THz Exposure of Whole Blood for the Study of Biological Effects on Human Lymphocytes

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Abstract. The aim of the present study is to investigate the genotoxic effect of THz radiation in human peripheral blood lymphocytes following 20 minutes exposure to 1 mW average power Free Electron Laser radiation in the frequency range 120–140 GHz. For this purpose 9 healthy donors were employed and cytokinesis block technique was applied to study micronucleus frequency and cell proliferation. The results obtained indicate that all the electromagnetic conditions adopted so far do not alter the investigated parameters, suggesting absence of direct chromosomal damage and alteration of cell cycle kinetics (two tailed paired Student's test: $p > 0.05$ in all cases).

Key words: Cell proliferation, genotoxic effects, human lymphocytes, micronuclei, THz radiation

1. Introduction

In this study the potential health hazard induced by electromagnetic radiation in the THz region, the portion of electromagnetic spectrum laying between 100 GHz and 20 THz at the boundary between the microwave and the infrared regions, has been considered. Despite the recent technological applications of THz radiation in biology and biomedicine, which are based on the specific spectroscopic fingerprints of biological matter in this spectral region, very little is known about its interaction with biological systems. Genotoxic effects are one of the most interesting aspects in the risk assessment of human exposure to ionising and non-ionising electromagnetic radiation, due to the close correlation between DNA damage and cancer occurrence [1]. Since lymphocytes are a well-known biological system playing a key role in the defence mechanisms and are easily obtainable from peripheral blood, they have been largely used as a biological model for studying the potential genotoxic effects of such radiation. Among the cytogenetic techniques allowing to test DNA damage at cellular and molecular level induced by various chemical and physical agents, the cytokinesis-block micronucleus (MN) technique is a very sensitive and simple indicator of chromosome damage, which also provides information on cell cycle progression [2–4].

The cytokinesis-block MN technique has been employed in this study to investigate the genotoxic effects on human peripheral blood lymphocytes exposed in Go phase for 20 minutes to 1 mW and 0.6 mW average power Free Electron Laser (FEL) radiation at the two frequency values of 120 and 130 GHz respectively.

2. Materials and Methods

2.1. THE ENEA COMPACT FREE ELECTRON LASER

In a Free Electron Laser (FEL) a beam of high energy electrons interacts with a suitable magnetic structure (undulator) to generate coherent electromagnetic radiation. A Compact FEL Facility is operating at the ENEA Research Center in Frascati, which utilizes a microtron as electron beam source at energies between 2.3 and 5 MeV [5].

For the experiments reported in this work, a permanent magnet undulator with 8 periods of 2.5 cm is used to generate coherent radiation in the frequency range between 90 and 150 GHz. The Compact FEL produces a 'train' of micropulses of about 50 ps duration, with 330 ps spacing between adjacent pulses. The overall duration of the train (macropulse) is 4 μ s. Macropulses can be generated up to a maximum repetition frequency of 20 Hz. Means are provided for adjustment and control of the peak and average power levels, as well as for frequency tuning. A maximum output power of about 1.5 kW in 4 μ s pulses has been obtained at 120 GHz.

The FEL spectrum consists of several emission lines, spaced at 3 GHz intervals corresponding to the period of the driving radio-frequency. The envelope of the emission lines shows a typical relative bandwidth of the FEL around 7%.

The FEL radiation is transported to a dedicated user room by means of a special mm-wave transmission line composed of an evacuated copper light pipe with 25 mm clear aperture and appropriate delivery optics.

2.2. IRRADIATION PROCEDURE

For the genotoxic evaluations on human lymphocytes, a specific THz Delivery System (TDS) has been designed to irradiate whole blood samples. Suitable containers transparent to the THz radiation were investigated, and absorption measurements on whole blood at 120 GHz were performed to determine the optimal thickness of the sample and the irradiation geometry [6]. The value of the absorption coefficient, $\alpha=75 \text{ cm}^{-1}$, measured for whole blood shows that less than 1% of the incident radiation penetrates through 1 mm thickness. The high value of α sets a severe condition on the minimum irradiation area to be used given a minimum useful whole blood volume of 2 ml. An upper limit to the irradiation area is set by the maximum feasible expansion of the THz beam needed to get a uniform and sufficient power density at the sample surface. A compromise between these two conditions was found for a diameter of the irradiation container of about 5 cm.

Table I. Irradiation parameters

Frequency	Bunch duration	Bunch interval	Pulse duration	Pulse rep. rate	Average power	Irradiation time	Delivered energy
120 GHz	50 ps	330 ps	4 μ s	2 Hz	1 mW	20 min.	1.20 J
130 GHz	50 ps	330 ps	4 μ s	2 Hz	0.6 mW	20 min.	0.72 J

Figure 1. Experimental set up showing the sample holder hosting the Petri dish containing human blood during the exposure. a) light pipe, b) TDS, c) right-angle bend, d) Pyroelectric detector.

Falcon Petri dishes (P/N 35.3004) with an internal diameter of 52 mm, containing 2 ml of whole blood, resulted the best choice to perform THz irradiation.

The samples were irradiated from the top of the Petri dish. In presence of blood sedimentation, lymphocytes settle about half way through the sample thickness, with the red blood cells laying underneath and the serum above.

From the absorption measurements carried out at 120 GHz it resulted that the absorption coefficient of whole blood ($\alpha_b = 75 \text{ cm}^{-1}$) is smaller than water and slightly higher than serum ($\alpha_s = 71 \text{ cm}^{-1}$). Since serum is composed for 90% by water, the measured absorption coefficient is consistent with the absorption coefficient of water at 120 GHz ($\alpha_w = 80 \text{ cm}^{-1}$). The above measurements also imply an absorption coefficient of about 80 cm^{-1} for hydrated red cells. One can therefore observe that, in a sedimented sample, the intensity of the radiation at the lymphocyte layer will be slightly higher when irradiating the sample from the top rather than from the bottom.

Two different FEL frequencies have been tested: 120 and 130 GHz (see Table I). The THz radiation is monitored by a calibrated pyroelectric detector that measures the transmitted power and allows to calculate the total energy delivered in a given irradiation time taking into account the macropulse repetition frequency. In Figure 1 a photograph of the experimental apparatus is shown, with the detector monitoring the radiation transmitted through the whole blood sample. Values of the electric field amplitude at the lymphocyte layer in the adopted irradiation conditions are reported in [6].

2.3. BIOLOGICAL PROCEDURE

Blood samples from 9 healthy subjects aged between 30 and 45 years (mean age: 39.33 ± 4.82 years) were collected at ENEA Occupational Health Unit. From each blood sample two Petri dishes were set-up containing 2 ml each: one to be exposed, the other to be sham exposed. From each of them a lymphocyte culture was set up; a third culture was also set up as a control. Blood samples from 6 donors were employed to perform experiments at 120 GHz, while blood samples from the remaining 3 donors were employed for exposure at 130 GHz.

Lymphocyte cultures were set-up by adding 0.8 ml whole blood to 9.2 ml of RPMI medium supplemented with 15% heat-inactivated foetal calf serum, 2 mM L-Glutamine and 100 μ l of Phytohemagglutinin as mitogen.

In order to block cytokinesis, 44 hours after PHA stimulation, Cytochalasin-B (2 mg/ml in DMSO) was added to lymphocyte cultures to give a final concentration of 6 μ g/ml. 72 h after PHA stimulation, cells were collected and processed for slides preparation as follows: after gentle homogenisation of the cell suspension, 1 ml cell culture was centrifuged and the cell pellet was resuspended in 3 ml of lysis buffer (10 mM EDTA, 155 mM NH_4Cl and 10 mM KHCO_3) and left for 7 min at room temperature, to break erythrocytes. Lymphocytes were then washed three times in RPMI + 2% FCS (wash medium), swelled by a soft hypotonic solution (one part of wash medium and four parts of distilled water) for 15 min and spun down onto slides using a cytocentrifuge (Cytospin, Shandon; 7 min at 1200 rpm). After fixation (80% methanol in aqueous solution for 10 min) and staining (5% Giemsa in phosphate buffer, pH = 6.8 for 10 min) coded slides were scored blindly and MN were counted in binucleated cytokinesis-blocked cells with a light microscope at $1250\times$ magnification following standard criteria. For each subject/treatment 1000 binucleated cells with well preserved cytoplasm were examined from various slides.

On the same slides cell proliferation was also evaluated by scoring the number of nuclei in 500 cells and determining the cytokinesis-block proliferation index (CBPI): it measures the mean number of cell cycles per cell and is defined as follows: $\text{CBPI} = [\text{M1} + 2\text{M2} + 3(\text{M3} + \text{M4})] / \text{N}$ where M1 to M4 indicate the number of cells with 1 to 4 nuclei respectively, and N the total number of cells scored [7].

Figure 2. Micronucleus frequency in control, sham exposed and exposed human lymphocyte cultures. Data are reported for 120 GHz (6 subjects) and 130 GHz (3 subjects) exposure frequency.

Figure 3. Cytokinesis block proliferation index in control, sham exposed and exposed human lymphocyte cultures. Data are reported for 120 GHz (6 subjects) and 130 GHz (3 subjects) exposure frequency.

In order to compare each exposed sample with its own control, two tailed paired Student's *t* test was applied to compare unexposed and exposed cultures as far as MN frequency and CBPI are concerned. Differences were considered to be significant at $p < 0.05$.

Results and Discussion

The effect of 20 minutes exposure on blood samples from 9 healthy subjects, 6 exposed to 120 GHz and 3 exposed to 130 GHz, are depicted in Figures 2 and 3 for micronucleus frequency and cell cycle kinetics, respectively, where results are reported for control, sham-exposed and exposed cultures. It appears that the environmental conditions at FEL laboratory do not influence the MN induction at both 120 and 130 GHz, as shown by comparing control and sham exposed cultures ($p = 0.380$ and $p = 0.260$ for 120 and 130 GHz respectively). Performing the same comparison, cell cycle kinetics also results unaffected ($p = 0.724$ and $p = 0.500$ for 120 and 130 GHz respectively).

Concerning the induction of genotoxic effects, the results obtained indicate that such radiation, in the experimental conditions adopted, does not affect micronucleus formation as shown by comparing sham exposed cultures with exposed ones, at both frequencies employed ($p = 0.182$ and $p = 0.43$ for 120 and 130 GHz, respectively). The same comparison doesn't show any effect also in terms of CBPI ($p = 0.133$ and $p = 0.50$ for 120 and 130 GHz respectively).

The study of the potential health hazards of EMF in this spectral region exerts a great relevance as a preventive instead of a reactive research, taking into account the present and rapidly increasing applications in biology and biomedicine such as THz imaging. The results presented here are particularly interesting since, to our knowledge, this is the first time that the THz radiation is investigated as potential genotoxic agent by applying the MN technique. The limited number of subjects considered, at least in the case of 130 GHz, does not yet allow definitive conclusions. Ongoing studies on a larger number of individuals should provide further, more complete information on the topic.

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References

1. Juutilainen, J. and Lang, S: Genotoxic, Carcinogenic and Teratogenic Effects of Electromagnetic Fields. Introduction and Overview, *Mutation Res.* **387** (1997), 165–171.
2. Fenech, M.: The Cytokinesis-Block Micronucleus Technique: A detailed Description of the Method and its Application to Genotoxicity Studies in Human population, *Mutation Res.* **285** (1993), 35–44.
3. Tice, R.R., Hook, G.G., Donner, M., McRee, D.I. and Guy, A.W.: Genotoxicity of Radiofrequency Signals. I. Investigation of DNA Damage and Micronuclei Induction in Cultured Human Blood Cells, *Bioelectromagnetics* **23** (2002), 113–126.

4. Scarfi, M.R., Lioi, M.B., Della Noce, M., Zeni, O., Franceschi, C., Monti, D., Castellani and G. Bersani, F.: Exposure to 100 Hz pulsed Magnetic Fields Increase Micronucleus Frequency and Cell Proliferation in Human Lymphocytes, *Bioelectrochem. & Bioenerg.* **43**(1) (1997), 77–81.
5. Gallerano, G.P. Doria, A., Giovenale, E. and Renieri, A.: Compact Free Electron Lasers: From Cerenkov to Waveguide FELs, *Infrared Phys. and Tech.* **40** (1999), 161–174.
6. Giovenale, E., D'Arienzo, M., Doria, A., Gallerano, G.P., Lai, A., Messina, G. and Piccinelli, D.: Absorption and Diffusion Measurements of Biological Samples using a THz Free Electron Laser, Tu-P21 these Proceedings
7. Surralles, J., Xamena, N., Creus, A., Catalan, J., Norppa, H. and Marcos, R.: Induction of Micronuclei by Five Pyrethroid Insecticides in Whole-Blood and Isolated Human Lymphocyte Cultures, *Mutation Res.* **341** (1995), 169–184.