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The Effects of Epigallocatechin Gallate and N-Acetylcysteine on Mobile Phone-Induced Oxidative Stress in Guinea Pigs

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Abstract: It has been reported that mobile phones induce free radical formation. Epigallocatechin Gallate (EGCG) and N-Acetylcysteine (NAC) were recently found to be potent antioxidants. The present study was carried out to determine the protective effects of antioxidants namely EGCG and NAC against the mobile phone-induced oxidative stress. Three-month-old male Guinea pigs were randomized into 6 groups; sham group, not exposed to mobile phone, NAC group (300 mg kg^{-1} ip, 7 days), 10 min mobile phone-exposed group with NAC, 10 min mobile phone-exposed group, EGCG group (25 mg kg^{-1} ip, 7 days) and 10 min mobile phone-exposed group with EGCG. During the exposure external E fields were measured by NARDA EMR 300 and type 8.3 probe. Guinea pigs were exposed to RFR averaged as 11.2±0.5 V m^{-1} for 10 min/day/7days. Malondialdehyde (MDA, an index of lipid peroxidation) were analysed as markers of oxidative stress. Plasma MDA levels increased in mobile phone exposed guinea pigs while EGCG causes a significant reduction of MDA levels, whereas NAC caused a significant increase in MDA levels. Also, MDA levels in mobile phone exposure group significantly increased with respect to sham exposure group. A number of studies have identified antioxidant species which show antioxidant effect in low-dose and pro-oxidant behaviour in high-dose. The increase of the lipid peroxidation at the treatment dose of NAC in guinea pigs may possibly be due to pro-oxidant behaviour. Besides, this study expressed that mobile phone exposure affect the level of lipid peroxidation of plasma.

Key words: Epigallocatechin gallate, n-acetylcysteine, oxidative stres, mobile phone, guinea pigs, MDA

INTRODUCTION

Oxidative stress is generated by an imbalance between oxidants and antioxidants in favour of oxidants, which may cause tissue damage. Oxidative stress associated can have detrimental effects mediated by the ability of Reactive Oxygen Species (ROS) to induce cell death in a number of different cell types. ROS include several molecules and radicals, such as the superoxide radical (O2˙), Hydrogen peroxide (H_2O_2), Hydroxyl radical (OH) and others. Under physiological conditions, ROS are constantly generated within cells by several intracellular oxidase enzymes and by mitochondrial respiration. H_2O_2, which easily diffuses inside and outside cells, is able to modulate multiple cellular processes: cell proliferation, signal transduction pathways, gene expression, DNA damage, apoptosis and necrosis (Estany *et al*., 2007).

N-Acetyl L-Cysteine (NAC) is a thiol, a mucolytic agent and a precursor of L-cysteine and reduced glutathione. NAC is a source of sulfhydryl groups in cells and scavenger of free radicals as it interacts with ROS such as OH- and H_2O_2. NAC can scavenge ROS, increase glutathione levels, undergo autooxidation (and produce H_2O_2) and serve as reducing agent. NAC has been an extensively utilized tool for investigating redox sensitivity of biological or pathological processes. NAC can interfere with cell adhesion, oxidative stress, smooth muscle cell proliferation by mitogens, stability of rupture-prone atherosclerotic plaques in the cardiovascular system and reduce lung inflammation, fibrosis, smoking-related changes and prolong transplants. In arthritis, it can reduce inflammation, synovial invasion and cartilage damage (Zafarullah *et al*., 2003).

Flavonoids are a ubiquitous group of polyphenolic substances which are present in a variety of plants (Zhang and Osborne, 2006). The antioxidant components in green tea include Epigallocatechin Gallate (EGCG), Epicatechin Gallate (ECG), Epigallocatechin (EGC) and Epicatechin (EC). All of these components have antioxidant properties but EGCG has been described as

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being a more active antioxidant than the other components (Graham, 1992). Catechins and polyphenols are effective scavengers of physiologically relevant reactive oxygen and nitrogen species *in vitro*, including superoxide, peroxyl radicals, singlet oxygen, peroxynitrite (Frei and Higdon, 2003).

Malondialdehyde (MDA) is a decomposition product of oxidized lipids and as an index of plasma and tissue lipid peroxidation. Lipid hydroperoxides may decompose to form aldehydes such as MDA and 4-Hydroxynonenal (4-HNE) (Frei and Higdon, 2003). Malondialdehyde (MDA) is the breakdown product of the major chain reactions leading to oxidation of polyunsaturated fatty acids and thus serves as a reliable marker of oxidative stress-mediated Lipid Peroxidation (LPO) (Ozguner *et al*., 2005b).

Widespread concerns have been raised about the possibility that exposure to the Radio Frequency (RF) fields from mobile telephones or their base stations could affect people's health (Repacholi, 2001). Electromagnetic Radiation (EMR) or radiofrequency fields of cellular mobile phones may affect biological systems by increasing free radicals, which appear mainly to enhance lipid peroxidation and by changing the antioxidant defense systems of human tissues, thus leading to oxidative stress (Ozguner *et al*., 2005a). Cellular telephones and their base stations produce Electromagnetic Radiation (EMR), the effect of which on the body depends on its frequency and power (Irmak *et al*., 2002).

The present study, was carried out to determine the protective effects of antioxidants namely EGCG and NAC against the mobile phone-induced oxidative stress.

MATERIALS AND METHODS

The experimental protocol was reviewed and approved by the Laboratory Animal Care Committee of Gazi University. All the animal procedures were performed in accordance with the approved protocol.

In this investigation, 56 each 3-month-old male Guinea pigs divided into six groups as mentioned at Table 1.

Animals were placed inside the cage just at the beginning of the experiment in order to reduce the stress factor. RF source, Nokia 3210 mobile phone with 0.81 W kg^{-1} digital SAR value was positioned on the cage, where the antenna of the mobile phone is above the head of the guinea pig. While, mobile phone is off mode as the sham exposure condition, it was in talking position during the exposure conditions. During the exposure of every Guinea pig, external E fields were measured by

Table 1: Experimental groups

Group 1	Sham exposed group as controls (All control groups injected intraperitoneally (i.p.) with 1 mL isotonic saline sol. in order to prevent the occurrence of stress from injection)
Group 2	Sham exposed with N-Acetyl L-Cysteine (NAC) administration group (NAC was treated (300 mg kg^{-1}) i.p. during 7 days)
Group 3	10 min mobile phone-exposed group with N-Acetyl L-Cysteine (NAC) administration (NAC was treated (300 mg kg^{-1}) i.p., 30 min before the exposure period during 7 days)
Group 4	10 min mobile phone-exposed group (10 minutes mobile phone-exposed groups injected intraperitoneally (i.p.) with 1 mL isotonic saline sol. before 30 min from exposure during 7 days)
Group 5	Sham exposed with (-)-Epigallocatechin-Gallate (EGCG) administration group (EGCG was treated (25 mg kg^{-1}) i.p., during 7 days)
Group 6	10 min mobile phone-exposed group with (-) Epigallocatechin-Gallate (EGCG) administration (EGCG was treated (25 mg kg^{-1}) i.p., 30 min before the exposure period during 7 days)

NARDA EMR 300 and type 8.3 probe. Measurements were taken for duration of 10 min per 2 sec and the data saved to the computer connected to device via fiber optic cable.

We had 300 instantaneous data for 10 min exposure. Averages and the statistical deviations of the whole instantaneous data were derived before statistical analysis. Guinea pigs were exposed to RFR averaged as 11.2±0.5 V m^{-1} for 10 min a day during 7 days.

MDA analysis: Blood was collected by cardiac puncture into heparinased tubes. Plasma levels of Malondialdehyde (MDA) were measured as TBA substance by the methods of Yoshioka *et al*. (1979).

Statistical analysis: Kruskal-Wallis test was carried out in order to find any differences between groups.

RESULTS AND DISCUSSION

Plasma MDA levels increased in mobile phone exposed guinea pigs while EGCG causes a significant reduction of MDA levels, whereas NAC caused a significant increase in MDA levels (Table 2).

EGCG inhibited iron-driven lipid peroxidation presumably not only by chelating to Fe ions but also by scavenging superoxide radicals, which are responsible for the reduction of Ferric (Fe_3) to Ferrous (Fe_2) that catalyzes the Fenton reaction. Chelating and radical scavenging activity of EGCG can be expected simultaneously in lipid peroxidations, which may explain the protective effects of EGCG against lipid peroxidations (Higuchi *et al*., 2003).

NAC can replenish non-protein cellular thiols and protect membrane lipids and proteins due to its direct radical-scavenging properties. NAC was a potent scavenger of hydrogen peroxide and hydroxy radicals, whereas the scavenging activity of NAC against

Table 2: Plasma MDA levels of guinea pigs (nmol mL^{-1})

Groups	N	Mean	SE	Min.	Max.
1	7	1.520c	0.47	0.16	3.48
2	7	3.300a	0.62	1.37	5.85
3	7	2.481b	0.61	0.10	4.38
4	9	2.082b	0.33	0.60	4.05
5	7	0.905c	0.19	0.33	1.55
6	8	0.852c	0.11	0.43	1.35

superoxide anion was found to be rather weak. On the other hand, NAC can generate thiyl radicals that may impact on pro-oxidant function (Maksimchik *et al*., 2008). The iron mediated oxidative metabolism of thiols would account for their pro-oxidant action. The antioxidant and pro-oxidant effects NAC depends on the nature of the radicals generated by the oxidative systems (Sagrista *et al*., 2002).

NAC can not be the protective effects on the hydroxyl radicals produced by the iron mediated Fenton reaction. The increase of the lipid peroxidation at the treatment dose of NAC in guinea pigs may possibly be due to pro-oxidant behaviour. The antioxidant and pro-oxidant effects NAC depends on the nature of the radicals generated by the oxidative systems. Besides, this study expressed that mobile phone exposure affect the level of lipid peroxidation of plasma.

CONCLUSION

To clarify the mechanism of mobile phone-induced lipid peroxidation further investigation of parameters like reactive oxygen metabolites is necessary.

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