



Aluminium foil dampened the adverse effect of 2100 MHz mobile phone–induced radiation on the blood parameters and myocardium in rats

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Abstract

Mobile phones emit a radiofrequency radiation (RFR) that might have adverse health effects. We aimed to investigate the possible protective effects of aluminium foil (AF) as a physical shield against the RFR from mobile phones on the blood parameters and the myocardium in rats. The effects of whole body 2100 MHz with 0.84–1.86 W/kg of SAR, 4 h/day for 30 days Global System for Mobile Communications (GSM)-RFR exposure for 4 h/day for 30 days on blood parameters (i.e. haemoglobin, leucocytes, thrombocytes, erythrocyte sedimentation rate, white blood cell differential count, corticosterone, CKMB), and the histology of myocardium were investigated. Three-month-old male rats ($n = 32$) were studied and randomised equally in the following four groups: K1 (non-AF non-RFR control), K2 (AF non-RFR control), P1 (non-AF RFR-exposed), P2 (AF RFR-exposed). Data were analysed with level of significance of $p < 0.05$. In P1, lower leucocytes and neutrophils counts with high corticosterone levels were found compared with the control groups, whilst a significantly higher CKMB was observed compared with P2 ($p = 0.034$). Lower cardiomyocyte counts congruent to the area fraction of the non-fibrotic myocardium were observed in P1 compared with the other groups ($p < 0.01$). AF might decrease the inflammatory-oxidative stress on rodent's blood cells and myocardium induced by the exposures of radiofrequency radiation of the mobile phones.

Keywords Electromagnetics · Cardiomyopathy · Haematology · Oxidative stress · Pollutant

Introduction

Mobile phones are one of most widely used gadgets worldwide nowadays. A mobile phone transmits electromagnetic radiations that may affect human's health adversely as an oxidative stressor (De Iuliis et al. 2009; Kovacic and Somanathan 2010). Biological tissue could absorb this radiofrequency radiation (RFR) according to the electrical conductivity and its permeability that is stated as specific absorption rate (SAR in W/kg). Absorption of RFRs would have a thermal and non-thermal effect to the tissue, which may cause a

resonance inside the body fluid and can increase the levels of the carbon monoxide in the blood circulation (Mahdavi et al. 2014; Sirswal and Dewan 2016). Mobile phone exposures have been reported to fluctuate the blood pressure and could modulate the myeloid and erythroid colony in the bone marrow of mice (Sirswal and Dewan 2016; Nadeljkovic et al. 2003). However, other study observed no significant effect on the cardiovascular and haematology of the rodents (Black and Heynick 2003).

In the current study, the potential effect of aluminium foil to shield mobile phone exposures on the blood parameters and the myocardium of rats would be investigated and compared with the control groups.

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Methods

This study was approved by the Komite Etik Penelitian Kesehatan (Ethic Committee on Health Research) of the Faculty of Medicine, Universitas Airlangga (No. 141/EC/

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KEPK/FKUA/2018). Thirty-two male rats of *Rattus norvegicus* were randomised into four groups equally ($n = 8$): K1 is control with no AF and no RFR, K2 is control with AF and no RFR, P1 is an RFR-exposed without AF, and P2 is an RFR-exposed with AF groups (Gumral et al. 2016; Mailankot et al. 2009). After acclimatisation, each rat was placed in a plastic container of $30 \times 25 \times 7$ cm size. A GSM mobile phone (Taiwan) was placed in a plastic bag at the floor of each cage in the P1 and P2 groups; it was covered with an aluminium foil in P2 (and only aluminium foil placed in K2) with thickness of 10×0.2 mm as this has been proven could decrease as much as half of the electromagnetic strength measured with an electromagnetic radiation tester (GM3120, Taiwan). The exposure was at the frequency of 2100 MHz with 0.84–1.86 W/kg of SAR, 4 h/day for 30 days (mobile phone was in a standby position and connected to the wifi M2Y source (Andromax, Taiwan) during the exposure). On day 31, all animals were sacrificed with decapitation and blood was collected from the atrium of the heart for analysis of levels of haemoglobin, leucocytes, thrombocytes, corticosterone, and CKMB (creatine kinase-MB) and also erythrocyte sedimentation rate (ESR) and differential count of the white blood cell (WBC) analysis. The heart was carefully processed into histology slides, cut diagonally in 5μ through the ventricles and stained using haematoxylin and eosin (H&E) that would show the normal cardiomyocytes and the non-fibrotic extracellular matrix of the healthy myocardium. Analysis of these slides was done using a light microscope with magnification of $\times 400$ and images were taken and studied using the Cell Sens (Olympus, Japan) and ImageJ software (NIH, USA) to measure the levels of cardiomyocytes and non-fibrotic extracellular matrix from each slide (by calculating the percentage of area fraction of each slide); the nuclei of the cardiomyocytes were also counted to confirm the prior semi-quantitative analysis of the non-fibrotic “healthy” percentage of the area fraction of the heart tissue in each slide ($n = 3$ of each group; 6–10 visual fields/slide) (Hales et al. 2011). The quantifications were done in duplicate (Daunoravicius et al. 2014; Kalanjati et al. 2017; Safer 2017). Data are presented as means and standard deviation. For the statistical comparison of data, the analysis of variance (ANOVA) with Tukey post-test or Kruskal-Wallis with Dunn post-test was applied after the Anderson-Darling normality test and Bartlett homogeneity test. The level of statistical significance is $p < 0.05$ (Max Stat Lite 3.60, Germany).

Results

In P1, the levels of leucocytes and neutrophils were lower compared with the other groups ($p > 0.05$), whilst the corticosterone level was higher compared with the control groups, although not statistically significant. The CKMB level in P1,

Table 1 The blood parameters of all animals from 4 groups, together with the percentage of the area fraction of non-fibrotic myocardium and the cardiomyocyte nuclei count (means \pm SD)

Group	Haemoglobin (g/dL)	Leucocytes (/mm ³)	Thrombocytes (/mm ³)	ESR (mm/h)	Differential count of the WBC			Corticosterone (ng/dL)	CKMB (μ /L)	% area fraction of normal myocardium	Cardiomyocyte count
					Eosinophils	Basophils	Neutrophils				
K1	13.78 \pm 0.61	13,000 \pm 4091.82	981,000 \pm 62,120.85	2 \pm 0	2.8 \pm 0.84	0 \pm 0	22.4 \pm 10.81	65.5 \pm 22.36	5.6 \pm 4.62	814.8 \pm 80.42	84,19 \pm 24.97
K2	14.02 \pm 0.48	13,860 \pm 6671.06	1,127,800 \pm 264,299.45	2 \pm 0	2.8 \pm 0.45	0.6 \pm 0.55	15.4 \pm 8.23	72.2 \pm 11.5	6.8 \pm 6.38	731 \pm 138.35	70,22 \pm 25.25
P1	13.45 \pm 2.12	10,100 \pm 3505.1	905,375 \pm 437,398.13	1.88 \pm 0.35	2.88 \pm 1.81	0.12 \pm 0.35	13.75 \pm 5.57	78 \pm 6.57	5.62 \pm 2.56	824.5 \pm 147.11 ^a	65.87 \pm 20.44
P2	13.02 \pm 1.31	1012 \pm 3992.65	639,000 \pm 282,119.02	1.5 \pm 0.53	2.5 \pm 0.93	0.12 \pm 0.35	17.75 \pm 3.99	72.62 \pm 8.02	7 \pm 6.23	596.25 \pm 232.1	71,07 \pm 19.29
<i>p</i>	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	< 0.05	< 0.01

^a Significant differences were observed amongst groups with P1 having the lowest CKMB levels. ^b Significant differences were observed amongst groups with P1 having the lowest % area fraction of non-fibrotic myocardium

however, was higher when compared with the other groups and was significantly higher when compared with the CKMB levels in P2 ($p = 0.034$). Interestingly, the haemoglobin, thrombocytes, and ESR in P2 were lower compared with those in P1, although not statistically significant. The blood parameter measurements are detailed in Table 1, i.e. levels of the haemoglobin, leucocytes, thrombocytes, corticosterone, and CKMB with the ESR and the differential count of the white blood cells (data are presented as means \pm standard deviation).

There were significant differences in the area fraction percentage of the non-fibrotic myocardium from the four groups; the lowest was observed in P1 ($p < 0.01$). These data were congruent with the cell nuclei counting of the cardiomyocytes in these four groups; the lowest count was in P1 ($p < 0.01$) (Fig. 1).

Discussion

In this study, we observed several modulations occurred in the group that was exposed to the RFR without the protection of the AF shield. The leucocytes and the neutrophils of the WBC differential count from this group were lower when compared with the other groups. These might cause higher vulnerability to infection risk, which closely correlated to the lower cellular immunity after unshielded mobile phone radiation exposures. However, previous study by Smialowicz et al. (1981) reported no significant alteration in the erythrocyte count, total or differential leucocyte counts, mean cell volume of erythrocytes, haemoglobin concentration, or haematocrit (Smialowicz et al. 1981). The contrast results in the current study may be due to the differences in the methodology, different source strength of the RFR, and the length of the study. Leucocyte is a vital component of the blood cells that plays various roles including

antibacterial immune system. Amongst these are neutrophils, which are parts of antibacterial immune system. In adult human, neutropenia would be diagnosed when the neutrophil levels are down below $1000/\mu\text{l}$ of blood, a condition which urgently needs a medical treatment. Neutrophils have a phagocytosis ability to eliminate the pathogens and/or infected cells that segmented neutrophils are the mature type that circulated in the blood whilst the banded neutrophils are the immature ones. Neutropenia may also due to other factors including taking specific drugs (anti-psychotics, immunosuppressants) and after serial chemotherapy. In neutropenia, activity of pro-inflammatory cytokines and growth factors to maintain the angiogenic and fibrogenic capacity could be disrupted. These would be the reasons behind mild changes shown in the myocardium of the rats (Black and Heynick 2003; Huang and Mold 1980; Sobrino-Marquez et al. 2018).

In this study, the corticosterone levels in the RFR-exposed group with and without the AF shielding were higher when compared with the control groups. The corticosterone is a representative cortisol hormone in human. This corticosterone closely correlated to the levels of the general adaptation stress (GAS) due to the physical stressors including mobile phone exposures. On the other hands, the corticosterone could increase the expression of the bcl-x1 without modulating the expression of the bax nor bcl-2; the final result would likely be depleting the cytoprotective effect of the corticosterones (De Iuliis et al. 2009; Neumann et al. 1982). The histopathology of fibrotic myocardium area and cardiomyocytes of the ventricles observed in the AF-unshielded RFR-exposed group could be due to the prior phenomenon, which was nicely represented by low percentage of the area fraction of the non-fibrotic myocardium and the cardiomyocyte count. Furthermore, the levels of the CKMB, a marker of cardiac and oxidative biomarkers, are significantly higher in the AF-unshielded group RFR-exposed from the mobile phones,

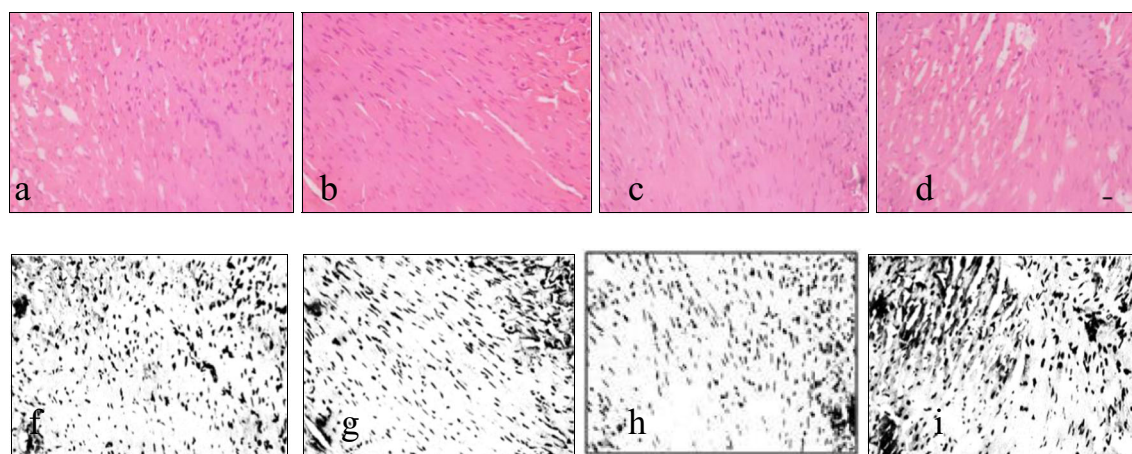


Fig. 1 a–d Myocardium of the ventricles of K1, K2, P1, and P2, respectively (H&E staining, $\times 400$ magnification, scale indicated 100μ). The hearts were sliced diagonally to follow the base of the cordis accordingly. f–i The quantification of the area fraction using

ImageJ (NIH, USA) of the K1, K2, P1, and P2, respectively. The non-fibrotic myocardium showed by the area fraction quantification in P1 was the least when compared with the other groups, which was corresponding to its cardiomyocyte count

when compared with the AF-shielded group. These may indicate higher inflammatory and oxidative stress occurs in the AF-unshielded RFR-exposed group compared with the AF-shielded RFR-exposed group (Willerson and Ridker 2004; Venkataraman et al. 2009). Chronic inflammatory process may result in the ventricle fibrosis, a condition that correlates to an excess deposition of extracellular matrix in the myocardium of the ventricles. In the current study, the healthy myocardium quantified by the percentage of the area fraction by ImageJ was stained well with H&E, whilst the interstitial myocardial collagen network could be clearly seen using other routine tissue staining methods including Mallory Azan-Trichrome staining. Ventricle fibrosis occurs when normal myocardium is replaced with fibrous connective tissue, which may result in the scarring and less compliant cardiac muscle. The process might be diffused and can be due to other pathology including hypertensive heart diseases, or localised such as in post-myocardial infarction. This condition may compromise the circulatory system or the heart itself (Daunoravicius et al. 2014; Frohlich 2001).

Conclusions

Aluminium foil, as a physical shield, dampened the inflammatory-oxidative stress on rodent's blood cells and myocardium induced by the exposures of radiofrequency radiation of the mobile phones.

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Compliance with ethical standards

This study was approved by the Komite Etik Penelitian Kesehatan (Ethic Committee on Health Research) of the Faculty of Medicine, Universitas Airlangga (No. 141/EC/KEPK/FKUA/2018).

Conflict of interest The authors declare that they have no conflict of interest.

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