of the cytoplasm, increased in it the number of

cytoplasmic organelles and on their apical surfaces are well defined by microvilli, cell with enlightened cytoplasmic matrix and edema of the organelle, especially mitochondria, are observed. These organelles have an enlightened intra-mitochondrial matrix and a destructive part of the cristae. Particular special attention is paid to the nucleus. They are larger than the similar cells of the intact animal.

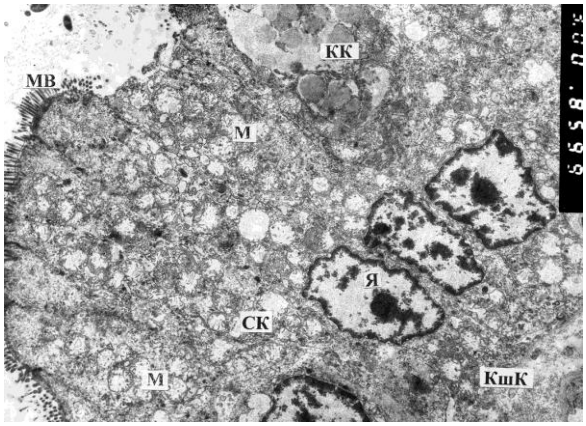

Fig. 5. Ultrastructure of the mucous membrane of the colon of rats after feeding with over-oxidized sunflower oil.

Fragment of epithelial layer: edema of mitochondria in columnar cells and absence of microvilli in their individual cells.

Electronic microphotography: X 3,000.

Conditional designation: N- nucleus, IC - intestinal crypt, CC - column cell, GC - goblet cell, M - mitochondria, MV - microvilli.

On other parts of the crypt, or in other crypts of the microvilli some columnar cells are reduced, their fragments are diffuse located in the cavity of the intestine. In their cytoplasm the elements of the hyaloplasm edema, pathological condition of separate mitochondria are observed. In separate columnar cages, located at the base of the cryptae. In this area well-defined large cells with shallow osmiophilic granules, which are located around the core, in cells of the Paneth, in which reactive changes of intracellular elements are shown. They consist in the edema of the internal mitochondrial matrix with the destruction of crystae, with the vacuolation of certain of these organelles, as well as in the extended profiles of GER cistern. It should be noted, that based on the crypts the ultrastructure of columnar cells more saved than on their apical surfaces.

In the own plate of the mucous membrane pay special attention to the large electron-transparent areas in the connective tissue, which indicates the swelling of its main substance. Bunches of collagen fibers in it occur seldom. In this area there are separate fragments of the destroyed cells, detritus and enlarged quantity, compared with the intact material, leukocytes and macrophages, separate burdens of collagen fibers have increased electron density. Among cells and fibers there are single blood and lymph microvessels. Endotiliary cells which also have signs of vacuolar degeneration.

Treatment of rats by the Quertulin on the background of feeding the over-oxidized sunflower oil.

After treatment with a Quertulin on the background of sunflower oil intake, the majority of columnar cells has a cylindrical shape, cells closely contacting each other. In the cytoplasm there is increased number of organell compared to the material of 2 groups. It contains short, narrow GER cistern, a large number of free ribosomes and polisomes, and many mitochondria. Mitochondria in cells are predominantly round form, have an electron-dense, internal mitochondrial matrix and an enlarged amount of crystae compared with the intact structure. Mitochondria are in contact with each other (Fig. 6).

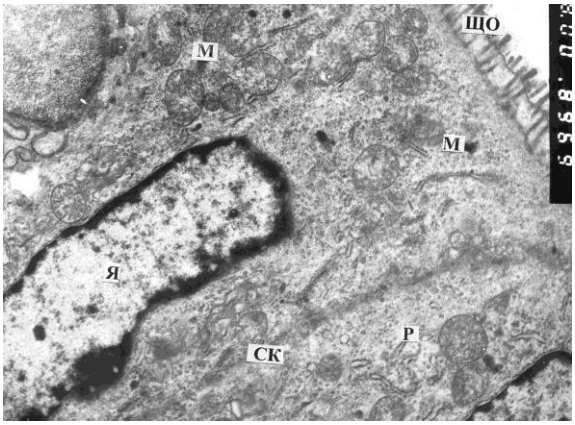

Fig. 6. Treatment of the colon of rats by Quertulin in the background of feeding with over-oxidized sunflower oil.

Column cells in the active state: in the cells a large number of mitochondria, free ribosomes and polysomes cells.

Electronic Microphotography: X 8,000.

Symbols: N- nucleus, M-mitochondria, CC-column cell, R-ribosome, BB-brush border.

In these cells, the Golgi complex is well expressed. The nuclei of these cells are large, round or oval, have a narrow electron-dense rim along the nuclear shell and there is a wave-like arrangement of the nuclear shell itself. In the karyoplasm diffusely placed fibrils of the chromatin, and granular material. Well-defined is nucleoli (Fig. 6).

On the surface of plasmolars of columnar cells a well-defined strip of glycocalyx with granular material. The border brush is also well expressed, the microvilli are thin and high. In a number of these cells in the apical region are located lysosomes, a visible accumulation of pinocytosis bubbles. At the heart of the crypt, most of the columnar cells have a normal ultrastructure, but these cells with focal degeneration of the organelles are found, and in the Paneth cells, there are extended GER and swollen mitochondria, that is, signs of hydropic dystrophy (Fig. 7).

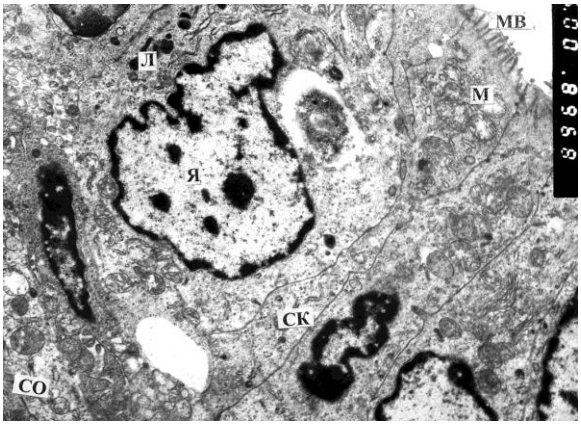

Fig. 7 Treatment of the colon of rats by Quertulin on the background of feeding with over-oxidized sunflower oil.

Column cells with a large nucleus and cariogenic invaginations with a large number of intracellular structures and a well-defined brush border.

Electronic micrograph: X 6,000.

Symbols: MM - mucous membrane, N- nucleus, SC - striated cell, L - lymphocyte, M - mitochondria, MV - microvilli.

The own plate contains some densely located cells that are characteristic of this plate and their number is increased in comparison with the material of intact animals. Among them, lymphocytes, macrophages, lymphocytes, plasma cells are determined. It should be noted that GER significantly expanded plasma cells, which are filled with secretion, are increased, and their

number is increased in comparison with the intact structure. Between cells of separate beams lies loose fibrous tissue.

At the same time, areas of the crypts of the mucous membrane are identified, on which columnar cells have lost microvilli and plasmalemma has been destroyed in them (Fig. 8).

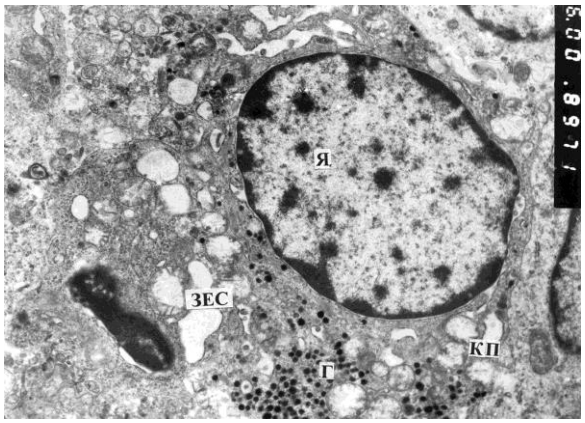

Fig. 8. Treatment of the colon of rats by Quertulin on the background of feeding with over-oxidized sunflower oil.

Paneth cell with extended elements of the granular endoplasmic reticulum and swollen mitochondria.

Electronic micrograph: X 6,000.

Symbols: N- nucleus, G - granules, GER - granular endoplasmic reticulum, PC – Paneth cell.

However, in the cytoplasm of these cells there are many mitochondria with electron-dense mitochondrial matrix and well-presented cristae. Certain crypts store the damage that was observed in the material of group 2, that is, with a significantly damaged apical surface (plasmalemma) of columnar cells. On the lateral surfaces and the basis of the crypt, the stem cells are more preserved.

The own plate is represented by single lymphocytes and macrophages, plasma cells, and others. There are also signs of edema of the main substance.

The results of an electron microscopy study showed that after administration of sunflower oil to rats, the structure of the mucous membrane in large areas is significantly damaged. A part of the columnar cells is subject to disruption in the epithelial layer and the destruction of intracellular organelles. Pathologically altered cells explode into the cavity. To a greater extent,

pathological changes in columnar cells are subject to mitochondria and the structure of the nucleus, as well as the brush border. Even in areas where the reservoir architecture is stored in the epithelial cells, the microvilli fringes are damaged, they become less and fragmented. The structure of the own plate is also pathologically altered. There are signs of edema of the main substance of the connective tissue, large nonstructural fields, detritus, remains of destroyed cells, more macrophages and leukocytes are detected than in intact structures.

After treatment with Quertulin, the structure of the epithelial layer of the crypt is more preserved. The columnar cells are more elongated, which indicates the increase of their area, cells closely contacting each other, places with the formation of interdigitation, which on the one hand increases the area of the surface of these cells, and on the other said the strengthening of metabolic processes between them. In addition, a well-defined brash border, which is suggests the possibility of intense absorption of substances and water.

Significant signs of increased metabolic activity are observed in the columnar cells, as evidenced by the large number of intracellular organelles and nuclei, which correspond to an increase in their functional activity, in particular, the synthesis of nucleic acids, protein, as well as the enhancement of adsorption properties, which is confirmed by a significant number of pinocytosis bubbles in the apical areas of cells with well-expressed microvilli rims and glycocalyx. Particularly expressed processes are directed to intracellular protein synthesis activity. Particular attention is paid to the mitochondria, there are many in the cell, most of them are large in size. Their condition indicates an increase in intra mitochondrial processes and accumulation of ATP, i.e. activation of energy processes in these cells. Conducting treatment is also beneficial for the structure of its own plate. The treatment with Quertulin in rats greatly contributes not only to the restoration of the ultrastructure of the mucous membrane of the large intestine of rats, but also greatly enhances the metabolic processes in them.

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