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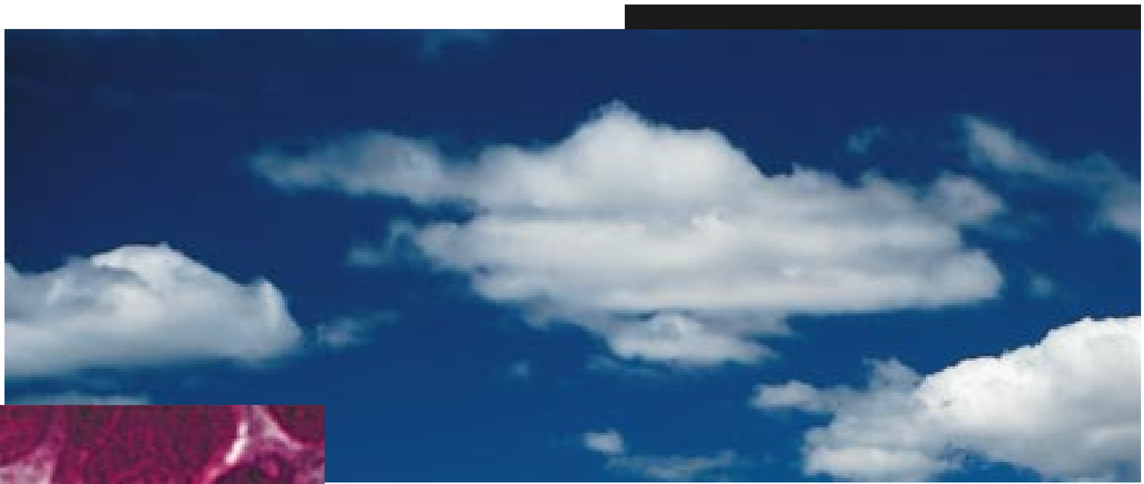

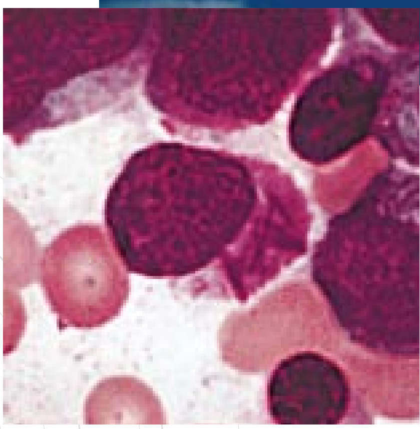

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Growth Behavior of Human Leukemia Cells

Influenced by High-Frequency Electromagnetic Fields (1.8 GHz and 900 MHz, pulsed with 217 Hz each time) for the Investigation Cancer Promoting Effects

R. Fitzner, E. Langer, E. Zemann,
U. Neibig, and K. Brinkmann

Forschungsgemeinschaft Funk

Editorial

Dear Readers,

in 1992 the „Forschungsgemeinschaft Funk“ was founded with the objective to do research on electromagnetic fields and their effects on human beings and our environment. At the same time we stress the importance of making objective information available to the public. Apart from the “Newsletter”, which deals with electromagnetic fields in general, we also publish the “Edition Wissenschaft” focussing on the current state of the scientific research.

The study “Growth Behavior of Human Leukemia HL-60 Cells…“ is part of a series of research projects on “Biological Effects of Radio-Frequency Fields“. The original study was published as “Edition Wissenschaft” No. 1 in 1995.

It is the content of the research project to find out, whether electromagnetic fields can also have athermal effects. These reports are not to be considered as „final reports“; they serve more as a basis for further studies.

Gerd Friedrich

Content

Edition Wissenschaft

Research Project “Biological Effects of Radio-Frequency Fields”

1st Edition:
Growth Behavior of Human Leukemia HL-60 Cells Influenced by High-Frequency Electromagnetic Fields (1.8 GHz and 900 MHz, pulsed with 217 Hz each time) for the Investigation of Cancer Promoting Effects

2nd Edition:
Influence of High-Frequency Electromagnetic Fields of Mobile Communication on the Calcium Homoeostasis of Heart Muscle Cells and Lymphocytes

3rd Edition:
Exposition Devices

4th Edition:
Cell Proliferation, Sister-Chromatide Exchanges, Chromosome Aberrations, Micronuclei and Mutation Rate of the HGPRT-Locus after the Influence of Electromagnetic High-Frequency Fields (440 MHz, 900 MHz and 1.8 GHz) on Human Peripheral Lymphocytes

Biological Effects of Radio-Frequency Fields

Prof. Dr.-Ing. Dr.-Ing. E.h. Karl Brinkmann,
Prof. Dr.-Ing. Rudolf Elsner

In the use of mobile communication equipment the user has to stand the effects of electromagnetic radiation. Therefore it has to be ensured, that he does not endure physical harm. The manufacturer therefore has to keep standard regulations. These standards are based on the knowledge of thermal effects of electromagnetic fields. It is known that with a specific absorption rate (SAR) of 4 W/kg the temperature of animal and therewith presumably also human tissue increases for 1°C [1]. To protect people against such damage, a SAR-limit value of 80 mW/kg is prescribed. If this value is kept thermal effects will certainly not occur.

Lately, however, it has been pointed out, that electromagnetic fields can also have athermal effects. It is the content of our research project to find out, whether such effects occur.

It is useful and usual to observe such effects in the biological behavior of animal or human cells. The cells are placed in a nutritive solution and put in a high-frequency electromagnetic field for a suitable duration. To eliminate environmental influences, a comparable sample is observed at the same time without being put in a high-frequency field. Different behavior of the cells with and without exposition can then only be caused by the influence of the electromagnetic field. Though it has to be left open in this investigation method, whether these effects also cause damage. This could only be solved by experiments at the whole organism. Yet it remains right, that no damage can be present, if no or just negligible effects occur. Examined were: lymphocytes of healthy human donors (workgroup Eberle, Braunschweig), heart muscle cells and lymphocytes (workgroup Meyer, Bonn) and human leukemia cells (workgroup Fitzner, Berlin). The exposition was made at 440 MHz (C-net), 900 MHz (D-net) and 1800 MHz (E-net). In D- and E-frequency band pulse-modulated signals were used. The nutritive solution was kept constant at 37°C ± 0.1°C. The sample holdings with cells, nutritive solution and white oil for keeping the temperature constant were relatively high with dimensions of 10 cm at the workgroups Eberle and Fitzner, at the workgroup Meyer with dimensions of 1 cm correspondingly smaller.

The electromagnetic fields should be utmost homogeneously in their structure without disturbing test objects. Therefore suitable wave guides (workgroup Elsner/Neibig, Braunschweig) were provided. These wave guides are metal closed space, so that the inner high-frequency fields cannot have a disturbing effect outwardly and other fields cannot penetrate and falsify the measuring space.

The capacity which is provided by the wave guide at the place of the test object, is essentially higher than the capacity which is adopted by the nutritive solution. This is due to the electromagnetic characteristics of the solution. The capacity density inside the nutritive solution and therewith also inside the cells cannot be measured. Therefore with the

Introduction of the test tube into the GTEM cell; on the left: equipment to generate and measure the high frequency electromagnetic fields; the permanent control and data recording of the field strenghts is safeguarded by a computer control device.

knowledge of the electromagnetic characteristics of the nutritive solution and the material of the sample holding the electric and magnetic field strength in the nutritive solution could be calculated. From that by means of the electric conductivity the SAR-values result as capacity density of the electromagnetic radiation inside the nutritive solution. The calculated values could only be derived by means of some simplifications. Hence they can differ from the real values for a factor two maximally. The SAR-values should be at 80 mW/kg respectively 80 W/cm³ for all experiments. This could only be obtained in that scale, as for temporal reasons the calculations were made while the biological investigations had already been running. Most of the calculated values are below these standard values.

For the electric and magnetic field strength the limit values are 100 V/m respectively 0.265 A/m (corresponding to a magnetic-flux density of 0.3 µT) according to DIN VDE 0848 part 2. Calculations further revealed, that in the used nutritive solutions the magnetic field strength within the wave guide is only varied a few by bringing in the test object, while the electric field strength becomes considerably lesser. This also happens inside the body in the same way.

In the following four partial treatises the biological basics, the experimental set-up and the results are described. All results do not indicate athermal effects.

- Partial treatise:
  Dipl.-Ing. Uwe Neibig, Technische Universität (Technical University) Braunschweig
  "Exposition Devices"

- Partial treatise:
  Dr. rer. nat. Susanne Diener, Prof. Dr. rer. nat. Paul Eberle, Technische Universität Braunschweig
  "Cell Proliferation, Sister-Chromatide Exchanges, Chromosome Aberrations, Micronuclei and Mutation Rate of the HGPRT-Locus after the Influence of Electromagnetic High-Frequency Fields (440 MHz, 900 MHz and 1.8 GHz) on Human Peripheral Lymphocytes"

- Partial treatise:
  Dr. rer. nat. Rainer Meyer, Universität Bonn
  "Influence of High-Frequency Electromagnetic Fields of Mobile Communication on the Calcium Homoeostasis of Heart Muscle Cells and Lymphocytes"

- Partial treatise:
  Dr. med. R. Fitzner, E. Langer, Freie Universität (Free University) Berlin
  "Growth Behavior of Human Leukemia HL-60 Cells Influenced by High-Frequency Electromagnetic Fields (1.8 GHz and 900 MHz, pulsed with 217 Hz each time) for the Investigation of Cancer Promoting Effects"

These research works were supported by means of the Forschungsgemeinschaft Funk e.V.

Literature

[1] Heinrich Baggenstoes: "Dosimetrische Untersuchungen zum Mobilfunk", Kleinheubacher Berichte, Band 37 (1993), S.589

Partial Treatise:

Growth Behavior of Human Leukemia HL-60 Cells

Influenced by High Frequency Electromagnetic Fields (1.8 GHz and 900 MHz, pulsed with 217 Hz each time) for the Investigation of Cancer Promoting Effects

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1. Introduction and Definition of the Task

For quite some time now, in the public the question has been discussed whether electromagnetic fields may cause or promote cancer. The cancerogenic effect of agents (either chemicals substances or physical influences such as electromagnetic waves) lies in either initiation or promotion of tumors. Initiation of tumors means a transformation of normal cells into malignant tumor cells (i.e. a change in the genetic factors of the cell), whereas tumor promotion describes the augmentative effects in transformed cells, which characterize the propagation tendency, the degree of malignity of the tumor and thus its aggressive potential. The more aggressive the behavior of malignant cells, the less effective protective mechanisms of the human organism become. The analytical assessment of quantitative parameters which describe the growth of human tumor cells appears to be a suitable procedure to investigate cancer development in vitro. When the effects of exposure to magnetic fields are studied, only a multiple of the changes occurring in unexposed control cells can be rated as a promoting effect.

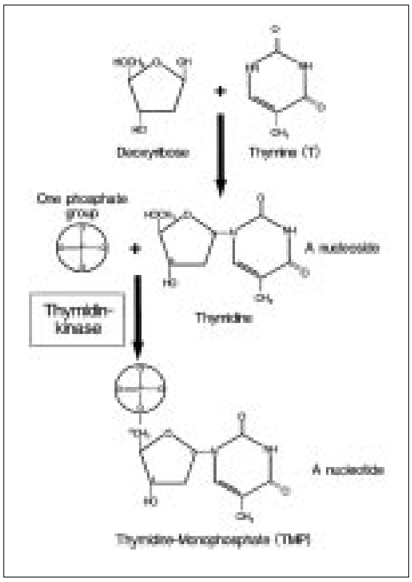


Figure 1: Biochemical mechanism of thymidine kinase
(Dr.med.R.Fitzner/Inst.f.Kl.Ch.u.Kl.Bioch./UKBF FU Berlin (August 1995))

Through numerous in-vitro-investigations using animal and human tumor cells already transformed, we were able to prove that a 50 Hz magnetic field exposition does not cause an additional promotion of such cells [1].

In the following in-vitro-investigations of human leukemia HL-60 cells in suspension cultures the question is pursued, whether an additional promotion of the growth behavior of already transformed human tumor cells and thus a cancerogenic effect can be proved for high frequency electromagnetic fields of 1.8 GHz pulsed with 217 Hz and 900 MHz pulsed with 217 Hz as well.

As critical analytical indicators of the growth rate of leukemia cells the doubling time and the synthesis and release of the enzyme thymidine kinase (TK) are determined in standardized suspension cultures. Cells exposed to the electromagnetic field are compared with identical controls not exposed to high frequency fields. A provable effect would lead to a multiple acceleration of cell division and to the generation and release of thymidine kinase in the suspension culture.

In a clinical setting, determination of thymidine kinase activity is a sensitive tool for the diagnostic evaluation of hematologic malignancies such as myeloid or lymphoblastic leukemia as well as solid tumors such as small cell lung cancer, breast cancer and brain tumors [3]. Extracellular thymidine kinase activity in serum is low in healthy adults (normal range: < 7 U/l) but markedly increased in patients with highly proliferative malignancies (up to hundred times the normal level in some cases).

Cytobiochemically, thymidine kinase is an intracellular enzyme

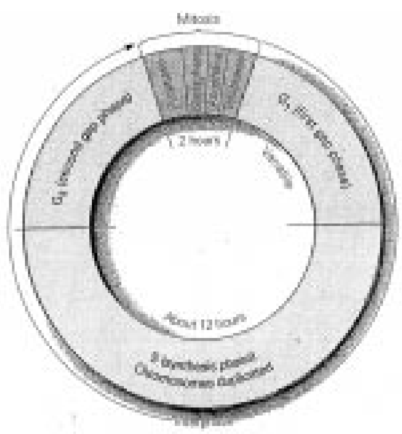


Figure 3: Cell cycle
(From: Rhoades & Pflanzer; Human Physiology)

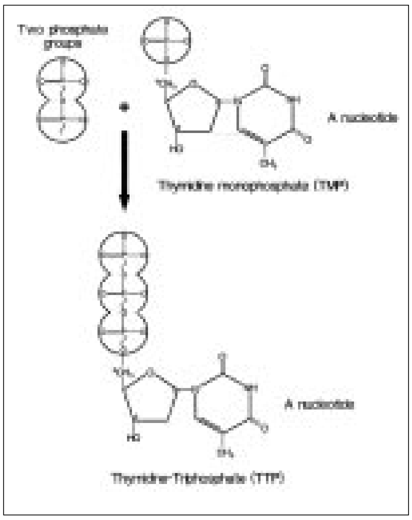


Figure 2: Transformation of TMP into TTP
(Dr.med.R.Fitzner/Inst.f.Kl.Ch.u.Kl.Bioch./UK BF FU Berlin (August 1995))

of mammalian cells which catalyses the phosphorylation of thymidine to thymidine monophosphate in presence of adenosine triphosphate (ATP) (Figure 1).

The thymidine triphosphate formed from thymidine monophosphate is used by the cell for DNA-synthesis during the S phase of the cell cycle (Figure 2).

Within the cell cycle (Figure 3) which consists of G1-, S-, G2- and M-phase (mitose phase), thymidine kinase activity is highest in the postmitotic resting phase (G1-phase) and the synthesis phase (S phase). Activity of the enzyme correlates with the mitotic cell division rate and thus with the identical reduplication of DNA.

In highly differentiated tissues with completed cell proliferation (e.g. renal parenchyma, nerve tissue) cells persist in the postmitotic resting phase. Therefore the G1-phase is referred to as the G0 phase in such cells. Since the latter are resting cells in terms of proliferation, their thymidine kinase activity is very low.

In mammal cells two different isoforms of this enzyme exist. Thymidine kinase 1 (TK1) is predominant in the cytosol ("cytosol-TK"), the second isoenzyme, TK2, was discovered in the mitochondria. Thymidine kinase uses endogenous thymidine from the cell metabolism as salvage enzyme or exogenous thymidine from the nutrition as a substrate.

2. Material and Methods

2.1. Experimental Device

The experiments using a so-called GTEM cell (gigahertz transverse electromagnetic cell, model 5302, manufacturer: EMCO, USA) as a field generator were performed with a type SMT 03 HF generator (Rhode & Schwarz, Germany) as a signal source postconnected with a band amplifier for the frequency ranges of 900 MHz and 1800 MHz respectively. The GTEM cell also served as a shielding against external influences. The shielding of the control cells consisted of a HF-box (Figure 4).

Maintenance of a constant temperature of 37°C in the experimental setup was ensured by an oil thermostat system with an imprecision of 0.1°C.

The test tubes with the cell suspensions were introduced into the GTEM cell in an acrylic glass specimen holder connected to the oil thermostat. Inside the exposition cell they were positioned at an angle of 45 degrees by means of a styrofoam holder. An identi-

Figure 4: Experimental setup

cal specimen holder was used to position the control cells.

The field strengths of the electromagnetic field inside the medium at a frequency of 1.8 GHz pulsed with 217 Hz, were 26 V/m for the electric respectively 0.8 A/m for the magnetic field referred to vertical sample position inside the GTEM-cell. With the required specimen position in an angle of 45 degrees, the field strength was below the above mentioned.

With 900 MHz, pulsed with 217 Hz as well, the field strengths were 19 V/m for the electric respectively 0.5 A/m for the magnetic field referred to the vertical positioning. The magnetic flux density was about 1 μT at 1.8 GHz and 0.6 μT at 900 MHz (referred to the vertical positioning).

The duration of the high frequency field exposition was 24 hours.

2.2. Cell Cultivation

The in-vitro experiments were performed with cells from the human leukemia stem HL-60 (American Type Culture Collection Certified Cell Lines 240). These cells had been obtained from a 35-year-old woman with acute myeloid leukemia. They are transformed promyelocytes. Normal promyelocytes are typically found only in bone marrow, whereas degenerated promyelocytes (large, polymorphic cells with a wide blue cytoplasm and coarse azurophilic granules, which may degenerate to form so-called "Auer's bodies") also enter peripheral circulation in the course of leukemia (Figure 5).

The cells were cultured in RPMI 1640 medium containing 8 % fetal calf serum and 2 % human serum at a temperature of 37°C. The cell cultures were transferred to new medium every 3-4 days.

The cell suspensions for all experiments were always prepared with a specific cell density (cell number/ml suspension, "initial cell count") and then distributed to the individual tubes required for the experiments in order to create identical initial conditions for all cell suspensions studied. (For more details about the cell density see "results".)

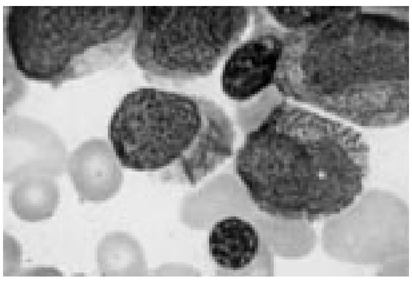


Figure 5: Promyelocytes leukemia (Bone marrow x 1500)
(From: Begemann H, Rastetter J; Atlas der Klinischen Hämatologie)

2.3. Determination of Thymidine Kinase Activity

Thymidine kinase activity in the cell culture supernatants was determined by radio-enzyme assay.

The substrate used in this test is ^{125}I-labelled deoxyuridine. The thymidine kinase present in the sample converts this substrate to ^{125}I-labelled deoxyuridine monophosphate, which is bound to a separation tablet. Residues of the remaining radioactively labelled substrate are removed by several washing steps. The remaining radioactivity is measured and enzyme activity calculated by means of a standard curve included in the experimental setup. The radioactivity level is directly proportional to the thymidine kinase activity.

3. Results

3.1. Doubling Times

Doubling times are calculated from the duration of growth and the initial cell count per ml and the number of cells at the end of the experiment. The doubling times of the human leukemia cells exposed to the electromagnetic field are tabulated as percentage of the doubling times of the control cells which were not exposed (they correspond to 100 %) (table 1 and 2).

High Frequency (HF) Expositions

(Dr.med.R.Fitzner/Inst.f.Kl.Ch.u.Kl.Bioch./UKBF FU Berlin (August 1995))

Figure 6: Doubling times at 900 MHz

Basic amount of cells: 40.000/ml; Student's T-Test (individual and mean values): p>0.05

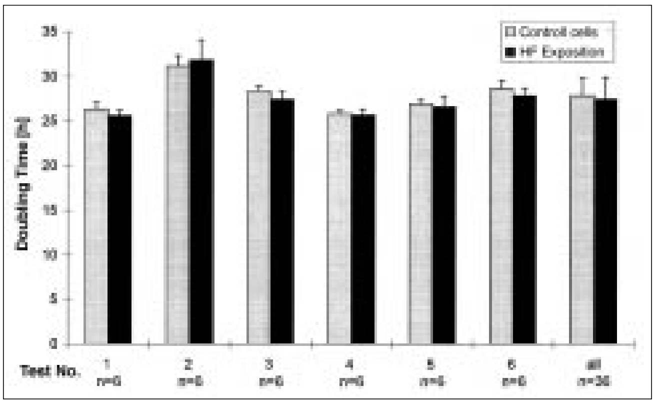


Figure 7: Doubling times at 1,8 GHz

Z.A.=Basic amount of cells: A=80.000/ml, B=40.000/ml; Student's T-Test (individual and mean values): p>0.05

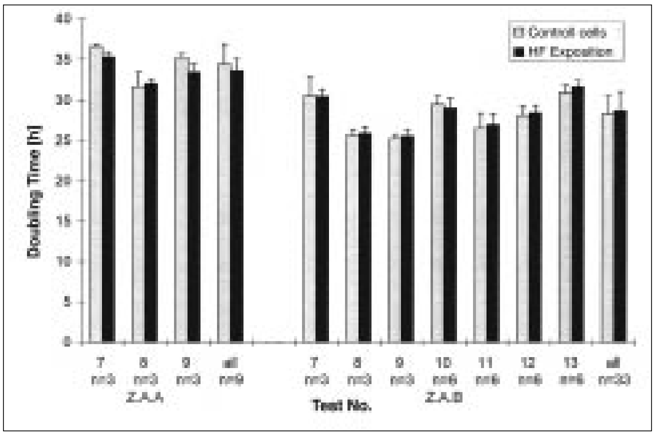


Figure 8: Thymidine kinase in cell supernatants at 900 MHz

Basic amount of cells: 40.000/ml; Student's T-Test (individual and mean values): p>0.05

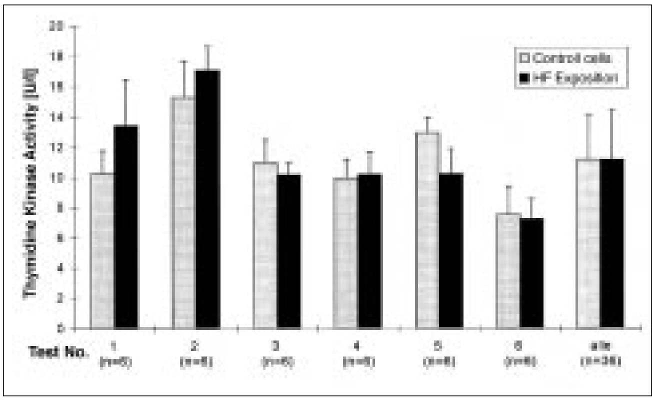
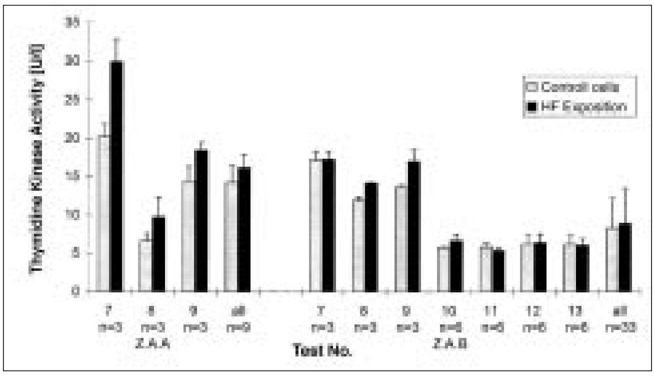


Figure 9: Thymidine kinase in cell supernatants at 1,8 GHz

Z.A.=Basic amount of cells: A=80.000/ml, B=40.000/ml; Student's T-Test (individual and mean values): p>0.05



As obvious from tables 1 and 2, there is no significant reduction of the doubling times under influence of the investigated electromagnetic fields compared to the controls. Otherwise percentage figures systematically and noticeably below 100 % would have been determined. The scattering width of the doubling times lies in the scope of the expected variation coefficient for cell cultures.

The mean values of the doubling times of the cells exposed to the high frequency field in contrast to the identically treated control cells without high frequency field influence are shown in Figure 8 and 9.

As obvious from the diagrams, the doubling times of the exposed cells are not statistically different from those of the unexposed control cells. All mean values and all individual values were tested for significance by means of the student's t-test using a level of 5 %. Since $p > 0.05$, the differences in doubling time between the cells exposed to a high-frequency field and the unexposed controls are not significant.

3.2. Thymidine Kinase Activity in Cell Culture Supernatants

Figures 8 and 9 show the thymidine kinase activities in the cell culture supernatants exposed to high frequency fields of 900 MHz and 1.8 GHz compared to the control cell cultures.

A multiple increase in the thymidine kinase activity of the exposed cells compared to the control cells is not recognizable. Thus, our

Doubling times of human leukemia cells (900 MHz)

Experiment	Exposition time	Initial cell number	DT in high frequency field referred to the DT of the controls in percent (mean value ± hrs)
1	24 h	4.0 x 10^4/ml	97.2 ± 1.6 (n=6)
2	24 h	4.0 x 10^4/ml	102.2 ± 6.7 (n=6)
3	24 h	4.0 x 10^4/ml	96.8 ± 5.3 (n=6)
4	24 h	4.0 x 10^4/ml	99.3 ± 3.6 (n=6)
5	24 h	4.0 x 10^4/ml	98.9 ± 6.2 (n=6)
6	24 h	4.0 x 10^4/ml	97.3 ± 5.1 (n=6)

Table 1: Doubling times (DT) of human leukemia cells in a high frequency field of 900 MHz pulsed with 217 Hz (electric field strength 19 V/m, magnetic field strength 0.5 A/m)

Doubling times of human leukemia cells (1.8 GHz)

Experiment	Exposition time	Initial cell number	DT in high frequency field referred to the DT of the controls in percent (mean value ± hrs)
1	24 h	0.8 x 10^4/ml	97.9 ± 6.0 (n=7)
2	24 h	4.0 x 10^4/ml	11.4 ± 6.7 (n=33)
3	24 h	8.0 x 10^4/ml	98.8 ± 4.1 (n=13)
4	8 h	0.8 x 10^4/ml	97.5 ± 3.5 (n=2)
5	8 h	4.0 x 10^4/ml	107.6 ± 11.6 (n=2)
6	8 h	8.0 x 10^4/ml	97.7 ± 8.1 (n=2)

Table 2: Doubling times (DT) of human leukemia cells in a high frequency field of 1.8 GHz pulsed with 217 Hz (electric field strength 26 V/m, magnetic field strength 0.8 A/m)

study demonstrated no field effect (i.e. an influence of high-frequency electromagnetic fields) on the cells investigated. The additionally made significance test of the mean values and all single values by means of the student's t-test resulted in $p > 0.05$. Hence the thymidine kinase activities in the cell culture supernatants between control cells without HF-exposition and exposed cells do not significantly differ from each other.

4. Conclusion

Already transformed human white blood cells (leukemia cells), exposed to high frequency fields (900 MHz and 1.8 GHz, pulsed with 217 Hz) showed no multiple increase in proliferation compared to unexposed but otherwise identically treated control cells. Neither doubling times nor thymidine kinase activities determined in the cell culture supernatants were found to markedly differ between exposed and unexposed cells. Additionally statistical testing of the differences in doubling times and thymidine kinase activities in cell supernatants between cells with and without high-frequency exposure revealed no statistical significance.

The results presented here show that there is no additional promotion of human leukemia cells as determined in terms of doubling time and thymidine kinase activity under the high-frequency exposure conditions of 900 MHz and 1.8 GHz, pulsed with 217 Hz (with an electric field strength of 19 respectively 26 V/m, a magnetic field strength of 0.5 respectively 0.8 A/m and a magnetic flux density of 0.6 respectively 1.0 µT).

In conclusion, the results of our study suggest that electromagnetic fields as they occur in cellular phone digital networks do not have a cancerogenic effect.

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