Ann Med Res 2021;28(8):1477-82

Annals of Medical Research

DOI: 10.5455/annalsmedres.2020.09.958

Original Article

Does GSM-like 1800 MHz radiofrequency cause KRAS and p53 mutations in colon? : Histopatologically and microbiologically changes in colon

Ezgi Ece Sag[1], Saadet Celikozlu[2], Hayri Dayioglu[1], Sibel Kokturk[3], Cengiz Ozzaim[4], Sinan Darcan[5]

[1]Department of Biology, Faculty of Arts and Sciences, Kutahya Dumlupinar University, Kutahya, Turkey
[2]Department of Food Tchnology, Altintas Vocational School, Kutahya Dumlupinar University, Kutahya, Turkey
[3]Department of Histolgy and Embriology, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey
[4]Depatment of Electrical-Electronic Engineering, Faculty of Engineering, Anadolu University, Eskisehir, Turkey
[5]Department of Medical Services an Techniques, Gediz Vocational School of Health Services, Kutahya Health Science University, Kutahya, Turkey

Abstract

Aim: The aim of this study was to determine effects of 1800 MHz magnetic field on amout of colonic *Bactroides and Fusobacterium* and colon pathology in rats and determination of KRAS and p53 gene mutation in colon.

Materials and Methods: In the present study, three groups were prepared as control, sham and RFR. The RFR group rats were exposed to 1800 MHz GSM-like RFR for 12 weeks as 45 minutes per day. An electromagnetic energy generator was used to generate 1800 MHz RF radiation in the RFR group. At the end of experiments, colons were dissected from rats for histopathological examination and determination of KRAS and p53 mutations. Fecal speciments were collected for bacterial detection. DNA was isolated from fecal bacterias and colon.

Results: No differences were observed between sham and control groups, histopathologically. Corrupted gland structure, mucosal edema and inflammatory cell infiltration were observed at mucosal epithelum in the RFR group. An increase in the amount of collagen and fibrosis were detected in the electromagnetic field group. Number of goblet cells showed a statistically significant decrease in electromagnetic field group compared to both the control and sham groups ($P<0.05$). The increase in the amount of *Fusobacterium* it was significantly higher in the RFR group compared to the control group. In the quantity of *Bacteroides*, no differences were observed between the groups. KRAS and Tp53 mutation analysis of all samples were found to be wild type, at the colon tissues. No significant difference observed between the control group and the electromagnetic field treated group.

Conclusion: These results support, for 12 weeks 45 min/day exposure to GSM-like RFR caused histopathological damage in rat colon. Also, the amount of *Fusobacterium* is increased. But RFR exposure did not caused to KRAS and P53 mutations in colon tissue.

Keywords: Colon; histology; microbiology; mutation; radiofrequency radiation

INTRODUCTION

Technological developments bring many innovations to our daily lives. One of these is mobile phones which emits radiofrequency radiation (RFR). Mobile phones are increasingly used. Nowadays, studies showed the harmful effects of RFR in human health, so that; this issue is becoming a major problem. Many studies have been carry out about the various effects of radiation emmissions regarding the behaviour, cancer, central nervous system, sleep, children, cardiovascular system, immun function, reproductive system (1). RFR of mobile phones may affect biological systems by increasing free radicals and by changing the antioxidant defense systems of tissues (2).

Recently it has been observed a rapid increase in the incidence of colon cancers. Colon cancer is the third most common cancer in the world. Some specific gut bacterias have been associated with the pathogenesis of colon cancer. At the previous studies, increased Fusobacterium (3-5) and Bacteroides (6,7) species were reported in colon cancer tumor tissues compared with controls. In addition, KRAS (is a proto-oncogene) (8,9) and p53 (is a tumor supressor gene) mutations have been detected at the majority of colon cancer cases (10).

In this study, we investigated effects of GSM-like 1800 MHz RFR (for 12 weeks as 45 minutes per day) on the amount of colonic *Bacteroides and Fusobacterium* and

Received: 16.09.2020 **Accepted:** 15.11.2020 **Available online:** 17.08.2021
Corresponding Author: Saadet Celikozlu, Department of Food Tchnology, Altintas Vocational School, Kutahya Dumlupinar University, Kutahya, Turkey **E-mail:** saadet.celikozlu@dpu.edu.tr

colon histopathology in rats and the determination of KRAS and p53 gene mutations in colon.

When looking at the literature, there is no study about effects of GSM-like 1800 MHz RFR on colon tissue and colonic microflora. The findings of this study are expected to shed light on relationship of GSM-like RFR with colon tissue damage.

MATERIALS and METHODS

Animal subjects

For this study, adult albino Spraque-Dawley male rats were used. Rats obtained from Dumlupınar University Experiment Animals Laboratory. Rats were housed individually in cages, maintained under standart conditions (12 light/12 dark cycle, 25±3 °C) and were fed with standart pellet and water ad libitum. Body weight measurements were recorded in every two days.

Experimental design

At this study, three groups were designed as;

1.Control group

2.Sham group: This group rats were kept in the same conditions with EMF group without exposure to RFR.

3.RFR group: This group rats were exposed to 1800 MHz RFR for 12 weeks as 45 min/day.

GSM-like RFR exposure procedure

GSM-like signals in 1800 MHz frequency were formed by using a signal generator (SET ELECO., Set Electronic Co., Istanbul, Turkey). For this generator, the modulation frequency was 217 Hz and the maximal peak power was 4W. The rats were confined in a RFR exposure apparatus (Figure 1). Dipole antenna of the generator was placed at the center of RFR exposure apparatus to provide ideal exposure (Figure 2). The rats were free to move.

Figure 1. Magnetic field exposure apparatus

Before RFR application, rats were acclimated to exposure apparatus for one week. Thus, rats were prevented from entering stress during experiments. Power density inside the apparatus was measured by EMR 300 (NARDA, Pfullingen, Germany).

Removal of the colon tissues and sample of feces

Rats in this study were sacrified with cervical dislocation after 12 week RFR exposure. Feces samples were collected from through the colon tissue of rats for determination of bacterias in colonic microflora. Feces samples were maintained in peptone water (ISO 6579-Merck) in sterile sample containers. Then, colon tissues of rats were taken. A portion of colon tissue was fixed with buffered 4% paraformaldehyde for immunohistochemical staining. The other portion of colon tissue kept at -80 ºC for DNA isolation.

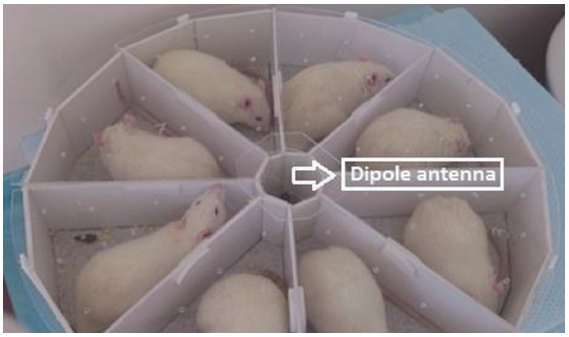

Figure 2. Dipole antenna

DNA isolation from stool samples

Fecal specimens in pepton water were inoculated to EO.ME Blue Agar (Biolife). Plates were incubated at 37ºC for 96 hours. DNA was isolated from obtained bacterias with High Pure PCR Template Preparation Kit (Version 2.0, Roche). Isolated DNAs kept at -20°C until the time of usage.

Fusobacterium and Bacteroides detection in the colon

Detection of bacterias was carry out by RT-qPCR analysis with SYBR Green I PCR Master Kit (Version 12, Catalog No: 04707 516 001, Roche). For this, the sequence given below was used as specific production primers. The obtained data was analysed using LightCycler 480 Instrument software version 1.5.1.

Fusobacterium Primer (Forward): 5′- GGA TTT ATT GGG CGT AAA GC -3′ (IDT-Integrated DNA Technologies ref. no. 68875457) (5).

Fusobacterium Primer (Reverse): 5′- GGC ATT CCT ACA AAT ATC TAC GAA -3′ (IDT-Integrated DNA Technologies ref. no. 68875458) (5).

Bacteroides Primer (Forward): 5′- GGA AGC ATT AAG TAT TCC ACC TC -3′ (PZR Bio Tech) (11).

Bacteroides Primer (Reverse): 5′- CGG TGA TTG GTA ACT GAC A -3′ (PZR Bio Tech) (11).

DNA isolation from colon tissue

Colon tissues were homogenizated before DNA isolation. DNA isolation from homogenizated tissue was carry out using High Pure PCR Template Preparation Kit (Version 2.0, Roche). The obtained DNAs kept at -20 °C until the time of usage.

KRAS and p53 mutation analyse

For KRAS (intron 4, rs number: 198298778) and p53 (exon 1 noncoding, rs number: 8151917), colonic DNA was analysed using RT-qPCR with LightCycler FastStart DNA Master HybProbe Kit (Version 15, Catalog No: 03 003 248 001, Roche). For this, the sequence giwen below was used as specific product primers.

Rat KRAS SP [G]: 5'- TTAAAATAXITCAgTgTCCCAgAgCT- -PH

Rat KRAS-R: 5'- gTgCTCTTAACCACTgAgCCA

Rat KRAS-F: 5'- gCCTgCTgTgAAAgCCA

RatTP53 SP [C]:5'- TAgCgACTXIACAgTTAgCgggTA- - PH

RatTP53-R: 5'- CgggCCCggATgCCAg

RatTP53-F: 5'- gCTAAAgTTCTgAAgCTCCAg

Histological staining

After rats were sacrified, colon tissues were fixed with buffered 4% paraformaldehyde solution. Following fixation, colon tissues were embedded in parafin after routin tissue fixation process and sectioned at 5-10 µm. The tissues were stained with Masson-tricrom staining method for detection of cell and connective tissue damage, cell infiltration and histopathological examination of lesions and numbers of goblet cells.

Statistical Analysis

RT-qPCR technique was used for both KRAS and p53 mutation analyzes and for bacteria detection. The obtained data was analysed using LightCycler 480 Instrument software version 1.5.1.

Histological stained preparats was examined under the microscope and it was analysed by image J analysis program after applying color deconvolution process two section (12,13). Obtained algorithms were evaluated statistically after color deconvolution processing. Statistical analysis was performed by using the computer software program SPSS for windows. Statistical differences between experimental and control groups were determinated by one-way analysis of variance (ANOVA) followed by Post-Hock multiple comparisons TUKEY tests. The level of significance was set to $p<0,05$.

RESULTS

SNP analysis results

Wild type mutations in the KRAS gene are observed at 52ºC and [A→C] mutation is observed at 56ºC. In the p53 gene, wild type mutations are observed at 56,6ºC, [G→C] mutation is observed at 57,6ºC.

According to results of this study, both KRAS and p53 mutations were found as wild type in all samples. Statistically significant difference was not observed between RFR group and neither control nor sham groups.

Bacteria detection results

The cp (crossing point) values give us an idea to determine the amount of bacteria in the RT-qPCR technique. According to tehe results of this study, the cp values of Fusobacterium in the RFR group werw determined in the range of 12,17 and 12,81. The cp values in the sham group were between 28,13 and 33,20, the cp values in the control group were between 29,05 and 39,07. The avarage cp values of Fusobacterium in the groups are shown in the Table 1.

Accordingly, the mean Fusobacterium concentration increased significantly when compared to controls. The avarage Fusobacterium concentrations of the groups are given in the Table 2. Looking at the Bacteroides results, the cp values of the groups were observed around 40. These values are considered unimportant.

Table 1. CP values of Fusobacterium in the groups according to PCR results

Control Group	Sham Group	RFR Group	Standart
32.20	31.60	12.49	11.21

Table 2. Mean Fusobacterium concentrations

Control Group	Sham Group	RFR Group	Standart
1.997×10^{1}	5.858×10^{1}	4.395×10^{7}	1×10^{8}

Histopathological results

Statistically significant differences were not observed between control and sham groups. In the control and sham groups, mucosal epithelium of colon tissue was intact, glands were regular and inflammatuar cell infiltration and edema were not observed (Figure 3a,b).

In the RFR group, erosions and partial losses were observed at mucosal epithelium. Gland structure was corrupted and the mucosal edema and the inflammatory cell infiltration were observed (Figure 3c).

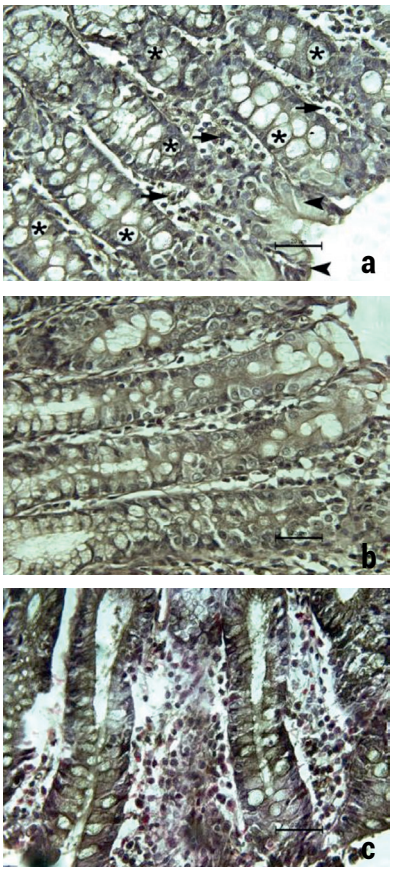

Figure 3. Repectively, intestinal sections of the control (a), sham (b) and RFR (c) groups are seen, at high magnification. Asterixes indicate goblet cells. The arrowheads show the intestinal surface epithelium and the arrows showthe connective tissue of the lamina propria located between the glands. Masson trichrome staining, bar 20 µm

Color deconvolution process was applied to colon sections stained by Masson tricrom with imaging J analysis program. Thus, amount of collagen were detected at images stained with aniline blue. By detecting an increase in the severity of collagen staining between the groups, development of fibrosis was detected. There was no statistically significant difference in the intensity of aniline staining in the control and sham groups. Dye intensity increased statistically significant in the RFR group compared to controls (Table 3).It was determined that there was an increase in the amount of collagen and fibrosis in the RFR group.

Table 3. Aniline blue intensity of staining and amount of collagen

	Control Group	Sham Group	RFR Group
Collagen	169.74478	169.84078	191.59678
Std Error Mean	0.95611807	1.0563857	1.0678512

The number of goblet cells in the mucosa was determined in the rats in each group. Goblet cell counts did not differ statistically in the control and sham groups. In the RFR group, the number of goblet cells showed a statistically significant decrease compared to both the control group and the sham group (Table 4). A paralellism was observed between the decrease in the number of goblet cells and the disruption of the gland structure in the RFR group.

Table 4. The number of goblet cells

	Control Group	Sham Group	RFR Group
Goblet Cels	28.592593	28.444444	14.722222
Std Error Mean	0.29260453	0.306768813	0.57029708

DISCUSSION

At this study, rats were exposed to GSM –like 1800 MHz RFR for 12 weeks as 45 minutes per day. Until today, many studies have investigated the effects of magnetic fields from mobile phones on biological systems. Especially, studies on the reproductive system are intensive. Many studies showed that, EMFs causes hyperthermia, inflammation; and apoptosis at male and female reproductive organs. Also, EMFs changes testosterone levels (14). Despite intensive researches on the reproductive system, there are no studies about the effects of magnetic field on bowels which are localized in the same area in the body. Therefore, in this study, we aimed to investigate the effects of GSM-like 1800 MHz RFR on colon tissue.

Mutations in KRAS are an early step in colorectal cancer carcinogenesis (15,16) and mutations in p53 are associated with the transition between late adenoma and carcinoma (17,18). According to the results of this study, both KRAS and p53 mutations were found as wild type in all samples. 1800 MHz RFR application for 12 weeks did not cause KRAS and p53 mutations at colon tissue in rats.

It is estimated that chronic inflammation associated with microbial infection directly contributes to the etiology of about 20 % of epitelial cancers. Microbial changes are also the most important resource in the colon cancer (19,20). *Bacteroides spp*. comprises a significant proportion of colonic commensal bacteria and the members of this group include symbionts and the leading human anaerobic pathogen, *B. fragilis* (19,20). Previous studies show that, increased numbers of *Bacteroides* have been associated with increased risk of colon cancer in humans (6,21,22). Ojetti et al. detected that *Bacteroides* and *Clostridium* species promote the growth incidence of colonic tumors in animals (22). When we look at the previous studies, there are no studies on whether or not the increase of the amount of *Bacteroides* of RFR on colon. According to the results of this study, the difference was not detected between groups in the amount of *Bacteriodes*.

Fusobacterium is part of the normal flora in the human gut mucosa. *Fusobacterium* species are higly heterogenous and some species have been recognized as opportunistic pathogens implicated in the inflammatory diseases such as appendicitis and inflammatory bowel diseases. Recent studies demonstrated that *Fusobacterium* species were abundant in colorectal cancer tissues compared to normal mucosa (3,4). According to this studies, *Fusobacterium* maybe associated with later stages of colorectal carcinoma but it is unknown if they play a role in the early stages of colorectal carcinogenesis. These results suggest *Fusobacterium* is a potential biomarker for colorectal carcinogenesis (4,5). In literature, there are no studies on the effect of GSM-like RFR on the amount of colonic *Fusobacterium* flora. We observed significant difference in bacterial abundance between RFR group and control-sham groups.

This finding is consistent with our histopathological results and explains the reason of cell infiltrations in the colon tissues. The fibrosis was developed in the RFR group. Exposure to RFR from mobile phone, causes the reduction of antioxidant enzyme activities in the body. Also, it leads to increased reactive oxygen species and tissue damages (23-29). Previous studies reported that RFR causes necrosis, thrombosis, dilatation, interstitial fibrosis, vacualization, inflammation, athrophy, sclerose at the tissues.

According to results of our study there is no difference between control and sham groups. In the RFR group erosions and partial loses were observed at mucosal epithelium, and gland structure was corrupted and the mucosal edema and the inflammatory cell infiltration was observed. Anilin dye intensity increased statistically significant in the RFR group compared to both control and sham groups. This suggests that the fibrosis developed and the amount of collagen increased. Furthermore, goblet cell numbers decreased significantly in the RFR group. In the study of Kostic et al. inflammation have been identified due to *Fusobacterium* growth in the colonic mucosa (3). Another study reported that there is a relationship between the amount of *Fusobacterium* and inflammations in the colonic mucosa (4). These findings support our results that we detected tissue damage associated with the growth of *Fusobacterium*.

CONCLUSION

In conclusion, in this study, the effects of 1800 MHz GSM-like electromagnetic field on the colon were examined. For this, KRAS and p53 mutations and amount of Fusobacterium and Bacteroides were determined as colon cancer marker.

According to obteined data, for 12 weeks 45 min/day exposure to GSM-like1800 MHz RFR did not cause to cancer and KRAS and p53 mutations in the colon tissue. In the study, both KRAS and p53 mutations were found as wild type in all samples. However, it caused histopathological damage in rat colon. Erosions and partial losses were observed at mucosal epithelium and gland structure was corrupted. Also, the amount of colonic Fusobacterium is increased significantly.

At the same time, the studies on the effects of RFR from mobile phone on the colon tissue must continue.

Competing Interests: The authors declare that they have no competing interest.

Financial Disclosure: This work was supported by the Scientific Research Project Unit of Kutahya Dumlupinar University, numbered 2014-81.

Ethical Approval: This work was supported by the Scientific Research Project Unit of Dumlupinar University (2014-81). Animal studies were performed after taking approval from Animal Experiments Local Ethics Committee of Dumlupinar University (No: 2015.02.04).

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