University of Perugia

DEPARTMENT OF MEDICAL AND SURGICAL SPECIALTIES AND PUBLIC HEALTH

Section of Public Health

– LABORATORY OF GENETIC TOXICOLOGY –

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Subject: In vitro evaluation of **DNA damage** on Jürkat cells exposed to a **mobile phone electromagnetic field**, in the presence and in the absence of the **Geoprotex** device – Result of tests performed on February 11, 2008 and February 12, 2008.

In order to perform an initial *screening* on the genotoxic potential of radiation from a mobile phone [redacted] and the extent of protection from such radiation provided by the **Geoprotex** device, the comet assay was applied to human lymphoblastoid cells (Jürkat).

Jürkat cells, grown in suspension, were cultured at 37°C in a 5% CO_2 humidified atmosphere, in a RPMI-1640 medium supplemented with 10% (inactivated) fetal bovine serum and antibiotics (100 IU/ml penicillin and 100 µg/ml streptomycin).

On February 12, 2008, six subcultures were made from exponentially-growing cultures by seeding $5x10^5$ cells/ml in 25 cm^5 flasks (containing 5 ml of complete RPMI).

Sixteen hours after seeding (on February 12, 2008), cell cultures were exposed continuously for 1 hour to the electromagnetic field of a mobile phone (A) switched on and transmitting (calling another phone number), and (B) switched on and transmitting (calling another phone number) in the presence of the **Geoprotex** device. During these tests, cell cultures were placed next to the receiver (Picture 1), where mobile emissions are maximal. The test also included a negative control (no treatment) and a positive control (1-hour treatment consisting of a known genotoxic compound: ethyl methanosulfonate).

After treatments, cell viability was assessed and the alkaline comet assay was applied (pH>13 pre-electrophoresis and electrophoresis). This procedure allows highlighting the genotoxic effects causing discontinuities in DNA phosphodiesteric backbone. In particular, such discontinuities can result from DNA single-strand breaks (SSBs), from double-strand breaks (DSBs), from the action of excision repair enzyme systems, and, lastly, from alkali-labile alterations (SAL) that are due to the breaks of the DNA molecule if the test is carried out in a basic environment.

Three slides were prepared for each test. Slide reading was performed by means of a fluorescence microscope (20× objective) equipped with a high-resolution CCD camera connected to a computerized image analysis system (Comet Assay III, Perceptive Instruments Ltd., Suffolk, UK). This system can provide an integrated profile of fluorescence intensity for each cell. This profile consists in parameters relating to the quantity of DNA migrated to the anode, i.e. tail length (TL),

tail intensity (TI, the percentage of fluorescence, hence of DNA, migrated to the tail) and tail moment (TM, a parameter resulting from the former two).

The results are shown in Table 1.

Remarks on the results

The investigations performed to evaluate any effects of a mobile phone electromagnetic field on Jürkat cell viability showed that such a field had no cytotoxic effects. As a matter of fact, viability values were always higher than 75% in all tests carried out.

The tests performed to investigate electromagnetic field genotoxicity, if any, in connection with the status of cell functioning, in the presence and in the absence of the **Geoprotex** device, showed the following:

1. TL did not prove to be a sensitive parameter in detecting any DNA damage resulting from mobile phone radiation. DNA breaks caused by radiation were likely to entail the creation of few but large fragments, which migrated to the anode with difficulty if subject to an electrophoretic field.
2. TI allowed instead to distinguish the effects of different exposure protocols:

- when cells were exposed to the electromagnetic field of a transmitting mobile phone (case A), in the absence of the **Geoprotex** device, an evident **genotoxic effect** emerged as **DNA damage** levels were comparable to the effect resulting in the positive control;
- when the mobile phone was switched on and transmitting, the presence of **Geoprotex** device (case B) **significantly reduced DNA damage** values as these were substantially **lower** than in case A and clearly approaching negative control values.

3. TM is an index that considered both tail length (TL) and the percentage quantity of DNA in the tail (TI). In this work, the trends of this parameter were perfectly superimposable to those of TI.

Final considerations

We deem it appropriate to highlight that:

- The **Geoprotex** device proved to be successful in **significantly reducing** the **genotoxic effects** resulting from exposure to the radio frequencies generated by a mobile phone. It reduced primary DNA damage caused by a transmitting mobile phone to levels that were virtually superimposable to negative control levels.

In witness whereof,

Dr. Massimo Moretti
(SIGNATURE)

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