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Effect of a Static Magnetic Field on Susceptibility to Ethanol-Induced Hepatic Dysfunction in Rats

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Abstract To determine whether alcohol-treated rat liver cells can be protected by a static magnetic field (SMF), we analyzed the blood chemistry and histology of hepatic tissue removed from alcohol-exposed rats that had been exposed to a static magnetic field. The rats were exposed to a 0.3 tesla (3,000 gauss) magnetic field (MF) for 24 hr daily for 5 weeks with appropriate controls. Glutamic pyruvic transaminase activity and the triglyceride levels in animals exposed to the north (N) or south (S) pole of the MF decreased significantly ($p<0.01$ and $p<0.05$, respectively) compared with negative control animals with alcohol exposure. A histological examination of hepatic tissue revealed a moderate to severe accumulation of fat vacuoles of various sizes in the cytoplasm of the hepatocytes of animals in the negative control group throughout the study; whereas in groups exposed to the MF poles, fewer fat vacuoles were seen compared with the negative control group. Electron microscopic observations showed that exposure to the N or S pole protected organelles, including the nucleus, from damage during exposure to this toxic agent, as indicated by the fact that the nucleus and the mithochondria virtually retained their shape throughout this study. These results suggest that exposure to a SMF could be an excellent way of protecting against alcohol-induced damage to the rat liver cell.

Keywords: static magnetic field (SMF), rat liver cell, glutamic pyruvic transaminase, triglycerides

Introduction

Researchers worldwide have attempted to evaluate the effect of magnetic fields (MFs) on the human body for a long time, but have not been able to develop a scientific mechanism for carrying out such an evaluation. Several apparatuses that can be used for this purpose have been developed recently, and many researchers have begun to report the results of scientific studies of mechanisms by which MF affects the human body. According to Weinber *et al.* (1), when 10 rats were inoculated with Zymosan, a yeast that can induce synovitis, then exposed to a static magnetic field (SMF) for 3 weeks, the rate at which they developed synovitis was significantly reduced compared to controls ($p<0.002$). Hong *et al.* (2) reported that 101 patients who had chronic pain in the neck and shoulders reported a significant reduction in pain when they wore a magnetic necklace. Vallbina *et al.* (3) reported on a double-blind pilot study in which 50 patients experiencing pain from poliomyelitis reported significantly more relief when exposed to an MF compared with patients who had not been exposed. Additionally, Nakagawa (4, 5) reported that pain in the head, shoulders, neck, and chest was alleviated without apparent reason and without any adverse effects in individuals who were exposed to an SMF. Considine (6) suggested that the mechanism by which MF affects the human body is associated with the movement of ions through the blood during exposure to an MF. Many investigators conducting research on the effects of MF on the body select two kinds of MF for study: the electromagnetic field and the static magnetic field (SMF), and recent studies (7-9) have predominantly focused on the SMF induced by magnetite (Fe_3O_4). Davis and Rawls (10) studied the physical aspects of exposure to the north (N) and south (S) poles and reported that the N pole promotes surface tension and increases the contraction and osmotic pressure of cells to activate metabolic pathways that reduce inflammation, while the S pole increases edema by pulling water into the tissues. The intensity of an SMF is expressed in teslas (T); 0.3 T (3,000 gauss) is considered appropriate for medical purposes (10). High-intensity MF (≥0.4 T) may induce the formation of cancer cells (11).

In this study, we administered ethanol to rats on a long-term basis to induce hepatic dysfunction and hyperlipidemia (12, 13). Chronic alcohol intake can impair the function of liver cells, which play an important role in the metabolism of nutrients. Hepatic injury can result in severe damage to the gastrointestinal tract, pancreas, brain, nervous system, endocrine system, and immune system, as well to the process of blood formation (14). It can be caused by a variety of conditions, including alcoholic hepatitis, hepatic cirrhosis, and fatty liver (15). Current findings indicate that ethanol-induced liver diseases are affected not only by the toxic effects of ethanol, but also by malnutrition associated with alcoholism, as well as genetics (16) and immunological factors; thus, ethanol can serve as a toxin in major body organs (17).

In this study, we monitored glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) levels, which correspond to the degree of liver dysfunction, in rats to identify the effects of exposure to the N and S poles within an SMF on metabolic activity in a liver that has been injured by chronic alcohol intake. We also monitored total cholesterol and triglyceride levels in the serum to determine the effect of such exposure on liver

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function and monitored the histopathologic status of the liver to identify changes in liver cell activity.

Materials and Methods

Preparation of an SMF and an SMF cage The N and S pole in a 0.3 T (3,000 gauss) SMF was used in this experiment. Each pole was attached to a rat cage that was specially made to expose the test animals to the SMF. The magnet, which had a thickness of 0.5 cm and a radius of 3 cm, was also attached to the cage. The MF range extended 18 cm from the bottom of the cage and 4 cm from the side of cage (Fig. 1).

Animal models Young adult male Sprague Dawley rats (initial weight: 150±10 g) were obtained from the Daehan Biolink Company, Ltd., in Seoul, Korea. The subjects were fed a standard diet (Samyang Assorted Diet produced by Samyang Co. Ltd., Korea) and were allowed free access to drinking water (distilled). Animals were housed in individual cages with a constant temperature (22±2°C) and humidity (55±5%). They were kept on a 12-hr light/dark cycle and were allowed 1 week to become acclimated to the housing environment before being subjected to any experiments. The rats were divided into 6 groups of 8 animals each. In the normal control group, the rats were given water. In the negative control group, the rats were given ethanol and water. Rats in positive-control group A were given ethanol and a solution containing a substance designed to cure liver disease (LCS) (Alcodex: Guju Pharmaceutical Co., Ltd., Seoul), and those in positive-control group B were given ethanol and a solution designed to increase blood circulation (BPS) (Vasoclean; Cho-A Pharmaceutical Co., Ltd., Seoul, Korea). Rats in experimental group A were given ethanol and water and were exposed to the N pole; those in experimental group B were given ethanol and water and exposed to the S pole. Ethanol/water intake of 5 g/kg/day was achieved. Either a treatment solution or a placebo (distilled water for the normal control group, alcohol and distilled water for the negative control group) were orally administered (syringe-fed) to all rats daily for 5 weeks (Table 1). The syringe feedings were performed at approximately the same time everyday. Food and water (or alcohol and water solution) consumption measurements were taken daily. Body weights were measured weekly.

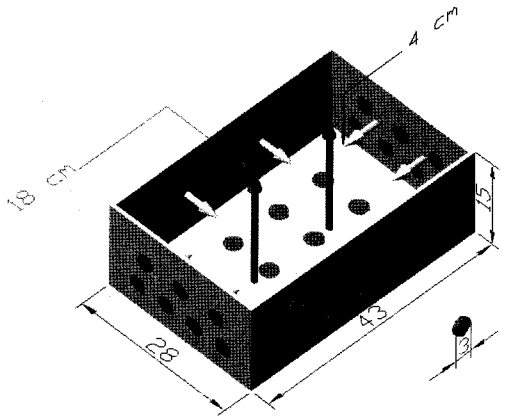

Fig 1. The rat cage was attached to the static magnetic field so that the animals would be exposed to the SMF N or S poles to test their effects on the rats during the study. The SMF was attached throughout the ethanol administration period (daily and continuously for 5 weeks).

Biochemical determinations Blood was set aside in an EDTA-free tube for half an hour. The serum was separated from the blood by centrifugation at 3,000×g for 15 min. GOT or GPT activity in the serum was determined using a GOT or GPT kit (Boehringer Mannheim, Germany). The triglyceride level was measured using the TG kit (Boehringer Mannheim), and the enzymatic colorimetric test for cholesterol content was applied using the total cholesterol kit (Boehringer Mannheim).

Liver histology Liver tissue (1 mm^3) was sliced and pre-fixed in a 4% paraformaldehyde solution with 0.1 M phosphate buffer (pH 7.3) and 2.5% glutaraldehyde for 2 hr at 4°C. The tissue was then rinsed using the same buffer solution and fixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.3). Fixation was followed by dehydration in ethanol; tissue samples were then embedded in Epon 812 followed by polymerization. Tissues were cut using an LKB 2088 ultramicrotome, then stained with 1% uranyl acetate and lead citrate and examined with a transmission electron microscope (TEM-2000 EXII, 80 KV; Jeol, Tokyo, Japan)

Statistical analysis All results are reported as the mean ±SD. The statistical analysis of data was performed using Duncan's multi-range test to make comparisons between

Table 1. Experimental designs

Groups	No. of rats treated	Treatments[1)]
Normal control	8	Distilled water
Negative control	8	Alcohol + distilled water
Positive control A	8	Alcohol + solution A (LCS)
Positive control B	8	Alcohol + solution B (BPS)
Experimental group A	8	Alcohol + distilled water + magnetic N pole exposure
Experimental group B	8	Alcohol + distilled water + magnetic S pole exposure

[1)]LCS, alcoholic liver disease cure solution; BPS, blood circulation promotion solution.

groups.

Results and Discussion

GOT and GPT activity It has long been hypothesized that MFs can have a positive effect on health. Specifically, it has been proposed that exposure to the polar aspects of an SMF may restore homeostasis by inducing physical and chemical changes in an intracellular environmental system that has been damaged through toxic exposure (10). However, the duration and intensity of MF exposure must be appropriate for the specific intracellular environment, so that the intracellular effects of such exposures can be reversed. When the intensity of an MF is too high, cellular receptors cannot be stimulated by kinetic energy to activate metabolic pathways that can help restore cell function to normal (10). The intensity of the stimulation and the timeframe within which the cell can be exposed to a MF should be determined so that the necessary intracellular changes can take place. This will involve a change in the arrangement of ions and, thus, a change in intracellular polarity; activation of such changes may create the appropriate conditions for inducing homeostasis.

To test this hypothesis, we performed a 5-week pilot study in which the response relationships among control rats and rats exposed to an SMF pole with simultaneous exposure to various combinations of nutrients, water, and ethanol were analyzed in order to establish the intensity of stimulation and the duration of polar exposure within an SMF that is necessary to restore homeostasis in hepatocytes that had been exposed to ethanol. Differences between N- and S-pole exposures were not determined under our study conditions, but the results of the experiment - gained from comparing liver weights following chronic ethanol exposure and monitoring the cells for specific changes in enzyme activity observed during the detoxification period - indicate that separate mechanisms of action are involved in N- vs. S-pole exposures. Further investigation of these differences may be an appropriate topic for further studies. As for the results of this study, in the normal group, the GOT level in the blood was 60.33±4.97 units; in the negative group (with induced hyperlipidemia), that value was doubled to 117.00±20.02 units. In positive groups A and B, it was 95.33±4.46 and 104.33±19.34 units, respectively, and in experimental treatment groups A and B, it was 113.00±16.37 and 107.00±15.62 units, respectively; these levels are similar to those found in positive group B. The change in GPT level in the experimental treatment groups was significantly less than in the other groups ($p<0.05$) (Table 2). The GPT level was 19.17±2.14 units in the normal group, but significantly higher in negative control group A and positive control group A at 45.83±7.14 and 43.50±8.62 units, respectively ($p<0.05$). The experimental treatment groups A and B demonstrated significantly lower GPT levels than the other control groups, with near-normal levels of 29.50±4.32 and 30.33±5.28 units, respectively. As a result, GPT activity, which is an indicator of hepatic dysfunction, decreased significantly more in animals with polar exposure than in the negative control group, while GOT activity and total cholesterol levels in both groups remained similar.

Table 2. Serum levels of GOT and GPT in alcohol-fed rats[1)]

Groups	GOT (U/L)	GPT (U/L)
Normal control	60.33±4.97	19.17±2.14
Negative control	117.00±20.02**	45.83±7.14
Positive control A	95.33±4.46#	43.50±8.62
Positive control B	104.33±19.34**	40.33±5.79
Experimental group A	113.00±16.37**	29.50±4.32#
Experimental group B	107.00±15.62**	30.33±5.28#

[1)]Each value represents mean±SD for 8 rats. Means with different superscripts within a column are significantly different from the normal control at $^{*}p<0.05$, $^{**}p<0.01$, and significantly different from negative control at $^{\#}p<0.05$.

Serum triglycerides and total cholesterol The serum triglyceride levels were monitored in all groups to determine the effects of polar exposure on lipid metabolism in animals with liver lesions. Animals treated in either pole had results that were distinct from the rest. The triglyceride level in the normal group was 14.17±6.79 mg/dL; in the negative control group, it was 71.33±44.27 mg/dL; in positive control group A, it was 76.33±23.59 mg/dL; and in positive control group B, it was 58.17±20.15 mg/dL. The results for the N-pole and S-pole groups were 12.5±4.28 and 10.67±1.03 mg/dL, respectively. These findings are significantly reduced compared to the other groups ($p<0.05$) (Table 3). Serum cholesterol levels were also reduced for animals with polar exposure compared to those that had not been exposed. In the normal group, it was 46.67±13.57 mg/dL; in the negative group, 70.5±6.19 mg/dL; in positive control group A, it was 81.17±6.90 mg/dL; and in positive control group B, it was 76.67±8.24 mg/dL. In experimental treatment groups A and B, it was 66.00±6.51 and 66.17±13.78 mg/dL, respectively. These results suggest an inverse relationship between polar exposure and both serum triglyceride and total cholesterol levels in the blood.

Histological studies The histological studies revealed the signs of a fatty liver that would be expected in animals that have undergone chronic ethanol exposure, i.e., an accumulation of fat - especially triglycerides - within the liver cells (18-20). The reasons for the fat accumulation include the following: triglyceride synthesis is increased because of the increased availability of NADH that is released during the metabolism of alcohol; the oxidation of fatty acids is inadequate because of mitochondrial impairment; or, alcohol induces the gradual mobilization of fat droplets to the periphery of the cell, i.e., from the hepatic central vein to the portal veins (21). The overall result is an increase in liver volume. Morphological changes seen in the fatty liver are caused by the nucleus being pushed toward the edge of the cell by the accumulating fat droplets, decreases in the number of intracellular organelles (e.g., the endoplasmic reticulum), and deformation of the mitochondria (22).

Figure 2 shows the central and portal vein regions of the liver in each group. The hepatocytes in animals in experimental treatment groups A and B were similar to

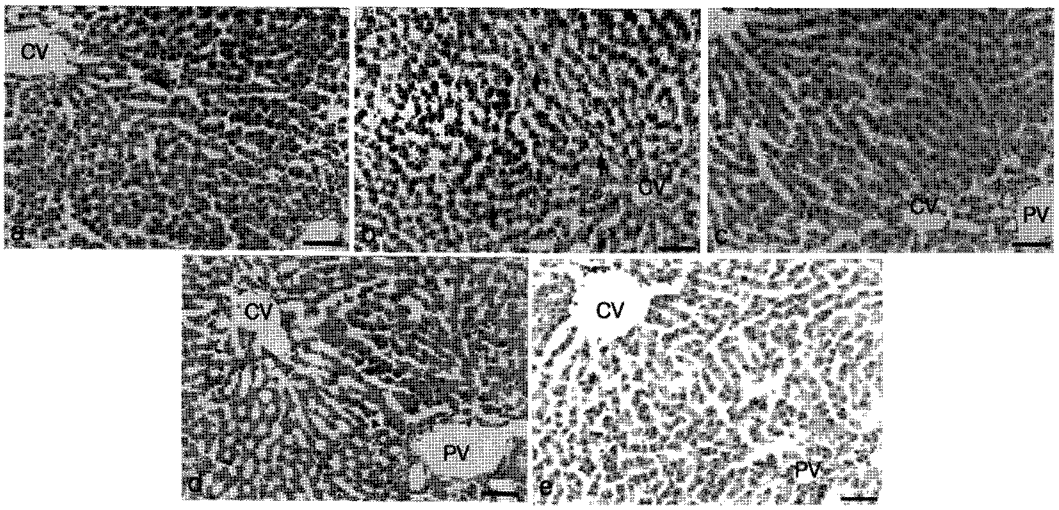

Fig 2. Histopathological changes in hepatocytes of rats given ethanol for 5 weeks. Central and portal vein regions are shown for animals in the normal control group (a); negative control group (b); positive control group A (c); and experimental treatment groups A and B (d and e, respectively), each at a magnification of ×100. The hepatocytes exposed to alcohol exhibited a diffuse accumulation of lipid droplets of various sizes (b, arrows) compared with the normal control group. This micrograph shows that (a) no cytoplasmic changes occurred in hepatocytes in the control group, (b) the number of lipid droplets increased in the negative control group, (c) the number of lipid droplets is increased in the positive control group, (d) the hepatocytes in animals exposed to the N pole are histologically similar to those in the normal control group, (e) the hepatocytes in animals exposed to the S pole are histologically similar to those in the normal control group. CV, central vein; PV, portal vein; arrows, lipid droplets. Scale bar: 50 μm.

those in the normal control group. We examined the morphology of the hepatocytes using transmission electron microscopy (Fig. 3). Figure 3a depicts the morphology of normal hepatocytes. Hepatocytes that had been exposed to alcohol exhibit a variety of degenerative changes (Fig. 3b), including swollen mitochondria in which the cristae are either missing or badly destroyed, an increased number of lysosomes, and the presence of myelin-like figures in the cytoplasm of many cells. Additionally, biliary canaliculi were generally dilated, there was a marked loss of luminal microvilli, and a diffuse accumulation of variously sized lipid droplets could be seen throughout the cell (Fig. 3b). By contrast, the hepatocytes of rats that were given both alcohol and LCS to reduce symptoms of liver disease exhibited less mitochondrial swelling and only moderately dilated biliary canaliculi with short microvilli (Fig. 3c). A small number of cytoplasmic myelin-like structures were still evident (Fig. 3c). In experimental treatment group B, the hepatocytes were similar to those in normal untreated rats (Fig. 3d and 3a), except that the Golgi cisterns were slightly dilated (Fig. 3d). These data suggest that polar exposure within an SMF may confer cytoprotection from the toxic effects of alcohol in the liver.

In our study, the histology revealed that, after ethanol administration, the rat liver cells contained fat droplets of various sizes spread diffusely throughout the cytoplasm in the negative control group, despite individual differences in ethanol consumption (Fig. 2). It also revealed that liver cells in the groups that underwent polar exposure were almost normal. Therefore, we feel safe in saying that the rate of accumulation of triglycerides within the cytoplasm of hepatocytes in animals exposed to the SMF poles was inhibited compared with the negative control group and that SMF showed a protective effect against hepatic dysfunction (i.e., fatty liver). These findings correspond

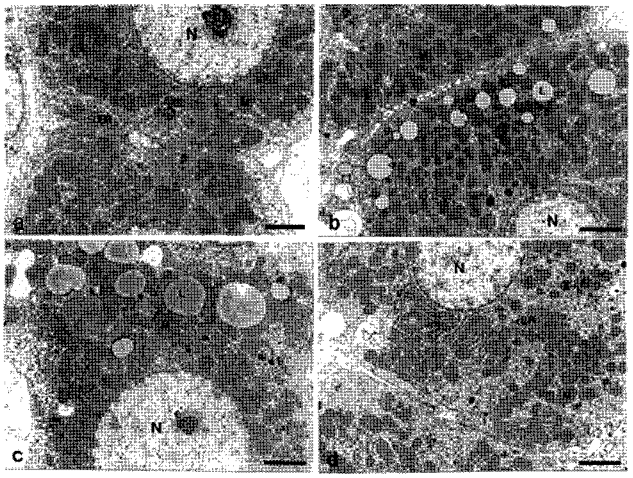

Fig 3. Changes in the ultrastructure of hepatocytes of rats given ethanol for 5 weeks. (a) no cytoplasmic changes in the control groups, (b) hepatocytes of negative control group show degenerative signs such as clear areas and dilated biliary canaliculi without microvilli, and the hepatocytes also exhibit a diffuse accumulation of lipid droplets (L) in the cytoplasm and increased collagen in the hepatic lobule, (c) lipid droplets are increased in the hepatocytes of the positive control group, and (d) the hepatocytes in animals exposed to the S pole exhibit normal hepatocyte morphology (as in the normal control group) with none of the degenerative signs displayed in the negative control group. CV, central vein; PV, portal vein; rER; rough ER; M, mitochondria; L, lipid droplets in the hepatocytes, Scale bar: 2 μm.

with those of the blood chemistry study, which showed that the serum triglyceride levels in the animals exposed to the SMF poles were similar to the levels in the normal group (see Table 3). The blood chemistry study performed

Table 3. Serum levels of total cholesterol and total lipids in alcohol-fed rats[1)]

Groups	Triglyceride (mg/dL)	Total cholesterol (mg/dL)
Normal control	14.17±6.79	46.67±13.57
Negative control	71.33±44.27**	70.50±6.19*
Positive control A	76.33±23.59**	81.17±6.90**#
Positive control B	58.17±20.15**	76.67±8.24*
Experimental group A	12.50±4.28#	66.00±6.51
Experimental group B	10.67±1.03#	66.17±13.78

[1)]Each value represents mean±SD for 8 rats. Means with different superscripts within a column are significantly different from the normal control at $^{*}p<0.05$, $^{**}p<0.01$, and significantly different from negative control at $^{\#}p<0.05$.

in animals with long-term exposure to alcohol showed that SMF and chemical stimulation may play important roles in maintaining homeostasis. Changes in lipid metabolism in the animals that underwent polar exposure following chronic alcohol exposure suggest that SMF exposure may be effective in preventing alcoholism and hyperlipidemia or treating individuals who develop these conditions. Studies of the relationship between SMF exposure and exposure to various biological substances may lead to the discovery of the mechanism of action by which SMF restores or maintains homeostasis in liver cells, and such studies are strongly recommended.

Acknowledgments

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