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Free radicals-induced morphological changes in the pancreas

Oxygen is indispensable for the life of aerobic organisms and acts as terminal oxidant in the mitochondrial respiratory chain. The univalent reduction of oxygen leads to the formation of one of reactive oxygen species. Several defense mechanisms have been developed by aerobic organisms against oxygen species (10). The functional components of these systems are called antioxidants and they are divided into three categories like: preventive antioxidants, radical scavenging antioxidants and repair compounds. Free radicals produced in incomplete oxygen reduction process are very detrimental (2). Small amounts (approximately 2-5%) can undergo an incomplete reduction. In this way the free radicals can be generated as the ultimate products (7). Under the physiological conditions these products are not dangerous for cell. Free radicals produced in biological systems are bound with the active center of the enzymes. Free radicals may cause cell damage, if the production of these compounds goes beyond the limit of the protective possibilities of enzymatic and nonenzymatic scavengers within a cell (3, 5). Free radicals play a decisive part in the pathophysiology of different diseases, such as: autoimmune disease, ischaemic disease, reperfusion, degenerate disease, acute pancreatitis, and also diabetes mellitus (1, 8, 11). It was shown that an activation of oxygen-derived free radicals occurred in tissues where the inflammatory process was noted. It seems clear that active neutrophilic granulocytes are the source of considerable amounts of oxygen free radicals (1). There are preclinical studies about the influence of normobaric hyperoxide process on the pancreas, but these studies ought to be tested clinically and criticized in the designing trials. This work was undertaken to determine the influence of normobaric hyperoxide process on morphological changes of the rat's pancreas. The authors are interested in knowing the degree of dependence between the intensification of pancreas changes and the duration of the hyperoxide process.

MATERIAL AND METHODS

Male Wistarstrain rats weighing 200-250g were used for all experiments. All the rats were given human care in compliance with institutional guidelines. The animals were fed ground chow *ad libitum*. The animals were subjected to reverse light cycling for 2 to 3 weeks before use. The middle dark point was set at 10 a.m. The rats were housed in standard laboratory cages (max. 6 pieces per cage). After

acclimation period the animals were gathered in four experimental groups. The animals lived in typical environmental conditions and breathed using atmospheric air. Inside the chamber as well as in the environment the average temperature was 23-25 °C. The exposure time of the chemically pure oxygen was the base of the classifications of the rats into four experimental groups labelled as follows: I, II, III and the K (control group). The time of exposure was 12, 24 and 48 hours, respectively. The oxygen passed as constant-oxygen flow ventilation at the rate of 2 dm^3 per minute. Oxygen concentration within the chambers was approximately 92%. It was measured using the Oxytest. Carbon dioxide concentration was determined using Capnograf. This concentration was less than 0.1%. The pressure within the chamber was analogous to barometric pressure.

The animals were dissected immediately after decapitation. Internal organs were investigated during autopsy. The whole pancreas with surrounding fatty tissue and peritoneum was cut out. The specimes for electron microscopy were fixed in 4% paraformaldehyde plus 1.5% glutaraldehyde in 0.1mol/l phosphate buffer (pH 7.2). For electron microscopy, the slices were washed overnight and they were post-fixed for 2 hours with osmium tetroxide in 0.1 mol/l cacodylate buffer (pH 7.4), dehydrated in increasing concentrations of ethanol, cleared in propylene oxide and embedded in Spurr's resin. Semithin and ultrathin sections were cut with a Reichert Cm-43 ultramicrotome. Semithin sections stained with methylene blue plus 1% Azur II in 15 sodium borate were examined in a Janowal Contrast Carl Zeiss-Jena electron microscope. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Tesla BS-500 electron microscope.

Ultrastructural identification of different cell types required the examination of several sections from each block and careful examination of numerous selected photographs.

RESULTS

In the control group the histological pictures of the pancreas were normal and referred to both parts of pancreas which have been responsible for exocrine and endocrine secretion. We observed the zymogen granules with the higher electron dense material into the exocrine cells. The dilated cisterns of endoplasmic reticulum were clearly visible. Within the lumen of some ductules the flucculent and microgranular protein substance and the single zymogen granules were found.

After 12 hours of hyperoxide process the histological pictures of the exocrine and the endocrine cells were normal. Inside the exocrine part of pancreas the numerous cells with a small amount of zymogen granules were observed. Sometimes, within the cells with numerous zymogen granules, we noted single granules with small dense material or others, with myelin figures. The latter were connected with the exocrine cell's cytoplasm. Occasionally, myelin forms were dispersed into the cytoplasm of endothelial capillaries cells. Inside the stroma, the "mature" zymogen granules were visible. Furthermore, the changed epithelial cells of the lipid granules were noted. The granules with small dense material and the myelin forms were revealed in beta-cells of pancreas.

After 24 hours of the study there was ultrastructurally noted the markedly decreased amount of zymogen granules. Numerous myelin figures have been noted in the cytoplasm of the exocrine cells as well as in stroma or within the lumen of the capillaries. Unexpectedly, large vacuoles with electron dense matrix, myelin figures and also zymogen granules were found (Fig. 1). A most intriguing aspect was intensive protein biosynthesis due to exocrine pancreas cells. These cells were deprived of zymogen granules. These features can lead to the dilatation of cisterns of RER. In this way, we observed cisterns of RER contained in the irregular nuclei, the open pores of the nuclear capsule, flucculent elements and heterochromatin deposits dispersed throughout the cytoplasm. Viewed with electron microscopy the mitochondria revealed striking and distinctive changes. They were variably

enlarged and swollen. The mitochondrial membranes were not damaged. No specific changes were noted in beta-cells of Langerhans' islets. Within the beta-cells, the amounts and electron density of the granules were decreased. Usually the endocrine cells, such as beta-cells and alfa-cells were compared with normal pictures. The dilated cisterns of SER were occasional. After 48 hours of the study, the histological hallmark of both endocrine and exocrine parts was similar to the control pictures. The decreased amounts of zymogen granules were more visible in this group than in the group of animals examined after 24 hours of the study. In addition to the myelin forms, they were collected within the exocrine cell's cytoplasm and within the stroma (Fig. 2). Generally, elimination process of myelin figures occurs due to exocytosis.

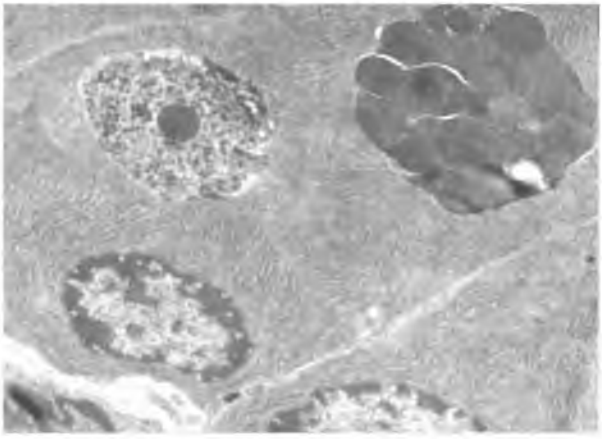

Fig. 1. Pancreas: Large vacuoles with electron dense matrix, myelin figures and zymogen granules

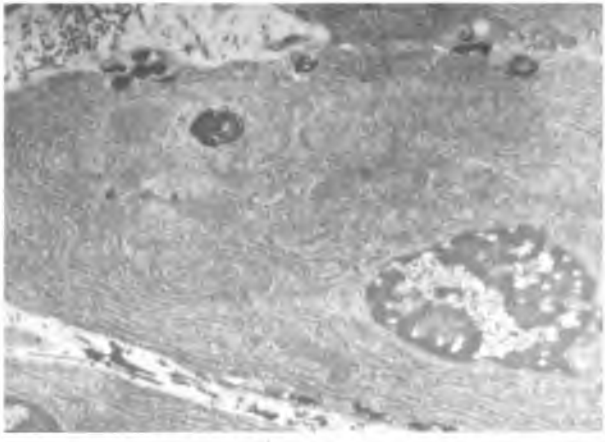

Fig. 2. Pancreas: Myelin forms collected within the exocrine cell's cytoplasm

DISCUSSION

The role of oxidative damage in pancreas disease has been proposed for many years. Both exocrine and endocrine parts of pancreas have metabolic possibilities and possibilities of inactivation of oxygen free radicals but it should be underlined that these processes are not so intensive, like the described ones in the liver (9). The importance of the former statement is supported due to the absence of smooth endoplasmic reticules aggregates. The observed changes in endocrine and exocrine parts of pancreas have adaptative and temporary character. The myelin figures are the index of bound lipid peroxidation. In the performed study, they were often visible within the exocrine cells. Sporadically, these forms were noted in the islet betacells dispersed in the cytoplasm of the endothelium cells. It is interesting that they were present within the intracellular spaces and could be sometimes noted inside the capillary vessel lumen. Therefore, the elimination process of myelin forms appears to have two ways: 1) through the injured cell's membrane due to peroxidative process, 2) due to the exocytosis. The latter way was ultrastructurally confirmed in the performed investigation (6).

The lipid vacuoles, probably resulting from the energetic disturbances are associated with myelin figures. The swelling of mitochondria observed after 24 hours of hyperoxide process confirmed this phenomenon. The mitochondrial oedema with uninjured membranes of mitochondria has got temporary character and is totally reversible. The corroboration of this fact were the ultrastructural pictures observed after 48 hours of hyperoxidation. The active protein biosynthesis in the exocrine cells and neighbouring changes of cellular nuclei testify to intracellular regeneration of damaged cells. The changes in size and electron density of endocrine islet beta-cells granules are the first symptom of their damage due to free radicals. The more intensive damages of islet beta-cells referring to other endocrine cells are typical of changes induced due to free radicals. Probably, this is the effect of smaller activity of islet beta-cells enzymes such as: superoxide dismutase-SOD, catalase-CAT, glutathione peroxidase and Cu, Zn-SOD (4).

CONCLUSIONS

1. The normobaric hyperoxide process causes the marked damage of beta-cells of Langerhans' islets.
2. The observed changes have adaptive and temporary character.

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SUMMARY

Oxidative stress occurs whenever there is an imbalance between the generation of free radicals and the antioxidant defense. Oxygen is cytotoxic if oxygen concentration is higher than its amount in the atmosphere. This phenomenon is called hyperoxide process.

This experiment was performed to determine the influence of normobaric hyperoxide process on peroxidation occurred in the rat's pancreas. Furthermore, the aim of this study was to estimate morphological changes in the pancreas.

The studies were carried out on 44 male Wistar strain rats with the initial body weight of 230-250g. After acclimation period the animals were divided into four experimental groups of 11 in a group. The exposure period of the influence of chemically pure oxygen was the base of the classification of rats into experimental groups labelled as follows: I, II, III. The time of exposure was 12, 24 and 48 hrs, respectively. The animals were dissected immediately after decapitation. The internal organs were investigated during autopsy. The whole pancreas with surrounding fatty tissue and peritoneum was cut out. Ultrastructural identification of different cell types required examination of several sections from each block and careful examination of numerous selected photographs. The histological preparations were evaluated in electron microscope. Histological evaluation revealed the presence of changes which seem to be adaptative and temporary. The B cells of Langerhans' islets were more damaged than other endocrine cells.

Zmiany morfologiczne trzustki indukowane wolnymi rodnikami

Tlen, który jest związkiem niezbędnym do życia organizmów, jest jednocześnie związkiem toksycznym. Skutki toksycznego działania hiperoksji są znane i udokumentowane od dawna. W organizmach aerobowych, poddanych działaniu stężeń tlenu wyższych niż w atmosferze, pojawiają się patologiczne zmiany mogące prowadzić do wielu chorób, a nawet śmierci. Celem pracy była ocena zmian morfologicznych w trzustce szczurów, powstałych w wyniku działania normobarycznej hiperoksji. Badano również zależność między nasileniem tych zmian a czasem trwania hyperoksji.

Badania przeprowadzono na 44 albinotycznych samcach szczura rasy Wistar o masie ciała 230--250g. Zwierzęta podzielono losowo na 4 grupy. Szczury grup I, II i III (po 11 szt.) były poddane ekspozycji czystego tlenu przez czas wynoszący odpowiednio 12, 24, 48 godz. Pozostałe zwierzęta stanowiły grupę kontrolną. Ocenę preparatów półcienkich i ultracienkich oraz dokumentację

fotograficzną wykonano przy użyciu mikroskopu elektronowego BS-500 Tesla.

Obrazy histologiczne trzustki szczurów grupy kontrolnej były prawidłowe, zarówno w odniesieniu do części zewnątrzwydzielniczej, jak też wysp Langerhansa. Obrazy ultrastrukturalne również nie wykazywały zmian. Po 12 godzinach hyperoksji w preparatach ultracienkich zaobserwowano w obrębie części zewnątrzwydzielniczej liczne komórki ze zmniejszoną ilością ziaren zymogenu lub też z zupełnym ich brakiem. Liczne pojedyncze figury mielinowe obecne były w cytoplazmie komórek części zewnątrzwydzielniczej, w komórkach endokrynnych, a zwłaszcza w komórkach B. Po 24 godz. zupełnie wyjątkowym zjawiskiem były duże wakuole autofagocytarne. W licznych komórkach części zewnątrzwydzielniczej trzustki obserwowano obrzęk mitochondriów oraz obecność ziarnistości „opustoszałych". Po 48 godz. nie obserwowano istotnych zmian histologicznych. Ultrastrukturalnie obserwowano zmniejszenie ilości zymogenu oraz obecność figur mielinowych. Częściej też obserwowano jądra z brzeżnie zlokalizowanymi skupiskami heterochromatyny i otwartymi porami otoczki jądrowej. W komórkach B stwierdzono zmniejszenie liczby, wielkości i gęstości elektronowej ziarnistości. Hyperoksja normobaryczna powoduje wyraźne głębsze uszkodzenie komórek B wysp Langerhansa w porównaniu z innymi komórkami endokrynnymi trzustki.