

Vorsorge aufgrund wiederholter Feststellung so genannter a-thermischer Wirkungen von HF-EMF

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In-vitro-Befunde

Die internationale Forschung erbrachte Befunde, die für den Fall der Exposition zu HF-EMF (hochfrequenten elektromagnetischen Feldern) Maßnahmen zur Risikoreduktion begründen.

Folgende Befunde und Erkenntnisse sind dafür relevant.

DNA-Brüche. Durch Exposition zu HF-EMF bedingte DNA-Brüche wurden erstmals bei Labortieren beschrieben [1, 2]. Bei Zellkulturen (*in vitro*) wirkte eine minutenweise unterbrochene Exposition stärker als kontinuierliche Bestrahlung [3, 4]. Zwar gibt es DNA-Brüche auch beim Gesunden, aber eine expositionsbedingte DNA-Bruch-Vermehrung wird allgemein als Risikoindikator für Krebserkrankungen gewertet.¹ Es gibt bereits erste Gerichts-Urteile, die das Risiko anerkennen.²

In einigen Fällen wurden Zellen mit zwei sich ergänzenden Methoden untersucht, um 1. DNA-Brüchen und 2. Proteinveränderungen festzustellen. Die DNA-Brüche in den sensiblen Zellen wurden durch die gefundenen Protein-Befunde plausibel bestätigt (siehe dazu Gerner *et al.* [5]). Mittlerweile sind die in HF-EMF-Expositions-bedingten DNA-Schäden international mehrfach repliziert [6, 7, 8].

Dass eine intermittierende Exposition (5 min „an“, 10 min „aus“), bzw. dass die Modulation der HF-EMF die DNA-Bruchrate erhöht, bricht mit einem wissenschaftlichen Dogma, weil trotz geringerer oder gleicher absorbierter Leistung pro Gewebemasse DNA-Brüche vermehrt auftreten.

Mit HF-EMF Exposition assoziierte DNA-Brüche wurden aber nicht nur *in vitro*, sondern auch an lebenden Tieren wiederholt beobachtet (siehe unten).

Konsistenz der Befunde. Bei der Interpretation alter Befunde führt die Beachtung biologischer Aspekte dazu, dass angebliche Widersprüche als scheinbar aufgelöst werden. Beispielsweise wurden bisher zumeist die Ergebnisse kurzer Expositionen (< 2 Stunden) publiziert [9, 10, 11, 12]. Diese negativen Befunde wurden auch beim EU-Reflexprojekt bestätigt, sie stehen aber nicht im Widerspruch zu jenen Untersuchungen, die nach längerer Exposition Effekte fanden [6, 8].

Die folgenden Befunde erklären angebliche Widersprüche in der Literatur als scheinbar.

Zelltyp-Abhängigkeit. Der am häufigsten untersuchte Zelltyp sind „Lymphozyten“ [11, 13, 14, 15, 16, 17, 18, 19, 20, 21]. In Übereinstimmung mit den Ergebnissen des EU-Reflex-Projektes und der Wiener Arbeitsgruppe um Ruediger zeigen Lymphozyten konsistent keine expositionsbedingten DNA-Brüche [15, 22]. Lymphozyten wurden also verschiedentlich als resistent gegenüber HF-EMF beschrieben. Die Resistenz eines Zelltyps hebt jedoch nicht die Empfindlichkeit eines anderen auf, wenn bei diesem anderen Zelltyp expositionsbedingte DNA-Brüche gibt. Beispielsweise wurden

¹ Der Vorsitzende des Ausschusses für „Nichtionisierende Strahlen“ der deutschen SSK (Alexander Lerchl) schrieb „Die Ergebnisse Besorgnis erregend. Sollten sie sich bestätigen, wäre dies nicht bloß ein Alarmsignal, sondern der Anfang vom Ende des Mobilfunks, da DNA-Schäden die erste Stufe zur Krebsentstehung sind“. (Quelle: Lerchl A (2008) Fälscher im Labor und ihre Helfer: Die Wiener Mobilfunk-Studien – Einzelfall oder Symptom? Books on Demand GmbH, ISBN-13: 978-3837063417, S. 43.

Anm. d. Verfassers: Besonders nachdem seither die Befunde zu DNA-Brüchen mehrfach, und sogar auch in Tier-Experimenten bestätigt sind, unterstreicht dies die Dringlichkeit von Prävention und Vorsorgemaßnahmen für einen sicheren Umgang mit HF-EMF abstrahlenden Geräten.

² In Italien wurde ein Gerichts-Urteil in zweiter Instanz gefällt, das mit der Krebs-Verursachung durch häufige Mobilfunk-Exposition begründet ist.

folgende Zelltypen als sensibel befunden: Fibroblasten, Neuronen, Trophoblasten, CHL-Zellen, lymphoblastoide Zellen [3, 4, 6, 8, 9, 23, 24].

Die Existenz weiterer sensibler Zellen oder Bedingungen ist sehr wahrscheinlich.

Latenzzeit. Im Gegensatz zu radioaktiver Bestrahlung findet man nach kurzer HF-EMF-Exposition keine Effekte. Bei sensiblen Zellen ist die Zeitdauer vom Beginn der Exposition bis zum Auftreten der positiven Befunde je nach Forschergruppe, Testmodell und Zelltyp zwanzig Minuten [7], 2 Stunden (beim EU-Reflex-Project), 4 Stunden [5], oder mit 16 Stunden [6] beschrieben.

Zell-Aktivität. Die Sensitivität der Zellen hängt von deren metabolischen Aktivität ab. Beim ATHEM-Projekt zeigte sich, dass die Erhöhung der metabolischen Aktivität selbst bei inaktiven (unsensiblen) Lymphozyten zu einer Erhöhung der Sensibilität führen kann [5].

Dies bedeutet, dass aktive Zellen vulnerabler sind als ruhende Zellen.

Erholungszeit. Nach der Exposition benötigen die Zellen eine bestimmte Zeit bis expositionsbedingte Effekte verschwinden (= Erholungszeit). Sowohl bei DNA-Brüchen wie bei den Proteinveränderungen wurden 2 Stunden beschrieben, bis der Ausgangszustand erreicht wird [5, 6]. Es bräuchte systematische Forschung, um zu prüfen, ob kürzere Zeiten eine ausreichende Erholung erlauben.

Effekt bei niedriger Intensität. An sensiblen Zellen wurden nach der Latenzzeit DNA-Brüche auch bei niedrigen Intensitäten (1,2 & 0,1 W/kg), die deutlich unter den aktuellen EU-Rats-Empfehlungen liegen, beobachtet [3, 4]. Eigene preliminäre Proteom-Befunde bestätigen die Sensibilität von Zellen bei geringen Feldstärken.

In-vivo-Befunde (Tierversuche)

Die *in-vivo*-Befunde bei verschiedenen Labortieren bestätigen und verstärken die Schlussfolgerungen aus der EU-REFLEX-Studie und der in jüngerer Zeit publizierten *in-vitro*-Untersuchungen zu DNA-Schäden.

Kesari *et al.* [25] exponierten junge Ratten 2 Stunden pro Tag (35 Tage) mit einem un-modulierten hochfrequenten elektromagnetischen Feld von 2450 MHz; die Leistungsflussdichte betrug 0,34 mW/cm² (Grenzwert: 1 mW/cm²); dies entspricht einer geschätzten Ganzkörper-SAR von 0,11 W/kg. Nach der Bestrahlung war die DNA-Strangbruchrate in den Hirnzellen der Ratten im Vergleich zu ihren Kontrollgruppen signifikant erhöht. Die Befunde zeigen, dass die gentoxische Wirkung von HF-EMF auch an Ganzkörper-bestrahlten Versuchstieren nachgewiesen werden kann.

Guler G. *et al.* [26] exponierten nicht-trächtige und trächtige Kaninchen 15 Minuten, 7 Tage lang mit GSM-ähnlichen 1800 MHz-Signalen bei einer elektrischen Feldstärke von 14 V/m (Grenzwert: 58 V/m). Nach der Bestrahlung wurde im Vergleich zu den Kontrollen im Hirngewebe beider Versuchsgruppen ein signifikanter Anstieg oxidativer Schädigungen bei Lipiden und DNA beobachtet. Bei den neugeborenen Tieren wurden derartige Veränderungen nicht beobachtet. Übereinstimmend mit den oben angeführten Befunden an Labor-Ratten wurde hier an einer weiteren Tierart beschrieben, dass modulierte hochfrequente elektromagnetische Felder weit unterhalb der geltenden Europäischen Richtwerte gentoxische Veränderung im Gehirn von Ganzkörper-bestrahlten Versuchstieren verursachen können. Die Befunde von Tomruk *et al.* stammen ebenfalls von Kaninchen und sind den oben angeführten (Guler *et al.*) ähnlich [27,26].

Bedeutung der Modulation

Da die spezifische Absorptionsrate (SAR) zwischen modulierten und nicht modulierten Feldern in der Stärke vergleichbar ist, belegen Unterschiede in der Wirkung von modulierten und un-modulierten Feldern, dass die bloße Begrenzung der eingestrahlt und absorbierbaren Energie zelluläre Reaktionen nicht zuverlässig verhindert. Die folgenden Publikationen zeigen, dass für die Entstehung

von DNA-Brüchen die Signal-Modulation (Funkanwendung) ein förderlicher Expositions-Parameter ist.

1. Franzellitti *et al.* [6] zeigten den gleichen Sachverhalt innerhalb einer Experimentserie. Die beobachteten DNA Brüche waren nicht mehr feststellbar, sobald mit der nicht modulierten Trägerfrequenz exponiert wurde.
2. Campisi *et al.* [7] fanden expositionsbedingt einen Anstieg von freien Sauerstoffradikalen assoziiert mit DNA-Brüchen nach 20 Minuten Exposition zu modulierten Feldern. Keine Effekte fanden sich nach der Exposition unter gleicher Feldstärke mit der nicht-modulierten Trägerfrequenz.

Zusammenfassend

Nach der Beschreibung der Vermehrung von DNA-Brüchen in Ratten-Gehirnen nach Mikrowellen-Exposition durch Henry Lai (USA) wurden vorzugsweise leicht zugängliche Lymphozyten zu Replikationsexperimenten herangezogen. Allerdings waren (und sind) in diesem Zelltyp die DNA-Brüche kaum feststellbar. Mittlerweile wurde wiederholt gezeigt, dass Lymphozyten gegen Mikrowellen-Exposition bei mäßigen Intensitäten eher robust sind. Hingegen reagieren andere HF-EMF exponierte Zelltypen mit messbar erhöhten DNA-Brüchen. Ob empfindliche Zellen nach Exposition DNA-Brüche aufweisen, hängt von mehreren Bedingungen ab:

1. Nach Expositionsbeginn vergeht eine so genannte Latenzzeit bis die DNA-Brüche feststellbar sind. Je kürzer die Expositionsdauer, umso weniger wahrscheinlich kommt es zu Schäden.
2. Zusätzlich zu der durch Energieabsorption pro Masseneinheit wirksamen Strahlungsleistung (SAR-Wert) sind weitere Expositions-Parameter relevant. Beispielsweise reagieren sensible Zellen bei rhythmischen Unterbrechungen (intermittierende Exposition) sensibler als auf kontinuierliche Exposition. Dies bedeutet, dass zelluläre Reaktionen auch unabhängig von der spezifischen Absorptionsrate (Wärmeentwicklung) auftreten können.
3. Es finden sich wiederholt Hinweise, dass bei gleicher Intensität die Exposition zu modulierten Signalen mehr DNA-Brüche erzeugt als die Exposition zu einer nicht modulierten Trägerwelle. Dies bestätigt, dass die zellulären Reaktionen nicht alleine von der spezifischen Absorptionsrate (Wärmeentwicklung) abhängen.
4. Nach Expositionsende verschwinden die DNA-Brüche ungefähr innerhalb von 2 Stunden Erholungszeit. Dieser Befund kann Expositionspausen zum Schutz vor Expositionsfolgen begründen. Für die exakte Feststellung der Erholungszeit bedarf es weiterer systematischer Forschung.

Schlussfolgerung

Bis die Expositions-Bedingungen, welche unerwünschte DNA-Brüche nach sich ziehen, zufriedenstellend erforscht sind, um als Grundlage für neue Grenzwerte dienen zu können, muss man, um ein mögliches Risiko zu minimieren, vorsorglich handeln. Bei der Installation und/oder Nutzung HF-EMF abstrahlender Geräte lässt sich das Risiko durch einfache Maßnahmen nach dem Prinzip „umsichtiger Vermeidung“ reduzieren. Dazu gibt es praktische Tipps im Projekt-Report 47 der Allgemeinen Unfallversicherungsanstalt http://esv-sva.sozvers.at/mediaDB/555261_R47.pdf, (Seite 171-173).

Wien, im Oktober 2010

Zitierte Literatur

- 1 **Lai, H and Singh, NP (1996). Single- and double-strand DNA breaks in rat brain cells after acute exposure to radiofrequency electromagnetic radiation. Int.J.Radiat.Biol. 69: 513-21.**

Abstrakt:

We investigated the effects of acute (2-h) exposure to pulsed (2-micros pulse width, 500 pulses s(-1)) and continuous wave 2450-MHz radiofrequency electromagnetic radiation on DNA strand breaks in brain cells of rat. The spatial averaged power density of the radiation was 2mW/cm², which produced a whole-body average-specific absorption rate of 1.2W/kg. Single- and double-strand DNA breaks in individual brain cells were measured at 4h post-exposure using a microgel electrophoresis assay. An increase in both types of DNA strand breaks was observed after exposure to either the pulsed or continuous-wave radiation, No significant difference was observed between the effects of the two forms of radiation. We speculate that these effects could result from a direct effect of radiofrequency electromagnetic energy on DNA molecules and/or impairment of DNA-damage repair mechanisms in brain cells. Our data further support the results of earlier in vitro and in vivo studies showing effects of radiofrequency electromagnetic radiation on DNA.

- 2 **Lai, H and Singh, NP (1995). Acute low-intensity microwave exposure increases DNA single-strand breaks in rat brain cells. Bioelectromagnetics. 16: 207-10.**

Abstrakt:

Levels of DNA single-strand break were assayed in brain cells from rats acutely exposed to low-intensity 2450 MHz microwaves using an alkaline microgel electrophoresis method. Immediately after 2 h of exposure to pulsed (2 microseconds width, 500 pulses/s) microwaves, no significant effect was observed, whereas a dose rate-dependent [0.6 and 1.2 W/kg whole body specific absorption rate (SAR)] increase in DNA single-strand breaks was found in brain cells of rats at 4 h postexposure. Furthermore, in rats exposed for 2 h to continuous-wave 2450 MHz microwaves (SAR 1.2 W/kg), increases in brain cell DNA single-strand breaks were observed immediately as well as at 4 h postexposure.

- 3 **Diem, E, Schwarz, C, Adlkofer, F, Jahn, O and Rüdiger, H (2005). Non-thermal DNA breakage by mobile-phone radiation (1800 MHz) in human fibroblasts and in transformed GFSH-R17 rat granulosa cells in vitro. Mutat.Res. 583: 178-83.**

Abstrakt:

Cultured human diploid fibroblasts and cultured rat granulosa cells were exposed to intermittent and continuous radiofrequency electromagnetic fields (RF-EMF) used in mobile phones, with different specific absorption rates (SAR) and different mobile-phone modulations. DNA strand breaks were determined by means of the alkaline and neutral comet assay. RF-EMF exposure (1800 MHz; SAR 1.2 or 2 W/kg; different modulations; during 4, 16 and 24h; intermittent 5 min on/10 min off or continuous wave) induced DNA single- and double-strand breaks. Effects occurred after 16 h exposure in both cell types and after different mobile-phone modulations. The intermittent exposure showed a stronger effect in the comet assay than continuous exposure. Therefore we conclude that the induced DNA damage cannot be based on thermal effects

- 4 **Schwarz, C, Kratochvil, E, Pilger, A, Kuster, N, Adlkofer, F and Rudiger, HW (2008). Radiofrequency electromagnetic fields (UMTS, 1,950 MHz) induce genotoxic effects in vitro in human fibroblasts but not in lymphocytes. Int.Arch.Occup.Environ.Health. 81: 755-67.**

Abstrakt:

OBJECTIVE: Universal Mobile Telecommunication System (UMTS) was recently introduced as the third generation mobile communication standard in Europe. This was done without any information on biological effects and genotoxic properties of these particular high-frequency electromagnetic fields. This is disconcerting, because genotoxic effects of the second generation standard Global System for Mobile Communication have been reported after exposure of human cells in vitro. METHODS: Human cultured fibroblasts of three different donors and three different short-term human lymphocyte cultures were exposed to 1,950 MHz UMTS below the specific absorption rate (SAR) safety limit of 2 W/kg. The alkaline comet assay and the micronucleus assay were used to ascertain dose and time-dependent genotoxic effects. Five hundred cells per slide were visually evaluated in the comet assay and comet tail factor (CTF) was calculated. In the micronucleus assay 1,000 binucleated cells were evaluated per assay. The origin of the micronuclei was determined by fluorescence labeled anticentromere antibodies. All evaluations were performed under blinded conditions. RESULTS: UMTS exposure increased the CTF and induced centromere-negative micronuclei (MN) in human cultured fibroblasts in a dose and time-dependent way. Incubation for 24 h at a SAR of 0.05 W/kg generated a statistically significant rise in both CTF and MN ($P = 0.02$). At a SAR of 0.1 W/kg the CTF was significantly increased after 8 h of incubation ($P = 0.02$), the number of MN after 12 h ($P = 0.02$). No UMTS effect was obtained with lymphocytes, either unstimulated or stimulated with Phytohemagglutinin. CONCLUSION: UMTS exposure may cause genetic alterations in some but not in all human cells in vitro

- 5 **Gerner, C, Haudek, V, Schandl, U, Bayer, E, Gundacker, N, Hutter, HP and Mosgoeller, W (2010). Increased protein synthesis by cells exposed to a 1,800-MHz radio-frequency mobile phone electromagnetic field, detected by proteome profiling. Int Arch Occup Environ Health. 83: 691-702.**

Abstrakt:

PURPOSE: To investigate whether or not low intensity radio frequency electromagnetic field exposure (RF-EME) associated with mobile phone use can affect human cells, we used a sensitive proteome analysis method to study changes in protein synthesis in cultured human cells. METHODS: Four different cell kinds were exposed to 2 W/kg specific absorption rate in medium containing ³⁵S-methionine/cysteine, and autoradiography of 2D gel spots was used to measure the increased synthesis of individual proteins. RESULTS: While short-term RF-EME did not significantly alter the proteome, an 8-h exposure caused a significant increase in protein synthesis in Jurkat T-cells and human fibroblasts, and to a lesser extent in activated primary human mononuclear cells. Quiescent (metabolically inactive) mononuclear cells, did not detectably respond to RF-EME. Since RF exposure induced a temperature increase of less than 0.15 degrees C, we suggest that the observed cellular response is a so called "athermal" effect of RF-EME. CONCLUSION: Our finding of an association between metabolic activity and the observed cellular reaction to low intensity RF-EME may reconcile conflicting results of previous studies. We further postulate that the observed increased protein synthesis reflects an increased rate of protein turnover stemming from protein folding problems caused by the interference of radio-frequency electromagnetic fields with hydrogen bonds. Our observations do not directly imply a health risk. However, vis-a-vis a synopsis of reports on cells stress and DNA breaks, after short and longer exposure, on active and inactive cells, our findings may contribute to the re-evaluation of previous reports.

- 6 **Franzellitti, S, Valbonesi, P, Ciancaglini, N, Biondi, C, Contin, A, Bersani, F and Fabbri, E (2010). Transient DNA damage induced by high-frequency electromagnetic fields (GSM 1.8GHz) in the human trophoblast HTR-8/SVneo cell line evaluated with the alkaline comet assay. *Mutat Res.* 683: 35-42.**

Abstrakt:

One of the most controversial issue regarding high-frequency electromagnetic fields (HF-EMF) is their putative capacity to affect DNA integrity. This is of particular concern due to the increasing use of HF-EMF in communication technologies, including mobile phones. Although epidemiological studies report no detrimental effects on human health, the possible disturbance generated by HF-EMF on cell physiology remains controversial. In addition, the question remains as to whether cells are able to compensate their potential effects. We have previously reported that a 1-h exposure to amplitude-modulated 1.8GHz sinusoidal waves (GSM-217Hz, SAR=2W/kg) largely used in mobile telephony did not cause increased levels of primary DNA damage in human trophoblast HTR-8/SVneo cells. Nevertheless, further investigations on trophoblast cell responses after exposure to GSM signals of different types and durations were considered of interest. In the present work, HTR-8/SVneo cells were exposed for 4, 16 or 24h to 1.8GHz continuous wave (CW) and different GSM signals, namely GSM-217Hz and GSM-Talk (intermittent exposure: 5min field on, 10min field off). The alkaline comet assay was used to evaluate primary DNA damages and/or strand breaks due to uncompleted repair processes in HF-EMF exposed samples. The amplitude-modulated signals GSM-217Hz and GSM-Talk induced a significant increase in comet parameters in trophoblast cells after 16 and 24h of exposure, while the un-modulated CW was ineffective. However, alterations were rapidly recovered and the DNA integrity of HF-EMF exposed cells was similar to that of sham-exposed cells within 2h of recovery in the absence irradiation. Our data suggest that HF-EMF with a carrier frequency and modulation scheme typical of the GSM signal may affect the DNA integrity.

- 7 **Campisi, A, Gulino, M, Acquaviva, R, Bellia, P, Raciti, G, Grasso, R, Musumeci, F, Vanella, A and Triglia, A (2010). Reactive oxygen species levels and DNA fragmentation on astrocytes in primary culture after acute exposure to low intensity microwave electromagnetic field. *Neurosci Lett.* 473: 52-5.**

Abstrakt:

The exposure of primary rat neocortical astroglial cell cultures to acute electromagnetic fields (EMF) in the microwave range was studied. Differentiated astroglial cell cultures at 14 days in vitro were exposed for 5, 10, or 20min to either 900MHz continuous waves or 900MHz waves modulated in amplitude at 50Hz using a sinusoidal waveform and 100% modulation index. The strength of the electric field (rms value) at the sample position was 10V/m. No change in cellular viability evaluated by MTT test and lactate dehydrogenase release was observed. A significant increase in ROS levels and DNA fragmentation was found only after exposure of the astrocytes to modulated EMF for 20min. No evident effects were detected when shorter time intervals or continuous waves were used. The irradiation conditions allowed the exclusion of any possible thermal effect. Our data demonstrate, for the first time, that even acute exposure to low intensity EMF induces ROS production and DNA fragmentation in astrocytes in primary cultures, which also represent the principal target of modulated EMF. Our findings also suggest the hypothesis that the effects could be due to hyperstimulation of the glutamate receptors, which play a crucial role in acute and chronic brain damage. Furthermore, the results show the importance of the amplitude modulation in the interaction between EMF and neocortical astrocytes.

- 8 **Xu, S, Zhou, Z, Zhang, L, Yu, Z, Zhang, W, Wang, Y, Wang, X, Li, M, Chen, Y, Chen, C, He, M, Zhang, G and Zhong, M (2010). Exposure to 1800 MHz radiofrequency radiation induces oxidative damage to mitochondrial DNA in primary cultured neurons. Brain Res. 1311: 189-96.**

Abstrakt:

Increasing evidence indicates that oxidative stress may be involved in the adverse effects of radiofrequency (RF) radiation on the brain. Because mitochondrial DNA (mtDNA) defects are closely associated with various nervous system diseases and mtDNA is particularly susceptible to oxidative stress, the purpose of this study was to determine whether radiofrequency radiation can cause oxidative damage to mtDNA. In this study, we exposed primary cultured cortical neurons to pulsed RF electromagnetic fields at a frequency of 1800 MHz modulated by 217 Hz at an average special absorption rate (SAR) of 2 W/kg. At 24 h after exposure, we found that RF radiation induced a significant increase in the levels of 8-hydroxyguanine (8-OHdG), a common biomarker of DNA oxidative damage, in the mitochondria of neurons. Concomitant with this finding, the copy number of mtDNA and the levels of mitochondrial RNA (mtRNA) transcripts showed an obvious reduction after RF exposure. Each of these mtDNA disturbances could be reversed by pretreatment with melatonin, which is known to be an efficient antioxidant in the brain. Together, these results suggested that 1800 MHz RF radiation could cause oxidative damage to mtDNA in primary cultured neurons. Oxidative damage to mtDNA may account for the neurotoxicity of RF radiation in the brain.

- 9 **Zhang, DY, Xu, ZP, Chiang, H, Lu, DQ and Zeng, QL (2006). Effects of GSM 1800 MHz radiofrequency electromagnetic fields on DNA damage in Chinese hamster lung cells. Zhonghua Yu Fang Yi.Xue.Za Zhi. 40: 149-52.**

Abstrakt:

OBJECTIVE: To study the effects of GSM 1800 MHz radiofrequency electromagnetic fields (RF EMF) on DNA damage in Chinese hamster lung (CHL) cells. METHODS: The cells were intermittently exposed or sham-exposed to GSM 1800 MHz RF EMF (5 minutes on/10 minutes off) at a special absorption rate (SAR) of 3.0 W/kg for 1 hour or 24 hours. Meanwhile, cells exposed to 2-acetaminofluorene, a DNA damage agent, at a final concentration of 20 mg/L for 2 hours were used as positive control. After exposure, cells were fixed by using 4% paraformaldehyde and processed for phosphorylated form of H2AX (gammaH2AX) immunofluorescence measurement. The primary antibody used for immunofluorescence was mouse monoclonal antibody against gammaH2AX and the secondary antibody was fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG. Nuclei were counterstained with 4, 6-diamidino-2-phenylindole (DAPI). The gammaH2AX foci and nuclei were visualized with an Olympus AX70 fluorescent microscope. Image Pro-Plus software was used to count the gammaH2AX foci in each cell. For each exposure condition, at least 50 cells were selected to detect gammaH2AX foci. Cells were classified as positive when more than five foci were detected. The percentage of gammaH2AX foci positive cells was adopted as the index of DNA damage. RESULTS: The percentage of gammaH2AX foci positive cell of 1800 MHz RF EMF exposure for 24 hours (37.9 +/- 8.6)% or 2-acetylaminofluorene exposure (50.9 +/- 9.4)% was significantly higher compared with the sham-exposure (28.0 +/- 8.4)%. However, there was no significant difference between the sham-exposure and RF EMF exposure for 1 hour (31.8 +/- 8.7)%. CONCLUSION: 1800 MHz RF EMF (SAR, 3.0 W/kg) for 24 hours might induce DNA damage in CHL cells

- 10 **Sakuma, N, Komatsubara, Y, Takeda, H, Hirose, H, Sekijima, M, Nojima, T and Miyakoshi, J (2006). DNA strand breaks are not induced in human cells exposed to 2.1425 GHz band CW**

and W-CDMA modulated radiofrequency fields allocated to mobile radio base stations. Bioelectromagnetics. 27: 51-7.

Abstrakt:

We conducted a large-scale in vitro study focused on the effects of low level radiofrequency (RF) fields from mobile radio base stations employing the International Mobile Telecommunication 2000 (IMT-2000) cellular system in order to test the hypothesis that modulated RF fields may act as a DNA damaging agent. First, we evaluated the responses of human cells to microwave exposure at a specific absorption rate (SAR) of 80 mW/kg, which corresponds to the limit of the average whole body SAR for general public exposure defined as a basic restriction in the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines. Second, we investigated whether continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) modulated signal RF fields at 2.1425 GHz induced different levels of DNA damage. Human glioblastoma A172 cells and normal human IMR-90 fibroblasts from fetal lungs were exposed to mobile communication frequency radiation to investigate whether such exposure produced DNA strand breaks in cell culture. A172 cells were exposed to W-CDMA radiation at SARs of 80, 250, and 800 mW/kg and CW radiation at 80 mW/kg for 2 and 24 h, while IMR-90 cells were exposed to both W-CDMA and CW radiations at a SAR of 80 mW/kg for the same time periods. Under the same RF field exposure conditions, no significant differences in the DNA strand breaks were observed between the test groups exposed to W-CDMA or CW radiation and the sham exposed negative controls, as evaluated immediately after the exposure periods by alkaline comet assays. Our results confirm that low level exposures do not act as a genotoxicant up to a SAR of 800 mW/kg

- 11 **Zeni, O, Romano, M, Perrotta, A, Lioi, MB, Barbieri, R, d'Ambrosio, G, Massa, R and Scarfi, MR (2005). Evaluation of genotoxic effects in human peripheral blood leukocytes following an acute in vitro exposure to 900 MHz radiofrequency fields. Bioelectromagnetics. 26: 258-65.**

Abstrakt:

Human peripheral blood leukocytes from healthy volunteers have been employed to investigate the induction of genotoxic effects following 2 h exposure to 900 MHz radiofrequency radiation. The GSM signal has been studied at specific absorption rates (SAR) of 0.3 and 1 W/kg. The exposures were carried out in a waveguide system under strictly controlled conditions of both dosimetry and temperature. The same temperature conditions (37.0 +/- 0.1 degrees C) were realized in a second waveguide, employed to perform sham exposures. The induction of DNA damage was evaluated in leukocytes by applying the alkaline single cell gel electrophoresis (SCGE)/comet assay, while structural chromosome aberrations and sister chromatid exchanges were evaluated in lymphocytes stimulated with phytohemagglutinin. Alterations in kinetics of cell proliferation were determined by calculating the mitotic index. Positive controls were also provided by using methyl methanesulfonate (MMS) for comet assay and mitomycin-C (MMC), for chromosome aberration, or sister chromatid exchange tests. No statistically significant differences were detected in exposed samples in comparison with sham exposed ones for all the parameters investigated. On the contrary, the positive controls gave a statistically significant increase in DNA damage in all cases, as expected. Thus the results obtained in our experimental conditions do not support the hypothesis that 900 MHz radiofrequency field exposure induces DNA damage in human peripheral blood leukocytes in this range of SAR

- 12 **Chemeris, NK, Gapeyev, AB, Sirota, NP, Gudkova, OY, Kornienko, NV, Tankanag, AV, Konovalov, IV, Buzoverya, ME, Suvorov, VG and Logunov, VA (2004). DNA damage in frog**

erythrocytes after in vitro exposure to a high peak-power pulsed electromagnetic field 5. Mutat.Res. 558: 27-34.

Abstrakt:

Till the present time, the genotoxic effects of high peak-power pulsed electromagnetic fields (HPPP EMF) on cultured cells have not been studied. We investigated possible genotoxic effects of HPPP EMF (8.8 GHz, 180 ns pulse width, peak power 65 kW, repetition rate 50 Hz) on erythrocytes of the frog *Xenopus laevis*. We used the alkaline comet assay, which is a highly sensitive method to assess DNA single-strand breaks and alkali-labile lesions. Blood samples were exposed to HPPP EMF for 40 min in rectangular wave guide. The specific absorption rate (SAR) calculated from temperature kinetics was about 1.6 kW/kg (peak SAR was about 300 MW/kg). The temperature rise in the blood samples at steady state was 3.5 +/- 0.1 degrees C. The data show that the increase in DNA damage after exposure of erythrocytes to HPPP EMF was induced by the rise in temperature in the exposed cell suspension. This was confirmed in experiments in which cells were incubated for 40 min under the corresponding temperature conditions. The results allow us to conclude that HPPP EMF-exposure at the given modality did not cause any a-thermal genotoxic effect on frog erythrocytes in vitro

- 13 **Vijayalaxmi, Mohan, N, Meltz, ML and Wittler, MA (1997). Proliferation and cytogenetic studies in human blood lymphocytes exposed in vitro to 2450 MHz radiofrequency radiation. Int.J.Radiat.Biol. 72: 751-7.**

Abstrakt:

Aliquots of human peripheral blood collected from two healthy human volunteers were exposed in vitro to continuous wave 2450 MHz radiofrequency radiation (RFR), either continuously for a period of 90 min or intermittently for a total exposure period of 90 min (30 min on and 30 min off, repeated three times). Blood aliquots which were sham-exposed or exposed in vitro to 150 cGy gamma radiation served as controls. The continuous wave 2450 MHz RFR was generated with a net forward power of 34.5 W and transmitted from a standard gain rectangular antenna horn in a vertically downward direction. The mean power density at the position of the cells was 5.0 mW/cm². The mean specific absorption rate calculated by Finite Difference Time Domain analysis was 12.46 W/kg. Immediately after exposure, lymphocytes were cultured for 48 and 72 h to determine the incidence of chromosomal aberrations and micronuclei, respectively. Proliferation indices were also recorded. There were no significant differences between RFR-exposed and sham-exposed lymphocytes with respect to; (a) mitotic indices; (b) incidence of cells showing chromosome damage; (c) exchange aberrations; (d) acentric fragments; (e) binucleate lymphocytes, and (f) micronuclei, for either the continuous or intermittent RFR exposures. In contrast, the response of positive control cells exposed to 150 cGy gamma radiation was significantly different from RFR-exposed and sham-exposed lymphocytes. Thus, there is no evidence for an effect on mitogen-stimulated proliferation kinetics or for excess genotoxicity within 72 h in human blood lymphocytes exposed in vitro to 2450 MHz RFR

- 14 **Vijayalaxmi, Pickard, WF, Bisht, KS, Prihoda, TJ, Meltz, ML, LaRegina, MC, Roti Roti, JL, Straube, WL and Moros, EG (2001). Micronuclei in the peripheral blood and bone marrow cells of rats exposed to 2450 MHz radiofrequency radiation 1. Int J.Radiat.Biol. 77: 1109-15.**

Abstrakt:

PURPOSE: To determine the incidence of micronuclei in peripheral blood and bone marrow cells of rats exposed continuously for 24h to 2450 MHz continuous wave radiofrequency radiation (RFR) at an average whole-body specific absorption rate (SAR) of 12W/kg.

MATERIALS AND METHODS: Eight adult male Sprague-Dawley rats were exposed to 2450 MHz RFR in circularly polarized waveguides. Eight sham-exposed rats were kept in similar waveguides without the transmission of RFR. Four rats were treated with mitomycin-C (MMC) and used as positive controls. All rats were necropsied 24h after the end of RFR and sham exposures, and after the 24h treatment with MMC. Peripheral blood and bone marrow smears were examined to determine the frequency of micronuclei (MN) in polychromatic erythrocytes (PCE). **RESULTS:** The results indicated that the incidence of MN/2000 PCE were not significantly different between RFR- and sham-exposed rats. The group mean frequencies of MN in the peripheral blood were 2.3+/-0.7 in RFR-exposed rats and 2.1+/-0.6 in sham-exposed rats. In bone marrow cells, the average MN incidence was 3.8+/-1.0 in RFR-exposed rats and 3.4+/-0.7 in sham-exposed rats. The corresponding values in positive control rats treated with MMC were 23.5+/-4.7 in the peripheral blood and 33.8+/-7.4 in bone marrow cells. **CONCLUSION:** There was no evidence for the induction of MN in peripheral blood and bone marrow cells of rats exposed for 24h to 2450 MHz continuous wave RFR at a whole body average SAR of 12 W/kg

- 15 **Vijayalaxmi, LBZ, Meltz, ML, Pickard, WF, Bisht, KS, Roti Roti, JL, Straube, W and Moros, EG (2001). Cytogenetic studies in human blood lymphocytes exposed in vitro to radiofrequency radiation at a cellular telephone frequency (835.62 MHz, FDMA). Radiation Research. 155: 113-21.**

Abstrakt:

Freshly collected peripheral blood samples from four healthy human volunteers were diluted with RPMI 1640 tissue culture medium and exposed in sterile T-75 tissue culture flasks in vitro for 24 h to 835.62 MHz radiofrequency (RF) radiation, a frequency employed for customer-to-base station transmission of cellular telephone communications. An analog signal was used, and the access technology was frequency division multiple access (FDMA, continuous wave). A nominal net forward power of 68 W was used, and the nominal power density at the center of the exposure flask was 860 W/m². The mean specific absorption rate in the exposure flask was 4.4 or 5.0 W/kg. Aliquots of diluted blood that were sham-exposed or exposed in vitro to an acute dose of 1.50 Gy of gamma radiation were used as negative or positive controls. Immediately after the exposures, the lymphocytes were stimulated with a mitogen, phytohemagglutinin, and cultured for 48 or 72 h to determine the extent of genetic damage, as assessed from the frequencies of chromosomal aberrations and micronuclei. The extent of alteration in the kinetics of cell proliferation was determined from the mitotic indices in 48-h cultures and from the incidence of binucleate cells in 72-h cultures. The data indicated no significant differences between RF-radiation- and sham-exposed lymphocytes with respect to mitotic indices, incidence of exchange aberrations, excess fragments, binucleate cells, and micronuclei. In contrast, the response of the lymphocytes exposed to gamma radiation was significantly different from both RF-radiation- and sham-exposed cells for all of these indices. Thus, under the experimental conditions tested, there is no evidence for the induction of chromosomal aberrations and micronuclei in human blood lymphocytes exposed in vitro for 24 h to 835.62 MHz RF radiation at SARs of 4.4 or 5.0 W/kg.

- 16 **McNamee, JP, Bellier, PV, Gajda, GB, Lavallee, BF, Lemay, EP, Marro, L and Thansandote, A (2002). DNA damage in human leukocytes after acute in vitro exposure to a 1.9 GHz pulse-modulated radiofrequency field. Radiation Research. 158: 534-7.**

Abstrakt:

Blood cultures from human volunteers were exposed to an acute 1.9 GHz pulse-modulated radiofrequency (RF) field for 2 h using a series of six circularly polarized, cylindrical

waveguides. Mean specific absorption rates (SARs) ranged from 0 to 10 W/kg, and the temperature within the cultures during the exposure was maintained at 37.0 +/- 0.5 degrees C. DNA damage was quantified in leukocytes by the alkaline comet assay and the cytokinesis-block micronucleus assay. When compared to the sham-treated controls, no evidence of increased primary DNA damage was detected by any parameter for any of the RF-field-exposed cultures when evaluated using the alkaline comet assay. Furthermore, no significant differences in the frequency of binucleated cells, incidence of micronucleated binucleated cells, or total incidence of micronuclei were detected between any of the RF-field-exposed cultures and the sham-treated control at any SAR tested. These results do not support the hypothesis that acute, nonthermalizing 1.9 GHz pulse-modulated RF-field exposure causes DNA damage in cultured human leukocytes

- 17 **McNamee, JP, Bellier, PV, Gajda, GB, Miller, SM, Lemay, EP, Lavallee, BF, Marro, L and Thansandote, A (2002). DNA damage and micronucleus induction in human leukocytes after acute in vitro exposure to a 1.9 GHz continuous-wave radiofrequency field. Radiation Research. 158: 523-33.**

Abstrakt:

Human blood cultures were exposed to a 1.9 GHz continuous-wave (CW) radiofrequency (RF) field for 2 h using a series of six circularly polarized, cylindrical waveguides. Mean specific absorption rates (SARs) of 0.0, 0.1, 0.26, 0.92, 2.4 and 10 W/kg were achieved, and the temperature within the cultures during a 2-h exposure was maintained at 37.0 +/- 0.5 degrees C. Concurrent negative (incubator) and positive (1.5 Gy (¹³⁷Cs gamma radiation) control cultures were run for each experiment. DNA damage was quantified immediately after RF-field exposure using the alkaline comet assay, and four parameters (tail ratio, tail moment, comet length and tail length) were used to assess DNA damage for each comet. No evidence of increased primary DNA damage was detected by any parameter for RF-field-exposed cultures at any SAR tested. The formation of micronuclei in the RF-field-exposed blood cell cultures was assessed using the cytokinesis-block micronucleus assay. There was no significant difference in the binucleated cell frequency, incidence of micronucleated binucleated cells, or total incidence of micronuclei between any of the RF-field-exposed cultures and the sham-exposed controls at any SAR tested. These results do not support the hypothesis that acute, nonthermalizing 1.9 GHz CW RF-field exposure causes DNA damage in cultured human leukocytes

- 18 **Tice, RR, Hook, GG, Donner, M, McRee, DI and Guy, AW (2002). Genotoxicity of radiofrequency signals. I. Investigation of DNA damage and micronuclei induction in cultured human blood cells. Bioelectromagnetics. 23: 113-26.**

Abstrakt:

As part of a comprehensive investigation of the potential genotoxicity of radiofrequency (RF) signals emitted by cellular telephones, in vitro studies evaluated the induction of DNA and chromosomal damage in human blood leukocytes and lymphocytes, respectively. The signals were voice modulated 837 MHz produced by an analog signal generator or by a time division multiple access (TDMA) cellular telephone, 837 MHz generated by a code division multiple access (CDMA) cellular telephone (not voice modulated), and voice modulated 1909.8 MHz generated by a global system of mobile communication (GSM)-type personal communication systems (PCS) cellular telephone. DNA damage (strand breaks/alkali labile sites) was assessed in leukocytes using the alkaline (pH>13) single cell gel electrophoresis (SCG) assay. Chromosomal damage was evaluated in lymphocytes mitogenically stimulated to divide postexposure using the cytochalasin B-binucleate cell micronucleus assay. Cells were exposed at 37+/-1 degrees C, for 3 or 24 h at average specific absorption rates (SARs) of

1.0-10.0 W/kg. Exposure for either 3 or 24 h did not induce a significant increase in DNA damage in leukocytes, nor did exposure for 3 h induce a significant increase in micronucleated cells among lymphocytes. However, exposure to each of the four RF signal technologies for 24 h at an average SAR of 5.0 or 10.0 W/kg resulted in a significant and reproducible increase in the frequency of micronucleated lymphocytes. The magnitude of the response (approximately four fold) was independent of the technology, the presence or absence of voice modulation, and the frequency (837 vs. 1909.8 MHz). This research demonstrates that, under extended exposure conditions, RF signals at an average SAR of at least 5.0 W/kg are capable of inducing chromosomal damage in human lymphocytes

- 19 **Zeni, O, Schiavoni, A, Perrotta, A, Forigo, D, Deplano, M and Scarfi, MR (2008). Evaluation of genotoxic effects in human leukocytes after in vitro exposure to 1950 MHz UMTS radiofrequency field. Bioelectromagnetics. 29: 177-84.**

Abstrakt:

In the present study the third generation wireless technology of the Universal Mobile Telecommunication System (UMTS) signal was investigated for the induction of genotoxic effects in human leukocytes. Peripheral blood from six healthy donors was used and, for each donor, intermittent exposures (6 min RF on, 2 h RF off) at the frequency of 1950 MHz were conducted at a specific absorption rate of 2.2 W/kg. The exposures were performed in a transverse electro magnetic (TEM) cell hosted in an incubator under strictly controlled conditions of temperature and dosimetry. Following long duration intermittent RF exposures (from 24 to 68 h) in different stages of the cell cycle, micronucleus formation was evaluated by applying the cytokinesis block micronucleus assay, which also provides information on cell division kinetics. Primary DNA damage (strand breaks/alkali labile sites) was also investigated following 24 h of intermittent RF exposures, by applying the alkaline single cell gel electrophoresis (SCG)/comet assay. Positive controls were included by treating cell cultures with Mitomycin-C and methylmethanesulfonate for micronucleus and comet assays, respectively. The results obtained indicate that intermittent exposures of human lymphocytes in different stages of cell cycle do not induce either an increase in micronucleated cells, or change in cell cycle kinetics; moreover, 24 h intermittent exposures also fail to affect DNA structure of human leukocytes soon after the exposures, likely indicating that repairable DNA damage was not induced

- 20 **Stronati, L, Testa, A, Moquet, J, Edwards, A, Cordelli, E, Villani, P, Marino, C, Fresegna, AM, Appolloni, M and Lloyd, D (2006). 935 MHz cellular phone radiation. An in vitro study of genotoxicity in human lymphocytes. Int.J.Radiat.Biol. 82: 339-46.**

Abstrakt:

PURPOSE: The possibility of genotoxicity of radiofrequency radiation (RFR) applied alone or in combination with x-rays was investigated in vitro using several assays on human lymphocytes. The chosen specific absorption rate (SAR) values are near the upper limit of actual energy absorption in localized tissue when persons use some cellular telephones. The purpose of the combined exposures was to examine whether RFR might act epigenetically by reducing the fidelity of repair of DNA damage caused by a well-characterized and established mutagen. METHODS: Blood specimens from 14 donors were exposed continuously for 24 h to a Global System for Mobile Communications (GSM) basic 935 MHz signal. The signal was applied at two SAR; 1 and 2 W/Kg, alone or combined with a 1-min exposure to 1.0 Gy of 250 kVp x-rays given immediately before or after the RFR. The assays employed were the alkaline comet technique to detect DNA strand breakage, metaphase analyses to detect unstable chromosomal aberrations and sister chromatid exchanges, micronuclei in cytokinesis-blocked binucleate lymphocytes and the nuclear division index to detect alterations in the speed of in

vitro cell cycling. RESULTS: By comparison with appropriate sham-exposed and control samples, no effect of RFR alone could be found for any of the assay endpoints. In addition RFR did not modify any measured effects of the x-radiation. CONCLUSIONS: This study has used several standard in vitro tests for chromosomal and DNA damage in Go human lymphocytes exposed in vitro to a combination of x-rays and RFR. It has comprehensively examined whether a 24-h continuous exposure to a 935 MHz GSM basic signal delivering SAR of 1 or 2 W/Kg is genotoxic per se or whether, it can influence the genotoxicity of the well-established clastogenic agent; x-radiation. Within the experimental parameters of the study in all instances no effect from the RFR signal was observed

- 21 **Vijayalaxmi, LBZ (2006). Cytogenetic studies in human blood lymphocytes exposed in vitro to 2.45 GHz or 8.2 GHz radiofrequency radiation. Radiation Research. 166: 532-8.**

Abstrakt:

Peripheral blood samples collected from healthy human volunteers were exposed in vitro to 2.45 GHz or 8.2 GHz pulsed-wave radiofrequency (RF) radiation. The net forward power, average power density, mean specific absorption rate, and the temperature maintained during the 2-h exposure of the cells to 2.45 GHz or 8.2 GHz were, respectively, 21 W or 60 W, 5 mW/cm² or 10 mW/cm², 2.13 W/kg or 20.71 W/kg, and 36.9 +/- 0.1 degrees C or 37.5 +/- 0.2 degrees C. Aliquots of the same blood samples that were either sham-exposed or exposed in vitro to an acute dose of 1.5 Gy gamma radiation were used as unexposed and positive controls, respectively. Cultured lymphocytes were examined to determine the extent of cytogenetic damage assessed from the incidence of chromosomal aberrations and micronuclei. Under the conditions used to perform the experiments, the levels of damage in RF-radiation-exposed and sham-exposed lymphocytes were not significantly different. Also, there were no significant differences in the response of unstimulated lymphocytes and lymphocytes stimulated with phytohemagglutinin when exposed to 8.2 GHz RF radiation. In contrast, the positive control cells that had been subjected to gamma irradiation exhibited significantly more damage than RF-radiation- and sham-exposed lymphocytes.

- 22 **Zhijian, C, Xiaoxue, L, Yezhen, L, Shijie, C, Lifen, J, Jianlin, L, Deqiang, L and Jiliang, H (2009). Impact of 1.8-GHz radiofrequency radiation (RFR) on DNA damage and repair induced by doxorubicin in human B-cell lymphoblastoid cells. Mutat Res.**

Abstrakt:

In the present in vitro study, a comet assay was used to determine whether 1.8-GHz radiofrequency radiation (RFR, SAR of 2W/kg) can influence DNA repair in human B-cell lymphoblastoid cells exposed to doxorubicin (DOX) at the doses of 0µg/ml, 0.05µg/ml, 0.075µg/ml, 0.10µg/ml, 0.15µg/ml and 0.20µg/ml. The combinative exposures to RFR with DOX were divided into five categories. DNA damage was detected at 0h, 6h, 12h, 18h and 24h after exposure to DOX via the comet assay, and the percent of DNA in the tail (% tail DNA) served as the indicator of DNA damage. The results demonstrated that (1) RFR could not directly induce DNA damage of human B-cell lymphoblastoid cells; (2) DOX could significantly induce DNA damage of human B-cell lymphoblastoid cells with the dose-effect relationship, and there were special repair characteristics of DNA damage induced by DOX; (3) E-E-E type (exposure to RFR for 2h, then simultaneous exposure to RFR and DOX, and exposure to RFR for 6h, 12h, 18h and 24h after exposure to DOX) combinative exposure could obviously influence DNA repair at 6h and 12h after exposure to DOX for four DOX doses (0.075µg/ml, 0.10µg/ml, 0.15µg/ml and 0.20µg/ml) in human B-cell lymphoblastoid cells.

- 23 **Kim, JY, Hong, SY, Lee, YM, Yu, SA, Koh, WS, Hong, JR, Son, T, Chang, SK and Lee, M (2008). In vitro assessment of clastogenicity of mobile-phone radiation (835 MHz) using the alkaline comet assay and chromosomal aberration test. *Environ.Toxicol.* 23: 319-27.**

Abstrakt:

Recently we demonstrated that 835-MHz radiofrequency radiation electromagnetic fields (RF-EMF) neither affected the reverse mutation frequency nor accelerated DNA degradation in vitro. Here, two kinds of cytogenetic endpoints were further investigated on mammalian cells exposed to 835-MHz RF-EMF (the most widely used communication frequency band in Korean CDMA mobile phone networks) alone and in combination with model clastogens: in vitro alkaline comet assay and in vitro chromosome aberration (CA) test. No direct cytogenetic effect of 835-MHz RF-EMF was found in the in vitro CA test. The combined exposure of the cells to RF-EMF in the presence of ethylmethanesulfonate (EMS) revealed a weak and insignificant cytogenetic effect when compared to cells exposed to EMS alone in CA test. Also, the comet assay results to evaluate the ability of RF-EMF alone to damage DNA were nearly negative, although showing a small increase in tail moment. However, the applied RF-EMF had potentiation effect in comet assay when administered in combination with model clastogens (cyclophosphamide or 4-nitroquinoline 1-oxide). Thus, our results imply that we cannot confidently exclude any possibility of an increased risk of genetic damage, with important implications for the possible health effects of exposure to 835-MHz electromagnetic fields

- 24 **Marinelli, F, La, SD, Cicciootti, G, Cattini, L, Trimarchi, C, Putti, S, Zamparelli, A, Giuliani, L, Tomassetti, G and Cinti, C (2004). Exposure to 900 MHz electromagnetic field induces an unbalance between pro-apoptotic and pro-survival signals in T-lymphoblastoid leukemia CCRF-CEM cells. *J.Cell Physiol.* 198: 324-32.**

Abstrakt:

It has been recently established that low-frequency electromagnetic field (EMFs) exposure induces biological changes and could be associated with increased incidence of cancer, while the issue remains unresolved as to whether high-frequency EMFs can have hazardous effect on health. Epidemiological studies on association between childhood cancers, particularly leukemia and brain cancer, and exposure to low- and high-frequency EMF suggested an etiological role of EMFs in inducing adverse health effects. To investigate whether exposure to high-frequency EMFs could affect in vitro cell survival, we cultured acute T-lymphoblastoid leukemia cells (CCRF-CEM) in the presence of unmodulated 900 MHz EMF, generated by a transverse electromagnetic (TEM) cell, at various exposure times. We evaluated the effects of high-frequency EMF on cell growth rate and apoptosis induction, by cell viability (MTT) test, FACS analysis and DNA ladder, and we investigated pro-apoptotic and pro-survival signaling pathways possibly involved as a function of exposure time by Western blot analysis. At short exposure times (2-12 h), unmodulated 900 MHz EMF induced DNA breaks and early activation of both p53-dependent and -independent apoptotic pathways while longer continuous exposure (24-48 h) determined silencing of pro-apoptotic signals and activation of genes involved in both intracellular (Bcl-2) and extracellular (Ras and Akt1) pro-survival signaling. Overall our results indicate that exposure to 900 MHz continuous wave, after inducing an early self-defense response triggered by DNA damage, could confer to the survivor CCRF-CEM cells a further advantage to survive and proliferate

- 25 **Kesari, KK, Behari, J and Kumar, S (2010). Mutagenic response of 2.45 GHz radiation exposure on rat brain. *Int J Radiat Biol.* 86: 334-43.**

Abstrakt:

PURPOSE: To investigate the effect of 2.45 GHz microwave radiation on rat brain of male wistar strain. **MATERIAL AND METHODS:** Male rats of wistar strain (35 days old with 130 +/- 10 g body weight) were selected for this study. Animals were divided into two groups: Sham exposed and experimental. Animals were exposed for 2 h a day for 35 days to 2.45 GHz frequency at 0.34 mW/cm² power density. The whole body specific absorption rate (SAR) was estimated to be 0.11 W/Kg. Exposure took place in a ventilated Plexiglas cage and kept in anechoic chamber in a far field configuration from the horn antenna. After the completion of exposure period, rats were sacrificed and the whole brain tissue was dissected and used for study of double strand DNA (Deoxyribonucleic acid) breaks by micro gel electrophoresis and the statistical analysis was carried out using comet assay (IV-2 version software). Thereafter, antioxidant enzymes and histone kinase estimation was also performed. **RESULTS:** A significant increase was observed in comet head ($P < 0.002$), tail length ($P < 0.0002$) and in tail movement ($P < 0.0001$) in exposed brain cells. An analysis of antioxidant enzymes glutathione peroxidase ($P < 0.005$), and superoxide dismutase ($P < 0.006$) showed a decrease while an increase in catalase ($P < 0.006$) was observed. A significant decrease ($P < 0.023$) in histone kinase was also recorded in the exposed group as compared to the control (sham-exposed) ones. One-way analysis of variance (ANOVA) method was adopted for statistical analysis. **CONCLUSION:** The study concludes that the chronic exposure to these radiations may cause significant damage to brain, which may be an indication of possible tumour promotion (Behari and Paulraj 2007).

- 26 **Guler, G, Tomruk, A, Ozgur, E and Seyhan, N (2010). The effect of radiofrequency radiation on DNA and lipid damage in non-pregnant and pregnant rabbits and their newborns. Gen Physiol Biophys. 29: 59-66.**

Abstrakt:

The concerns of people on possible adverse health effects of radiofrequency radiation (RFR) generated from mobile phones as well as their supporting transmitters (base stations) have increased markedly. RFR effect on oversensitive people, such as pregnant women and their developing fetuses, and older people is another source of concern that should be considered. In this study, oxidative DNA damage and lipid peroxidation levels in the brain tissue of pregnant and non-pregnant New Zealand White rabbits and their newborns exposed to RFR were investigated. Thirteen-month-old rabbits were studied in four groups as non-pregnant-control, non-pregnant-RFR exposed, pregnant-control and pregnant-RFR exposed. They were exposed to RFR (1800 MHz GSM; 14 V/m as reference level) for 15 min/day during 7 days. Malondialdehyde (MDA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels were analyzed. MDA and 8-OHdG levels of non-pregnant and pregnant-RFR exposed animals significantly increased with respect to controls ($p < 0.001$, Mann-Whitney test). No difference was found in the newborns ($p > 0.05$, Mann-Whitney). There exist very few experimental studies on the effects of RFR during pregnancy. It would be beneficial to increase the number of these studies in order to establish international standards for the protection of pregnant women from RFR.

- 27 **Tomruk, A, Guler, G and Dincel, AS (2010). The influence of 1800 MHz GSM-like signals on hepatic oxidative DNA and lipid damage in nonpregnant, pregnant, and newly born rabbits. Cell Biochem Biophys. 56: 39-47.**

Abstrakt:

The aim of our study is to evaluate the possible biological effects of whole-body 1800 MHz GSM-like radiofrequency (RF) radiation exposure on liver oxidative DNA damage and lipid peroxidation levels in nonpregnant, pregnant New Zealand White rabbits, and in their newly borns. Eighteen nonpregnant and pregnant rabbits were used and randomly divided into four

groups which were composed of nine rabbits: (i) Group I (nonpregnant control), (ii) Group II (nonpregnant-RF exposed), (iii) Group III (pregnant control), (iv) Group IV (pregnant-RF exposed). Newborns of the pregnant rabbits were also divided into two groups: (v) Group V (newborns of Group III) and (vi) Group VI (newborns of Group III). 1800 MHz GSM-like RF radiation whole-body exposure (15 min/day for a week) was applied to Group II and Group IV. No significant differences were found in liver 8 OHdG/10(6) dG levels of exposure groups (Group II and Group IV) compared to controls (Group I and Group III). However, in Group II and Group IV malondialdehyde (MDA) and ferrous oxidation in xylenol orange (FOX) levels were increased compared to Group I ($P < 0.05$, Mann-Whitney). No significant differences were found in liver tissue of 8 OHdG/10(6) dG and MDA levels between Group VI and Group V ($P > 0.05$, Mann-Whitney) while liver FOX levels were found significantly increased in Group VI with respect to Group V ($P < 0.05$, Mann-Whitney). Consequently, the whole-body 1800 MHz GSM-like RF radiation exposure may lead to oxidative destruction as being indicators of subsequent reactions that occur to form oxygen toxicity in tissues.