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ARTICLE

Investigation of inflammatory markers after electrostimulation

Daniel Crabtree[1,4], Orsolya Sípos[2], Kata Filkor[2], Ernő Duda[2], Csaba Vágvölgyi[3], Ferenc Somogyvári[4]*

[1]Université Joseph Fourier, Grenoble, France, [2]Department of Medical Biology, University of Szeged, Szeged, Hungary, [3]Department of Microbiology, University of Szeged, Szeged, Hungary, [4]Department of Medical Microbiology and Immunobiology, University of Szeged, Szeged, Hungary

ABSTRACT Electric and magnetic fields are known to interact with living systems. Our aim was to determine the impact of electric stimulation on human immune cells at the level of gene expression. THP-1 human monocytes were treated with direct electric impulses and with the electric field of a commercial cell phone. The relative expression of 21 genes was determined by real-time quantitative PCR and calculated by ΔΔCt method with GAPDH control. The smaller electric field and electromagnetic field of cell phone have a slight, not significant but consequent down-regulation of the pro-inflammatory genes. The higher electric field induced significant up-regulation of same genes.

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The electric fields generally act as environmental stress factors. In practice, these stimuli are able to induce cell activation (Liang et al. 2011) or differentiation (Haneef et al. 2012). Furthermore, the electromagnetic field is even used in therapy (Tran et al. 2013). The effects of the cell phones' electromagnetic field were previously investigated in context of the interference with electric implants (Kainz et al. 2003) or with the sensory cortex (Yuasa et al. 2006). In our laboratory the changes in the activities of several enzymes and surface antibody pattern in response to electric impulses were previously tested (Filipic et al. 2000).

One of the most frequently used model systems for monocytes are the THP-1 cultures. They have more uniformed genetic background and phenotype than other monocytic cell lines, for example U937. It is easy to study their activity, migration, phagocytic capacity or cytokine production. The cytokine response of these cells have been investigated after chemical stress (Michée et al. 2013), lipopolisaccharide of *E. coli* (Sharif et al. 2007) or after infectious stress of *Streptococcus pneumoniae* (Zhang et al. 2013).

Our aim was to determine the cytokine response of THP-1 monocytes at the level of gene expression after electric stimulation.

Materials and Methods

Culture of THP-1 human monocytic cells

THP-1 human monocytic cells were propagated in RPMI-1640 medium (Lonza, Basel, Switzerland) supplemented with 10% heat-inactivated foetal bovine serum (FBS Gold; PAA Laboratories, Cölbe, Germany), 2 mM L-glutamine (Invitrogen, Carlsbad, USA) and 100 µg/ml kanamycin (Sigma-Aldrich, St Louis, USA) under standard conditions (humidified atmosphere of 5% CO_2 at 37°C). The cells were plated into 6 cm diameter Petri-dishes (Orange Scientific, Braine-l'Alleud, Belgium) at 10^6 cells/ml in the same medium.

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*Corresponding author. E-mail: somogyvari.ferenc@med.u-szeged.hu

Electrostimulation

After 24 hours of incubation, cells were treated by direct electric impulses and by the electric field of a commercial cell phone. The direct field electrostimulation was performed by a CFA 400 Electrofusion Equipment (Krüss, Hamburg, Germany) which consist of a combined frequency and pulse generator. The frequency generator part was not used. Three impulses of direct current of 80 and 160 V/cm were applied in a home-made chamber formed from a plastic Petri-dish. The electrodes made from platinum wire in 0.1 mm diameter, the connection between the chamber and the equipment was oscilloscope test wire (Philips Electronics, Amsterdam, Netherlands). The duration of the impulses was 2 msec and the repetition period was 2 sec.

Two commercial cell phones (Nokia 2730 and Samsung Galaxy2 Mini) were tested by Electromagnetic Radiation Detector DT1130 (Wing International, Guangdong, China). As the relative electromagnetic emission was larger in the case of Samsung Galaxy2 Mini, this was used for the electrostimulation. The cell phone fitted on the top of the Petri-dishes was turned on for 20 minutes with continuous calls.

The investigations are repeated three times, and in all cases parallel, untreated cells were used as a control.

Table 1. Genes, primers and the resulted relative gene expression of the monocytic THP-1 cells after different electric treatment. The investigations are repeated three times, and in all cases parallel, untreated cells were used as a control. The housekeeping gene was the GAPDH. Three squared impulses of direct current of 80 and 160 V/cm were applied with the duration of 2 msec and the repetition period was 2 sec. The cell phone was the Samsung Galaxy2 Mini because its relative electromagnetic emission was the largest.

Genes Name	Primers	Relative expression					
		Direct pulses (80 V/cm)		Direct pulses (160 V/cm)		Cell phone	
		Mean	SD	Mean	SD	Mean	SD
CCL1	CTTCACCAGGCTCATCAAAGCTG TCTGGAGAAGGGTACCTGCATG	0.63	0.47	4.6[ab]	1.00	0.38[a]	0.14
TNFAIP6	GCTGGATGGATGGCTAAGGG TCCTTTGCGTGTGGGTTGTAG	0.34[a]	0.16	2.66[ab]	0.22	0.18[a]	0.15
IL-17A	ATTGGTGTCACTGCTACTGCT TTCCAAGGTGAGGTGGATCG	0.26[a]	0.15	2.46[ab]	0.85	0.13[a]	0.11
IL-1B	CCACCTCCAGGGACAGGATA TTTGGGATCTACACTCTCCAGC	0.78	0.17	1.74[ab]	0.82	0.94	0.23
CXCL10	ACACTAGCCCCACGTTTTCT GAGAGGTACTCCTTGAATGCCA	0.29[a]	0.10	1.38[b]	1.35	0.21[a]	0.19
Tyro3	CTCACGGTAGAAGGTGTGCC AAACATGGTGCTCTGGGTCA	0.72[a]	0.06	1.27[b]	0.10	0.82[a]	0.19
Axl	GTATCAAGGCCAGGACACCC GAAGGTTCCTTCACTGGGCG	0.80	0.01	1.17[b]	0.14	0.76	0.03
ICAM1	TGTGACCAGCCCAAGTTGTT TGGAGTCCAGTACACGGTGA	0.82	0.08	1.09	0.06	0.79	0.10
IL-12	AGGGACATCATCAAACCTGACC GCTGAGGTCTTGTCCGTGAA	0.60[a]	0.21	1.03	0.16	0.29[a]	0.23
IL-27	TGGTTCAAGCTGGTGTCTGG GGTGAGATTCCGCAAAGCG	1.45[a]	0.42	0.71[b]	0.24	1.30[a]	0.14
CXCL9	AGTGCAAGGAACCCCAGTAG TCACATCTGCTGAATCTGGGTT	1.28[a]	0.20	0.75[ab]	0.25	0.29[a]	0.16
TGF-β	ACCAACTATTGCTTCAGCTCCA CTTGCTGTACTGCGTGTCCA	1.07	0.04	0.09	0.95	1.44[a]	0.28
TNF	GCCTCTTCTCCTTCCTGATCG GCTTGAGGGTTTGCTACAACAT	1.41[a]	0.10	1.90[a]	0.64	1.49[a]	0.31
IL-8	ACTCCAAACCTTTCCACCCC TTCTCAGCCCTCTTCAAAAACTTC	1.11	0.11	1.52[ab]	0.25	1.39[a]	0.04
Gas6	AAAAACTCAGGCTTCGCCAC TCGTTGACATCTTTGTCGCAG	0.82	0.13	1.12	0.13	1.18	0.14
Kynu	GCTGAGGCACTTCAGGGAGTG TGACCATAGGCTGCTATTTTGG	1.04	0.02	0.92	0.05	0.92	0.15
ProS	TGCCCCGAAGGCTACAGATA GCACACTGAAACAACCTCACAA	0.95	0.07	0.81	0.09	1.04	0.10
CKIP1	ACCTGCAACCGACGATTCTT CATTCCATGAAGTCAGCGATATGT	1.08	0.07	1.04	0.16	1.25	0.07
TNFAIP3	GCTGAAAACGAACGGTGACGG AGAGACTCCAGTTGCCAGCGG	1.01	0.07	0.99	0.11	1.22	0.13
TNFAIP6	GCTGGATGGATGGCTAAGGG TCCTTTGCGTGTGGGTTGTAG	1.09	0.16	1.18	0.22	1.39[a]	0.15
IL23	TGCCAGCAGCTTTCACAGAA GCAAGCAGAACTGACTGTTGTC	0.93	0.11	0.81	0.12	1.27[a]	0.05
GAPDH	CAGTCAGCCGCATCTTCTTTTG CGCCCAATACGACCAAATCC						

[a] $p<0.05$. The expression were compared with the GAPDH control
[b] $p<0.05$. The expression were compared with the expression after 80 V/cm

Quantitative reverse transcriptase polymerase chain reaction (QRT-PCR)

Total RNA was extracted from THP-1 cells after 4 hours incubation using GeneJet RNA Purification Kit (Thermo Fisher, Waltham, USA) according to the manufacturer's instructions. The quality and the quantity of the extracted RNA were determined by spectrophotometric measurement (BioPhotometer; Eppendorf, Hamburg, Germany). cDNA was synthesized from 500 ng of total RNA by using Maxima First Strand cDNA Synthesis Kit for RT-QPCR (Thermo Scientific)

according to the manufacturer's instructions. The relative abundance of selected mRNAs was determined by QRT-PCR by using a CFX96 system (Bio-Rad, Hercules, USA). Reactions were performed by using IQ SybrGreen Supermix qPCR mastermix (Bio-Rad). Reaction mixtures without cDNA were used as negative control. All of the measurements were performed in duplicate from 3 biological repetitions.

For this investigation, pro-inflammatory and anti-inflammatory genes were chosen. All of the primers were constructed with the help of the Integrated DNA Technologies homepage (www.eu.idtdna.com) and the chromosomal sequences of the target genes were previously collected from the National Center for Biotechnology Information homepage (www.ncbi.nlm.nih.gov). The genes and the sequence of the primers are listed in Table 1.

Data representation and statistical analysis

The ratio of each mRNA is relative to GAPDH and calculated by using the ΔΔCt method.

Data show average ± standard deviation of the mean (SD). The significance of the differences between different sets of data was determined by Student's paired t test using Graph Pad Prism for Windows. A probability value (p) of less than 0,05 was considered significant.

Results and Discussion

Beside the direct stimulation with ionic current the electric field can be generated between two plates of a capacitance, or a magnetic field with coils or antennas. The issue is complicated by the fact that electric fields can be applied as long-lasting continuous DC fluxes, AC waves or impulses. Technically the impulses are the most easily achievable and more often used. Now and previously (Kovacs et al. 2000) we had used impulses for the direct stimulation. The electric field created capacitively is not able to induce a characteristic cytokine response (Caputo et al. 2013). For magnetic treatment the electric coils are used, but cell phones are operated with the same impulses. The magnetic field created by coils can easily be calculated and controlled. The electric force, the carrier frequency and the shape of the cell phone impulses are known, but the repetition frequency not. In practice, we have daily practice with the phones, and the electric field of the commerce phones was used for further investigations. We are compared the effect of the direct impulses and the magnetic field.

The relative expression data are listed in Table 1.

The 80 V/cm pulses decreased significantly the transcription of IL17, CXCL10 and TNFAIP6. The down-regulation was not significant in the case of other cytokines related by inflammation but the tendency was very same (CCL1, IL1β, Tyro3, AXL, IL12).

The 160V/cm impulses increased the transcription of CCL1, TNFAIP6 and IL17. No other transcriptions are changed significantly.

Although there are no significant changes of other inflammatory cytokines the tendency is in concordance with the previous investigations, when THP-1 cells were stimulated with lipopolysaccharides (Schildberger et al. 2013). Although, this study examined the cytokine release and thus the translational regulation can modify the outcome, the observed effect was same. The TNFα, IL8 and IL1β cytokine release increased after four hours while there were no IL6 and IL10 production. (The IL6 and IL10 are excluded from Table 1 because of the lack of the biological parallels.) Accordingly these findings the protein production was measurable after four hours. We are tested the mRNA release after the same period, and the reaction looks quicker than we expected. Probably we have to check earlier the transcription itself.

Most of the inflammatory cytokines are induced with 160 V/cm high impulses –at least in tendency-, except IL27. Previous papers are reported about the contradictory effect of this cytokine (Carl and Bai 2008). The IL27 is a member of the IL6/IL12 cytokine family and it has both pro- and anti-inflammatory properties. Other possible reason is, that whilst the THP-1 cells are one of the best model systems of the monocytes, there are differences between the primary cells and this cell lines (Qin 2012).

The IL12 and the IL27 are tested in immunological regulatory therapies, although the exact mechanism of this opposite action is still unknown. One attempt was the electroporation of the IL12 and IL27 genes for eliminating aggressive tumours (Zhou et al. 2010). The applied electric field was 320 V/cm. This energy is able to disrupt the cell membrane. On the basis of the observations above, the electric field can modify the cytokine expression and, probably the effect of the therapy.

The magnetic impulses of the cell phone have the very same effect as the lower (80 V/cm) direct impulses. There were three differences. Two another inflammatory cytokines are down-regulated significantly (CCL1 and IL12), but the tendency was same, as with the lower direct impulses. In the case of the CXCL9 the down-regulation was in the opposite direction. It is well known that CXCL9 together with CXCL10 and CXCL11 are IFN-γ inducible and by cross-linking their cognate receptor, namely CXCR3 they have central role in the mediation of cell migration (Broxmeyer et. al. 2006, Moser et. al. 2001). Moreover, elevated CXCL9 level is also involved in the pathogenesis of acute graft-versus-host disease (Bouazzaoui et. al. 2009) and significantly enhanced secreted CXCL9 level in the urine of kidney transplanted patients is characteristic for acute rejection (Hirick et. al. 2013).

In conclusion, it looks that the electric impulses have a threshold, and over this the impulses work as a stress which induced the release of the inflammatory cytokines. The question is whether the same threshold exists in the case of

the electromagnetic field, or, if there is, than the changes of the gene expression as definite as in the case of the direct impulses.

Finally, further investigations are necessary because the reaction for electric and magnetic impulses looks more rapid than we are expected.

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