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RESEARCH ARTICLE

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The effect of exposure to radiofrequency LTE signal and coexposure to mitomycin-C in Chinese hamster lung fibroblast V79 cells

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Abstract
This study aims to investigate the cellular effects of radiofrequency exposure, 1950 MHz, long-term evolution (LTE) signal, administered alone and in combination with mitomycin-C (MMC), a well-known cytotoxic agent. Chinese hamster lung fibroblast (V79) cells were exposed/sham exposed in a waveguide-based system under strictly controlled conditions of both electromagnetic and environmental parameters, at specific absorption rate (SAR) of 0.3 and 1.25 W/kg. Chromosomal damage (micronuclei formation), oxidative stress (reactive oxygen species [ROS] formation), and cell cycle progression were analyzed after exposure and coexposure. No differences between exposed samples and sham-controls were detected following radiofrequency exposure alone, for all the experimental conditions tested and biological endpoints investigated. When radiofrequency exposure was followed by MMC treatment, 3 h pre-exposure did not modify MMC-induced micronuclei. Pre-exposure of 20 h at 0.3 W/kg did not modify the number of micronuclei induced by MMC, while 1.25 W/kg resulted in a significant reduction of MMC-induced damage. Absence of effects was also detected when CW was used, at both SAR levels. MMC-induced ROS formation resulted significantly decreased at both SAR levels investigated, while cell proliferation and cell cycle progression were not affected by coexposures. The results here reported provide no evidence of direct effects of 1950 MHz, LTE signal. Moreover, they further support our previous findings on the capability of radiofrequency pre-exposure to induce protection from a subsequent toxic treatment, and the key role of the modulated signals and the experimental conditions adopted in eliciting the effect.

KEYWORDS
coexposure, in vitro, LTE, radiofrequency

1 | INTRODUCTION

Over the last decades, human exposure to electromagnetic fields emitted by wireless communication systems has exponentially increased, and it will further expand in the near future, as a result of technological innovation, and of the increasing demand for wireless connectivity that has become of crucial importance for the modern lifestyle.

Currently, human population and workers are exposed to radiofrequency electromagnetic fields emitted by wireless technologies based on multiple standards, from the second generation (2G) global system for mobile communication (GSM), to the third

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generation (3G) systems based on technologies such as the wideband code-division multiple access (WCDMA, and the 4th generation [4G]) mobile service. The latter relies on a fully digital Internet protocol-based technology called long-term evolution (LTE) operating in the frequency range from 800 to 2600 MHz. Moreover, despite the very recent transition from the 4G to 5G network, 4G LTE is still the most frequently used signal in wireless communication (Rappaport et al., 2013; Souffi et al., 2022).

Over several decades, scientific research into the possible adverse effects deriving from radiofrequency (RF) exposure has been carried out, covering mostly 2G and 3G signals, while a limited number of investigations addressed 4G signal. The available literature has been analysed by many expert groups within national and international organizations. Most of the studies did not report effects in the analysed outcomes/endpoints, while other studies reported effects although not confirmed when replicated by independent research groups. As a whole, in most cases, the general opinion is that the health risk for humans is weak, although the need of additional studies fulfilling quality criteria of both electromagnetic and biological aspects is always highlighted (ICNIRP, 2020; IEEE, 2019; SCENIHR, 2015).

By referring more specifically to investigations addressing 4G systems, only a few experimental studies are available in the literature, and the majority of them has been carried out in vivo. Most of them report on the effects on brain function in both rat models (Ozdemir et al., 2021; Souffi et al., 2022) and human subjects (Lv et al., 2014; Vecsei et al., 2018; Wei et al., 2019; Yang et al., 2017, 2021). Alterations on behavior (Broom et al., 2019), fertility (Hasan et al., 2021; Oh et al., 2018; Yu et al., 2020), and hematological parameters (Hasan et al., 2021) have also been reported in mice models.

To the best of authors' knowledge, only a few in vitro studies have been carried out addressing the effect of 4G LTE exposure at frequencies around 1750 MHz on cancer-related endpoints (Choi et al., 2020; Jin et al., 2021; Kim, Jeon, et al., 2021; Kim, Kang, et al., 2021).

A further, very interesting but less investigated, aspect is the possible enhancement or reduction of the effect induced by physical or chemical treatments given in combination with RF. In this respect, we demonstrated in previous studies that RF exposure, given at several frequencies, modulated signals of 2G and 3G standards, and specific absorption rate (SAR) levels, does not affect key cellular endpoints in mammalian cell cultures when administered alone. Conversely, RF pre-exposure was capable of inducing protective effect against a subsequent chemical or physical damaging treatment.

Specifically, RF exposure reduced the DNA damage induced by treatments with mitomycin-C (MMC), menadione, and X rays in different cell models exposed under several conditions, as summarized in Sannino et al. (2022). The protective effect exhibited some features of ionizing radiation-induced adaptive response (AR), including the capability to propagate via bystander mechanisms to cells not directly exposed to RF (Zeni et al., 2021).

Moreover, the occurrence of modulation-specific effects was demonstrated with a relation between the bandwidth and the power absorbed by samples, while exposure to continuous wave (CW) did not induce the effect (Romeo et al., 2020).

In this study, we aimed at examining the cellular effects of 4 G LTE signal at the frequency of 1950 MHz in Chinese hamster lung fibroblasts (V79). Cell cultures were subjected to exposure of 3 and 20 h at SAR levels of 0.3 and 1.25 W/kg to analyze chromosomal damage, ROS formation, cell proliferation, and cell cycle progression. The same cellular endpoints were also analyzed in coexposure protocols in which MMC treatment was given after the RF exposure to investigate possible modification of the chemical-induced damage. The induction of chromosomal damage was also investigated after CW exposure.

Highlights

- A cytotoxicity study in Chinese hamster lung fibroblasts exposed to 1950 MHz 4G LTE signal is presented.
- RF exposure alone does not induce DNA damage, ROS formation, and cell cycle progression under the experimental conditions adopted.
- RF pre-exposure reduces the MMC-induced DNA damage and ROS formation under selected experimental conditions.
- CW exposure does not exert any variation in both RF exposure and co-exposure protocols highlighting the key role of modulated signal in interacting with mammalian cells.

2 | MATERIALS AND METHODS

2.1 | Reagents

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and phosphate buffer were from Domenique Dutscher (Brumath), L-glutamine, trypsin-EDTA, and penicillin/streptomycin were from Biowhittaker. 2′,7′-Dichlorofluorescin diacetate (DCFH-DA), cytochalasin-B, mitomycin-C (MMC), Triton X-100, and propidium iodide (PI) were from Sigma. Dimethyl sulfoxide (DMSO), sodium citrate, methanol, and Giemsa were from Baker.

2.2 | Radiofrequency exposure system set up

The exposure set up, sketched in Figure 1, was used for the experiments. Four cell cultures were exposed simultaneously to 1950 MHz, LTE or CW signal, 0.3

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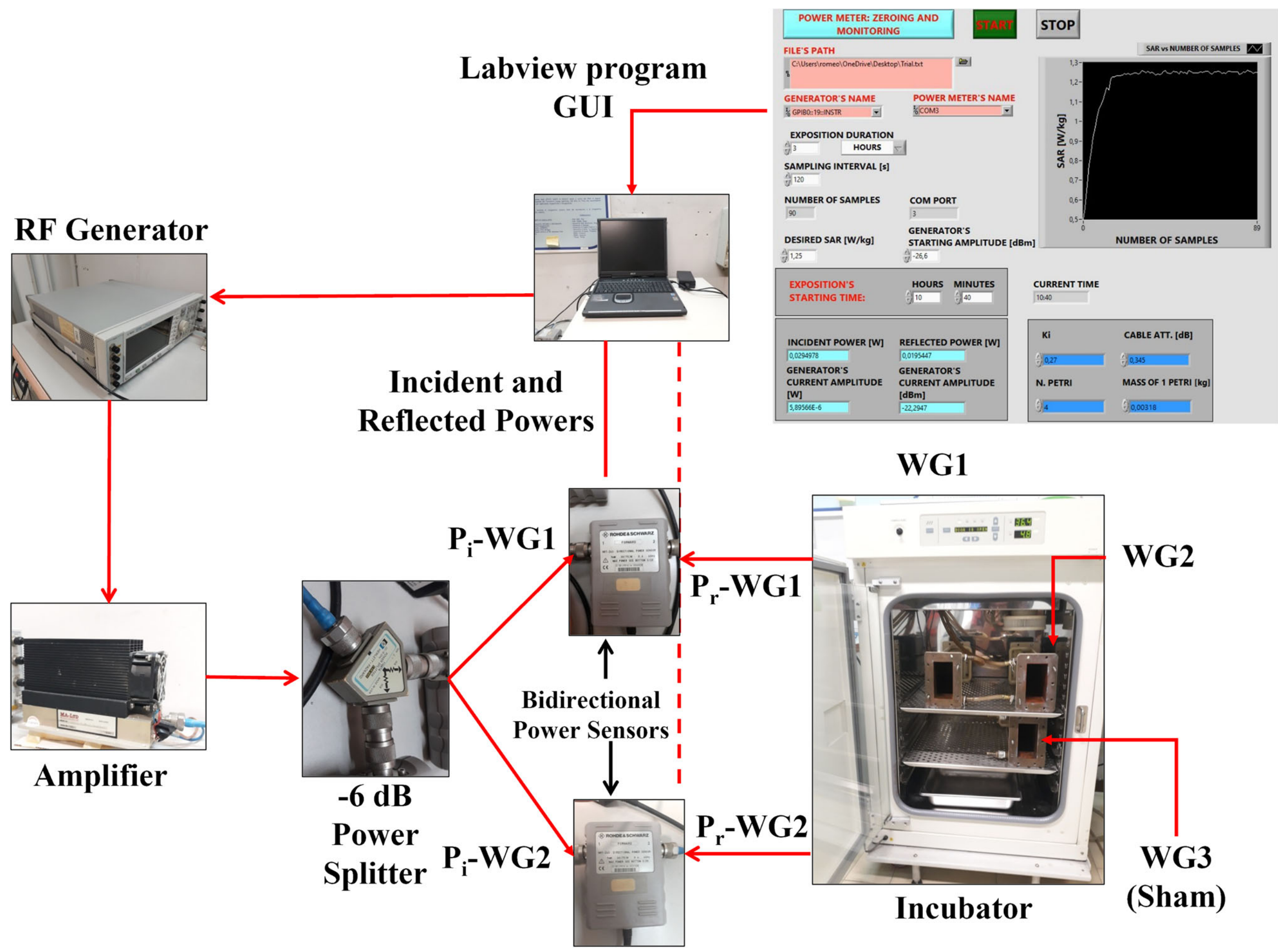

FIGURE 1 Configuration and components of the set up employed to expose cell cultures to 1950 MHz, LTE or CW, electromagnetic field (GUI, graphic user interface; P_i, incident power; P_r, reflected power; WG, waveguide).

and/or 1.25 W/kg SAR levels. The LTE signal was generated through a code built in-house in Matlab 2019 (MathWorks) environment, and provided to the RF signal generator (Agilent, E4432B ESG-D series) via GPIB interface. CW signal was generated by setting the frequency of 1950 MHz with no modulation applied.

The signal was sent to a microwave amplifier (MA-LTD, AM38A-0925-40-43), then to a −6 dB power splitter (HP, 11667 A), and conveyed, through a couple of bidirectional power sensors (Rohde & Schwarz, NRT-Z43), to two identical RF applicators consisting of rectangular, short-circuited waveguides (WG, WR430, 350 mm long), connected to the feeding side by means of a coaxial-to-waveguide adapter (Maury Microwave R213A2, VSWR: 1.05). A program was set up in Labview (National Instruments) with a graphic user interface, in such a way to set the exposure parameters (frequency, desired SAR, exposure duration, and initial time of exposure), and to remotely control and drive the signal generator and the power sensors. The incident and reflected power levels were continuously monitored throughout the exposure time, and adjusted to keep the required SAR constant.

The spectral power density of LTE and CW signals were monitored and acquired through a FPH Spectrum Rider (Rohde&Schwarz, 5 kHz to 31 GHz frequency range) spectrum analyzer (Figure 2).

The two waveguides were placed inside a cell culture incubator (Forma Scientific, model 311) to guarantee a 37°C, 95% air, and 5% CO_2 atmosphere, together with a third, identical one, disconnected from the RF feeding and used for sham-exposures.

As in our previous papers, the waveguide configuration and samples aspect have been optimized through numerical and experimental dosimetry to obtain high efficiency and uniformity of electric field (and SAR) distribution in the biological samples at 1950 MHz (overall efficiency >70%; coefficient of variation <30%) (Romeo et al., 2013; Sannino et al., 2006).

To rule out possible thermal effects, in separate experiments, the temperature of the sample exposed to the highest SAR was measured by a fiber-optic thermometer (FISO Technologies, UMI4, equipped with fiber-optic temperature probe, FOT-M/2 m), and temperature variations in the sample never exceeded the accuracy range of the instrument (±0.3°C).

2.3 | Cells, culture conditions, and experimental protocol

V79 cells (Sigma) were cultured as monolayer in DMEM medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin,

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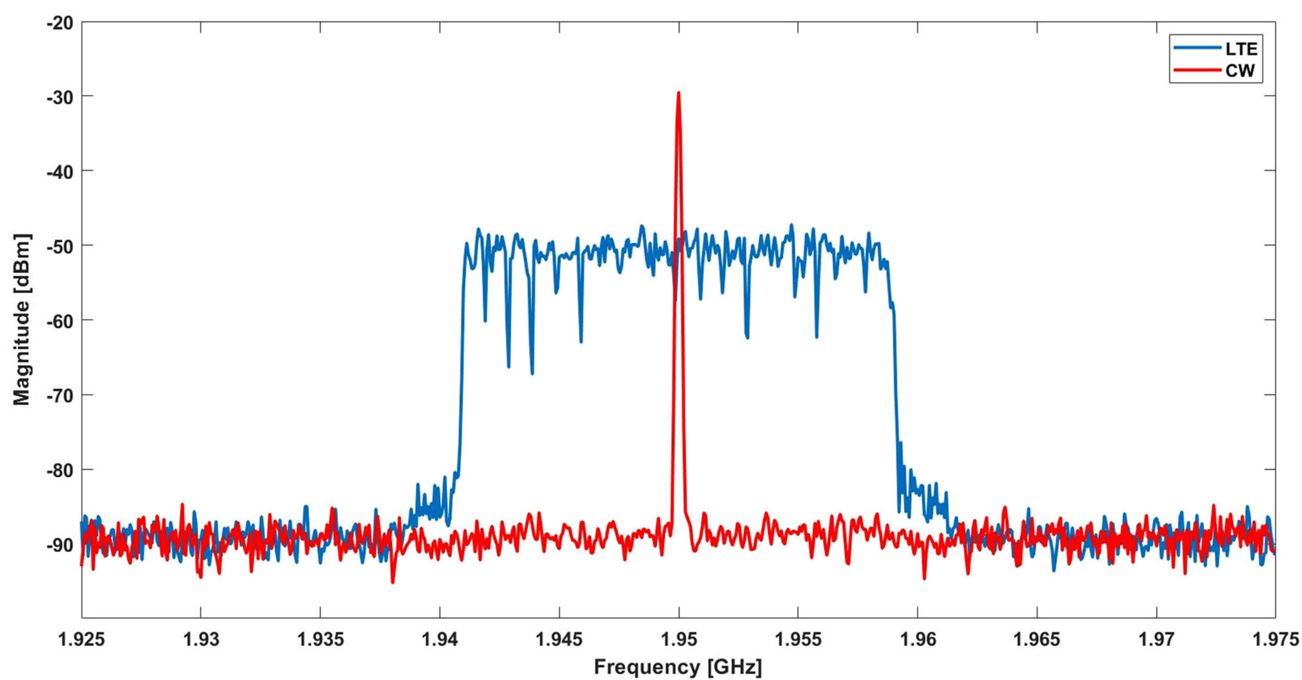

FIGURE 2 Spectral power density of LTE and CW signals at 1950 MHz. CW, continuous wave; LTE, long-term evolution.

and 100 mg/mL streptomycin in an atmosphere of 95% air and 5% CO_2 in a commercial incubator (model 311; Forma Scientific), and routinely checked for mycoplasma infection. Cells were supplied with fresh culture medium every 48 h and kept exponentially growing by splitting them twice a week by 200 mg/mL trypsin treatment.

For experiments, 1.5×10^5 cells were seeded in 3 mL complete medium, in coded Petri dishes (Corning, catalog no. 430165), and grown for a total of 48 h.

For consistency and reproducibility, the same batch of reagents was used, and experiments were carried out on cells from passages 3–15.

Five sets of experiments were carried out to evaluate the effect of RF exposure alone and in combination with MMC on: (1) chromosomal damage after 20 h LTE exposure; (2) chromosomal damage after 3 h LTE exposure; (3) chromosomal damage after 20 h CW exposure; (4) ROS formation after 20 h LTE exposure; (5) cell cycle progression after 20 h LTE exposure.

For each set of experiments, each experimental run was carried out on cells harvested from the same parent flask, and included eight randomly assigned cultures as follows: (1) untreated control (incubator); (2) sham control (Sham); (3) RF-exposed at 0.3 W/kg; (4) RF-exposed at 1.25 W/kg; (5) MMC-treated (MMC); (6) sham-exposed and MMC-treated (Sham + MMC); (7) RF-exposed at 0.3 W/kg and MMC-treated (0.3 W/kg + MMC); (8) RF-exposed at 1.25 W/kg and MMC-treated (1.25 W/kg + MMC).

RF was given from 8 to 28 h or from 25 to 28 h after cell seeding to test 20 and 3 h exposure, respectively. MMC (500 ng/mL), was given 32 h after seeding, where requested.

Although the experiments involving treatments with MMC were designed to investigate the effect of combined exposures, samples treated with MMC alone can be regarded as positive control, providing evidence of controlled experimental conditions and assurance that the assay methodology is responding adequately to a well known damaging agent.

All the experiments were carried out blinded, that is, samples were coded in such a way that the operator involved in the analysis was not aware of the treatment.

2.4 | Evaluation of chromosomal damage

The cytokinesis block micronucleus (MN) assay was applied following the protocol previously described in detail (Sannino et al., 2017). Briefly, 28 h after cell seeding, cytochalasin-B (3 μg/mL final concentration) was added to cell cultures to block cytokinesis. At the end of culture period (48 h), cells were trypsinized and washed with DMEM plus 2% FBS. Cells were collected on microscope slides by using a cytocentrifuge (Cytospin) at 1200 rpm for 5 min. Slides were fixed by 10 min treatment with 80% methanol and stained with 10% Giemsa solution in phosphate buffer (pH 6.8). Slide scoring was carried out by using a light microscope (Leitz, Dialux 22) at ×1250 magnification. A total of 2000 binucleated cells (BC) was scored to record the number of BC with MN (BCMN) which provides information on genotoxicity (Kirsch-Volders et al., 2003). Identification of BCMN followed the morphological criteria described in detail in Fenech et al. (2003). On the same slides, the proliferation index (PI) was derived as $[M_1 + 2M_2 + 3(M_3 + M_4)]/N$, where M_1 to M_4 represent the number of cells with one to four nuclei, respectively, and N is the total number of scored cells. PI provides information on cytotoxicity (Fenech, 2000; Fenech et al., 2003).

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2.5 | Measurement of intracellular ROS levels

The fluorescent probe DCFH-DA was used. It easily passes the cell membrane and is hydrolysed by intracellular enzymes to the nonfluorescent polar derivative, DCFH which, in presence of ROS, is oxidised to fluorescent dichlorofluorescein (DCF) (LeBel et al., 1990). Briefly, cell monolayers were treated for 30 min at 37°C with a solution of DCFH-DA (10 µM final concentration in absolute DMEM medium). After trypsinization, cell suspensions were washed in cold PBS, DCF fluorescence was measured by acquiring 15,000 events by flow cytometry (FACSCalibur™; Becton Dickinson), and the raw data were quantitatively analyzed using FlowJo analysis program (TreeStar). The results were expressed as the percentage of DCF-positive cells, that is, cells expressing DCF fluorescence above a threshold value, which was set on the base of the background DCF fluorescence in the control sample.

2.6 | Analysis of cell cycle

Propidium iodidie (PI) staining of permeabilized cells was used according to the protocol of Cotugno et al. (2012). After trypsinization, 5×10^5 cells were collected by centrifugation (5 min at 1200 rpm), washed in cold PBS and treated for 30 min at 4°C with a permeabilizing staining solution (50 µg/mL PI; 33 mM sodium citrate, pH 8, and 0.1% Triton X-100) diluted 1:2 in DMEM medium. PI fluorescence was analysed by acquiring 25,000 events by flow cytometry the percentage of cells in G0/G1, S, and G2/M stages of the cell cycle was calculated using FlowJo analysis program.

2.7 | Statistical analysis

For each parameter investigated, results were expressed as the mean ± standard deviation (SD) of three to four independent experiments. The statistical analysis was performed on raw data by one-way analysis of variance by using Matlab software, with post hoc comparisons carried out by Tukey test. $p < 0.05$ were considered statistically significant.

3 | RESULTS

3.1 | RF exposure alone does not induce chromosomal damage while reducing the MMC-induced damage under selected experimental conditions

We examined MN formation and PI in V79 cells exposed to 1950 MHz LTE signal either alone or in combination with MMC. The results are reported in Table 1 as mean ± SD of three and four independent experiments for 3 and 20 h exposure, respectively.

RF exposure for 3 h at both 0.3 and 1.25 W/kg SAR did not alter the spontaneous MN formation and PI of sham-exposed controls. Statistically significant increase of MN and decrease of PI were detected in the MMC-treated samples with respect to the corresponding control samples, as expected (MMC vs. control; Sh + MMC vs. Sh, $p < 0.001$). Pre-exposure to RF at both SAR levels did not change the MMC-induced damage.

When the same experimental conditions were tested after 20 h exposure to LTE signal, again the RF exposure at both SAR levels did not exert any effect on MN formation and PI, whereas only the effects of MMC treatment were recorded. Combined treatments showed that pre-exposure to 0.3 W/kg did not vary the MMC-induced damage. On the contrary, a statistically significant reduction (46% decrease) of MMC-induced MN was detected in cultures pre-exposed to 1.25 W/kg (1.25 W/kg + MMC vs. Sham + MMC; $p < 0.001$).

These findings prompted us to test the effects of RF exposure and coexposure under the same experimental conditions by applying the CW signal. In four independent experiments, CW exposure did not exert any

TABLE 1 Number of binucleate cells with micronuclei (BCMN) on 2000 BC cells and proliferation index (PI) on 500 cells in V79 cultures exposed for 3 h (three independent experiments) and 20 h (four independent experiments) to 1950 MHz, LTE, and/or mitomycin-C (MMC, 500 ng/mL).

	3 h RF exposure		20 h RF exposure	
Treatment	BCMN (mean ± SD)	PI (mean ± SD)	BCMN (mean ± SD)	PI (mean ± SD)
Control	10.7 ± 1.03	1.86 ± 0.02	10.5 ± 0.53	2.00 ± 0.02
Sham	10.7 ± 1.37	1.85 ± 0.03	11.0 ± 1.31	1.97 ± 0.05
0.3 W/kg	12.3 ± 1.37	1.92 ± 0.04	11.3 ± 0.89	1.99 ± 0.07
1.25 W/kg	12.0 ± 0.89	1.92 ± 0.04	10.8 ± 1.91	2.03 ± 0.05
MMC	42.3 ± 3.39[a]	1.48 ± 0.04[a]	44.8 ± 1.58[a]	1.58 ± 0.07[a]
Sham + MMC	43.0 ± 4.47[a]	1.47 ± 0.04[a]	43.5 ± 3.59[a]	1.53 ± 0.01[a]
0.3 W/kg + MMC	41.0 ± 2.68[a]	1.46 ± 0.05[a]	41.0 ± 3.12[a]	1.54 ± 0.02[a]
1.25 W/kg + MMC	42.3 ± 3.61[a]	1.48 ± 0.01[a]	23.5 ± 7.35[a,b]	1.59 ± 0.07[a]

Note: One-way ANOVA for repeated measurements followed by post hoc Tukey test.

Abbreviation: ANOVA, analysis of variance.

[a]Significantly different from the corresponding control (MMC vs. Control; Sham + MMC vs. Sham; RF + MMC vs. RF; $p < 0.001$).

[b]Significantly different from Sham + MMC ($p < 0.001$).

TABLE 2 Number of binucleate cells with micronuclei (BCMN) on 2000 BC cells and proliferation index (PI) on 500 nuclei in V79 cultures exposed for 20 h (four independent experiments) to 1950 MHz, continuous wave, and/or mitomycin-C (MMC, 500 ng/mL).

Treatment	BCMN (mean ± SD)	PI (mean ± SD)
Control	14.5 ± 1.20	1.86 ± 0.06
Sham	15.0 ± 2.00	1.90 ± 0.03
0.3 W/kg	14.3 ± 1.39	1.87 ± 0.04
1.25 W/kg	13.5 ± 1.60	1.88 ± 0.04
MMC	39.0 ± 2.93[a]	1.41 ± 0.05[a]
Sham + MMC	37.5 ± 0.53[a]	1.44 ± 0.05[a]
0.3 W/kg + MMC	37.3 ± 2.55[a]	1.44 ± 0.04[a]
1.25 W/kg + MMC	38.3 ± 1.91[a]	1.44 ± 0.06[a]

Note: One-way ANOVA for repeated measurements followed by post hoc Tukey test.

Abbreviation: ANOVA, analysis of variance.

[a]Significantly different from the corresponding control (MMC vs. control; Sham + MMC vs. Sham; RF + MMC vs. RF; $p < 0.001$).

variation in MN frequency in all cases, while an increase in MMC-treated cultures was recorded compared to untreated samples, as expected. The results are shown in Table 2.

3.2 | RF exposure does not affect ROS formation while reducing the MMC-induced effect

Results of ROS formation after 20 h exposure and co-exposure to MMC are shown in Figure 3. The percentage of DCF-positive cells for all the treatment groups is presented as mean ± SD of four independent experiments in Panel A. The relative DCF fluorescence of MMC-untreated and -treated samples is reported in Panel B for a representative experiment. LTE exposure alone did not induce ROS formation at both SAR levels, whereas a statistically significant increase ($p < 0.001$) was detected in MMC-treated samples compared to the corresponding controls, as expected.

FIGURE 3 Reactive oxygen species formation in V79 cells exposed for 20 h to 1950 MHz, LTE, and/or mitomycin-C (MMC; 500 ng/mL). (a) Percentage of DCF-positive cells for each condition tested (mean ± SD of four independent). (b) Relative DCF fluorescence of MMC–untreated (left side) and MMC–treated (right side) samples for representative. *$p < 0.001$; one-way ANOVA for repeated measurements followed by post hoc Tukey test. ANOVA, analysis of variance; DCF, dichlorofluorescein; LTE, long-term evolution.

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TABLE 3 Cell cycle analysis in V79 cells exposed for 20 h (three independent experiments) to 1950 MHz, LTE, and/or mitomycin-C (MMC, 500 ng/mL) expressed as relative percentage of cells in different stages of the cell cycle.

Treatment	G0-G1 (mean ± SD)	S (mean ± SD)	G2-M (mean ± SD)
Control	51.73 ± 4.88	33.30 ± 3.82	17.47 ± 2.32
Sham	49.40 ± 5.20	30.27 ± 7.95	18.63 ± 22.93
0.3 W/kg	44.93 ± 4.02	29.43 ± 6.85	18.87 ± 2.08
1.25 W/kg	51.23 ± 5.31	29.17 ± 7.37	18.40 ± 1.40
MMC	15.43 ± 0.90[a]	37.73 ± 4.19[a]	44.93 ± 2.00[a]
Sham + MMC	13.93 ± 1.69[a]	38.00 ± 6.04[a]	45.27 ± 3.48[a]
0.3 W/kg + MMC	14.97 ± 1.30[a]	38.27 ± 5.60[a]	43.87 ± 2.08[a]
1.25 W/kg + MMC	14.90 ± 0.79[a]	39.00 ± 3.29[a]	43.23 ± 1.07[a]

Note: one-way ANOVA for repeated measurements followed by post hoc Tukey test.

Abbreviation: ANOVA, analysis of variance.

[a]Significantly different from the corresponding control (MMC vs. Control; Sham + MMC vs. Sham; RF + MMC vs. RF; $p < 0.001$).

In the case of combined treatments, pre-exposure at both SAR levels gave a statistically significant reduction in the MMC-induced ROS compared to sham exposed and MMC-treated samples ($p < 0.001$). The reduction was of 37% at both SAR levels investigated.

3.3 | RF exposure does not affect cell cycle progression and does not modify the MMC-induced effect

The effect on cell cycle progression was investigated after 20 h LTE exposure either alone or in combination with MMC. The results are presented in Table 3, where the relative percentage of cells in different stages of cell cycle is provided as mean ± SD of three independent experiments. RF exposure at both SAR levels did not affect cell cycle progression. MMC induced an arrest at G2-M phase, which did not vary when RF was given before MMC, indicating absence of combined effect.

4 | DISCUSSION

The fourth generation of cellular technology, 4G LTE, employs new digital signal processing and modulation to increase the speed of telecommunication networks. It was introduced more than a decade ago and used worldwide but, with respect to the previous wireless communication technologies, a limited number of investigations has been devoted to assess its potential biological effects, and thus the possible association of human diseases with exposure.

In this study, we focused on V79 cells as they are a widely recognized sensitive model towards a broad spectrum of chemical and physical agents, including nonionizing radiation (Ballardin et al., 2011; Jagetia & Adiga, 2000; Xu et al., 2013). They are generally accepted in the literature as a suitable model for cytotoxicity studies (Bianchi, 1995).

Moreover, we have previously reported on their sensitivity to evidence either adverse or beneficial effects under RF exposure to 1950 MHz, UMTS signal, depending on the experimental conditions applied (Sannino et al., 2017).

Here we investigated the chromosomal damage, ROS formation, cell proliferation, and cell cycle progression following exposure to 1950 MHz, LTE signal, and coexposure to MMC, a quinone anticancer drug that exerts its activity via ROS induction and resulting in DNA cross-linkage (Koedrith & Seo, 2011).

These cellular parameters have a key role in the carcinogenesis process. Moreover, activation of the ROS system, and the consequent oxidative stress is deemed to be the main critical condition that could provide evidence of a mechanism by which RF exposure might affect human health (Schuermann & Mevissen, 2021).

We first observed that 3 and 20 h exposure to 1950 MHz, LTE signal, did not induce MN formation in V79 cells at the SAR the levels investigated. The lack of genotoxic effects was also detected after 20 h exposure to CW.

This result confirms most of the findings reported in the literature, including ours, on the absence of DNA damage of RF exposure in several cell models under frequencies and signals of 2G and 3G technologies at exposure levels below the limits set by ICNIRP (2020). For a review on this topic see (Manna & Ghosh, 2016; Vijayalaxmi & Prihoda, 2012, 2019). In addition, the lack of DNA damage was also reported following 4G LTE exposure on different cell types, although in some cases a decrease in cell proliferation and increase in ROS formation were detected (Choi et al., 2020; Jin et al., 2021; Kim, Jeon, et al., 2021).

At the same time, our findings highlight the different sensitivity of V79 cells to 4G and 3G signals when exposed to the same SAR levels.

Indeed, in a previous investigation 20 h exposure to 3G 1950 MHz, UMTS signal did not induce MN formation at SAR level of 1.25 W/kg, accordingly to the findings here reported, while exposure at 0.3 W/kg induced a slight but statistically significant increase in MN formation (Sannino et al., 2017).

We also demonstrated here that 20 h LTE exposure was not able either to induce ROS formation and to alter cell cycle progression at both SAR levels investigated.

At variance, when RF was followed by MMC treatment, different biological responses were detected depending on the experimental conditions applied.

In particular, the modulated LTE signal induced a 46% reduction of MMC-induced MN formation in samples pre-exposed for 20 h at 1.25 W/kg SAR, while 0.3 W/kg did not. Pre-exposure for 3 h did not exert any effect at both SAR levels.

These results are in agreement with our previous findings. As a matter of fact, in earlier investigations, our research group collected evidence that exposure to RF with modulation schemes employed for second- and

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third-generation wireless technologies, is able to protect mammalian cell cultures of different origin from the damage induced by a subsequent treatment with chemical or physical agents. Such phenomenon, resembling the ionizing radiation-induced AR (Vijayalaxmi et al., 2014), has been observed under different electromagnetic conditions and by evaluating different biological endpoints (Falone et al., 2018; Romeo et al., 2020; Sannino et al., 2009, 2019, 2014, 2017, 2011; Zeni et al., 2021, 2012). Experimental results published by independent research groups confirmed our findings both in vitro (He et al., 2016, 2017; Ji et al., 2016) and in vivo (Cao & Tong, 2014; Gapeyev & Lukyanova, 2015; Mortazavi et al., 2017; Zong et al., 2015) and extended the variety of experimental models and electromagnetic conditions able to elicit similar response in different assays.

4.1 | Protective effect was also reported following exposure to 4 G LTE

Jin and coworkers investigated the effects of 24 h exposure to 1762 MHz, 8 W/kg SAR, in murine melanoma (B16), human melanoma (MNT-1), and human keratinocytes (HaCaT) cells in combination with ionizing radiation or bleomycin. A reduction of DNA damage induced by ionizing radiation in HaCat and B16 cells and by bleomycin in HaCat and MNT-1 cells was recorded, after 3 and 24 h LTE exposure, although differently from the protocol we applied in this study, RF was given after the damaging treatment (Jin et al., 2021).

Here we also reported that pre-exposure to CW signal did not alter the DNA damage induced by MMC in all the experimental conditions tested, confirming our earlier results on human peripheral blood lymphocytes pre-exposed at 1950 MHz and treated with MMC, while a significant reduction of MN frequency was detected when either Wideband Direct-Sequence Code Division Multiple Access (WCDMA) or additive white Gaussian noise (AWGN) signals were tested (Romeo et al., 2020).

Therefore, the presence of a modulated signal seems to be a crucial factor in eliciting a protective effect, as also reported by Gapeyev and Lukyanova (2015).

Our findings also indicated a statistically significant reduction of MMC-induced ROS in coexposed samples at both SAR levels investigated, compared to sham-exposed and MMC-treated ones. Although RF exposure under the experimental conditions here adopted did not result in oxidative stress measured as ROS production, it can be hypothesized that RF may act via oxidative stress, as suggested by several authors and summarized by Schuermann and Mevissen (2021). Therefore, we can argue that RF can trigger a stress response that prepares the cells to better cope with the stress induced by MMC treatment. This is in accordance to our previous findings showing a variation of gene expression of some antioxidant enzymes also involved in DNA repair in human neuroblastoma cells treated with menadione (Falone et al., 2018).

When the cell cycle of V79 cells was analysed under the same experimental conditions, we failed to find effect of RF exposure alone, or combined with MMC. In accordance with data reported in the literature, the only effect detected was a cell cycle arrest in samples treated with MMC (Nakayama et al., 2020).

On the whole, the results here presented confirm the ones of our previous investigations on the lack of genotoxic and cytotoxic effects induced by RF exposure at different frequencies and modulation schemes. In addition, they extend to the LTE signal the existence of a nonthermal, complex interaction between modulated RF fields and biological systems whose effects become detectable when the latter are appropriately sensitized, as in combined exposure protocols.

Further investigations are mandatory to clarify the mechanisms underneath the observed effects by exploring the phenomenon at molecular level, with pathways involved in DNA repair and oxidative stress as primary focus.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ETHICS STATEMENT

The authors have nothing to report.

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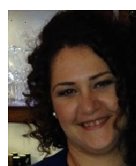

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