Effect of high frequency electromagnetic field on beta cells of langerhans islets in pancreas of rats

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Taking into account the poor and contradictory data on the effects of high frequency electromagnetic fields on both human and animal pancreas, especially its endocrine part, this paper aims to test the animal model for morphological features of pancreas beta cells under the impact of a high frequency electromagnetic field (HF EMF) featuring 1.9 GHz, 4.79 V/m, 0.24 A/m and 2.0 W/m2, for a 30-day period. The experimental animal group consisted of Wistar rats and the pancreas tissue of both control and radiated animals underwent a series of standard and immunohistochemical coloring as a preparation for stereological analysis. In our radiated group, HF EMF caused an increase in the number, volume density, and nucleocytoplasmic ratio and a decrease in beta cell surface in comparison with the control animal group, all of which indicated enhanced activity. Briefly summarizing the results of this paper, we may infer that the experimental animals subjected to HF EMF suffered morphological and morphometric changes in the beta cells. As insulin, the hormone beta cells excrete, decreases glucose levels, these results indicate a potential diabetogenic action of HF EMF in rats.

Key words: endocrine pancreas, immunohistochemistry, volume density

Introduction

The pancreas is of utmost relevance for the organism and a high frequency electromagnetic field (HF EMF) is a typical environmental feature that might be regarded as a potential health risk factor. To gain more knowledge on potential health consequences of HF EMF (1, 2) new studies are needed. Bearing in mind the complexity of the pancreas, the goal of our research was to determine the effect of HF EMF on the beta cells of the endocrine part of this organ (3, 4, 5). We were prompted by the fact that bibliographic data indicate a possible connection between EMF exposure and the constant increase in the number of diabetic patients (6). The application of highly specific immunohistochemical techniques on endocrine pancreas enables precise functional cell identification. A combination of methods confirms the existence of five major cell types: alpha, beta, D, PP, and EC cells in the endocrine part of the pancreas (7). Beta cells make 60-

70% of all compact islet cells, whereas the diffuse islets contain only 20%. Most islet cells in the cytoplasm are beta cells which contain many secretory granules the size of 200 nm containing crystallized insulin. The position of beta cells and the polarity of the insulin granules within the cell confirm the endocrine nature of their activity (8, 9, 10).

Being exposed to HF EMF, biological tissues are under the impact of magnetic flux and electric fields, which might induce new electromagnetic fields (3). The mechanisms of HF EMF activity on biological systems suggest modification of the cell membrane and protein receptors by the transfer of energy to proteins of the cell membrane (11). Furthermore, balance perturbation between free radicals production and anti-oxidative protection by electromagnetic fields has been proven (2, 11, 12). As a stress factor (4), the HF EMF affects beta cells by inducing synthesis and insulin granule release. The increased insulin synthesis causes beta cells to form a syncytium, i.e. the cell membrane fades (13).

The World Health Organization set the maximum values of HF EMF intensity and frequency within the human environment. Thus, 10 GHz is the maximum frequency, 45 V/m is the maximum intensity, and specific absorption rate (SAR) is 5 W/m^2. These are indeed high values and there still has not been a worldwide agreement on them (4, 5, 14).

Method

The research was performed with the approval of the Ethic Committee for experimental animals of the Faculty of Sciences, University of Banja Luka. The base station of the Republic of Srpska mobile phone network at the top of the Banja Luka Telecom building provided the HF EMF. The station generated the HF EMF for its own purposes, in accordance with the policy of „ZASTITA" Ltd. Banjaluka company (Report number 066/09, report date June 8, 2009, report time 10:05, air temperature at the moment of HF EMF intensity measurement was 20 C^o). Measurement equipment HF 6080 Rev2 No. 01099, HyperLOG 6080, manufactured by AARONIA Germany was used; the instrument frequency and measurement range was 1MHz – 7MHz, -90dBm – 10dBm, antenna 680 MHz – 6 GHz, manufactured in 2005.

The testing of the effect of HF EMF on the endocrine pancreas was performed on Wistar white male laboratory rats bred at the Banja Luka Faculty of Sciences vivarium, starting with the 60th up to the 90th post-natal day. The two-month old animals were divided into two experimental groups – the radiated group consisted of 21 animals exposed to HF EMF and the control group of 20 animals that were not exposed to any kind of HF field. During the experiment, 5 animals per cage were placed in both experimental groups. Both were subjected to controlled day-night light rhythm (14 hours of daylight and 10 hours of dark) with an air temperature of 20±3 °C. They were fed standard pellet lab food (purchased from Subotica Institute of Animal Health), and water was consumed as needed. The animals selected for the HF EMF treatment were exposed to it for 2 hours a day, 5 days a week. The control group was kept in a similar environment, but completely isolated from any kind of artificial HF EMF.

Once the thirty-day exposure was completed, in the early morning on the 31st day the animals were decapitated under diethyl ether narcosis. 12 animals from the control group and 15 from the radiated group were decapitated, and their pancreases were submerged in the puferized Bouin solution. The paraphine moulds were then cut into serial cross sections of 4 μm using the Reichert slide microtome and Leica Mikrotom RM 2165 (Wetzlar, Germany). The histological content of both control and exposed beta cells was studied with a light microscope,

using different cross section coloring methods such as hematoxylin eosin, Malori Azan, Mason, and Victoria blue 8GX-floxin light green. The cross sections were also colored using immunohistochemical methods typical for beta cells of the endocrine pancreas. DAKO LSAB$^+$/HRP technique was used for the presentation and localization of certain endocrine cells of the islet, in the DAKO LSAB$^+$ kit, streptavidin was marked by peroxidase. In accordance with our starting point, the number, volume density, nucleocytoplasmatic ratio, and beta cell surface results of the control and exposed animals were compared. The photographs of beta cells were taken using a Leica 8000D microscope with a MEGA VIEW camera and a digital transfer and photo analysis software system.

The reliability of differences in pancreas, cells, and cell elements between the control and radiated groups was analyzed by statistical tests using statistical software SSPS 2007 and Excel 2007. The existence and level of differences in the mean values of the monitored stereological parameters between the two groups were analyzed via the t-test. P value less than 0.05 was considered statistically significant. All the figures were displayed as means in interval high, average and low values.

Results

Once we completed our experiment with lower values of 1.9 GHz, 4.79 V/m and 2.0 W/m^2, the pancreases of both control and radiated animal groups were submitted to standard biochemical, immunohistochemical, cytological, histological, and stereological methods. The analysis of beta cells indicated that they had been activated under the impact of HF EMF, which might be considered an adaptive, anti-diabetogenic response, i.e. the diabetogenic effect of insulin. The stereological analysis of the endocrine pancreas beta cell density illustrated the increase of interval values of volume density in the animals exposed to HF EMF in comparison with the control group (Fig.1). The exposed animals displayed a 43% increase of interval values of the beta cell number (Fig. 2) which was statistically higher ($p<0.0032$) in comparison with the control group. Compared to the control group, the results of the stereological analysis of the endocrine beta cell surface illustrated the

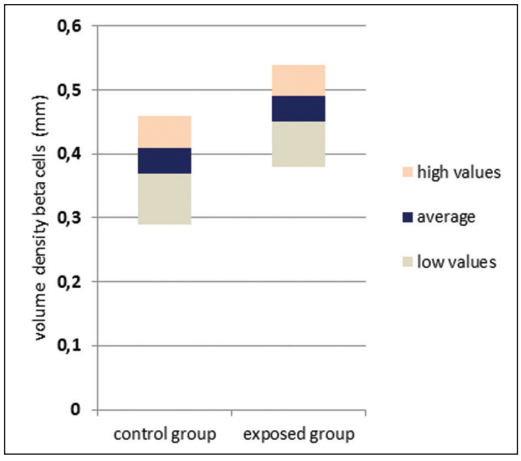

Fig. 1 – Interval values of volume density of beta cells of control group animals and animals exposed to HF EMF

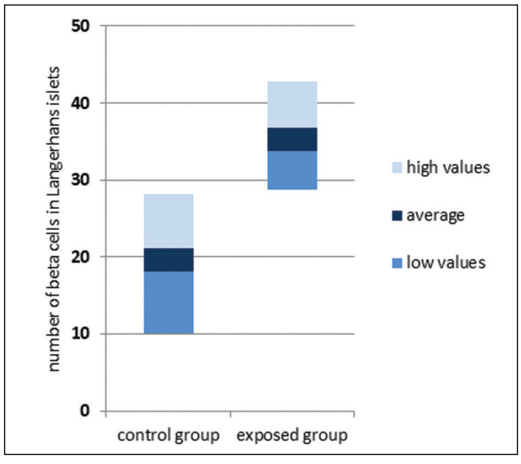

Fig. 2 – Interval values of the number of beta cells in Langerhans islets of control group animals and animals exposed to HF EMF

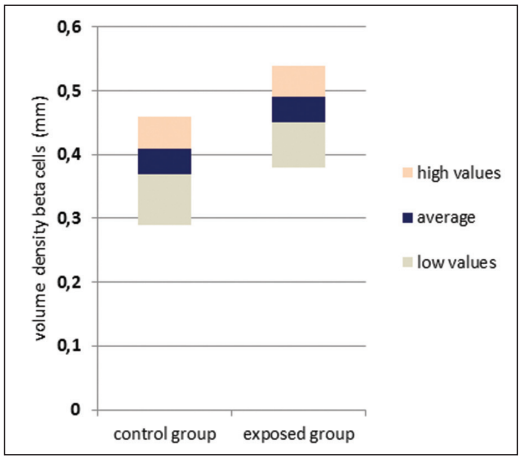

Fig. 3 – Interval values of beta cells surface of control group animals and animals exposed to HF EMF

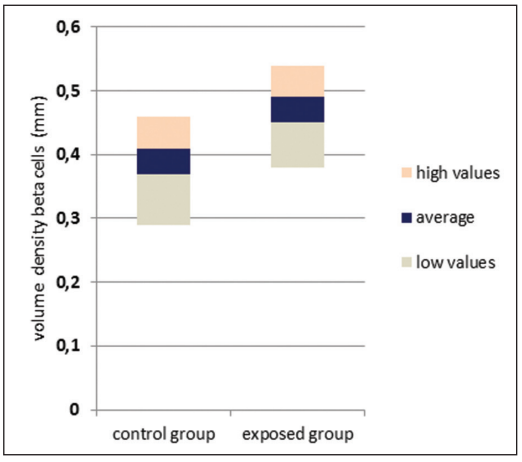

Fig. 4 – Interval values of nucleo-cytoplasmic ratio of beta cells of control group animals and animals exposed to HF EMF

decrease of this parameter in the animals exposed to HF EMF, which was statistically relevant ($p<0.0149$) as well (Fig. 3). The decrease of 42.96 μm^2 was most evident with beta cell surface mean values. The interval values of the nucleocytoplasmic ratio (Fig. 4) increased by almost 8% in exposed beta cells in comparison with the control group, which was statistically significant ($p<0.05$).

Discussion

The application of highly specific immunohistochemical techniques on endocrine pancreas enables precise functional cell identification. Our experiment established the increase in beta cell volume density in the animals exposed to HF EMF, in comparison with the control group. Furthermore, the experimental environment indicated the growth of beta cells (34.6 cells per pancreas cross section with the exposed animals and 19.8 cells per pancreas cross section with the control group), which was statistically relevant. Simultaneously, the beta cell surface decreased in the exposed animals in comparison with the control group, which was also statistically relevant. At the same time, beta cell surface decreased per 42.96 μm^2 in comparison with other Langerhans islet cells. This decrease was accompanied by a significant increase of nucleo-cytoplasmic ratio in beta cells that increased from 0.30 (the control group) to 0.33 (the HF EMF exposed animals).

Furthermore, it was shown that beta cells may result from the transformation of acinus and centroacinus cells, epithelial cells of small canals, from the existing stem cells localized in the epithelium of small outlet canals (15), or from the transformation of alpha cells. Nonetheless, nowadays, the belief is that beta cells of adult rats are multiplied via the self-duplication of the existing beta cells (16). As our experiment helped infer, the increased number of beta cells followed by the decrease of the surface upon the HF EMF exposure indicates a fast proliferation, which prevented the cells from reaching the optimum size. Such results are in compliance with the earlier data on the heterogeneity of Langerhans islet beta cells (16). The previous research proved there were subpopulations among the beta cells that differ according to glucose sensitivity. Centrally-located cells are more sensitive to lower glucose concentration than the peripheral ones. In our experiment, we noticed a stronger light

in the central beta cells within the Langerhans islet of animals exposed to HF EMF, in comparison with the control group. Such a result might indicate either the disturbance of insulin secretion, or its fast synthesis due to which insulin granules are piled within the cells. According to some researches, HF EMF causes intensive secretion of insulin from beta cells, which might be the case with our experiment as well (12, 13).

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