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Original article

Low-frequency electromagnetic field influences human oral mucosa keratinocyte viability in response to lipopolysaccharide or minocycline treatment in cell culture conditions

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ABSTRACT

The aim of the current study was to investigate the influence of low-frequency electromagnetic field (LF-EMF) exposure on viability parameters of oral mucosa keratinocytes cultured in in vitro conditions. The effect of LF-EMF stimulation on cell viability was also specified in the simultaneous presence of lipopolysaccharide (LPS) infectious agent or minocycline (Mino) anti-inflammatory agent. Viability parameters such as early-, late apoptosis and necrosis of keratinocytes were analysed by the flow cytometry method (FCM).

The exposure of human oral keratinocyte cell cultures to LF-EMF acting alone or combined with LPS/minocycline agents caused changes in the percentage of cells that undergo programmed or incidental cell death. The overall obtained results are compiled in a graphical form presented in Fig. 1.

1. Introduction

Recently, growing interest can be seen in studying the possible effects of non-ionizing electromagnetic radiation on different types of cells. Human skin keratinocytes or oral mucosa keratinocytes seem to be appropriate model systems to analyse the biological effects of external stress factors like electromagnetic fields (EMFs) in a wide range of frequencies. The intracellular changes evoked by electromagnetic fields are crucial to understanding the bio-mechanism underlying their interaction with living cells and to determine the possible therapeutic potential of EMF.

Oral keratinocytes and fibroblasts are the first line of defense against oral microorganisms and pathogens. Keratinocytes are the dominating cell type in the oral mucosa epithelium. They recognize microorganisms via Toll-like receptors (TLRs) and participate in the oral inflammatory response [4]. The protective role of oral mucosa can be weakened in some clinical states e.g. tumor resection, periodontitis, mucosal barrier injury (mucositis). For instance, periodontitis, an uncontrolled bacteria-induced inflammatory destruction of tooth-supporting tissues and alveolar bone, is a leading cause of tooth loss in adults. It has been shown that development of periodontal diseases to a great extent can be triggered by the release of lipopolysaccharide (LPS), a bacterial endotoxin [17,22,37,52,63]. In terms of etiopathology, periodontitis also increases the risk of cardiovascular disease and diabetes mellitus [30,31,34,35,43,58].

In turn, oral mucositis, a painful ulceration of the oral mucosa, is the most common complication of cancer treatment. It develops when cancer treatments break down the rapidly divided epithelial cells, leaving the mucosal tissue open to ulceration and infection. According [59], the majority of cancer patients receiving radio- or chemotherapy or undergoing stem cell transplant suffer from mucositis. It has also been shown in numerous studies that severe oral mucositis hinders definitive

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cancer therapy [5,50,51].

The oral mucosa is also sensitive to other stress factors such as mechanical stimuli, high temperature, or chemical irritants. Maintaining the integrity of the oral mucosa is critically important not only for oral cavity function, but also for general health status [1,8,41,57].

Currently, oral mucosa loss is treated with the patient's own (autologous) skin or mucosa grafts. Generally, the proliferation capacities of oral mucosal cells and a specific preselection of cells with stem cell properties can help support the rapid development of autologous tissue transplants [20]. Tissue engineering offers important tools for the regeneration of oral mucosa, potentially minimizing the most common problems occurring in clinical treatment [6,9].

Recently, host modulatory therapy (HMT) has been proposed as a promising complement to the conventional periodontal treatment [46,53]. Some examples of HMT in the treatment of periodontitis include sub-antimicrobial doses of doxycycline, lipoxins and resolvin E1 [32,56,60]. Some other chemical factors have been examined in the treatment of periodontal disease. Minocycline (Mino) is a broad-spectrum semi-synthetic tetracycline derivative which exhibits anti-inflammatory properties independent of its antibiotic activity [7,11,33,38,45,65]. The anti-inflammatory effects of minocycline in human monocytes have revealed that it inhibits convergence points of interacting signaling pathways, thus mediating multiple inflammatory signals [42]. It is also one of the most active antibiotics against most of the microorganisms associated with periodontal disease [2,36].

Taking into consideration the cell physiology of mucous cell, an especially important phenomenon seems to be the cell death of abnormal epithelium of the oral cavity, which is responsible for pathogenesis of many infectious dental diseases, autoimmune processes and cancer development.

According to many studies, electromagnetic fields may modulate many cellular processes e.g. cell viability, proliferation activity of cells, production of cytokines, phosphorylation of proteins or calcium homeostasis, in this way influencing regenerative mechanisms [15,25,29,47,55,61]. Depending on the parameters of the applied EMF itself, the model cell type or simultaneous treatment with various chemical factors (synergistic effects), the evoked bio-effects can differ, regardless, the underlying biophysical mechanism(-s) is still unknown.

The aim of our study was to examine the influence of low-frequency electromagnetic fields on the viability parameters (early-, late apoptosis and necrosis) of oral mucosa keratinocytes cultured in vitro in cell culture conditions, when acting alone or simultaneously with the LPS infectious agent/minocycline (Mino), an anti-inflammatory agent.

2. Materials and methods

2.1. Materials

The following chemicals were purchased from the sources indicated as follow: keratinocyte cell basal medium (KBM-Gold) supplemented with KBM-Gold Single Quots (containing bovine pituitary extract - BPE, hEGF, insulin, hydrocortisone, transferrin, epinephrine, gentamycin); fetal bovine serum - FBS (LONZA, Clonetics, Walkersville, MD, USA); phosphate buffered saline w/o Ca^{2+} and Mg^{2+} (Dulbecco's modified PBS); Dulbecco's Modified Eagle's Medium (DMEM); dispase; trypsin/EDTA solution; penicillin/streptomycin/fungizon solution (PSF); gentamycin (Sigma-Aldrich, Germany); AnnexinV-allophycocyanin conjugate (APC); propidium iodide, and binding buffer from (BD Biosciences, USA). Cell culture flasks and 96-well cell culture plates were purchased from Corning (London, UK).

2.2. Patients' characteristics

Before the study began, it was approved by the Bioethics Committee of the Jagiellonian University No. KBET / 205 / B / 2011 dated 30 September 2011.

The studies included (18 ÷ 50) year-old patients who were generally healthy and consented to participation in the study. The patient inclusion criterion for the study was the need for surgical tooth extraction. The exclusion criteria were as follow: concurrent systemic disease, age below 18 years or over 50 years, pregnancy, diseases of the oral mucosa and inflammation of the oral mucosa in the area of the tooth removed. During the surgical tooth extraction treatments from 26 patients at the Central Ambulatory, University Dental Clinic, Jagiellonian University Medical College (Montelupich 4 street, 30–155 Cracow), for the purposes of this research, small portions of the oral mucosa (approx. 5 × 5 mm) were collected. This was formed after angioplasty of the surrounding soft tissues mucosa in the area of the removed tooth. In the standard procedure, these tissues are treated as medical waste, but in our study were used as the raw material for tissue culture. A small fragment of mucosa was placed immediately after surgery in cold PBS (Dulbecco's phosphate buffered saline) supplemented with penicillin/streptomycin/fungizon (PSF, 1%).

2.3. Cell culture

Human oral mucosa keratinocytes were isolated from the oral tissue of healthy patients after the tooth extraction process and plastic surgery of the surrounding tissues. A small piece of the oral mucosa was obtained for the oral keratinocyte isolation procedure. A fragment of the mucus tissue was placed into cold PBS w/o calcium and magnesium ions, supplemented with 1% PSF. After washing, the biopsy was placed in 1% PSF rich Dulbecco's modified PBS containing dispase (12 U/ml), for 16 h at 4 °C. After detachment, subcutaneous tissue was removed and the epithelium was treated with 0.05% trypsin-EDTA solution for 10 min to isolate epithelial cells. The enzyme activity was eliminated by dilution (1:5) with Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and gentamycin (5 μg/ml) to arrest the trypsin reaction, and centrifuged at 100 × *g* for 5 min. The supernatants were removed and the cells were suspended in a DMEM medium containing 10% FBS and gentamycin. After 24 h, the medium was changed to serum-free keratinocyte basal medium KBM-Gold supplemented with BPE, hEGF, insulin, hydrocortisone, transferrin, epinephrine, and gentamycin.

When the primary culture of keratinocytes had reached 70–80% of confluence (after 7–8 days), the cells were passaged to expand the culture. The keratinocytes were detached by incubation with 0.05% trypsin, and the cells were suspended in the supplemented keratinocyte serum-free medium KBM-Gold. Then, the cells were counted with a hemocytometer, seeded on a 96-well plate at a density of 0.5×10^6 cells/ml and cultured at 37 °C in a 5% CO_2 incubator with 90% humidity. The cells were used for experiments after 72 h of culture following the cell culture medium replacement.

2.4. Keratinocyte cell culture exposure to low-frequency electromagnetic fields (LF-EMF)

A low-frequency sinusoidal electromagnetic field generator (7 Hz and 30 mT; Institute of Electron Technology, Cracow, Poland) was placed inside the cell culture incubator. The 96-well plates with human oral keratinocytes cells were exposed to LF-EMF for four hours per day. The LF-EMF exposure was repeated three times, with 24-h intervals between stimulations. The control cells were kept in the same incubator, being shielded and placed at a sufficient distance from the electromagnetic field source. The rationale for choosing the specified parameters of the applied electromagnetic field was mainly based on its bio-activity in the range of low frequencies and the negligibility of the thermal effects.

2.5. Lipopolysaccharide (LPS) stimulation of keratinocyte cell cultures

Human oral keratinocytes after 72 h duration of cell cultures were stimulated with *E. coli* LPS (*Escherichia coli* serotype 0111: B4; Sigma-

Aldrich, Germany) at a final concentration of 1 μg/ml, and exposed to LF-EMF strictly according to the experimental schedule.

2.6. Minocycline (Mino) keratinocyte cell culture treatment

Keratinocytes after 72 h of cell culture were treated with minocycline at a final concentration of 100 μg/ml, three times for 4 h parallel to exposure to LF-EMF. The minocycline stimulated keratinocytes were washed three times with PBS, centrifuged and resuspended in the original volume of 200 μl culture medium.

2.7. Cell viability assessment by means of flow cytometric analysis

Twenty-four hours after the last exposure to LF-EMF, the human oral keratinocytes were detached from the culture plates by trypsinization, washed three times with cold PBS (Sigma-Aldrich, Germany) and then stained strictly according to the FCM manufacturer's procedure. The proportion of apoptotic cells was determined with allophycocyanin (APC) conjugated annexin V (AnV-APC). Propidium iodide (PI) was used as a standard flow cytometric viability probe to distinguish necrotic cells from viable ones. AnV-APC-positive, PI-negative cells (AnV+) were classified as early apoptotic, AnV-APC- and PI-positive cells (AnV+ PI+) as late apoptotic, and AnV-APC-negative, PI-positive cells (PI+) as necrotic. For staining, keratinocytes were washed twice with cold PBS and resuspended in 1x binding buffer at a concentration of 1×10^6 cells/ml. Then, the solution (100 μl) was transferred to a 5 ml culture tube, and AnV-APC and PI were added (5 μl each). The cells were gently vortexed and incubated in darkness, at room temperature for 15 min. After adding 1 × binding buffer (400 μl), the cells were analyzed on a FacsCalibur flow cytometer (Becton Dickinson, San Jose, CA) equipped with Cell-Quest software. Unstained cells and cells stained with AnV-APC or PI alone were used as controls to adjust the proper fluorescence compensation signal. To obtain reliable statistics, a minimum of 10^4 events were acquired for each sample.

2.8. Statistical analysis

Database management and statistical analysis were performed with TIBCO Statistica for Windows, version 13.3 PL (TIBCO Software Inc., Palo Alto, CA, USA; license owned by the Jagiellonian University in Cracow, Poland). Normal distribution of the experimental data was verified with the Shapiro-Wilk test, not all data presented normality. A one-way ANOVA on ranks (Kruskal-Wallis test) was done to evaluate the differences between all the investigated group in variables. The significance of intergroup differences was verified with the Mann-Whitney *U*-test, which was chosen to assess the differences among analyzed parameters in both groups. The results were expressed as a mean ± SD and/or median [min-max] for 26 independent experiments, each carried out in triplicates. The threshold of statistical significance for all the tests was set at $p < 0.05$.

3. Results

Human oral keratinocytes cultured in in vitro conditions and exposed to LF-EMF alone or simultaneously with minocycline/lipopolysaccharide revealed changes in their viability parameters. The overall results are summarized in Table 1 and presented separately for different populations of cells, early- and late apoptotic or necrotic ones, in Figs. 1 and 2A,B,C.

In the case of the applied electromagnetic field acting alone, we observed a decrease in the percentage of cells in the early phase of apoptosis, in comparison to the control group (Table 1, $p = 0.017$, Fig. 2A). For cells undergoing late apoptosis or necrosis, LF-EMF stimulation did not exert any significant changes (Table 1, $p > 0.05$, Fig. 2B, C).

The application of Mino or LPS alone influenced keratinocytes viability in various ways, in accordance with their anti- or pro-inflammatory role, respectively. For instance, in Mino-treated samples, we observed an elevated level of early apoptotic- and necrotic cells compared to the control group. Results for both populations of keratinocytes are statistically significant (Table 1, $p \leq 0.008$, Fig. 2A,C).

In turn, treatment of human oral keratinocyte cultures with LPS was responsible for a decrease in the percentage of early apoptotic cells and a significant elevation of the number of cells that undergo necrosis in comparison to the control group (Table 1, $p = 0.004$ and $p < 0.001$, respectively). Data are presented in Fig. 2A and C. However, the late apoptotic cell group was unchanged compared to the evaluation of this population of cells in the control group (Fig. 2B).

To summarize the influence of these physical- and chemical factors on keratinocytes, when acting separately, it can be stated for low-

Table 1
Keratinocytes viability parameters for low-frequency EMF and/or Mino/LPS stimulation: AnV+ - early apoptosis; AnV+PI+ - late apoptosis; PI+ - necrosis; C - control cells; EMF - low-frequency electromagnetic field (30 mT; 7 Hz); CMino - cells treated with minocycline; CLPS - cells treated with lipopolysaccharide; Mino-EMF - cells stimulated with electromagnetic field and minocycline; LPS-EMF - cells stimulated with electromagnetic field and lipopolysaccharide; p^1- statistical significance between C and EMF, p^2- statistical significance between C and CMino; p^3- statistical significance between C and CLPS; p^4- statistical significance between CLPS and LPS-EMF; p^5- statistical significance between CMino and Mino-EMF, p^6- statistical significance between EMF and LPS-EMF (not shown in the graphs); p^7- statistical significance between EMF and Mino-EMF (not shown in the graphs). Data are presented as Mean ± SD; Me[min-max]; (Kruskal-Wallis test and Mann-Whitney *U*-test, threshold of statistical significance set at $p < 0.05$).

Cell population	C	EMF	CMino	Mino-EMF	CLPS	LPS-EMF	p^1	p^2	p^3	p^4	p^5	p^6	p^7
AnV+													
Mean±SD Me [min-max]	9.74±5.20 7.94 [3.60-19.60]	7.68±4.18 6.77 [2.10-17.54]	16.05±8.92 13.01 [3.95-37.90]	21.65±9.43 18.40 [10.10-42.40]	5.86±3.30 5.60 [0.80-12.87]	7.81±3.00 8.16 [2.40-12.80]	***0.017***	***0.005***	***0.004***	*0.106*	***0.049***	*0.588*	***<0.001***
AnV+PI+													
Mean±SD Me [min-max]	12.39±8.99 10.74 [1.02-39.50]	9.12±6.88 6.60 [0.70-24.20]	8.17±3.62 7.85 [3.50 -16.20]	10.81±2.28 10.40 [7.60-14.10]	10.82±8.91 7.70 [2.07-33.90]	6.73±2.65 7.40 [1.00-10.90]	*0.068*	*0.248*	*0.423*	*0.458*	***0.038***	*0.883*	***0.033***
PI+													
Mean±SD Me [min-max]	14.77±5.64 13.04 [7.70-30.90]	12.46±4.49 12.20 [3.20-20.70]	25.66±16.71 19.90 [10.00-64.19]	16.69±9.94 17.90 [3.40-44.50]	31.12±10.53 26.65 [21.30-55.80]	24.17±7.33 24.40 [10.30-39.70]	*0.089*	***0.008***	***<0.001***	*0.076*	*0.108*	***<0.001***	*0.104*

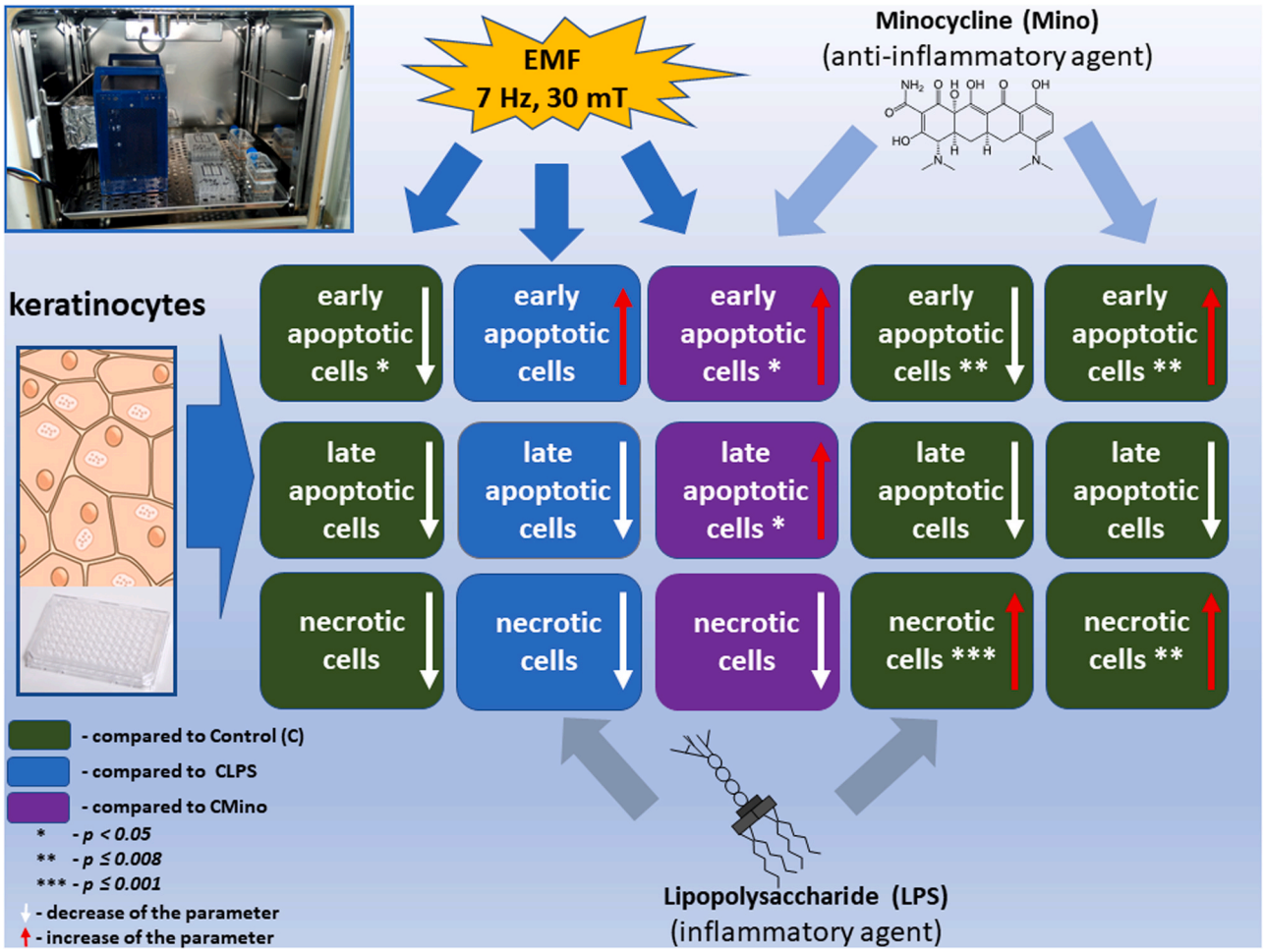

Fig. 1. A graphical presentation of the observed bio-effects in keratinocytes for LF-EMF and/or LPS/Mino stimulation.

frequency EMF stimulation or LPS treatment that the same trend of changes is evident in two subpopulations undergoing programmed cell death (decrease of this viability parameter). Conversely, the effect exerted by minocycline on the percentage of early apoptotic cells was the opposite.

The combination of LF-EMF and pro- or anti-inflammatory agents provoked further changes in the level of cells undergoing programmed cell death. For instance, when LF-EMF acted simultaneously with minocycline (Mino-EMF), an elevated number of cells in early- and late apoptotic phase was observed, in comparison to cells only treated with minocycline (CMino). Also, in contrast to the action of minocycline alone, the necrotic cell level was slightly diminished, however this effect was not statistically significant (Table 1, $p = 0.108$). This result indicates that the applied LF-EMF enhanced apoptosis in keratinocytes, thus amplifying the minocycline-induced effect (Fig. 2A and B). In the case of simultaneous stimulation of keratinocytes with LF-EMF and lipopolysaccharide (LPS-EMF), no significant changes in cell viability in any subpopulation of cells were noticed, in comparison to LPS treatment alone (CLPS), (Fig. 2A,B,C). Thus, the applied LF-EMF was found not to play a role in the activity of the LPS as an infectious agent.

The obtained results for simultaneous treatment of keratinocytes with minocycline/LPS and applied LF-EMF are an example of a synergistic effect of interaction between chemical- and physical stimulus and are not a direct additive outcome evoked by two factors acting independently.

4. Discussion

Our hypothesis assumed an anti-inflammatory effect of electromagnetic field exposure exerted on keratinocytes isolated from the mucous membrane of the oral cavity and cell cultured in in vitro conditions. Some experimental data indicate the influence of EMFs on inflammatory engaged tissues isolated from dental patients and their therapeutic role in the healing process of pathogenic tissues [40,66].

The possibility to apply EMF exposure alone or in combination with chemical treatment seems to be a non-invasive prospective therapy in dentistry and many other branches of regenerative medicine [14,16,19,39,48,49]. For instance, in a study by Haddad et al. [16], the authors discussed the possible intracellular bio-mechanisms responsible for bone repair due to electric- and electromagnetic field stimulation. They consider these to involve the most probable signaling pathways related to changes in expression of morphogenetic bone proteins, transforming growth factor-beta, and the insulin-like growth factor II, which results in an increase of the extracellular matrix of cartilage and bone.

In our study, we focused on oral mucosa keratinocytes, which are thought to be the first line of defense against pathogens similarly to immune competent cells (e.g. peripheral blood mononuclear cells), fulfilling their function through expression of TLRs on their surfaces. The obtained results showed that the application of low-frequency EMF and/or chemical agents (minocycline or LPS) affected both cell death types: programmed - apoptosis and incidental - necrosis. The changes in cell viability are dependent on the type of stimulation (e.g. only physical (LF-EMF)-, only chemical (pro- or anti-inflammatory agents), or combined ones). For instance, in contrast to the majority of scientific studies

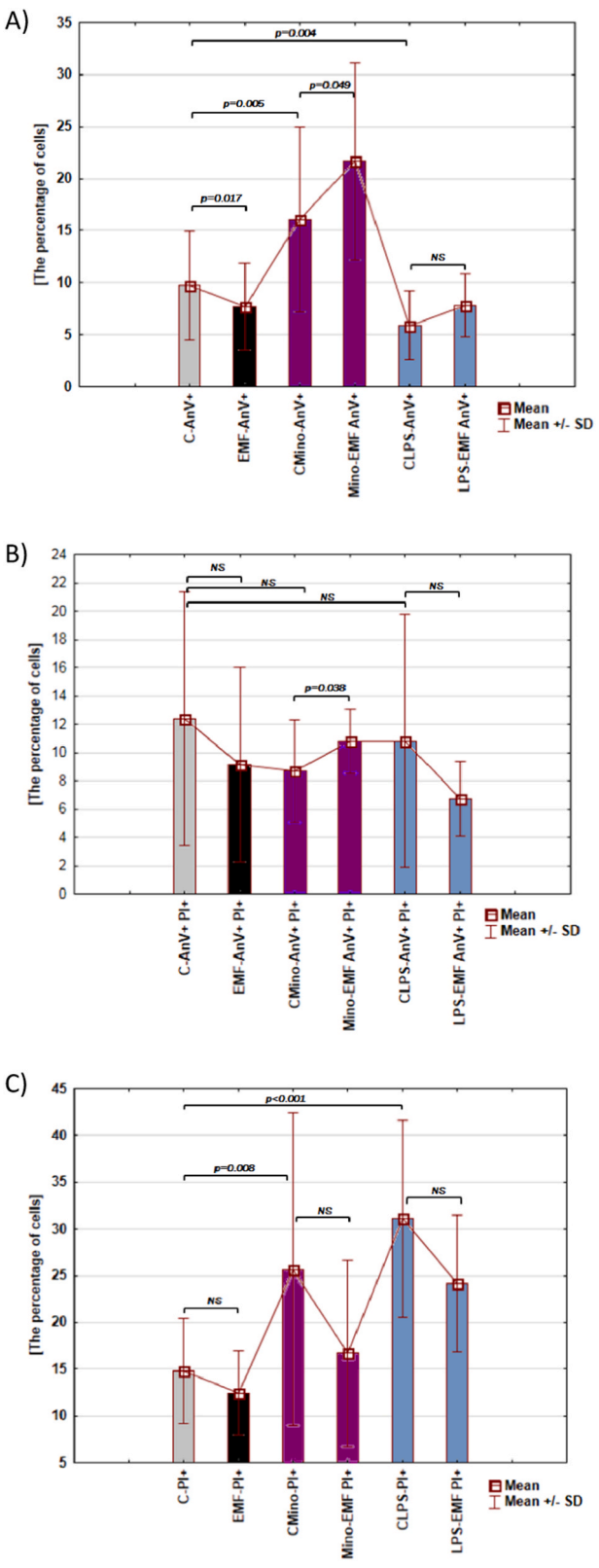

Fig. 2. Analysis of viability parameters of human oral keratinocytes exposed to low-frequency electromagnetic field and/or minocycline(Mino)/lipopolysaccharide(LPS): A) early apoptotic cells (AnV+); B) late apoptotic cells (AnV+PI+); C) necrotic cells (PI+); C - control/sham cells; EMF - low-frequency electromagnetic field (30 mT; 7 Hz); CMino - cells treated with Mino; CLPS - cells treated with LPS; Mino-EMF - cells exposed to EMF combined with Mino; LPS-EMF - cells exposed to EMF combined with LPS; NS - non statistically significant ($p > 0.05$).

performed on various cell types, the presented results show a (Mino)-triggered induction of programmed cell death in keratinocyte cell cultures which is in accordance with our previous data obtained for MonoMac6 cells [27]. From the pathophysiological point of view, the increased level of the population of early apoptotic keratinocyte cells might promote an anti-inflammatory response of these cells, since the mechanism of apoptosis is involved in quenching and resolution of the inflammation, preventing the development of a chronic process. The anti-inflammatory effects of minocycline on various cells can be manifested through the inhibition of the points of convergence of intracellular pathways mediating multiple inflammatory signals. There are some experimental results showing the effects of minocycline on expression of the interleukin 8 (IL-8), as a crucial chemokine for inflammatory process development. However, this effect takes place in a minocycline dose-dependent manner in epidermal keratinocytes [14,18]. In comparison to a study by Ishikawa et al. [18], the used dosage of minocycline in our experiments was much higher (approx. 20×) in cell culture conditions, thus the cell response might differ e.g. some percentage of cells can undergo necrosis. The simultaneous treatment of oral mucosa keratinocytes with Mino and LF-EMF increased the percentage of both early- and late apoptotic cells, meaning that the applied LF-EMF enhanced the minocycline-induced effect. Regarding the effects evoked by lipopolysaccharide (LPS) as a highly pro-inflammatory agent (also present in gram positive bacteria in the oral cavity such as *Porphyromonas gingivalis*), in our studies it triggered an increase in necrosis and decrease in the percentage of apoptotic cells in cell cultures. The obtained results are in agreement with studies carried out by Jaffer et al. [21]. However, when the keratinocytes were LPS-stimulated and exposed to LF-EMF simultaneously, no significant changes in cell viability were noticed. This means that the application of LF-EMF did not influence infectious activity of the LPS. The obtained results for simultaneous treatment of keratinocytes with Mino/LPS and applied LF-EMF are an example of a synergistic effect of interaction between chemical- and physical stimulus, thus this cannot be interpreted directly as an additive value, when two factors act independently e.g. Wójcik-Piotrowicz et al. [61,62].

However, a comprehensive interpretation seems to be even more complex because the effects of EMF exposure strictly depend on the parameters of the EMF itself (e.g. signal waveform, frequency, magnetic flux density, time of exposure) and type of cells as the biological "receiver" of the EMF dose, e.g. naive cells such as keratinocytes, peripheral blood mononuclear cells, or cell lines commonly used in studies. Both of these factors are important and cannot be separated from each other, because the same EMF as a physical factor can influence various cell types differently [13,16,39]. Even cells originating from the same donor but at a different phase of the cell cycle might react differently to an externally applied stress factor. Regarding keratinocytes, it is difficult to state unambiguously the advantages of applied EMF exposure (mainly due to the non-homogenous population of cultured keratinocytes originating from different donors), as well as about the bio-mechanism(s) behind these advantages. Nevertheless, what can be undoubtedly confirmed in reference to these studies is that the application of EMF exerts effect on viability of oral mucosa keratinocytes cultured in in vitro conditions. However, the detailed bio-mechanism underlying the interaction between the applied electromagnetic field and oral mucosa keratinocytes is not fully understood and needs further studies focused on e.g. determination of the main pro-inflammatory cytokine level (IL-8, IL-6, etc.) and investigations of downstream signaling pathways. Similar EMF-induced effects have already been shown in previously published studies by our group. For instance, the viability of mononuclear leukocytes isolated from patients with Crohn's disease and exposed to a low-frequency electromagnetic field (50 Hz, 45 mT) was changed. The EMF-exposed leukocytes showed a shorter lifespan in comparison to cells in the control group, and also revealed a changed profile of the cytokines produced from pro- to anti-inflammatory [25]. The anti-inflammatory effects induced by LF-EMF (7 Hz, 30 mT) were also

achieved in experiments carried out with a co-culture composed of a human bladder microvascular endothelial cell line (HMVEC-Bd) and MonoMac 6 (MM6) cell lines which was LPS stimulated and EMF exposed. In the co-culture stimulated with EMF, a diminished production of some pro-inflammatory agents (e.g. IL-8, ICAM-1 and VEGF-A) released in response to LPS activation was noticed compared to control samples [28]. EMF-triggered modulation of cell viability was also observed for an MM6 cell line simultaneously treated with puromycin, a known apoptosis-inducer [27]. The application of low-frequency EMF affected MonoMac 6 cells by activation of genes belonging to the two apoptosis induction pathways: the intrinsic pathway and that related to endoplasmic reticulum. Previously, Fanelli et al. [12], showed 6 mT static magnetic field inhibited apoptosis in U937 and CEM cells, due to an increase in ion Ca^{+2} influx from the extracellular medium to the cells. In a study by Manni et al. [38], the effect of low-frequency EMF (2 mT, 50 Hz) on the bio-chemical properties of human oral keratinocytes was noticed. Exposed cells revealed a modification in shape and morphology associated with different actin distribution, as has been shown by scanning electron microscopy. They exhibited a decrease in cellular growth and of EGF receptor production, together with an increase in involucrin protein expression, suggesting that the application of LF-EMF influences cell differentiation. Many experiments have been carried out in in vivo and in vitro conditions to investigate the effect of EMFs on tumor cell growth. However, the various parameters of the electromagnetic stimulation applied result in completely different biological effects [3,10,26,54,64].

The problem of odontogenic pain is a very common problem among dental patients. The treatment of pain caused by local inflammatory process is mainly carried out with non-steroidal anti-inflammatory drugs (NSAIDs), through their inhibitory effects on COX-1 and COX-2. Nevertheless, NSAIDs have negative side effects on the digestive system, causing the formation of ulcers that can lead to life-threatening bleeding, and which can also be nephrotoxic [59]. Therapy with variable electromagnetic fields appears to be an alternative to NSAIDs, thanks to its anti-inflammatory and anti-edema effects, inhibiting the destructive processes on cellular and tissue levels, and most importantly its analgesic effect via its effect on β-endorphin release. Currently, it is known that various electromagnetic fields greatly reduce the quality and intensity of the pain sensation in post-operative patients after surgical procedures, and also in patients with toothaches or post-traumatic patients, where damage of tissue structure causes strong long-lasting pain of high value, often accompanied by edema and local inflammation. Therefore, it is advisable to use various electromagnetic field therapies in such cases where pain seriously disturbs the patients' quality of life [23,24,44]. Treatment with magnetic fields is an area that has recently begun to develop dynamically, with an increasingly widespread use in various branches of medicine and dentistry. Data from the literature are consistent with our results, confirming the potential therapeutic role of EMF treatment during the healing process of oral mucosa inflammation, via EMF-triggered changes in cell death parameters. Thus, electromagnetic fields as physical external factors combined with chemical treatment seem to be a promising tool for modulation of various cell signaling pathways (e.g. favoring cell survival or cell death). In the authors' opinion, studies concerning the involvement of EMFs and pharmaceuticals are part of the mainstream of interests of EMF-related research in modern medicine.

Conflict of interest statement

The authors declare no conflicts of interest.

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