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L-type calcium channel blocker effects on electromagnetic field exposed lung histology

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Abstract

Electromagnetic field is produced by electrically charged objects and is defined as the combination of both electric field and a magnetic field. Wireless (wifi) networks, cell phones, bluetooth devices, power lines are some of the examples which emit electromagnetic field. Calcium channel blockers are a group of medicine used to lower blood pressure. They stop calcium from entering the cells of the heart and arteries. Our aim is to observe the possible histopathologic events of lungs from rats which was given a calcium blocker, amlodipine, meanwhile received a prolong electromagnetic field. To grade the lung histopathology, we stained the slides with hematoxylin and eosin and examined them under a camera attached light microscope.

Keywords: electromagnetic field, calcium channel blocker, lung, rat

1. Introduction

Popular use of devices which works through electromagnetic field (EMF) facilitates our lives but is it almost harmless? Mobile phones emit radiation; they use radiofrequency radiations to send signals The International Agency for Research on Cancer classifies radiofrequency radiations as possible human carcinogens (1). Besides, mobile phone base stations also emit EMF. Unavoidably, mankind is all surrounded by this invader unseen EMF haziness. A simple radiation energy cannot break down a molecular bond, but it may induce a molecular movement. On the other side, a high EMF (1 mT) will affect the cells growth or lead them to apoptosis or promote differentiation; decrease in T cells growth and release of Ca^{2+} are possible examples (2).

Lowering blood pressure is possible with the use of a calcium channel blockers. This class of drug stops the calcium to enter to the cells, for example the arteries (3). Amlodipine is an oral calcium channel blocker drug (blocks the voltage-dependent L-type calcium channels) which inhibits the influx of calcium ions. Hypertension, chronic stable angina, and vasospastic angina are some of the cases using this prolong half-life drug (4, 5).

Our aim of this study was to observe the possible histopathologic events of lungs from rats which administered with calcium blocker amlodipine meanwhile received a prolong EMF.

2. Material and Methods

Fifty adult *Wistar albino* male rats weighing 180-200 g were quarantined for one week and housed in cages for bedding at 21 - 24°C and 45 - 55% humidity and with a 12-h light-dark cycle at the "Animal Breeding Laboratory and Experimental Research Unit". The experimental procedures of the current study were approved by the "Experimental Animal Ethics Committee" of Ondokuz Mayıs University (2019/05). All experiments were performed under the ethical guidelines from the "Ethics Commission on Animal Use" and "National Institutes of Health Guidelines".

2.1. Group design

The groups of this study were designed as; control group which subjects were not exposed to EMF or received amlodipine, sham group where subjects were only placed at the area of which place of assembly of EMF for 1 hour 28 days. Group of EMF subjects were placed at the area of which they had EMF for 1 hour 28 days. Group EMF +AML subjects were placed at the area of which they had EMF for 1 hour 28 days meanwhile treated with amlodipine. Finally, the last group AML subjects were positive control receiving only amlodipine.

This was a double-blind randomized study in which the subjects took 2 doses of 1 mg/kg amlodipine per day for 3 weeks (6, 7) meanwhile exposed to 2100 MHz EMF (8).

The live animal studies were all undertaken in "Animal Breeding Laboratory and Experimental Research Unit" at Ondokuz Mayıs University. Histopathology studies were performed in "Histopathology Research Laboratory" at Istinye University.

2.2. EMF application

EMF generator (SynthUSBII, USB RF Signal Generator) operating at 34 MHz–4.4 GHz and with 1–2-Watt (W) output (pulse wave) was used. The probe was fixed to the middle part

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of the mechanism where the rats were placed and received 2100 MHz EMF. The signal generator was operated at 2 W power and the power density in the area close to the monopole antenna was measured precisely with an electric field probe (Extech RF EMF strength meter). Measurements was recorded every 6 minutes and at the end of the day 11 measurements was obtained. This application was carried 28 days (8).

2.3. Histopathologic examination

Lung samples were maintained in 10% formol saline for 24 hours. After washing the samples in tap water, they were dehydrated using serial dilutions of alcohol, cleared in xylene, embedded in paraffin at 58 ºC. A microtome was used to cut the paraffin tissue blocks at 10 µm thickness with a sample interval of 1/10. Tissue sections were collected on glass slides, deparaffinized and stained with hematoxylin and eosin (H & E). Finally, the preparations were examined by using a microscope with attached camera connected to a computer.

Histopathology results were classified into 4 grades as; Grade 1: normal lung tissue histology; Grade 2: few neutrophil leukocyte infiltrations; Grade 3: moderate neutrophil leukocyte infiltration, perivascular edema formation, partial destruction of pulmonary structure; Grade 4: dense neutrophil leukocyte infiltration, abscess formation, complete destruction of pulmonary structure (9, 10).

3. Results

Lung tissues of the control group was absent of histopathological alteration records (grade 1) (Fig 1). Intra bronchial erythrocyte aggregates, presumably due to sacrification, was observed in only one subject. Sham group subject slides of lung tissue parameters were similar as the control group. Some slides showed us that some of the subjects had grade 2 events where few neutrophil leukocyte infiltrations were seen: but not so dramatically.

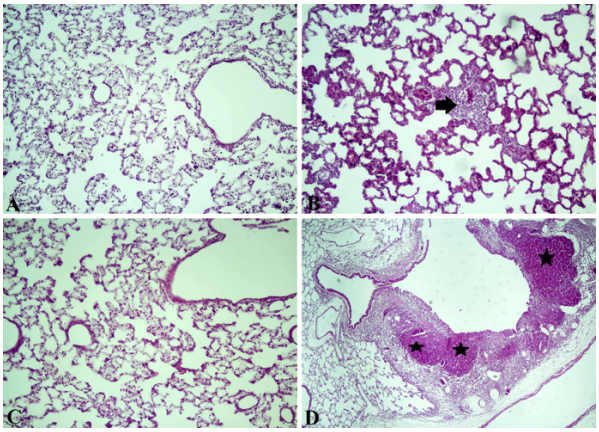

Fig.1. Photomicrographs of rat lung sections stained with H&E. Control group (A) grade 1, normal lung tissue histology. Sham group (B) grade 2, few neutrophil leukocyte infiltrations (arrow). EMF group (C) grade 2, few neutrophil leukocyte infiltrations. AML group (D) grade 4, dense neutrophil leukocyte infiltration (asterix)

There was none of complete destruction of pulmonary structures and was absent of formation disruption. Only EMF exposed group lungs were similar as sham group where only few places in the slides were seen as effected leaving few neutrophil leukocyte infiltrations behind. The most advanced pathological views were obtained from the subjects which was affected by EMF meanwhile treated with amlodipine (grade 4). Dense neutrophil leukocyte infiltration was general at all slides. Abscess formation of bronchiole epithelial structure was observed (Fig 3); group AML showed similar histopathologic structures (Fig 2).

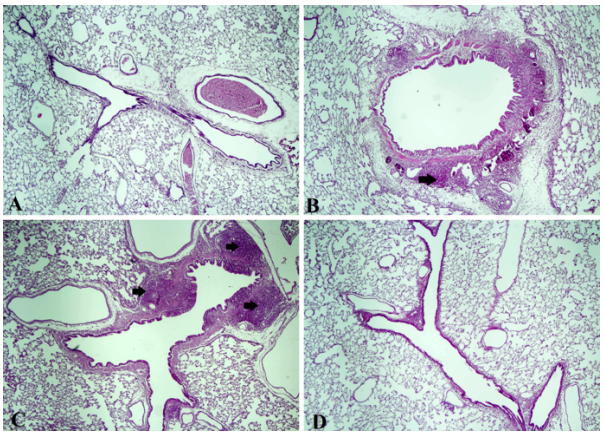

Fig.2. EMF group (A) pulmonary vessels in the view. AML group (B) moderate neutrophil leukocyte infiltrations in the peribronchiolar area (arrow). EMF +AML group (C) dense neutrophil leukocyte infiltration in the peribronchiolar area (arrows). Control group (D) normal lung tissue histology (H&E)

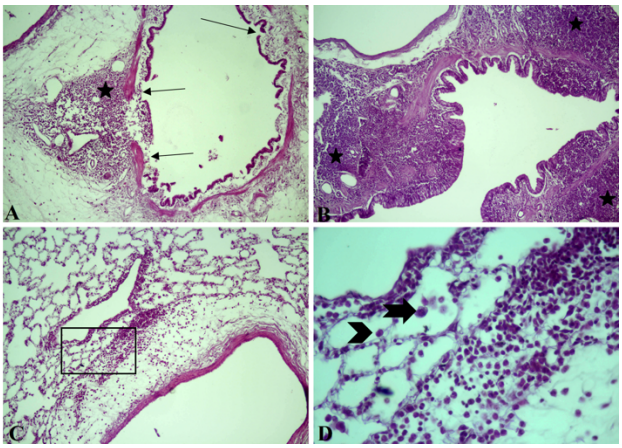

Fig.3. EMF + AML group micrographs. (A) neutrophil leukocyte infiltration (asterix); abscess formation, complete destruction of pulmonary structure (arrows). (B) neutrophil leukocyte infiltration (asterix). (D) higher magnification from (C; box), plasma cell (arrowhead) with alveolar macrophage (thick arrow) and dense neutrophil leukocyte infiltration (H&E)

4. Discussion

Histopathological results from this study indicates that calcium channel blocker disrupted the structural integrity of the rat lung while subjects were exposed to 2100 MHz EMF. EMF itself did not affect the lungs to the extent of causing lung damage. Only little amounts of neutrophil leukocyte infiltration were seen at few slides, but we can point out that disruption was out of the question. Only amlodipine receiving group subjects had also equivalent results as EMF meanwhile amlodipine receiving group. This brings us to the conclusion that whenever there is an amlodipine apply, lung structures are negatively affected by its mechanism. Calcium channel blockers suppress macrophage cells by effecting their receptor leading to an anti-

inflammatory process. Alveolar macrophages play role at neutrophil infiltration via releasing chemokines. Therefore, activation of neutrophiles is mediated by these chemokines. Hence, if calcium channel blockers suppress alveolar macrophages, neutrophil infiltration existence cannot be observed under this circumstance (11-13). On the other hand, calcium is necessary for muscle contraction; its inhibition will affect blood vessels. Calcium channel blockers may cause arterial vasodilation (14); and vasodilation may lead to fluid exudation and neutrophil infiltration (15). Neutrophil infiltration is an overly complex system. Therefore, more experimental animal studies are needed in this field.

Neutrophil plays a significant role in immune defense (16) but also can lead to tissue damage ending up with an inflammatory process (17). Here, we noticed that there was a dense neutrophil leukocyte infiltration at the lung tissues when subjects received a calcium channel blocker. A limitation of this study may be the duration of use of the drug AML. Subjects were not kept alive for a long time after possible neutrophil infiltration. This study covers a period of 28 days, it may be useful to extend this period and look at the results afterwards. In fact, are aim was to focus on the EMF usage and the possible effects with alongside using AML. The histopathological view in subjects with EMF alone was not as dramatic as in those with AML; for us, this was an unexpected result.

There are very limited studies on the topic of calcium channel blockers effects on lung structure, more is needed. To fully elucidate the mechanism, it is necessary to work with additional multiple parameters: a study plan can be designed by distinguishing between the case of infiltration caused by the inflammatory process and the cascade of vasodilation.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: B.A., Design: B.A., Data Collection or Processing: B.A., A.K., Analysis or Interpretation: B.A., A.K., Literature Search: B.A., A.K., Writing: A.K.

Ethical Statement

Approval was obtained from Ondokuz Mayıs University Animal Research Ethics Committee, the study started. The ethics committee decision date is 05/03/2019 and the number of ethical committee decisions is 2019/05.

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