

Induction of RhoGAP and Pathological Changes Characteristic of Alzheimer's Disease by UAHFEMF Discharge in Rat Brain

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Abstract: Novel experiments with Ultrasound Associated with High Frequency Electromagnetic Field (UAHFEMF) irradiation on rats and mice found evidences of characteristic Alzheimer's disease (AD) degenerations including neurite plaques, beta-amyloid, TAU plaque and deposition in cells, Neuro-Fibrillary Tangle and Paired Helical Filament (PHF) with rats and mice irradiated up to 2454 hours. Concomitant passive avoidance test was performed on six mice, and all showed signs of visual and auditory agnosia and lost cognition of threatening condition. The post section Thioflavin-S fluorescent microscopy found dilated ventricles and dense amyloid-deposition in Ca3 and dentate gyrus. In addition, PHF was identified in the 2454 hours-irradiated rat brain by electron microscope. A human T-cell activation RhoGTPase-activating protein (TAGAP) isoform b homolog (GenBank accession # P84107) induced in the UAHFEMF-treated rat brain was identified using electron spray ionization (ESI) liquid chromatography tandem mass spectrometry (LC/MS/MS). We hypothesized that one of the causes of AD can be the UAHFEMF discharges in human brain.

Keywords: Alzheimer's disease, UAHFEMF, paired helical filament, beta-amyloid, TAU, RhoGAP, mass spectrometry.

INTRODUCTION

Due to the bewildering heterogeneity of senile plaques [1], available data could not satisfactorily explain their generalized etiology. The length of a myelin sheath between two Ranvier's nodes is approximately one mm and the conduction velocity of an impulse in the nerve fibers varies from a few to 120 m per second [2]. Dividing 120 m by one mm, the resulting frequency suggests that nerves with the most rapid conduction velocity could generate a 120 KHz frequency during normal conduction. This suggestion led to the hypothesis that ultrasound with a frequency of as high as 120 KHz could be generated by action potentials in axons. In this study, this hypothesis was verified by scalp EEG using an instrument called the Medelec Sapphire II (data not shown, collected from ERHLIN HAPPY CHRISTIAN HOMES, Taiwan), which detected higher ultrasound in older persons and milder mental deteriorated cases, however on the contrary in the advanced cases the Ultrasound potentials were found remarkably got down, perhaps due to the large amount loss of neurons in the brain of patient with Alzheimer's disease (AD).

Ultrasound can be transmitted through air, water, minerals and tissues. When ultrasound passes through liquid it generates vacuoles of varied size from ultra-structural to visible bubbles [4]. Enhanced ultrasound associated with high frequency electromagnetic field (UAHFEMF) potentials can also charge the metallic particles in the brain tissues to generate electric discharge [23] with a very high heat, and thus can change phosphate into white phosphorus that will cause phosphorylation, which reacts strongly with O₂,

Halogens and other substances except N₂ and Carbon [6]. Thus it could change MAP to phosphorylated TAU protein [5]. Discharge of UAHFEMF can evoke water explosion by water critical temperature mechanism [3] so that many phenotype destructions and deformation of cells and altered cells arrangement were attributed to its effects. Ultrasound also produces H₂O₂, HNO₃, HNO₂, and O₃ upon transmission through water to kill brain cells. The very fine vigorous vibration generated by ultrasound can also degrade high weight molecular protein into unrespectable many lower weight molecular peptides, amyloid and amino acids [7] and even to NO, or to free radicals [8]. Ultrasound also gives rise to oxidation, reduction and other chemical changes, emulsification or aggregation of some type of droplets or particles [9].

Elevated risk of AD among workers with likely electromagnetic field exposure (in the thesis of E. Sobel. *et al.* 1996) [25] is consistent with previous findings regarding the hypothesis that electromagnetic field exposure maybe etiologically associated with the occurrence of AD. Remarkable epidemiological study of dementia in a rural community in Kerala, India [26] indicated that from 2067 persons aged 60 and above, 1.38 % were diagnosed as AD, which satisfied the criteria for ICD-10 dementia. The most prominent etiological differences between the lowest AD prevalence people and higher prevalence people is that those who are living in Kerala India are in the majority walking on bare feet so that any elevated electromagnetic field in their bodies the excess electrical charges which can be leaded into the ground by walking on bare feet as (data not shown). However, those who are living in civilized country such as in North America their prevalence of AD is approximately 8 to 13% if the milder cases are included [27], (D.A. Evans *et al.*). Prevalency of AD were calculated for a geographically defined US community, of 3623 persons over 65 years old, an estimated

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10.3 % had probable AD [28]. They were walking on boots or traveling by motor cars, or on the floor seated with carpets or electrically insulated materials. Accordingly in any time the accumulated electromagnetic charges in human bodies could not be leaded into the ground, thus the accumulated electrical charge are inevitably be charged in the iron particles in their brain tissues and then discharge would occurs to cause neuronal damages to develop AD.

We determined whether UAHFEMF irradiation can cause formation of beta-amyloid deposits, TAU, neuro-fibrillary tangle (NFT), paired helical filament (PHF), plaques similar to senile plaques, sponge form degeneration, congophilic amyloid angiopathy, neuronal death and signs or symptoms characteristic of those in human dementia in rats and mice brain. Recently, mass spectrometry (MS) was widely used in proteomic study [13]. We performed a MS analysis by using electron spray ionization (ESI) liquid chromatography tandem mass spectrometry (LC/MS/MS) and identified the UAHFEMF treatment induced protein, a RhoGTPase-activating protein (RhoGAP), in rat brain tissues. The identification of induced proteins may help us understand the pathology of AD on biochemical level. Here we hypothesized that the UAHFEMF discharges in human brain can be one of the causes of AD.

MATERIAL AND METHODS

Material

During 1997~1999, eight male Straque Dawley Rats and 80 male ICR mice were purchased from the Animal Experimental Center of China Medical and Pharmacological College in Taiwan. The average weight of the rats was 200grms ~250 grams. The average weight of the mice was 30gram and the age was nine week old when bought. These animals were housed at 23°C~26k in a room and illuminated for 12 hours daily. During 2002 May 3 ~ 2003 June 20, 20 male Straque Dawley Rat were bought from the same place. They were two months age, 200 grams weight.

Discovery of Amyloid Development from Human Plasma by Discharge of UAHFEMF *In Vitro*

Thick human plasma smear was placed on a slide glass and let it be half dried. It was treated with electrical discharge from a surgical coagulator for about 25 seconds, fixed with alcohol and then stained with 1% congo red solution for one hour followed to examine with polarized birefringent microscope for the presence of amyloid.

Experiment with UAHFEMF Irradiation on Rats or Mice *In Vivo*

An ultra-short wave therapy unit (input voltage 110 volt, 120 watts, 27.12 M Hz) was applied with parallel connection of 106 micro-Farad capacitor to the power source to make UAHFEMF in five compartment rooms made of insulated plastic materials (each could contain two rats) apparatus in which at most 10 Rats could be allowed to be irradiated simultaneously with a considerably strong UAHFEMF that could be tested with a 20 cm fluorescent lamp to make alight. The apparatus was shield with one mm thick copper plate leaded grounding to protect from leakage of UAHFEMF

(Fig. 1a). Every irradiation was not exceed 30 minutes following about five minutes pause, and the accumulated irradiation was usually less than 12 hours a day. Automatic ventilation was devised. The daily and total accumulated irradiation hours and the used accumulated KW H were recorded.



Fig. (1a). Facility for UAHFEMF treatment.

Apparatus in which at most 10 Rats could be allowed to be irradiated simultaneously with a considerably strong UAHFEMF that could be tested with a 20 cm fluorescent lamp to make alight. The apparatus was shield with one mm thick copper plate leaded grounding to protect from leakage of UAHFEMF.

Rat Post UAHFEMF Irradiation Pathological Examination

Any died rat was sectioned instantly and fixed in 10% formalin for paraffin embedded blocks preparation. Died date, final accumulated irradiation hours, autopsied numbers, preparation and paraffin embedded blocks were stocked and recorded for pathological study. For Bielschowsky's silver staining, Palmgren's silver impregnating technique, pyrogallol, glycin, gold chloride, Anillin oil and Silver nitrate were prepared. The post section pathological examination included immuno-histochemical stainings with antibody against TAU, beta amyloid and ubiquitin (Novo Castra Laboratory, Canada), citrate buffer solution for antigen recovery, Histo Kit (Zymed Laboratory Inc., Sanfrancisco USA), Diphenilamin reagent, congo red, Thioflavin-S and AuCL₃ (Japan), light microscopy. (CHK 2 -F3-100, Olympus Optic Co. Ltd. Taiwan), birefringent polarizing accessory, and Fluorescence microscopy (BX40 F-3, Olympus Optic Co. Ltd., Japan).

Passive Avoidance test Combined with Irradiation of UAHFEMF

The purpose of this experiment was to determine the effect of UAHFEMF treatment on behavior changes in ICR mice by a biological research apparatus with which using the inborn light phobic habit of mice. Mice were placed in the light chamber and then let the mouse enter the dark chamber to get an electric shock of 1.0 mA current for a duration of 3 second. As a training trial, 24 hours later the same procedure was repeated. By measuring the time between been placed in the light chamber and entered the dark chamber to test the

avoidance of mice to re-enter the chamber. The longer the time, the higher the probability of the damage is to the brain. A total of 40 male mice aged nine months weighing from 25-35 grams were purchased (Experimental Animal Center of China Medical and Pharmacological Collage). A plastic cage (30 cm, 20 cm, 15 cm height) was devised to make an environment of UAHFEMF equipped with a specially designed ultra-short-wave therapy unit (Model K-50A, ac 110 V 50/60 Hz 120 W, 27.12 M Hz NBR 76430010) purchased from ITO Com. Japan in which could allow to put 6 mice at a time, and a microwave oven from Sharp Com. Ltd. Model RE 8062 (ac 110 V, 1000 W, 2450 M Hz, Capacity 16 L. 47263283.). Normal microwave oven could not be used as an instrument for irradiating mice because it is too powerful that would kill the mice at once. So that the input voltage of alternative current were set down with a transformer to 90 volt AC instead of 110 volt AC, and the intensity should be as low fire, the irradiation were controlled with a tween timer as ten second "on" followed by a three seconds "off" of irradiation alternatively. The irradiation was not exceed over 15 minutes at a time, by using passive avoidance test apparatus (UGO BASILE, Type 7553 BIOLOGICAL RESEARCH APPARATUS, 21025 COMERIO-VARESE ITALY).

Transmission Electron Microscopic Observation for PHF in Brain Tissues of the Rat Irradiated by UAHFEMF for 2454 Hours with an Age Matched Non-Irradiated Rat Brain as a Control

Rat brain tissues were fixed in 0.1 M sodium phosphate buffer containing 2% Osmium tetroxide for two h. The tissues were dehydrated in an ethanol series and embedded in Spurr resin. Three percent uranyl acetate in 70% ethanol was applied for two h during dehydration. Tissues were observed under a Philips CM 300 electron microscopy (FEI, Netherlands).

Rat Brain Soluble Proteins Fractionation and Mass Spectrometric Analysis

Mass spectrometry analysis was performed as previously described [12, 29]. Two hundred mg of soluble proteins of 19-month-old rat brain tissues (both in control and UAHFEMF treatment groups) were extracted in one mL extraction buffer [0.05 M Tris (pH 6.8), 1 mM EDTA, 2% SDS, 20 μ M PMSF]. The homogenate was centrifuged at 4°C for 10 min at 7,740 x g and supernatant proteins were precipitated by addition of five volumes of acetone, storage at -20°C overnight. After two washes of acetone followed by one wash with 99% (v/v) ethanol, the centrifuged pellet was dried under a vacuum. The proteins were separated by 15% Laemmli SDS-PAGE [18] [15% (w/v) acrylamide, 0.5% (w/v) N', N'-methylene-bis-acrylamide, 0.375 M Tris (pH 8.8), 0.1% (w/v) SDS, 0.5% (w/v) ammonium persulfate and 0.5% (v/v) TEMED] at 100 Volt for two h. Protein gels were stained by SYPRO Ruby stain and scanned for image analysis. Protein band on the gel was excised out with a sterile scalpel, placed in an Eppendorf tube, immersed in 400 μ L destain solution [50% (v/v) acetonitrile (ACN), 0.025 M ammonium bicarbonate, pH 8.0], vortexed for 15 min at room temperature three times, and vortexed in 100 % ACN for 5 min. Gel slices were dried in a vacuum and hydrated in

60 μ L trypsin solution [60 μ g/ml trypsin and 25 mM ammonium bicarbonate, pH 8.0]. In-gel digestion was performed for 16 h at 37°C, peptides were eluted from the gel slice with 50% (v/v) ACN, 5% (v/v) trifluoroacetic acid (TFA), and the elutant was dried in a vacuum. MS analysis was performed using LC/MS/MS (Waters, Milford, MA) in Analytical Chemistry Instrumentation Facility at UC, Riverside. Database searching by use of amino acid sequence tag was performed as previously described [30]. After LC/MS/MS analysis, the mass value of y and b ions was obtained and the peptide sequence was predicted by use of the MassLynx algorithm (Waters, Milford, MA). The MS-Pattern algorithm (<http://prospector.ucsf.edu/ucsfhtml4.0/mspattern.htm>) [12] was used to match derived peptide sequence against de novo s the National Center for Biotechnology Information (NCBI) mammals database.

RESULTS AND DISCUSSION

UAHFEMF Discharge Produced Amyloid in Thick Human Plasma Smear *In Vitro*

The spark of Superfricator was applied on thick human plasma smear for 25 seconds then air dried and fixed with alcohol and stained with 1% congo red solution for an hour, and visualized with polarized birefringent microscope (Fig. 1b). The green colour indicated newly developed amyloid and red color indicated that plasma protein is congo-red phil when it was heated with electrical discharge.



Fig. (1b). UAHFEMF discharge produced Amyloid in thick human serum smear *in vitro*.

Bar 150 micron.

The Cause of Senile Plaque Formation

Bielschowsky's silver stain showed a large plaque similar to a senile plaque with a discernible core to which many tangled astrocytes and debris of processes were aggregated. The outer circle consisted a halo (Fig. 2a), (Fig. 2b), (Fig. 2d). The puzzling structure of SP might be modulated by the magnetic property of UAHFEMF without any enzymes. This pathology was proved in this experiment. Because iron and ferrite such as SP contents [20, 21] are strong magnetic bodies which is strongly aggregated towards the core of SP. O₂, N₂O, N₂, vanadium, Cr, and Mn are positive magnetic bod

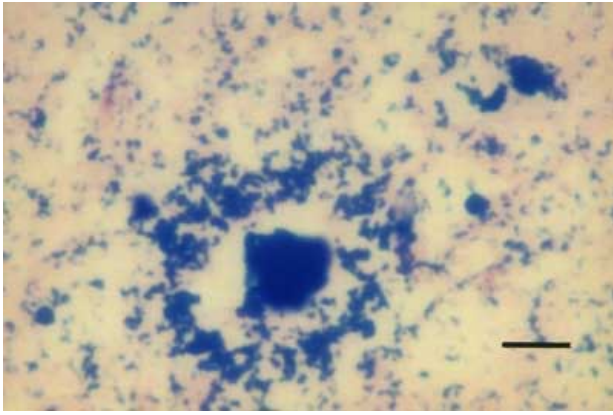


Fig. (2a). Neuritic plaque.

Bielschow'sky-PAS silver staining on brain tissue from rat 1644 hours irradiated with UAHFEMF revealed a neuritic plaque with a distinct halo. Bar 30 micron.

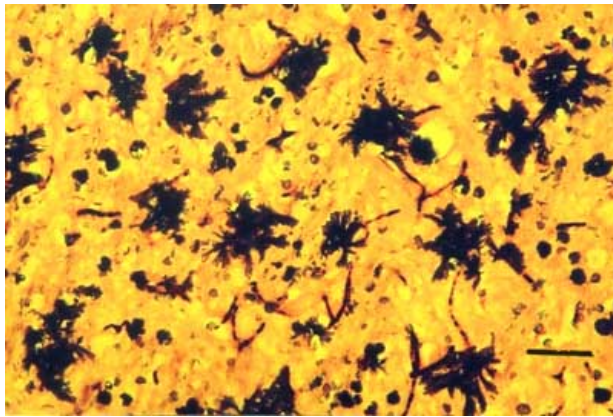


Fig. (2b). So many Neuritic plaques in a field.

Bielschow'sky-PAS silver staining from rat 1644 hours irradiated with UAHFEMF. Bar 30 micron.

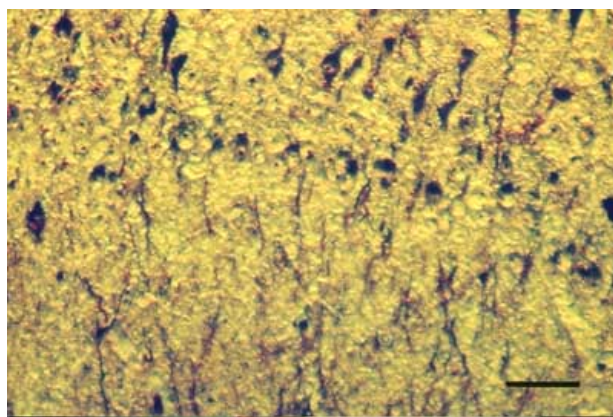


Fig. (2c). Neurofibrillary degeneration with distorted nerve fibrils in Hippocampus.

Bielschow'sky PAS silver staining on brain tissue from rat 928 hours irradiated with UAHFEMF showing distorted and distended dystrophic neurites referred to as "curly fibers" and several tangled-pyramidal neurons were seen. Bar 30 micron.

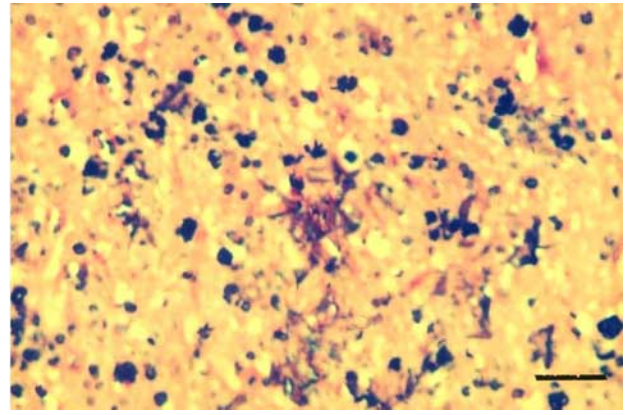


Fig. (2d). Neuritic plaque with a red core.

Bielschowsky PAS silver staining on brain tissue from rat 1644 hours irradiated with UAHFEMF, red core indicate presence of polysaccharides which is a substance specific for SP Bielschowsky silver stain. Bar 30 micron.

ies. On the contrary, organic substances, copper, silver, gold, zinc and mercury are negative magnetic bodies. These negative magnetic bodies were repulsed by the north pole of the magnet [10] when they are placed near the magnet that was made by discharge of UAHFEMF, which developed at the core of the SP. At the same time from the central core vacuoles as well as H_2O_2 mediated vacuoles develops, which push out the negative magnetic substances. These processes were included in the course of the disease and during the staining of the preparations. By this mechanism the halo of SP might be developed.

The Pathogenesis of Neurofibrillary Degeneration with Distorted Nerve Fibrils in Hippocampus

Bielschow'sky PAS silver staining on brain tissue from Rat 928 hours irradiated with UAHFEMF showed distorted and distended dystrophic neurites referred to as "curly fibers", and several tangled pyramidal neurons were observed (Fig. 2c). Discharge of UAHFEMF has a faculty to yield heat to make nerve fiber soft and bendable. By its screwing ultrasound wave it make the fibrils twisted because the wave lengths are so short that can function in the ultrastructural space of nerve fibers. (Fig. 5a) showed that Thioflavin-S fluorescent microscopic finding from Rat 928 hours UAHFEMF irradiated Rat showed many distended nerve fibers with amyloid deposition. Fig. 5b showed that the same Rat preparation, HE stained, in hippocampus was found granulo-vacuolar degeneration that might be caused by the faculty of UAHFEMF that can make vacuoles in the protoplasm or in the nucleus of brain cells. Fig. 5c showed that Lewy Body was found in the same preparation. Whereas in "Lewy body variant AD" diffuse Lewy bodies could be found diffusely.

Amyloid Plaque Formation by the Discharge of UAHFEMF Irradiation on Rat

The most important reason of beta-amyloid formation is that enhanced UAHFEMF would induce polarization on a metallic conductor as a nidus and charge the conductor in-

cessantly till over charged then discharge would occur [23]. The discharge has a vigorous shaking power and very short varied wavelength with which degrade high molecular weight protein to many low molecular weight peptides with high heat of the UAHFEMF discharge in this experiment (Fig. 3a), (Fig. 3b). Amyloid peptide was produced extracellularly as well as intra-cellularly [7]. The process was done actually non enzym-atically. In (Fig. 3b) a large matured beta amyloid plaque from Rat 1000 hours irradiated showed very similar to (Fig. 3c). That is a matured beta amyloid plaque of an AD patient as a positive control. Both figures showed as if there were discharging sparkling from the center of the plaque with very high temperature intermittently for a very long period of time and degraded the serum protein to beta amyloid at the same time the water was heated up to critical temperature 374k to make very small water explosion. In such way the matured amyloid plaque might be developed in such a peculiar form.

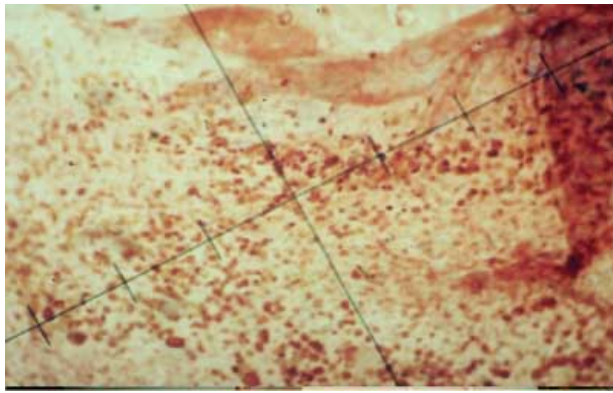


Fig. (3a). Profuse beta amyloid deposits.

Immuno-histo chemical stained on brain tissue from rat 928 hours irradiated with UAHFEMF. Scale 40 micron.

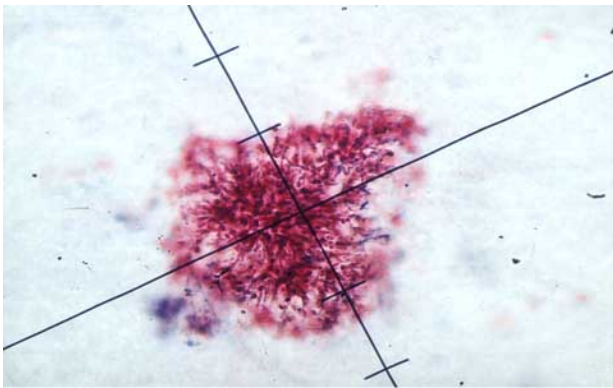


Fig. (3b). A large single beta amyloid plaque.

Immuno-histo chemical stained on brain tissue from rat 1000 hours irradiated with UAHFEMF. This beta-amyloid plaque looks very similar to the following positive control amyloid plaque in the brain tissue from Alzheimer's patient brain that obtained from Novocastra Laboratories Ltd UK. Scale 40 micron.

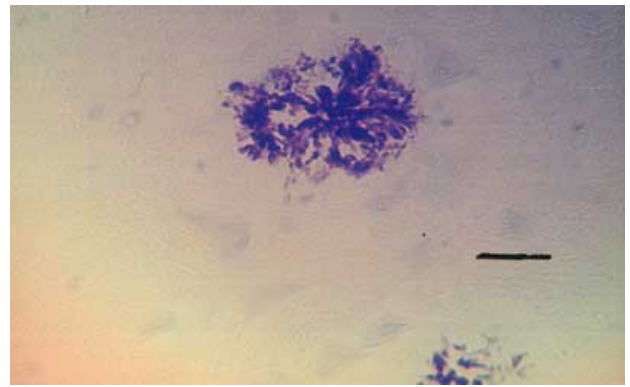


Fig. (3c). Beta amyloid plaque.

Immuno-histo stained on brain tissue from Alzheimer brain as a positive control. Bar 30 micron.

TAU Plaque Formation by the Discharge of UAHFEMF Irradiation on Rat

Antibody against TAU densely stained TAU plaque (Fig. 4a) and profusely stained in (Fig. 4b) The cause of the TAU protein formation was by phosphorylation from MAP (Microtubule Associated Protein). Whereas in this study the phosphorylation was not enzymatically done, but done by white phosphorus that was mediated by discharge of UAHFEMF [6]. Namely the discharge mediated by UAHFEMF has over 1000k temperature high enough to change any phosphate in brain tissues into white phosphorus that has a peculiar property to cause phosphorylation with any substance except nitrogen and carbon. [6]. UAHFEMF contains very fine wavelength such as less than few nanometers that it can penetrate into the cells even into the chromosome and DNA structure to make small electrical discharge to make mutation or phosphorylation. Thus hyper phosphorylated tau protein is found intra-cellularly.

Another very important event concerning the white phosphorous is its toxicity, it is extremely toxic, the lethal dosis is 0.05~0.15g, its characteristic properties are to damage liver, cerebro-nervous systems, kidney, like in Alzheimer's Disease it is involved in hyper phosphorylation of Tau protein in neuro-fibrillary degeneration, dementia, even to the apraxia that is probably due to hypoglicosemia mediated by liver atrophy caused by long lasted chronic white phosphorous intoxication.

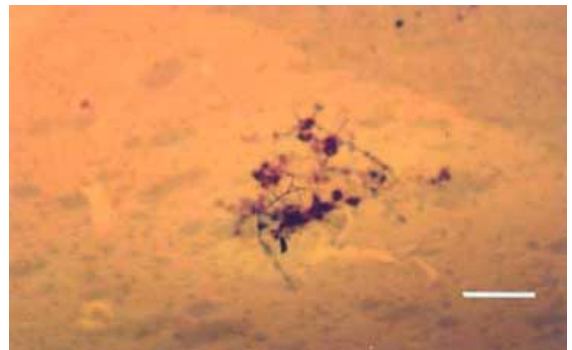


Fig. (4a). Tau plaque.

Immuno Histo-stain with antibody to Tau on brain tissue from rat 1644 hours irradiated with UAHFEMF. Bar 30 micron.

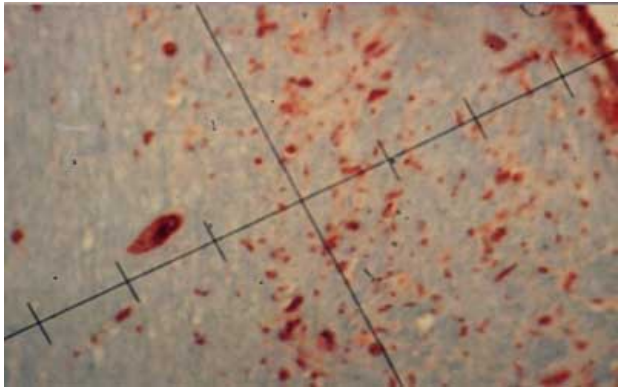


Fig. (4b). Profuse small Tau plaques and a large tangle.
Immuno-histo stained with antibody to Tau on brain tissue from rat 2148 hours irradiated with UAHFEMF. Scale 40 micron.

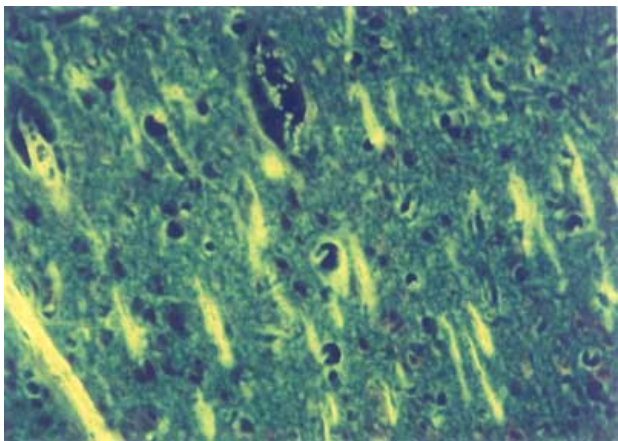


Fig. (5a). Profuse elongated degenerated neurons shows fluorescence.
Thioflavin-S stained fluorescence microscopic examination on brain tissue from Rat 928 hours irradiated with UAHFEMF. Bar 30 micron.

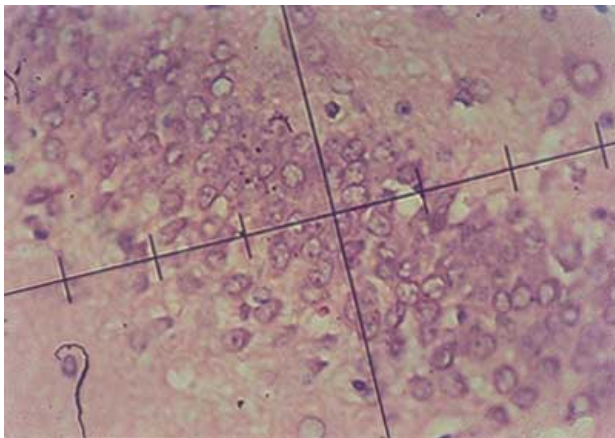


Fig. (5b). Granulo-vacuolar degeneration in hippocampus tissue HE stained.
Rat 928 hours irradiated with UAHFEMF. Scale 40 micron.

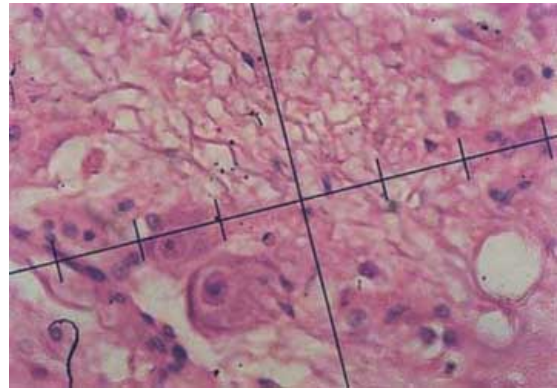


Fig. (5c). HE stain on brain tissue of rat 928 hours irradiated with UAHFEMF.
Lewy Body was found in this Figure, in some variant of AD has Lewy Body. Scale 40 micron.

H₂O₂ was Produced by the Discharge of UAHFEMF Irradiation on Rat for 928 Hours

H₂O₂ was stained with AuCl₃ and Thioflavin-S solution fluorescence microscopy demonstrated distinct amyloid and gold deposited H₂O₂ in the NFT neurons of the 928 hours UAHFEMF irradiated rat brain (Fig. 6).

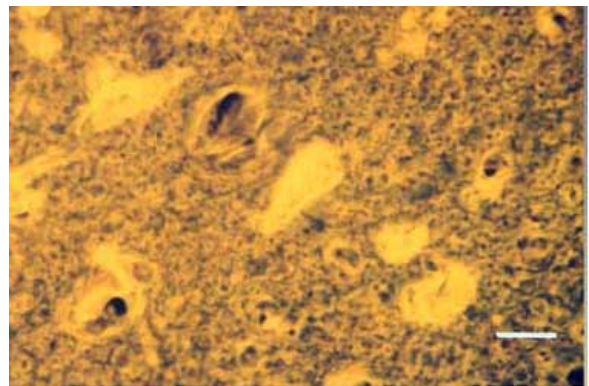


Fig. (6). H₂O₂ was produced in degenerated neurons.
H₂O₂ was stained with 1 % AuCl₃ showing gold deposition in the NFT neurons in the 928 hours UAHFEMF irradiated rat brain. Bar 30 micron.

Passive Avoidance Test

All of the 40 mice undergone passive avoidance screening test and 6 mice were then selected for use. These six mice were 23 weeks old, during the first experiment all six mice entered the dark chamber in less than 23 seconds in the training trial, then 24 hours later, three of the six mice did not enter the dark chamber, other three mice entered the dark chamber in the test trial. Then in the following two months these six mice were irradiated for 660 hours, and in the second experiment three of the six mice entered the dark chamber in less than 23 seconds in the training trial, the 24 hours later, non of the six mice entered in 298 seconds in the test trial. The following two months these six mice were irradi-

Table 1. Passive Avoidance Test on Six MICE with UAHFEMF after 0 Hour, 660 Hours and 901 Hours Accumulative Irradiation

| * date | 0 minutes* 1999. 12. 20 | | 660 hours* 2000. 2. 24 | | 900 hours* 2000. 4. 25 | | 901 hours* 2000. 6. 8 | |
|-----------|----------------------------|-------|----------------------------|-------|----------------------------|-------|----------------------------|--------|
| | 1 st experiment | | 2 nd experiment | | 3 rd experiment | | 4 th experiment | |
| 1 | 12.8 | 298.0 | 16.6 | 298.0 | 235.3 | 298.0 | 298.0 | 298.0. |
| 2 | 10.8 | 93.0 | 55.3 | 298.0 | 210.7 | 298.0 | 298.0 | 298.0 |
| 3 | 16.7 | 111.8 | 27.6 | 298.0 | 113.8 | 298.0 | 298.0 | 298.0 |
| 4 | 17.1 | 298.0 | 12.9 | 298.0 | 298.0 | 298.0 | 298.0 | 298.0 |
| 5 | 4.4 | 169.1 | 12.2 | 298.0 | 298.0 | 298.0 | 298.0 | 298.0 |
| 6 | 23.0 | 298.0 | 134.4 | 298.0 | 298.0 | 298.0 | 298.0 | 298.0 |

* total dose of UAHFEMF irradiated.
electric shock current: 1.0 mA, duration: 3 seconds.

ated for 900 hours cumulative irradiation. At the third experiment, of the six mice only three mice entered the dark chamber within 113.8~235.3 seconds and the other three mice did not enter in 298seconds in the training trial. 24 hours later all of the six mice did not enter the dark chamber in 298 seconds in test trial. After the third experiment, ferrous fumarate 25 mg rubbed in a slice of sweet potato was added to the diet of the six mice and some tea was added in the water bottle for daily drinking for the last two months to see if the effect of the irradiation may be more enhanced. Before the fourth experiment these six mice received 900 hours cumulative irradiation including ultra short wave 238 hours and microwave two hours accumulative irradiation. In the fourth experiment, six months later, none of the mice entered the dark chamber in 298 seconds in the training trial and 24 hours later, also none of the six mice entered the dark chamber in 298 seconds in test trial (Table 1). These results suggested that cognition of the threatening situation was so impaired by the adding ferrous fumarate that these rats had become almost no sense of fear. Before the fourth experiment, these mice had undergone a total of 900 hours of cumulative irradiation, including radiation from an ultrashortwave therapy unit at 27.12 MHz for 238 hours and a microwave oven at 2450 MHz for two hours. All six mice Brain sections prepared and examined by Thioflavin-S fluorescent microscopy of Mouse treated with 900 hours were suggestive of auditory and visual agnosia corresponding to the results of the passive avoidance test.

Visual Agnosia Development of Mice by UAHFEMF Irradiation and with Ferrous Fumarate Added in their Diet

In the result of post passive avoidance test pathological examination by Thioflavin-S fluorescence microscopic (Fig. 7a) findings there showed that prominent amyloid deposition at the Ca3 and the dentate gyrus, distinctly dilated inferior horn of lateral ventricle, and the Ca3 and Dentate gyrus were faced with Nuc. dorsalis corporis geniculati medialis and Nuc. dorsalis corporis geniculati lateralis they are the auditory and visual relay station so that auditory and visual signals cannot be accepted to the severely degenerated Ca3 and the Dentate gyrus to be stored as proximal memory engrams also by the dilated ventricle mediated failure of ultrasound resonance . There fore auditory and visual agnosia might be

developed. Accordingly cognition of threatening condition was impossible. Perpetual memory loss might also be mediated by disappearance of magnetic engrams in the neurons ferrite particles by the heating of UAHFEMF over Currie point 770k. These six mice were fed with adding ferrous Fumarate and tea for two months during passive avoidance test so that all of the rats showed profuse amyloid plaques expression in post tests section suggested that much iron intake combined with UAHFEMF irradiation will enhance amyloid deposition. In (Fig. 7b) photograph from one of 4 age matched 11 months old control rats that was not received UAHFEMF irradiation and have not been fed with ferrous fumarate and tea, in their thioflavin-S fluorescence microscopic findings there are scarce of amyloid deposition showed that the contrast between (Fig. 7a) and (Fig. 7b) is very distinct. These findings suggests that tea contains much tannic acid when it was taken in rat body it may be combined with proteins and easily traps iron substance in rat brain cells such as astrocytes, micro-glias cells or substantia nigra and some specific region in the brain to become targets of electrical charging in an environment of enhanced UAHFEMF. Then discharge developed neuronal degeneration and finally cause dementia.

PHF Development in AD Brain and in Experimental Rat

PHF was produced by UAHFEMF 2454 hours accumulative irradiation on rat for as long as 17 months period.of time which consumed 240 KWH electricity. The rat died at 19 months of age. The specimen was sent to Stephen McDaniel in Center for Advanced Microscope and Microanalysis, 1432 Geology Bldg, UC Riverside, Ca 92521 for determining of the presence of PHF. PHF is the most characteristic delicate expression of NFT in AD pathology. Its etiology is still unknown. Evidence that UAHFEMF irradiations on a rat for accumulated 2454 hours in a course of 17 months could produce PHF suggest that UAHFEMF discharges in human brain could mediate PHF (Fig. 8c). Recently the mechanism of PHF formation in AD brain has been upon debate mainly through biochemical pathway. However reasonable approaching is still not yet obtained. The UAHFEMF multiple functions may provide a good idea that Ultrasound waves have longitudinal wave, transverse



Fig. (7a). One of the six Post Passive avoidance test autopsies Thioflavin-S Fluorescence microscopic examination.

Thioflavin-S Fluorescence microscopy, from six mice 900 hours irradiated with UAHFEMF and received passive avoidance test. The result showed that prominent amyloid deposition at the Ca3 and the dentate gyrus. In each mice we found both distinctly dilated inferior horn of lateral ventricles, the third ventricle is also dilated and partial ependyma membrane was separated. The *Fistura longitudinalis cerebri* was very dilated. The Ca3 and Dentate gyrus are strongly affected by amyloid deposition and bulked. They are faced with *Nuc. dorsalis corporis geniculati medialis* and *Nuc. dorsalis corporis geniculati lateralis*. Several large amyloid plaque fluorescence shadows and numerous mid size amyloid shadows were profusely covered the whole brain field especially miliary amyloid shadows are seen in the *Cortex cerebri, area cingli, cortex cerebri, ventro lateral area tempolaris* and *Nuc. medialis tr. Optici* were affected with miliary amyloid deposition. Bar 720 micron.



Fig. (7b). One of the 4 age matched non irradiated 11 months old rats control brain preparation all showed scarce of amyloid deposition. Bar 360 micron.

wave, screwing wave, slipping wave and plate waves form [22], of course have 20nm and 80nm wave lengths, therefore in the brain cell protoplasm numerous microtubules may sub-

ject to the pressure of the plain waves going forward and back ward intermittently to make helix thread of microtubules as the manner of making a two helix thread rope with right palm skin and right thigh skin. Another screwing longitudinal waves with 80nm wavelength will break the paired helix thread into 80nm length intervals cooperated by the UAHFEMF that mediated high heat and could make the microtubules appropriately fragile. Thus the peculiar form of PHF might be fabricated by the super faculties of UAHFEMF. Concomitant Thioflavin-S fluorescence microscopic examination was performed after fixation of rat with UAHFEMF 2454 hours irradiation (Fig. 8b), and another Thioflavin-S fluorescence microscopic examination after fixation with 10% formalin from rat with UAHFEMF 2458 hours irradiation (Fig. 8a). Both figures finding showed that abundant Thioflavin-S fluorescence positive degenerated neurons can be detected in rat brain that was treated with UAHFEMF over 2450 hours net irradiation within a long period of time 17 months.

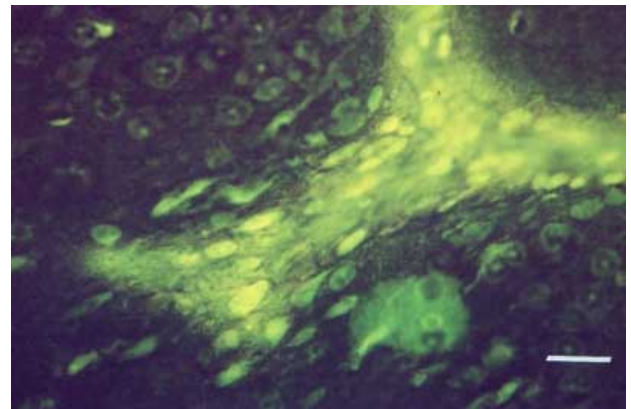


Fig. (8a). Thioflavin-S fluorescence microscopic examination rat 2458 hours irradiated by UAHFEMF.

Many tangled neurons showed strong fluorescence under Thioflavin-Fluorescence microscopy, some curly neurons and a Lewy Body is seen. This rat was the last UAHFEMF irradiated sample, almost neurons showed amyloid deposited and Thioflavin-S fluorescence strongly positive under fluorescence microscopy. Bar 30 micron.

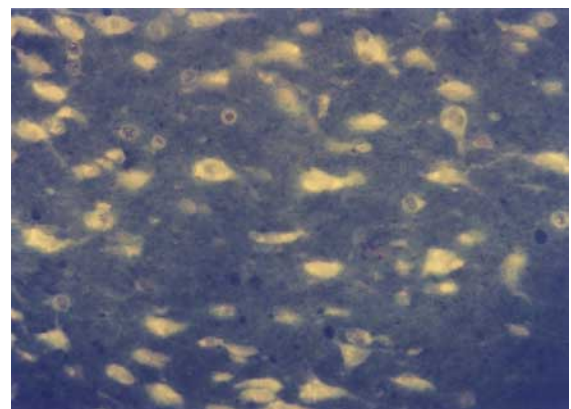


Fig. (8b). Amyloid deposition was proved in degenerated large neurons in the brain tissue of a rat 2454 hours irradiated with UAHFEMF.

The preparation was fixed with (4FiG), stained with 1% Thioflavin-S solution looked with fluorescence microscopy. Bar 30 micron.

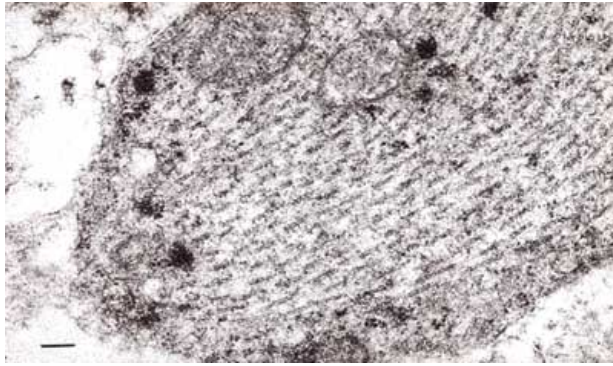


Fig. (8c). PHF was produced in Rat brain tissue by UAHFEMF irradiation for 2454 accumulated irradiation hours.

The specimen was sent to Stephen Mc Daniel Center for Advanced Microscope and Microanalysis 1432 Geology Bldg UC Riverside Ca 9252 for determining of the presence of PHF. Bar 0.2 micron.

Identification of UAHFEMF-Induced Human T-cell Activation RhoGTPase-Activating Protein (TAGAP) Isoform b Homolog in Rat Brain by Mass Spectrometry

Rat brain (both Rat control and UAHFEMF-treated for 2454 hours) soluble proteins were extracted and separated by a 15% Laemmli SDS-PAGE and stained with Ruby stain (Fig. 9a). A protein band of apparent mass around 80-90 kD was detected in the ultrasonic-treated group. LC/MS/MS analysis was performed on protein band of treatment group

and relative position of control following in-gel trypsin digestion. A double charged peptide with molecular mass of 1941.3 D was detected in UAHFEMF-treated sample (Fig. 9b) and control (Fig. 9c). Since there is only one protein identified, the protein level in the treatment group is at least 10 times of that of the control based on the gel image. The derived amino acid sequence tag was [IL] FEENGGA [IL] (GenBank accession # P84107). A human T-cell activation Rho GTPase-activating protein (TAGAP) isoform b homolog (accession # NP_473455) was identified with sequence very similar to the sequence tag after database searching. The mass spectrometry profile of the peptide is shown in Fig (9b). According to a BLAST search [14], the identified peptide was located in the Rho GTPase-activating protein (RhoGAP) domain.

RhoGAP is the negative regulator of Rho GTPase, which is one of the small GTPase family members. It released the GTP from GTP-bound Rho, which is the active form of Rho, into non GTP-bound form. These small GTPases all act as molecular switches in cells. Our identification of RhoGAP domain suggested a Rho GTPase-dependent signal transduction pathway might be induced under the UAHFEMF-treated condition. Since a link between Rho GTPase-activating protein (RhoGAP) and mental retardation has been characterized [16], it is possible that RhoGAP is involved in the pathology of Alzheimer's disease. In addition, a link between Rho GTPase-activating protein (RhoGAP) and cytoskeleton has also been characterized [17]. It is possible that RhoGAP is also involved in the Tau protein formation. On the other

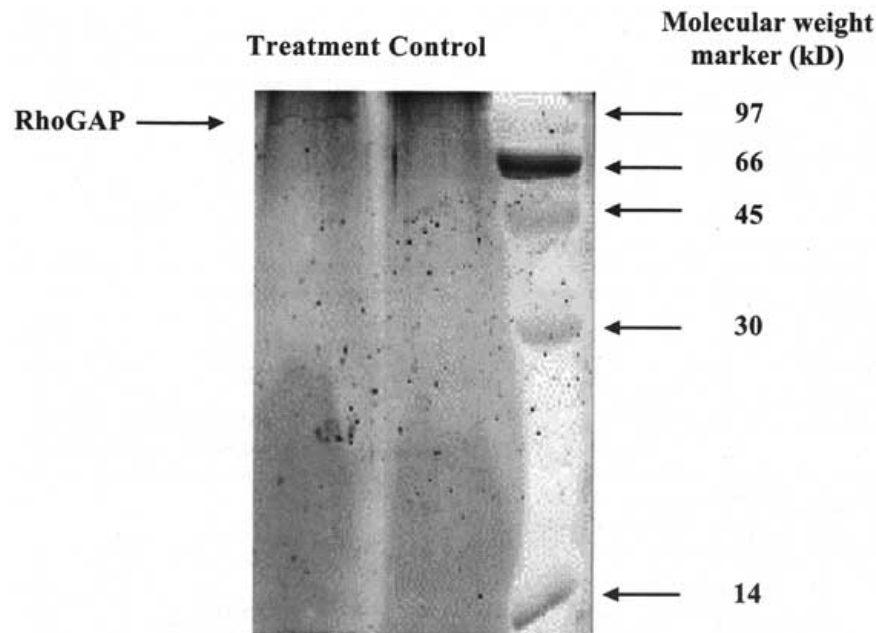


Fig. (9a). Identification of UAHFEMF-induced Human T-cell activation RhoGTPase-activating protein (TAGAP) isoform b homolog in Rat brain by mass spectrometry.

Rat brain (both rat control and UAHFEMF-treated for 2454 hours) soluble proteins were extracted and separated by a 15 % Laemmli SDS-PAGE and stained with SYPRO Ruby stain. A protein band of apparent mass around 80-90 kD was detected in the ultrasonic-treated group. LC/MS/MS analysis was performed on protein band in the treatment group and the relative position in the control following in-gel trypsin digestion. A double charged peptide with molecular mass of 1941.3 D was detected in UAHFEMF-treated sample and control. The derived amino acid sequence tag was [IL]FEENGGA[IL]. A human T-cell activation RhoGTPase-activating protein (TAGAP) isoform b homolog (accession # NP_473455) was identified with sequence very similar to the sequence tag by database searching.

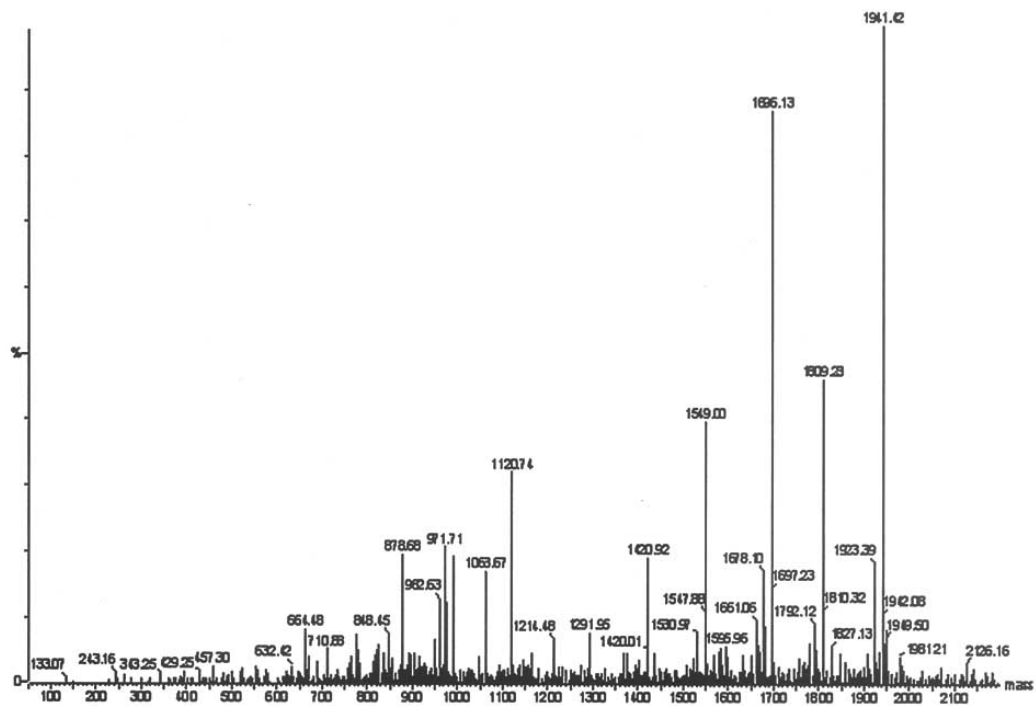


Fig. (9b). The MS/MS spectrometry profile of RhoGAP in the treatment group.

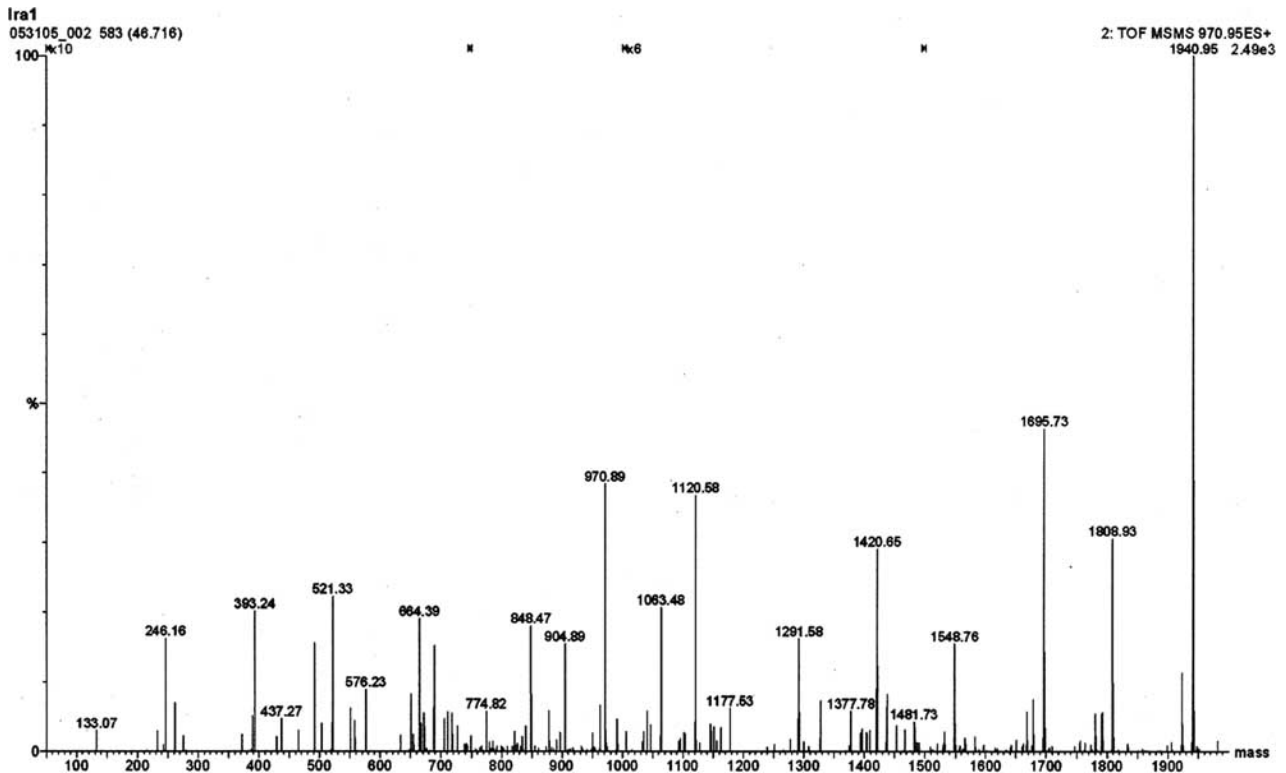


Fig. (9c). The MS/MS spectrometry profile of RhoGAP in the control.

hand, RhoGAP (RhoGAP in plant) regulated production of hydrogen peroxide [15,19]. The involvement of RhoGAP in the production of hydrogen peroxide under ultrasony treatment in rat is possible. However, there remained further evidences to prove these possibilities.

CONCLUSION

The characteristics of pathological phenotype expressions of AD are senile plaques, neuro-fibrillary degeneration, amyloid deposition, and phosphorylated Tau protein deposi-

tion etc. Their causes up to the present were taken as heterogeneous. The discovery of the induced characteristics of AD in rat brain treated with UAHFEMF led us to the hypothesis that AD development may be attributed to the UAHFEMF energy in human brain. In conclusion, we proved a good model system for AD study using rat brain under UAHFEMF treatment condition. By use of this system, we may elucidate the pathology of AD.

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