In-vivo Study on the Harmful Effect of the Extremely Low Frequency Unipolar Pulsating Magnetic Field in Mice

We studied the biological effect of a magnetic field on murine brain and kidney. Magnetic field we used was generated by Magno-DR apparatus (Hanil Co., Korea) which produced a high density unipolar square pulsating magnetic field, about 0.3~0.5 Tesla at 7 Hertz. Animals were placed in the chamber of the machine for various times from 4 hour to 24 hours. Histological sections of brain and kidney were made after perfusion fixation with paraformaldehyde. The light microscopic examination showed eosinophilic change of cytoplasm and positive immunohistochemical reaction to amyloid precursor protein in the neurons of the cerebral cortex. However, the thalamus and brain stem were less affected. The changes in the brain was seen in the mouse exposed more than 12 hours. The renal tubular epithelium showed degenerated tubules scattered in cortical area but little change was noted in glomeruli in the cortex and collecting tubules in the medulla. Immunohistochemistry of the kidney showed weakly positive reaction for the amyloid precursor protein in the distal tubular epithelium after 4 hours of exposure. These data suggest that strong pulsating magnetic fields could induce deleterious effect on the murine brain tissue and renal cortical tubules. ***(JKMS 1997; 12: 128~34)***

Key Words : *Magnetic field, Extremely low frequency, Amyloid precursor protein, Heat shock protein.*

Gyoung Hoon Kang, M.D., Chong Heon Lee, D.D.S.,*
Jeong-Wook Seo, M.D., Ro Hyun Sung, M.D.,**
Young Hae Chung, Ph.D.,* Suk Keun Lee, D.D.S.,**
Yoo-Hun Suh, M.D.,* Je G. Chi, M.D.**

Departments of Pathology and Pharmacology,***
Seoul National University College of Medicine, Seoul,
Department of Oral Pathology, Dankook University College of Dentistry, Cheonan,* Department of Pathology, Choongbook University College of Medicine, Cheongjoo,** Korea

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Address for correspondence
Jeong-Wook Seo, M.D., Department of Pathology,
Seoul National University College of Medicine,
28 Yongon-dong, Chongno-gu, Seoul 110-799, Korea.
Tel : 82-2-760-3541, Fax : 82-2-745-7966

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INTRODUCTION

A significant increase in the quantity of the magnetic field is inevitable by the rapidly increasing use of electric power. Many machines used in the medical field and household produce electromagnetic field in their complex working systems. In the household and industrial conditions, human beings are often exposed to extremely low frequency (ELF) magnetic fields (1). It is known that alternating current is more harmful than the static magnetic field. However, the question of whether weak, low-frequency magnetic fields are able to influence living organisms has long been one of the most controversial subjects in any field of science due to the apparent lack of knowledge on the biophysical mechanism (2, 3).

We had developed a unipolar pulsating magnetic field machine. It was originally designed for a pathology laboratory in their use to facilitate the decalcifying procedure (4). This machine is also useful in the evisceration procedure of bone from soft tissue. We found recently this machine was useful in the assessment of the biological hazard of electromagnetic field in a living animal tissue. In preliminary studies there was a lethal effect when the mouse was exposed for a long period in this machine. Our previous studies included ultrastructural study on the effect of extremely low frequency magnetic fields on the central nervous system, assessment on the apoptotic cells in the testis in the mouse (5, 6). The current study aims to reveal the comparative morphological study on the effects of magnetic fields in brain and kidney.

MATERIALS AND METHODS

We used the Magno-DR apparatus (Hanil Co., Korea) which was made for the enhancement of decalcification and resolution of bony tissues. This machine can produce high density of unipolar square pulsating magnetic field, about 0.3~0.5 Tesla, in changeable frequencies from 1 to 99 Hertz.

Mice of C3H strain were supplied from the animal

Table 1. Mean and standard error of mean value of percentage of pyknotic cells in each area of brain from each group of exposure time.

	Cerebral cortex		Thalamus & brain stem	
	Experimental	Control	Experimental	Control
4 hours	2.5 ± 0.7	2.3 ± 0.3	1.2 ± 0.4	1.7 ± 0.3
8 hours	5.8 ± 1.9	4.0 ± 0.5	2.0 ± 0.7	1.7 ± 0.7
12 hours	29.5 ± 3.6	9.0 ± 0.8	11.3 ± 4.4	2.3 ± 1.0
18 hours	37.2 ± 5.4	11.3 ± 1.1	10.0 ± 3.8	3.3 ± 0.5
24 hours	32.7 ± 4.7	10.7 ± 1.4	16.8 ± 6.9	4.0 ± 0.8

facility of Seoul National University College of Medicine. Male mice at 6~7 weeks old were used and their body weight varied from 18.5 to 26.3 gm. Three mice were housed in the magnetic field chamber. Six mice were exposed to magnetic field for 4, 8, 12, 18, and 24 hours and three additional mice for each group were housed in the apparatus with the machine switched off. The basic parameters of the mice induced by this stress model is assessed by checking the behavioral change and body weight. During exposure in the magnetic field the Magno-DR chamber was maintained room temperature by fan ventilation.

To remove the brain and visceral organs, mice were anesthetized by intraperitoneal injection of chloral hydrate. After opening the thoracic cavity the left ventricle was cannulated by 21 gauze needle tip. Paraformaldehyde in phosphate buffer was infused slowly and the tip of the right atrial appendage was removed to drain the venous return. After trimming the scalp, the brain in skull was fixed for 6 hours in paraformaldehyde solution. The brain was then carefully removed from the skull. Abdominal organs were removed and fixed in 10% buffered neutral formalin and embedded in paraffin. The brain was sliced into coronal planes with razor blade at the levels of prefrontal region, middle of the parietal lobe, cerebro-cerebellar junction, mid-cerebellum and medulla oblongata. Kidneys, liver, intestine were sampled and processed to make paraffin blocks.

Four μm thick microsections were stained by hematoxylin and eosin stain and immunohistochemistry. Immunohistochemical stain was done using primary monoclonal antibody against amyloid precursor protein raised in mice and secondary antibody against murine IgG raised in swine.

Five representative areas from each of cerebral cortex, hippocampus, thalamus, cerebellar cortex and brain stem were chosen to count the neuronal cells with cellular changes. The number of cells with cytoplasmic eosinophilia and nuclear pyknosis among 100 cells was counted. The mean value and standard error of mean (SEM) of percentage of cells with morphological change at the cerebral cortex and the central part of the brain were calculated (Table 1).

For the renal cortical change, the qualitative change was observed by hematoxylin and eosin stains and immunohistochemical stain using the same antibody.

RESULTS

The animals were slightly aggressive after the exposure but they became calm down after 1 hour. The food intake was minimal during the first three hour period, and then food intake was normal after 3~4 hours. Histologic studies of the brains revealed significant increase of the cytoplasmic eosinophilia in the granular cell layer of the cerebral cortex. The nucleus of the neuron was pyknotic and the cytoplasm was shrunken and eosinophilic. These degenerated neurons were scattered in the cerebral cortex, however, there was no significant abnormalities in intervening neurons. There was no evidence of edema, hemorrhage or inflammatory change. The severest histologic change was found in the cerebral cortex and hippocampus (Fig. 1) but thalamus and basal ganglia were less affected. The distribution of the degenerated neurons was diffusely scattered but more pronounced in the cerebral cortex (Fig. 2). Cerebellum showed eosinophilic degeneration of the Purkinje cells. But sections from thalamus, basal ganglia and brain stem showed less morphological change than cerebral cortex (Fig. 3). Brain change was seen in the mice exposed for more than 12 hours.

Histologic change of the mouse kidney exposed to magnetic field was pronounced in the cortical tubules, however, medullary tubules were less affected. The distribution of the degenerated tubules was diffusely scattered but more pronounced in cortical area. There was a slight increase of the cytoplasmic eosinophilia in cells of the proximal and distal tubules, but glomeruli in the cortex and collecting tubules in the medulla did not show any significant changes. While the nuclei of tubules and glomeruli were pyknotic and coarsely clumped, less well defined cytoplasm were shrunken and eosinophilic. These degenerated tubules and glomeruli

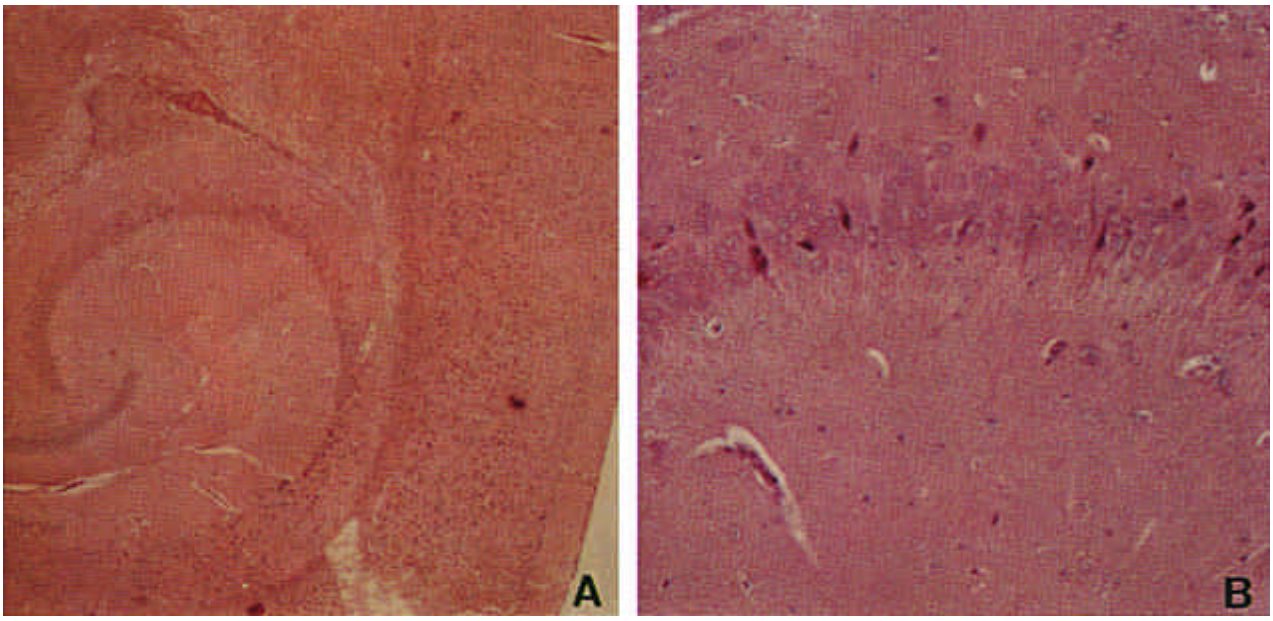

Fig. 1. Low power view of the histological section of hippocampus of the mouse after the exposure of magnetic fields for 18 hours (A : × 40). High power view of the hippocampal area showed a pyknosis of neurons (B : × 200).

were scattered but intervening tubules showed no abnormalities. There was no evidence of edema, hemorrhage or inflammatory change. Immunohistochemistry of the kidney showed trace positive staining for amyloid precursor protein (APP) in tubules of control group. The eosinophilic degenerated cells in the tubules showed more prominent APP positivity than any other portion. There was trace positivity in glomeruli, but mild positivity in collecting tubules of medulla after 2~4 hours. The distal convoluted tubules showed more increased APP positivity than that of the proximal convoluted tubules after 4 hours. Occasionally the enhanced APP positive cells in the distal tubules were observed after 10 hours. The APP positivity in the proximal tubules showed similar features from 2 to 24 hours (Fig. 4). There was no significant change in the liver and intestine by histologic examination and immunohistochemistry using antibody against amyloid precursor protein.

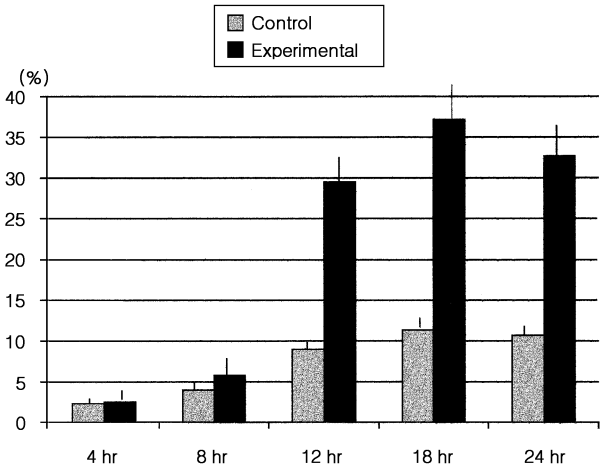

Fig. 2. Percentage of neurons with pyknotic nucleus in the cerebral cortex at 4, 8, 12, 18 and 24 hours of exposure comparing to the control.

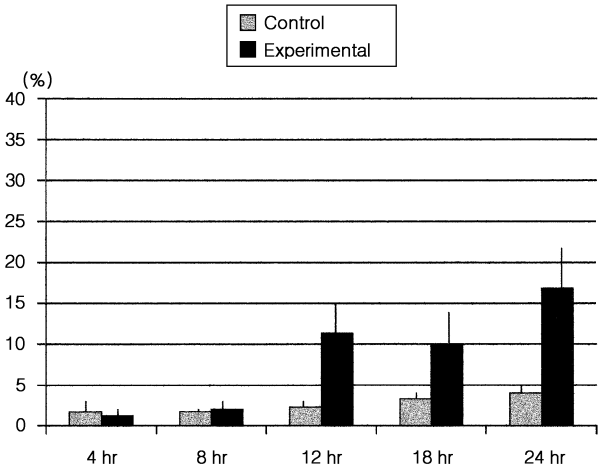

Fig. 3. Percentage of neurons with pyknotic nucleus in the thalamus and the brain stem at 4, 8, 12, 18 and 24 hours of exposure comparing to the control.

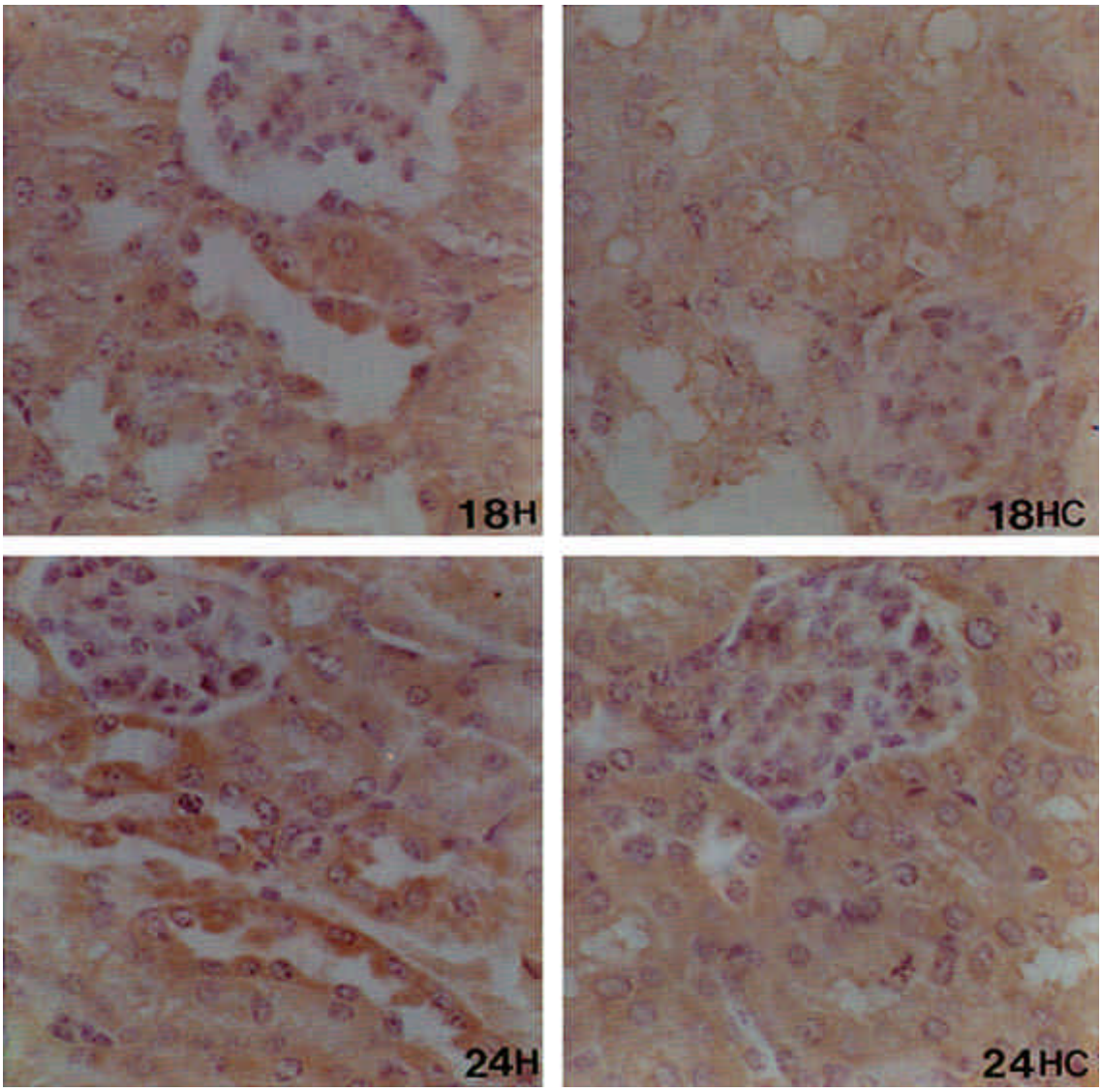

Fig. 4. Immunohistochemical staining against amyloid precursor protein in the renal cortex at 18 and 24 hours of exposure (18H and 24H) and controls (18HC, 24HC). There was increased APP positivity in distal tubules after 24 hours, but glomeruli were not affected (×400).

DISCUSSION

Magnetic field is a potential cause of chronic environmental hazard inducing degenerative change of adult morbidity. Electric charge could be the source of magnetic field but only electric charge in physical motion produces a magnetic field. The magnetic fields are specified by two vector quantities, the magnetic flux density (B) and magnetic field strength (H). B and H have units of tesla (T) and ampere/meter (A/m), and they are related by the formula ; 0.01 μT=8 mA/m. Natural phenomena, such as thunderstorms and solar activity, produce time-varying magnetic field of extremely low frequency. The magnetic flux density is 0.01~0.5 μT. In the usual home and office environment, the magnetic flux density is 0.3 μT. In the communication and power transmission systems, magnetic fields can approach a level of up to about 15 μT (7). Considerably higher flux densities up to 8~70 mT can occur in the immediate proximity of industrial processes using induction motor and heating devices. The pulsed magnetic fields, with its flux densities ranging from 1 to 10 mT, has been allowed for various diagnostic and treatment procedures of the medical care (8, 9). The magnetic flux density in our machine produces 200 mT, which is 20 times or even higher than the level produced in the magnetic resonance imaging system.

The frequency of the pulsating magnetic field is ex-

emplified by those produced by alternating current of power line, which is either 50 or 60 hertz. The definition of electric or magnetic field of extremely low frequency (ELF) is 30~300 hertz in Europe but in the United States the ELF is designated as 0~100 Hertz (10). In contrast to the high frequency electromagnetic fields, ELF magnetic fields have a strong biological effect (7). Varieties of therapeutic or diagnostic applications have been introduced, including healing of bone fractures, promotion of nerve regeneration, acceleration of wound healing, and magnetic resonance imaging devices. Hazardous effect has also been reported in the nervous system including neuronal excitability, neurochemical change (11), altered hormonal level, change of the behavioral response (12) and biorhythm (7, 11). The biological effect at the cell level is largely understood as to the membrane activity particularly on the calcium metabolism. The effect on the calcium metabolism is windowed in frequency at 16 Hz (13) and there is a strong frequency dependence of cellular activity in responses to extracellular currents that included synchronization with the applied field (14). Measurements of serum corticosteroid level have resulted in a confusing picture (15). There is a possibility of synergistic effects from the magnetic fields and a stressful environment cannot be ruled out (7).

We used a unipolar square pulsating magnetic field made by Magno-DR apparatus. This machine can produce a strong magnetic field, 0.3~0.5 Tesla, and has frequency controller from 1 Hertz to 99 Hertz. Because the unipolar magnetic field can induce more electric polarity than the bipolar magnetic field, and square pulsating magnetic field can produce more acute inductance and reluctancy than the sinusoidal pulsating magnetic field, we suggest the unipolar square pulsating magnetic field seems to be the ideal method to apply for the hazardous effect on the cells. However, we found an enhanced magnetic resonance effect in low frequency magnetic field, which was maximum in 7~10 Hertz, and examined the hazardous effect on the murine organs.

Structural changes in the hepatocyte of guinea pig occur after 0.005 Tesla and 0.3 Tesla constant magnetic field for 3 to 7 weeks (16), and calcium ion efflux from brain tissue occurs by different kinds of electromagnetic fields (13, 17, 18). On the other hand, the pulsating electromagnetic field could be used for the treatment of un-united fractures and failed arthrodeses (8, 19). More recently, magnetic fields at 50 Hertz could inhibit spermatogenesis in the mouse by affecting the spermatogonia (5). The biological effect of this ubiquitous power still has to be evaluated, although many suggest they have significant hazardous effect on bone, neural, endocrine and reproductive system (7). Our observations showed increased cytodegenerative changes in brain, kidney and testis. The basic mechanism is still to be elucidated and the current experimental setting is much stronger than any environmental situation. It is worthwhile to note that the electromagnetic fields can produce diverse hazardous effects in the environment in the future. The probable mechanisms of these injuries by the pulsating magnetic field would be one of solutions for these problems.

Most cells undergo an adaptive protective response in reaction to an environmental stress, producing stress proteins (20). The best studied example is heat shock proteins (HSP). They are induced by a variety of physical and chemical agents and by ischemia other than heat. A transiently upregulated family of proteins, HSP, are present in normal cells, which are intimately involved in intracellular protein folding and translocation as well as targeting of proteins to specific cellular organelles (21). Characterization of the APP promoter revealed the presence of a HSP site (22). A twofold increase in APP mRNA was observed in Epstein Barr virus transformed human B lymphocytes following heat shock (23). Several studies suggested that APP might have a protective role in stressed neurons. When soluble exogenous APP was added to cultured neurons, it lowered the levels of intracellular calcium, and when cultured neurons were subjected to hypoglycemic shock, treatment with APP appeared to stabilize the neurons (24). Since the APP gene contains a HSP, increased APP mRNA and protein expression is expected in response to cellular stresses and APP seems to be a stress protein.

APP is the precursor of a series of soluble proteins and peptides, including the amyloid peptide (25) which is the major component of the β amyloid plaques, one of the neuropathological hallmarks of Alzheimer's disease. Several lines of evidence (26~28) implicate that overexpression and/or abnormal accumulation of APP may play a central role in the pathogenesis of Alzheimer's disease. A combination of many environmental factors such as head trauma and exposure to neurotoxins has been proposed as risk factors for the development of at least the sporadic or late onset type of Alzheimer's disease. Interestingly, recent studies have demonstrated that APP is increased following different models of injury to the central nervous system (29) probably at least as a part of the protective cellular response under stress conditions. In this regard, our immunohistochemical study presented here demonstrating significantly increased APP level in response to the exposure to magnetic field suggest that APP could be a general marker for injury to the central nervous system in response to various insults of stresses as HSP or α-1 antichymotrypsin. However, this increased APP level

may overload the nonamyloidogenic processing pathway and lead to the disrupted APP metabolism with the utilization of excessive amyloidogenic processing pathway which may link to the amyloid deposition and neurodegeneration characteristic of Alzheimer's disease.

Many environmental factors such as heat trauma and exposure to chemotoxins have been proposed as risk factors for the development of at least the sporadic or late onset type of kidney disease. Since the APP gene contains an HSP, increased APP mRNA and protein expression is expected in response to cellular stresses and APP seems to be a stress protein. Interestingly, recent studies have demonstrated that APP is increased following different models of injury to the kidney (30) probably at least as a part of the protective cellular response under the stress condition. In this regard, our immunohistochemical study presented that significantly increased APP level in response to the exposure to magnetic field suggested that APP could be a general marker for kidney thermal injury in response to various physical insults of stresses as HSP. Increased APP levels may overload the nonamyloidogenic processing pathway and lead to the disrupted APP metabolism with the utilization of excessive amyloidogenic processing pathway which may link to the amyloid deposition (31) and tubular degeneration of kidney. Our data, therefore, presented that the enhanced APP level particularly in the degenerating cortical tubules, especially distal tubule could postulate a magnetic stress as one of environmental risk factors possibly linked to pathogenesis in kidney disease. Thus, it will be interesting to investigate a possible link of increased APP level by chronic magnetic stress to an aberrant proteolysis of APP generating excess A-β or A-β bearing amyloidogenic peptides which may be toxic to the kidney tubules causing further metabolic and cytoskeletal changes (32) and slowly developing amyloid deposition in the degenerating tubules (31). Interestingly, increased staining for beta APP in cortical pyramidal neurons was evident in the majority of renal patients (33). A better understanding of the HSP and the APP induced by exposure of this magnetic field may provide important insight on renal pathophysiology associated with Alzheimer's disease and suggest paradigms for therapeutic interventions.

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