German Mobile Telecommunication Research Programme

"Genotoxic effects of HF-signals on human cells"

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HF-induced effects

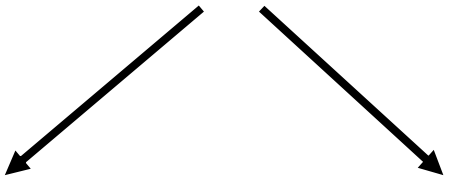
thermal:

established mechanism for health effects (at high exposure levels)

nonthermal:

photon energy at 1 GHz is 4 µeV

but ~ 1eV is required to ionize molecules



no direct DNA lesions

mechanisms leading indirectly to genotoxic events

Mechanisms for interaction between RF fields and biological tissue [Challis, 2005]

- changes in protein conformation
- changes in ligand binding
- resonant absorption of HF energy by cellular components
- demodulation by cellular membranes

EF effects

- radical pairs (dissociation/recombination)

MF effects

HF-induced genotoxic effects in studies between 1990 - 2003 [Vijayalaxmi & Obe, 2004]:

58 % negative
23 % positive (strand breaks, chrom. aberrations, micronuclei, SCE)
19 % inconclusive

HF-induced genotoxic effects in studies of the REFLEX project:

Prof. R. Tauber, Universitätsklinikum Benjamin Franklin, Berlin

1.8 GHz CW, intermittent exposure	HL-60 cells	comet assay, micronucleus test	time- and dose-related window effects

Prof. H.W. Rüdiger, Universitätsklinik für Innere Medizin, Vienna

1.8 GHz CW, intermittent exposure	fibroblasts	comet assay,	dose-related window effects (SAR ≤ 1 W/kg)

HF-induced genotoxic effects in studies since 2003:

McNamee, 2003	CW, PW	lymphocytes	negative
Koyama, 2003		CHO-K1	positive but no clear dose-response
Mashevich, 2003	CW	lymphocytes	SAR-dependent
Chemeris, 2004	PW	frog erythrocytes	negative
Komatsubara, 2005	CW, PW	mouse m5S cells	negative
Baohong, 2005	CW	lymphocytes	negative
Zotti-Martelli, 2005	CW	lymphocytes	time- and frequency-dependent, no clear dose-response
Diem, 2005	CW, intermitt., div. mod.	fibroblasts, rat granulosa cells	time-dependent
Zeni, 2003, 2005	div. mod.	lymphocytes	negative
Sakuma, 2006	CW, mod.	fibroblasts, glioblastoma cells	negative
Speit, 2006	confirmatory study to Diem, 2005		negative
Schär, still unpublished	same study design as Diem, 2005		(weak but significant time window effect)

HF-induced genotoxic effects in recent studies:

- seem to be cell type specific (underlying mechanism?)

- seem to depend on an appropriate time or dose window (no clear dose-response)

- seem to be hardly reproducible (Due to technical pitfalls? Statistics?)

Assessment of genotoxic effects:

- applying standard procedures (guidelines, published recommendations, ...)

- appropriate technical equipment
  (e.g. image analysis software for comet assays: *CASP*, University of Wroclaw/Poland [Konca, 2003; Garcia, 2007], *NIH Image* based system, University of Vienna/Austria [Helma, 2000])

- calibration of comet assay data to Gy equivalents

- samples/slides processed for long-term storage (enabling reanalysis)

Exposure:

- dosimetry based on SAR values
- concurrent positive controls:
  - > suitable for the genotoxic endpoint (e.g. not MMC for comet assays)
  - > at concentrations/doses yielding submaximum effects
  - > preferebly NIR (UV-A/-B), IR (gamma-rays), radiomimetics (bleomycin, 4-NQO)
- continuous temperature assessment
- standardised (and thoroughly tested) wave guide set-ups

Post-exposure:

- immediately: volume of culture medium (evaporation, hypertonic effects)
- at time-point of preparation for genotoxicity analysis:
  - > % viable cells
  - > % apoptotic cells

Proposed mechanisms leading to genotoxic effects:

- disturbed protein/protein interactions: cell cycle control, mitotic spindle, ... ?
- disturbed protein/DNA interactions: replication machinery, DNA repair, mitosis, histone structure, ... ?
- imbalanced cellular redox status:
  - > elevated ROS formation?
  - > impaired radical recombination?

Proposed mechanisms leading to genotoxic effects:

- disturbed protein/protein interactions: cell cycle control, mitotic spindle, ... ?
- disturbed protein/DNA interactions: replication machine
  mitosis, histone struc
- imbalanced cellular redox status:

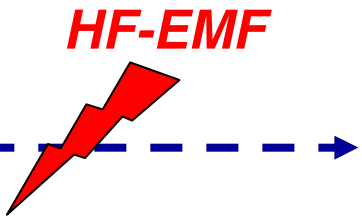
DNA repair

elevated ROS formation

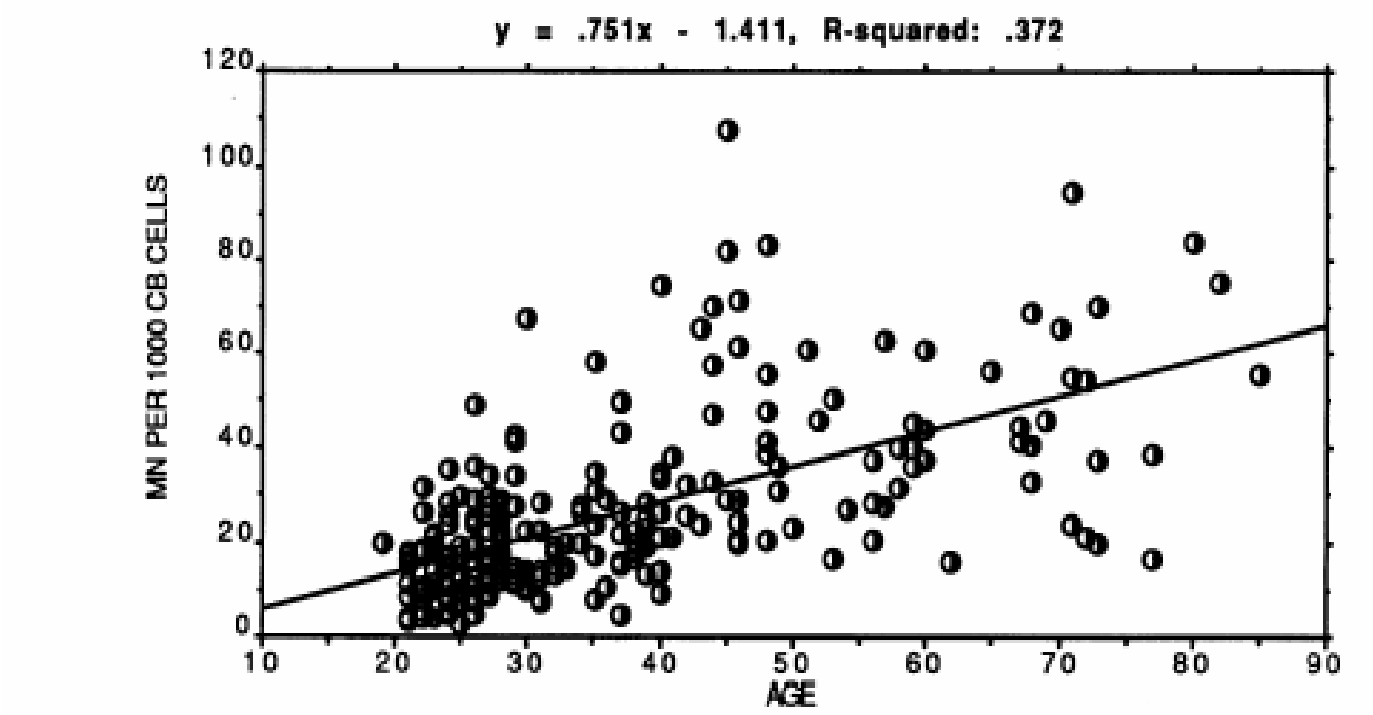
> impaired radical recombination?

Considering impaired DNA repair:

DNA damage (spontaneous or experimentally induced)



DNA repair



Spontaneous micronuclei frequency in cytokinesis-blocked lymphocytes of 224 healthy Australians plotted in relation to the age of the donor. [Fenech, 1993]

Considering impaired DNA repair:

- introducing defined types/levels of DNA damage:

  HF-EMF exposure after treatment with environmentally relevant genotoxicants (UV, oxidants, alkylating agents, ...)

  [Maes, 1997; Koyama, 2003; Baohong, 2005]

Considering free radical formation:

- better defined culture conditions necessary:

  > antioxidants / serum in culture medium?

  > culture conditions limiting oxygen supply:

    culture vessel dimensions
    depth of medium
    cell titer

- more studies necessary involving different oxygen concentrations or antioxidants / free radical scavengers
  (e.g. Lai & Singh, 1997: *Melatonin and a spin-trap compound block RF-EMF radiation-induced DNA strand breaks* ..."

Considering free radical formation:

... this is hardly supported by biophysical model calculations:

Adair, 2003: "... *no significant effects could be expected from weak fields* [*< 10 mW/cm²*] *through* [*magnetic field interaction*]."

Challis, 2005: "*It seems unlikely ... that RF radiation at frequencies of 1 GHz ... could lead to a significant increase in the concentration of free radicals*."

Athermal effects of HF exposure:

Michaelson & Elson, 1996:

"... *the nonreproducibility of results and the nonrobustnes of effects ... seem especially vexatious*."

Postow & Swicord, 1996:

"*There is no consistent repeatable pattern ... of responses*."

Crumpton & Collins, 2004:

"*The lack of independent replication has been a persistant feature of experimental studies looking for biological effects of ... EMF. It remains to be determined whether or not the present reports on DNA damage will be substantiated and whether it will be possible to draw any conclusions* ..."

Athermal effects of HF exposure:

and in 2007?