Global Veterinaria 5 (4): 204-208, 2010
ISSN 1992-6197

Influence of Electromagnetic Fields of Two Phases Square Wave with Low Frequency on Serum Alt and Ast Levels and Histochemistry of Hepatocytes' Glycogen

[1]Nafisi Saeid, [2]Bafande Yusof, [3]Hobbenaghi Rahim, [4]Athari Seyyed Shamsadin, [5]Hasanpour Fateme, [5]Yahyaee Fatere and [5]Majedi asl Leili

[1]Department of Physiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
[2]Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Iran
[3]Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
[4]Member of Young Researchers Club, Azad University of Tabriz Branch, Iran
[5]Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

Abstract: Long term exposition to wave generator and radiowaves, has been associated with external and internal disorders in people. Human liver contains a complex of paranchymic cells that perform different vital functions. Clarification of the effects of such waves on the liver function, seems quite interesting. In the present study, the amount of Glycogen storage in the liver lobules and cytoplasm of hepatocytes in addition to the level of ALT and AST enzyme of liver in serum were evaluated. 24 male Newzeland white rabbits allocated into 4 equal groups as follows: control group (No treatment) and 3 experimental groups which were exposed to waves with frequencyies of 1,10 and 100 HZS. AT the end of experiment, serum ALT and AST levels were assayed by using an auto analyzer device. Histopathological sections of liver were prepared of stained with PAS FOR DETERMINATION OF GLYCOGEN STORES. Results were analyzed using spss (version 9) and t test. No significant changes were observed in enzymes levels. In microscopic control samples, a lot of Glycogen was found in liver lobules. Offloading was moderate at frequency 1 (HZ). In 10 HZ frequency offloading was enhanced ($P<0.05$), however in frequency of 100 HZS no offloading was present. It was concluded that, waves with 1 to 10 Hzs frequency can decrease liver glycogen stores, with no significant effect on serum ALT and AST levels. This needs more research.

Key words: Electromagnetic · Hepatic enzymes · Glycogen

INTRODUCTION

Published reports in 1996 by the academy of scientist society showed that electromagnetic fields are able to affect on the biologic tissues, but its relation to cancer and creating mutation has not been approved yet [1]. Daily increase usage of Radio waves for different purposes has increased the number of people exposed to such waves people exposed to UHF wave producers and radio waves for a long time, have shown internal and external disorders. The most important effect of UHF is on eyes and genital tissues in men, because these organs situated on the surface of the body and are easily exposed to these waves. In addition, cardio vascular and nervous tissues are sensitive to these waves since not only are close to the surface of the body, but also have conducting abilities [2]. Lewy and Massot, discovered in 2003 that electromagnetic fields of 50 (Hz) increase activity of NAT (N- Acetyl Transferase) enzyme and HIOMT (Hydroxy Inidphel O Methyl Transfrase) and Melatonin excretion through indirect pathways. Human liver containing complex of parenchymal cells that perform different vital function. Liver is the most important place for catabolism of thyroid, steroid and other hormones and it takes part in adjusting serum hormone levels. Liver can respond to nervous and hormonal signal to set blood glucose, moreover it has a role in human Immune system [3].

Correspoding Author: Athari Seyyed Shamsadin, Member of Young Researchers Club, Azad University of Tabriz Branch, Tabriz, Iran, E-mail: ss.athari@gmail.com.

Liver is made up of a lot of lobule or practical units. Classic lobule of liver is multidimensional prism of connective tissue and biliary and vascular ducts. Central vein is placed at the center of the lobule and epithelial parechymal cells are located in a radial arrangement around the lobule [4,5]. Therefore, this work aimed to study the effect of electromagnetic fields with low frequency on the liver tissue.

MATERIALS AND METHODS

For liver histochemistry, we studied the amount of glycogen storage in liver lobules and in the cytoplasm of hepatocyts. Level of ALT and AST liver enzymes in serum were determined. A function generator set (PHYWE, Germany) was used both as a power supply and signal generator. In this study 8 (volt) power AC with 12 (A) and 2 phase square wave used.

Source or Field Producer: The produced current in function generator is conducted to two winding coils which are placed in front of each other by 20 cm distance and have a special soft metal core to amplify the field (layer structure of metal core will prevent "foko" waves). Winding coils are made by PHYWE factory, Germany, with rotation of 600 and internal resistance of 2.5 ohm which can transfer 12 A for 8 hours continuously without getting hot. The A box made of thin fiber with minimum amount of absorption is considered for waves and electromagnetic fields with 35 cm length, 20 cm width and 30 cm height, which no metal is used in it. The goal of making this box is restraining of the animal during radiation. Two square symmetrical holes with dimension of 4 × 4 cm are placed on two longitudinal walls of box to place metal cores in front of each other, so that, their distance from each other is 20 cm. for more protection of the field and prevention of its dispersal a protection strip made of pure soft metal with 3mms thickness and 5cms width is used which is placed behind the coils and covers the whole box except ceiling of the box, which is open. Design of the box is so that a matured rabbit with 2.6 kg weight is placed comfortably in it and is it not possible to exit from it. Also it provides a calm environment without stress. In the present study rabbit is used as a model and 24 white Newzeland male rabbits here are used with approximate weight of 2000 ± 100 g. male rabbits were used to exclude sex-dependent hormonal changes. All rabbits were kept in the same separate boxes for one week as an adaptation period . At the beginning of the study, animals were divided into 4 groups of 6, one group as a control and the others as experimental. Of each four groups of animals under test firstly 2ml blood was taken through ear marginal vein without using anticoagulant substance and serum was kept in freezer immediately. Then the second group of animals were radiated in an electromagnetic field with 2 phase square wave and 700 MG (Mily Gaus) intensity and 1 (Hz) frequency (for two hours and after 24 hours again for 2 hours separately). Then blood sampling was done in the same way in amount of 2ml and its serum was kept separately in freezer with .For the third and fourth group, method was the same except for in the third group, frequency changed to 10 (Hz) but other factors such as shape of electric wave of field producer, intensity of the field and timing were kept the same. At the fourth group the used frequency was 100 Hz. The serum samples of were sent to the lab and AST and ALT enzymes were assayed using auto analyzer set (Model: U.S.A. RA1000).After the second blood sampling animals were killed immediately with using CO2 gas without pain and immediately the whole liver was removed then samples were put in cold 10% formalin (with temperature). Samples were used for histochemistry evaluation and to determine the amount of glycogen storage in liver. The samples were kept in fixator solution until the usage time in . after passageing with 6- m slides were prepared from paraffin embedded samples. (the melting point of paraffin was 25°C) then for the histochemical study of changes in glycogen storage of liver, samples were stained with PAS [6,7].

RESULTS

The Results of Alt and Slt Enzymes Are Shown in Tables 1-4: In each table, the no.1 refers to enzyme level before irradiation and no.2 refers to the post irradiation level.

t-test shows no meaningful change in amount of enzymes

Table 1:Results of Ast and Alt in Control group:

Result average	Number	Control groupe
28 (I.U./L.)	6	AST
47.7 (I.U./L.)	6	ALT

Table 2: Results of Ast and Alt in 1Hz frequency :

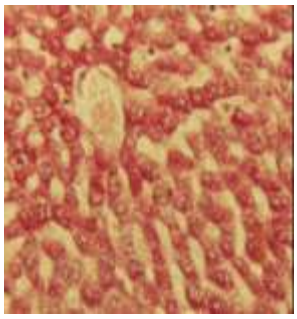
Result average	Number	Frequency with 1 Hz
29 (I.U./L.)	6	AST1
27.5 (I.U./L.)	6	AST2
49 (I.U./L.)	6	ALT1
49.7 (I.U./L.)	6	ALT2

Table 3: Results of Ast and Alt in 10 Hz frequency:

Result average	Number	Frequency with 10 Hz
29.7 (I.U./L.)	6	AST1
28.5 (I.U./L.)	6	AST2
45 (I.U./L.)	6	ALT1
47 (I.U./L.)	6	ALT2

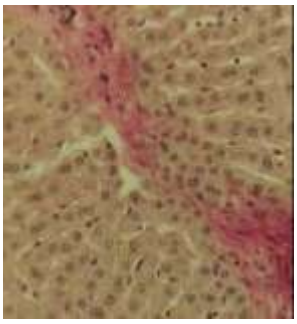
Table 4: Results of Ast and Alt in 100 Hz frequency:

Result average	Number	Frequency with 100 Hz
33.3 (I.U./L.)	6	AST1
25.8 (I.U./L.)	6	AST2
49 (I.U./L.)	6	ALT1
51.7 (I.U./L.)	6	ALT2

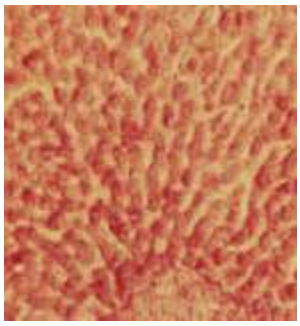


Pictures 1

Pictures 2



Pictures 3



Pictures 4

Liver tissue photomicrography in Rabbit: (Stain with PAS method. ×20)

Results of Liver Glycogen Storage of Microscopic Samples:

- In control samples stained with PAS techniques, glycogen was observed in liver lobules and was more in cells around the central vein. The peripheral lobular cells had less glycogen. It should be mentioned that red granules in the cytoplasm of hepatocytes was considered glycogen.(Picture 1)
- In samples irradiated in frequency field with 100 mg intensity and 1Hz frequency, offloading was done moderately. This discharge in histopathological sections was recognized with reduction of limitation of positive PAS cells around lobular central rein.(Picture 2)
- Study of microscopic sections of liver of exposed animals to magnetic-field with intensity of 700 (MG) and frequency of 10 (Hz) shows a higher Glycogen discharge In a manner that cytoplasm of cells of around central vein of lobule nearly has been discharged of Glycogen in many cases. (Picture 3)
- In samples taken from animals under 700 (MG) magnetic field and 100 (Hz) frequency, some cells were seen around lobular central vein that almost had high amount of glycogen and red granules. concentration of these granules near central vein is more and it shows no glycogen discharging.

DISCUSSION:

Most researches in pulsed electromagnetic fields with high or low frequency, have used sinus electric waves. Therefore, in this paper, we studied the effects of pulsed electromagnetic fields of 2 phase square electromagnetic currents in biological systems. Liver is an important metabolic tissue and the main organ of detoxification. Another reason for selection of liver was its sensitivity to waste products. Further more markers of liver function could be measured more easily than the other organs. Since 1997, most researches of electromagnetic fields have been about nerve science, immunology and oncology and physiology of liver has not been studied. It should be mentioned that, in most published studies, maximum biologic effects of sinus pulsed magnetic fields have been seen in the region of 1 Hz frequency. Therefore in this research, electromagnetic field of 2 phase square is designed according to the 1, 10 and 100 Hz frequencies to compare them with sinus fields besides doing research about these fields. One of the most important features of pathologic changes of liver cells is the change of the level of ALT and AST enzymes in serum. During cell injury, because of higher permeability of hepatocyte membrane, these two enzymes penetrate to sinusoids and then enter into the peripheral blood and an increase in the level of such enzymes is observed. [8].therefore in the present study an increase in ALT and AST level in serum of irradiated rabbits was expected. but there hasn't been seen meaningful statistical difference between control group and rabbits under field frequency of 1,10,100Hz. Therefore, it is possible that electromagnetic fields of 700 (MG) made of 2 phase square waves has not hurt the hepatocytes membrane. Glycogen storage of liver is an important factor of pathological liver changes.

The electromagnetic fields from 2 phase square waves with 700 (MG) intensity and 1 Hz frequency can significantly decrease glycogen storage of liver. Increasing the frequency of the fields to 10 Hz, resulted in enhancement of this process, but more increase of frequency to 100 Hz, not only didn't have more discharge of glycogen but also it seems frequency didn't make any change in storage of glycogen and the irradiated samples and the control samples were the same. The melatonin hypothesis is the only acceptable and explanatory hypothesis of the influence of sinus electromagnetic fields. According to this theory, pulsed electromagnetic fields with intensity of 12 (MG) and low frequency, are able to suppress melatonin .It has been clarified that melatonin can increase storage of glycogen in liver and muscular cells in rabbit and this process is dependent to the change of carbohydrate and peptid consumption [9]. Since the effects of melatonin in muscular cells and hepatocyts are through MTI receptors, so it is plausible that effect of Melatonin on the MTI receptor has resulted in a reduction of glycogenolysis and subsequently this has caused excessive amount of it in the liver cells [10]. Regarding to these facts, we concluded that the 2 phase square electromagnetic fields just like sinus fields can suppress excretion of melatonin which leads to higher rate of glycogenolysis in liver. Then storage of glycogen in liver reduces. It has been demonstrated that Intraperitouneal administration of 4 mg/kg melatonin dissolved in ethanol (0.1gr/100ml), daily for two weeks, Will result in returning of glycogen storage of liver in animals that were faced with electromagnetic fields [11]. Another hypothesis relates this phenomenon which results from effect of the low frequency EMFs fields to the increase in the amount of Epinephrine [12].

Pulsed electromagnetic fields are related to gen expression and activation of enzymes that excite DNA. In this research, we could not rely on them because animals were irradiated only for 4 hours a day and it is unlikely that in this short irradiation time, field factor would be able to make change in DNA level and gen expression [13].

Another important point about analysis and discharge of glycogen is that catecoulamines can be stayed on the alpha-1 adrenergic receptors of liver and discharge glycogen. This discharge of glycogen can be done in terms of intracellular calcium. On the other hand electro magnetic field with 15 Hz frequency and 1 (Mili Tesla) intensity can significantly increase intracellular calcium during 4 hours. So, it seems plausible that increase in calcium level can be an important factor of discharging liver glycogen too [14]. Although the possibility of increase in vasopressin and angiotensin II and their effect on glycogenolysis in should be considered too [12]. In a study in 2008, effect of electromagnetic waves with very low frequency for 50 days radiation on the metabolism of some liver factors was observed [15].

In another study, short term effect of EMS with 50 Hz frequency on the rat, showed a decrease in serum lipoprotein level and an increase in serum HDL and liver AST enzymes but ALT was not changed and it had no effects on the cytochrom p 448 / p450 enzymes.This was also the case in our reasearch but contrary to the above results ,AST didn'y have any significant change in our research [16].

Use of sinus field with 50 Hz frequency for 2 weeks and 2h/day in rats caused significant increase in ALP, AST and bilirubine and slight increase in amount of ALT and albumin and peroxidation of liver lipids. Harmful effect of this field was a decrease in glothation (GSH/GSSG)and Nicotinamide dinnucleotide (NADH/NAD). In our study the liver glycogen storage had significantly decreased in 1 and 10 Hz frequencies [17].

According to the results, we can use electromagnetic fields in the mentioned frequencies to decrease glycogen of liver. However it needs more researches especially the effect of fields during longer time and more times and its effect on the hatches, channels and cellular pumps and different variety of fields.

ACKNOWLEDGMENT

The authors gratefully acknowledged Faculty of Veterinary Medicine of Urmia university for support.

REFERENCES

1. Mercola, J., 1998. Electromagnetic fields Exert Effect on and throgh Hormons, Janet Rallof Sciena NEWS Jan; 10: 29-31.
2. Marha, 1971. Electromagnetic fiols and the life Environment. Tuh, Hana, San Francisco, pp: 29-38.
3. Burtis, C., 2007. Tietz textbook of clinical chemistry, W. B. Sanders Co., pp: 1449-1451.
4. Boncroft, 1990. Theory and practice of histological techniques, Churchill Livingston Co., pp: 186-191.
5. Shelok, S., 1981. Disease of the liver and biliary systems 6th. ED. Blackwell Co.,
6. Gretohea, L.H., 1979. Animal Tissue Tecniques, W. H. freeman Co., pp: 208-215.

7. Lewy, H. and O. Massot, 2003. Magnetic field 150Hz increases N-acetyltranse, hydroxy-indol -o-metyltransforce activity and melation releese through an indirect pathway. Int Journal Radiat Rio., l79: 431-435.
8. Fridel, R. and E. Shimidt, 1997. Release and exteracellar turnover of cellular enzymes: in Advances in Clinical Enzymol., 2: 121-128
9. Mazepa, R.C., 2000. Melatonin increases muscle and liver glycogen contetin nononexercised and exercised rats. Life Sci., 66: 153-160.
10. Naji, L., 2004. Experssion of memberane and nuclear melation receptorsin in mous pripheral organs, Life Sci., 74: 2227-2236.
11. Chang, W.H. and Chen, 2004. Effect of pulse-burst electromagnetic field stimulation on osteoblast cell activity. Bioelectromagnetics, 25: 457-465.
12. Ganong, W.F., 1999. Review of medical physiology, 19Ed. Appleton and LANG Co., pp: 274-278.
13. Blank, M. and M. Goodman, 2001. Stimulation of the cellular stress Response by Low Frequency Electromagnetic fields: Possibility of Direct Intraction with DNA IEEE. Trans Plasma Sci., 28: 168-172.
14. Spadaro, J.A. and W.H. Bergestrom, 2002. Invivo and Invitro Effect of a pulsed Electro magnetic Field on Net Calcium Flux in Rat Calvarial Rone. Cacif Tissue Int., 70: 496-502.
15. Gabriele, G., D.N. Antonella, M. Prosdocimi, V. Ferrari, F. Barbaro, S. Mazzariol, D. Bernardini and G. Talpo, 2008. Effects of electromagnetic fields of low frequency and low intensity on rat metabolism. BioMagnetic Research and Technol., 6: 10-16
16. Yaghmaei,p., K. Parivar, D. Doranian, M. Hashemi. and F. Torkaman, 2009. Study the effect of extremely low frequency electromagnetic fields on some blood serum's lipoproteins, liver enzymes and P448/P450 cytochrome enzyme system in NMRI female mice. J. Paramedical Sci. (JPS), 1: 74-80
17. Ibrahim, M., M. EL-Ashry. and E. Ali, 2008. the influence of 50 Hz Magnetic field on Liver function. Romanian J. Biophys, 18: 113-122.