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Journal of Photochemistry and Photobiology B: Biology 64 (2001) 21–26

Journal of Photochemistry and Photobiology B:Biology

www.elsevier.com/locate/jphotobiol

Photodynamic effect on cancer cells influenced by electromagnetic fields

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Received 4 May 2001; accepted 7 August 2001

Abstract

The synergism of low-frequency electromagnetic field treatment and photodynamic effect on killing of human cancer cells is presented. The weak pulsating electromagnetic field (PEMF) generated by Helmholtz coils in the mT range influences the permeability of cell membranes for photosensitizers. Several types of sensitizers were excited by visible light during incorporation without and with two kinds of PEMF treatment. In the first part suitable photosensitizers were selected in the absorption range between 400 and 700 nm against human myeloid leukaemia K562 and human histiocytic lymphoma U937 cells by treatment of PEMF consisting of rectangular pulse groups. In the second part amplitude and frequency dependencies were measured using sinuous PEMF and white light with the result that after 12 min the PEMF treatment enhanced photodynamic effectivity by more than 40% over the control value. Taking into account the influence of many parameters, an additional optimization will be possible by photodynamic PEMF synergism for an increased drug delivery in general.

Keywords: Cancer cells; Photodynamic effect; Electromagnetic field; Synergism

1. Introduction

Stimulation or inhibition of cellular processes by electromagnetic fields are of considerable interest in cell biology and biotechnology, not only with respect to basic mechanisms, but also for its practical applications. Different processes including proliferation, enzyme reaction, biopolymer synthesis and membrane transport have been investigated with respect to their alteration by weak pulsing electromagnetic fields (PEMF) [1–3]. In contrast, high single electric pulses cause electroporation of membranes for fast incorporation of drugs [4–6], however, the viability of cancer cells and tissues decreases.

Cytotoxicity of photodynamic effects, especially against tumor cells, has been evaluated since the 1960s. According to historical development [7] the first generation of photosensitizers show absorption maximum mostly below 600 nm, e.g., simple porphyrins, rose bengal, anthrachinon-2-sulfonic acid, acridine orange, thiopyronine [8–12], not only for basic research but also for therapy of cancer, psoriasis, etc. [13–17]. Later effective dyes of higher molecular weight with absorption maximum beyond 600 nm have been used, e.g., porphyrin derivatives [18], Zn–phthalocyanine [19–22], its derivatives, and many other cyclic structures such as naphthocyanines [17], classified as 'second generation'.

In order to find out the possibility of synergism between electric pulses and light for improving photodynamic effects by facilitating the uptake of photosensitizers, we combined at first the electroporation with photodynamic effects on yeast [23] and cancer cells. In Fig. 1 [24] is shown the usual photodynamic effect and in comparison to it the action, but after a single electric pulse.

Mechanisms of light excitation have been analysed since the pioneering work of Jori and Spikes [7,15]. There are two main photochemical pathways:

Type I: photo-oxidation of cell components by excited sensitizers being reduced.

Type II: singlet oxygen — produced by energy transfer to O_2 — oxidizes also cell compounds.

Whereas type II mechanism destroys many compounds out and inside the cell, the type I mechanism needs penetration of the sensitizer through the cell envelope for complex formation with nucleic acids or proteins before local oxidation [9,10]. Recently, we tried a second possi-

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1011-1344/01/$ – see front matter
PII: S1011-1344(01)00185-3

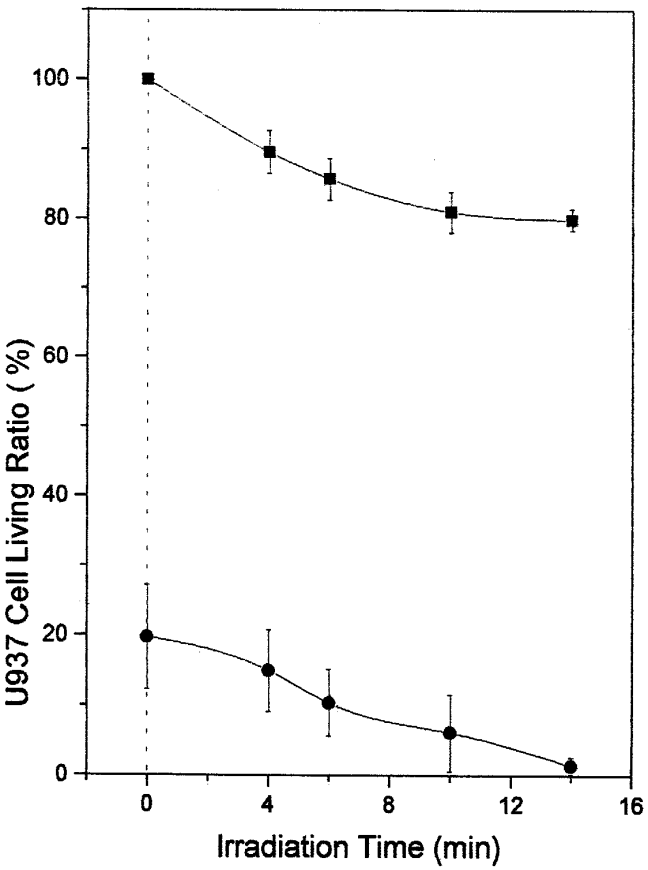

Fig. 1. Synergism of electroporation and photodynamic effect on U937 cells for thiopyronin (1×10^{-5} M) in 1:1 nutrition medium and 0.6 M mannitol, ambient temperature 18–22°C. Upper curve control: irradiation only without electroporation. Lower curve experiment: irradiation 30 s after the pulse treatment (2 kV cm^{-1}, pulse length 11–12 ms) [24]. Four minutes irradiation corresponds to 13.2 J/cm^2.

bility — application of single high electric pulses, pulsating electromagnetic fields (PEMF) generated by Helmholtz coils [25–28] for influencing cell membrane stability and protein channels [20] in order to stimulate dye penetration in a noninvasive way. We observed the first successful synergism of PEMF and increased photodynamic effect after treatment of yeast cells [30]. Corresponding results will be presented for the enhancement of photodynamic action of some photosensitizers of the first and second generation against human U937 and K562 cells by two techniques of PEMF treatment.

2. Materials and methods

2.1. Dyes

The following sensitizers were used: methylene blue (MB, Merck, Darmstadt, Germany, Mw 319.86, abs. max. 656 nm), thiopyronine (TP, Merck, Mw 318.8, abs. max. 560 nm), protoporphyrin IX (PP, Fluka, Mw 562.6, abs. max. 370 nm), Zinc–phthalocyanine (Zn–pc, Merck, Mw 577.92, abs. max. 673 nm), copper(II)phthalocyanine-sulfonate (Cu–pc, Merck, Mw 984.24, abs. max. 610 nm), Cibacronblue-3G-A-dextran (CB, Pharmacia, Uppsala, Sweden, Mw 3300, dye itself Mw 773, abs. max. 610 nm), daunomycin–HCl (DA, Serva, Heidelberg, Germany, Mw 564.0, abs. max. 481 nm), actinomycin-C (ACM, Zimet, Jena, Germany, Mw 1300, abs. max. 440 nm).

Trypan blue (TB, Sigma, St. Louis, USA, Mw 960.8, abs. max. 592 nm) was used for determination of the viability of cells.

2.2. Cells

The human chronic myeloid, leukemic K-562 cells [31] were from Fujisachi Cell Center (Japan). The culture medium was 90% RPMI (Gibco) with 10% CO_2, 37°C, cultivated in the FG Molecular Cytology (IMB, Jena).

The human histiocytic lymphoma U937 cells with mononuclear phagocytic characteristics were from the American Cell Culture Collection, and cultivated by the Institute of Virology (FSU, Jena), in RPMI 1640 medium supplemented with 10% fetal calf serum (Gibco Life Science, USA), 100 $\mu g\ ml^{-1}$ streptomycin and 100 U ml^{-1} penicillin (Sigma, USA) at 37°C in a 5% CO_2 incubator of 90% humidity.

2.3. Design of electromagnetic fields

2.3.1. Rectangular pulse bursts (RPB)

The exposure system to produce PEMF of rectangular shape (RPB) consisted of Helmholtz coils (mean diameter: 9 cm; hollow cores: 5 cm; copper wire: 1.2 mm diameter; 0.3 Ohm resistance) connected on a Bi-Osteogen System 204 (Electrobiology Inc., Fairfield, NJ, USA). This PEMF condition produced 5.5 ms bursts of 25 single 220 μs pulses with 60 ms intervals in between (used originally for enhanced Ca transport for bone healing [32]). The mean magnetic flux density B in the center area of the coils was approximately 15 mT for the plus tip and 2 mT for the minus tip.

Cell suspension in the presence of sensitizer were exposed to PEMF for 6, 12 or 24 min simultaneously with visible light irradiation: 19.8, 39.6 and 79.2 J cm^{-2}, respectively.

2.3.2. Sine waves (SW)

Mostly $f=50$ Hz and $B=0$–15 mT, generated either by a transformer (Conrad Electronics, Hirschau) or a frequency generator (Tesla Oscillator B492) combined with the Amplifier PA940 (Conrad Electronics, Hirschau) and different Helmholtz coils with resistances up to 22 Ohm, were used.

2.4. Light irradiation

2.4.1. Tungsten–halogen lamp irradiation

Irradiation of the 100 μl cell suspension plus 10–40 μl dye stock solution was arranged in two ways (A and B).

2.4.1.1. Simultaneous irradiation

The experimental (E) and the control (C) Acryl cuvettes (REF, Sarstedt, Germany) were positioned on an 'optical bench' (Carl Zeiss) at both sides of the tungsten halogen lamp (24 V and 150 W, Osram 64465) behind two identical concave glass lenses. Furthermore, for heat absorption two glass chambers containing a 7 cm water layer were positioned between the lamp and the lenses. By this means two nearly identical light beams of an area 4×3 mm could be obtained for E and C. The white light beams had about 55 mW/cm^2 irradiance measured by a thermopile from Laser 2000 (SL-Microtest GmbH, Jena).

2.4.1.2. Alternate irradiation (sham condition)

Only the beam of one side of the lamp (Section 2.4.1) – directed into the Helmholtz coil – was used for irradiation of E and C cuvettes in turn.

2.4.2. Laser

Laser of 650 nm of 1 mW (Conrad Electronics, Hirschau) and 670 nm of 3 mW (Laser Components) produce irradiance of 8 mW/cm^2 and 25 mW/cm^2, respectively, however, the irradiated area on the cuvette is only 12 mm^2.

2.5. Measurements and calculation

After mixing cell suspension (100 μl) with the sensitizer (10–40 μl) light irradiation started simultaneously with the PEMF experiments. Experiments (with PEMF) and controls (without PEMF) were carried out on cells washed once in 0.3 M mannitol solution after centrifugation for 8 min (rotation centrifuge factor (RCF)=172 *g*).

The viability of the cells was tested by TB (0.4% stock solution) dyeing for 5 min, otherwise an 'after-effect' caused by more dead cells was found. The ratio of nonviable/viable cells was ascertained by an inverted light microscope (Olympus, Tokyo, Japan) equipped with a CCD–IRIS video camera (Sony, Tokyo, Japan). About 100–150 cells were counted from the E and C cuvettes. The nonviable cells of C by photodynamic action were between 15 to 25%. Three to seven independent experiments have been performed.

The effectivity (E_f) of this synergistic treatment was determined according to:

$$E_f = (N_E/N_C - 1) \times 100\%$$

where N_E is the percentage of dead cells in the experiment and N_C is the percentage of dead cells in the control. The amount of dead cells $N_0 < 10\%$ in the culture medium was subtracted before E_f determination.

3. Results

3.1. Selection of photosensitizers

Before the combined experiments started the viability of cells have been checked by 5 min TB treatment:

- U cells in the presence of 0.2% TB are stable for 1 h.
- U cell illumination only did not change the percentage of dead cells from the beginning.
- K cells show no more dead cells after 1 h exposure by RPB field.

3.1.1. Thiopyronine (TP; first generation; Type I action)

On U cells: The final concentration of TP was 1.8×10^{-5} M and the exposure 6 min. One result given in Table 1 demonstrates synergistic kinetics until $E_f = +40.5\%$.

On K cells: They are more stable than U-cells: 1.8×10^{-5} M and the 20 min exposure reaches $E_f = +34\pm2.5\%$. Table 1 shows: 4.6×10^{-5} M, but only 12 min exposure yielding $E_f = +28.6\pm6.6\%$.

3.1.2. Protoporphyrin IX (PP; second generation; Type II action)

On U cells: [PP] 0.9×10^{-5} M, 12 min exposure: $E_f = +20.2\pm6.2\%$ (Table 1).

On K cells: [PP] 0.45×10^{-5} M, 6 min (Table 1).

3.1.3. Zn–phthalocyanin (Zn–pc; second generation; Type II action)

On U cells: [Zn–pc] $=1.5\times10^{-5}$ M, exposure 6 min. The result is given in Table 1, demonstrating the relatively strongest effect $E_f = +42.4\%$. Contrary to this result Cu–pc shows unexpectedly a much lower activity.

3.1.4. Daunomycin (DA; cytostatic drug; Type I action)

On U cells: [DA] 0.45×10^{-5} M and 12 min treatment (Table 1).

Table 1
Selected photosensitizers, tested with U and K cells at room temperature (22°C) by BRP, showing mean values of C, E and E_f and standard deviations

Photosensitizer/Cell	C (%)	E (%)	E_f (%)
TP/U	29.0±3.4	40.8±5.7	40.5±5.7
PP/U	32.3±4.1	38.6±3.6	20.2±6.2
DAU/U	34.5±4.6	42.9±4.1	25.2±11.3
Zn–pc/U	29.8±3.5	42.0±3.3	42.4±23.2
TP/K	34.9±3.2	44.9±4.7	28.6±6.6
PP/K	31.2±3.3	37.8±2.7	21.5±5.7

For concentrations and times, see text.

Table 2
Frequency dependence of E_f (white light, 39.6 J/cm^2) for Zn–pc with U cells and for ACM with K cells shows a maximum at 50 Hz

Frequency (Hz)	B (mT)	E_f (%), Zn–pc	E_f (%), ACM
10	8.5	8.9	
20	8.5	22.4	25
50	8.5	41.3	47
60	8.5	22.0	
100	8.5	18.9	21

3.1.5. Actinomycin (ACM, cytostatic drug; Type I action)

In spite of its higher molecular weight than DA it shows the same order of activity (compare Table 2).

3.1.6. High-molecular weight compound

The high-molecular dye, CB, an anthraquinone derivate bound to the dextran chain, has been tested as a sensitizer with the result that its photodynamic activity is smaller than that of all other sensitizers because the pulsating electromagnetic field (PEMF) was not strong enough to increase its permeation.

3.2. Amplitude dependence at 50 Hz

The following PEMF experiments were performed at 50 Hz (Fig. 2), by a sine-wave generator.

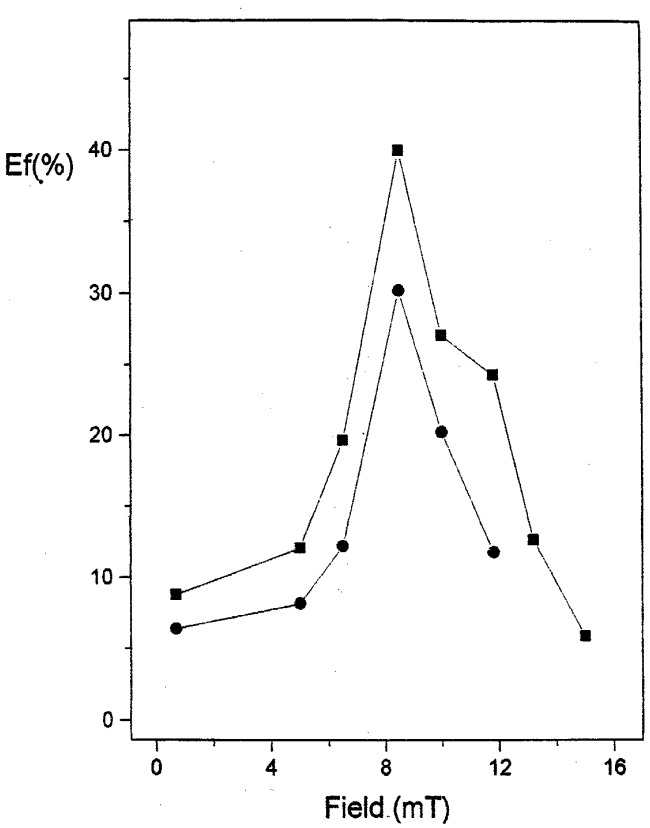

Fig. 2. Amplitude dependence of E_f for U937-cells with Zn–pc (■) and PP (●), 50 Hz. For details see the text.

3.2.1. Zn–pc and U cells: (in mannitol 0.3 M)

[Zn–pc] = 1.4×10^{-5} M. After 12 min exposure, the ratio of nonviable/viable cells was ascertained again by the trypan blue test. The amplitude of PEMF were fixed at: B = 0.7, 5, 6.5, 8.5, 10, 11.8, 13.2 and 15 mT, respectively (Fig. 2).

The results in Fig. 2. show a main 'electromagnetic window' resulting in $E_f = +40\pm16\%$ at 8.5 mT in comparison with lower and higher amplitudes.

3.2.2. PP and U cells

The suspension of cells in mannitol (0.3 M) contains 0.9×10^{-5} M PP. The parameters of PEMF were set at: B = 0.7, 6.5, 8.5, 10, 11.8, 13.2, 15 mT. After 12 min exposure and TB test the results are also shown also in Fig. 2. One similar 'electromagnetic window' of $E_f = +31\%$ appeared around 8.5 mT at the same position as for the Zn–pc peak independently of the quite different light absorption curves of both sensitizers.

3.3. Frequency dependence (Table 2)

In the low frequency range there is again a maximum for 8.5 mT at 50 Hz, which decreases to 60 Hz shown in Table 2 for the Zn–pc action on U-cells. Further measurements in the direction of higher frequencies are in progress to ascertain if only one or more 'electromagnetic window' occurs.

3.4. Dependence on irradiation time

Suspensions of U-cells with Zn–pc were exposed to PEMF of amplitudes 1.5 mT or 8.5 mT for 6, 12, and 24 min light irradiation. Ascertained by TB test an exponential enlargement of numbers of dead cells was found.

3.5. Dependence on coherent light

For some sensitizers it can be more suitable to irradiate by Lasers [33]. Therefore the effect of our 670 nm Laser has been tested (20 min ≅ 30 J/cm^2) preliminarily on K cells for a first comparison (Table 3).

Of course nowadays a broad range of light sources and light conductors is available in order arrange optimal conditions for therapy.

Table 3
Comparison of effectivity of Laser (670 nm) and the white light lamp

	Laser (%)	White light (%)	
[MB] = 5.7×10^{-5} M	40.0	80	Dead cells
[Zn–pc] = 1.6×10^{-5} M	45.5	67	Dead cells
[PP] = 0.9×10^{-5} M	40.0	82	Dead cells

4. Discussion

Our results for improvement of photodynamic action by easier penetration of external photosensitizers through disturbed membranes by PEMIC can be summarized as follows:

(a) As shown in the literature [1–3] each kind of cell seems to respond in the frame of a specific 'electromagnetic window' depending on frequency, amplitude and wave form. However, not only the membrane channels will be affected, but also certain metabolic processes, e.g., the increase of DNA crosslinking by malondialdehydes may occur according to [41].

(b) Some photosensitizers of the first and second generation and white light irradiation have been chosen for testing this principle of synergism. The PEMF treatment yields higher effectivity, the lower the control values are. That means for practical purposes this method can be useful if the photosensitizer or in general a drug does not penetrate sufficiently to reach its maximal impact [34,35] (e.g., the cytostatic bleomycin needs electroporation for higher efficacy [4]). Higher E_f values may be possible for the combination of stronger Laser and long wavelength PEMF equipment for membrane destabilization in the depth of cancer tissue.

(c) A further possibility is to utilize a cytostatic drug as a sensitizer [34,35]. In the case of anthracyclines (e.g., DA and ACM), the photo-oxidation is combined with nuclease hindrance by their intercalation into the DNA double helix.

(d) Besides possibilities of high direct currents between electrodes or extremely high magnetic flux densities $B>$ 1T [39] for destroying malignant cells, the photodynamic efficacy can be increased by:

(i) single strong electroporative pulses via inert electrodes [23,24] (Fig. 1),
(ii) capacitively coupled alternating currents via inert electrodes [36,37],
(iii) PEMF for drug transport stimulation, e.g., by conformational coupling [38] of channel proteins, carrying electric or magnetic moments [29].

So far as a comparison is possible, the highest delivery (uptake of sensitizers) is caused by electric pulse treatments (i), whereas (ii) and (iii) don't reach the same efficacy up to now. However, method (iii) induces apoptosis besides necrosis in a noninvasive manner. The easier penetration of propidium iodide — a weaker sensitizer — was demonstrated by flow cytometry after 10 mT treatment [40].

5. Conclusions

A systematic research of this synergism between PEMF and light is still in its beginning. Nevertheless, it can be concluded that some promising strategies should be tested in future.

- Preference of water soluble photosensitizers absorbing $>$600 nm because the long wavelength Laser techniques can irradiate deeper cancer tissues without using light conductors.
- Combination of photo-oxidation by dye complexes inside the cell (type I) and also membrane destruction by singlet oxygen (type II) may be possible by a mixture of two sensitizers, e.g., methylene blue and protoporphyrin IX.
- Application of higher PEMF frequencies because the impedance of tissues is lower in the MHz range and therefore deeper cancer tissues can be treated.
- This synergism of PEMF and white light or stronger Laser irradiation offers the chance to develop a noninvasive, improved treatment of a localized tumor [15] as well as of psoriasis [16] — spreading out over the skin — by such photosensitizers enriched preferentially in cancer cells.

Acknowledgements

Lijun Pang, Cristina Baciu and Nelly Traitcheva are grateful to the Gesellschaft für Biologische Krebsbekämpfung (Heidelberg) for a grant to work in the Lab. Bioelectrochemistry at Campus Beutenberg, Jena. For cell cultivation we like also to thank Mrs G. Gothe (IMB, Jena) and Mrs V. Güntzschel (Instit. Virology, FSU, Jena).

References

[1] H. Berg, Possibilities and problems of low frequency weak electromagnetic fields in cell biology, Bioelectrochem. Bioenerg. 38 (1995) 153–159.
[2] C. Polk, E. Postow, Handbook of Biological Effects of Electromagnetic Fields, CRC Press, Boca Raton, 1996.
[3] H. Berg, Problems of weak electromagnetic field effects in cell biology, Bioelectrochem. Bioenerg. 48 (1999) 355–360.
[4] S.B. Dev, D. Rabussay, G. Widra, G. Hofmann, Medical application of electroporation, IEEE Trans. Plasma Sci. 28 (2000) 206–223.
[5] D. Miklavcic, T. Jarm, M. Cemezar, G. Sersa, D. Arz, J. Belehradek, L. Mir, Tumor treatment by direct electric current. Tumor perfusion changes, Bioelectrochem. Bioenerg. 43 (1997) 253–256.
[6] M. Jaroszeske, R. Heller, R. Gilbert, Electrochemotherapy, Electrogenetherapy/electrically Mediated Delivering of Molecules to Cells and Transdermal Drug Delivery, Humana Press, Totowa, 2000.
[7] J. Spikes, Photodynamic action; from paramecium to photochemotherapy, Photochem. Photobiol. B55 (1997) 142–147.
[8] H. Berg, F.A. Gollmick, H. Triebel, E. Bauer, G. Horn, J. Flemming, L. Kittler, Redox processes during photodynamic damage of DNA I. Results obtained by several physico-chemical methods; II. A new model for electron exchange and strand breaking, Bioelectrochem. Bioenerg 5 (1978) 335–356.
[9] H. Triebel, H. Bär, H.-E. Jacob, E. Sarfert, H. Berg, Sedimentation

analysis of DNA photooxidized in the presence of thiopyronine, Photochem. Photobiol. 28 (1978) 331–337.
[10] H. Berg, H.E. Jacob, Biologische Wirkungen photochemischer Wasserstoffperoxidbildung. 2. Mitt. Intensivierung der photodynamischen Wirkung von Anthrachinon-2-sulfonsäure in Gegenwart von Glycerin und anderen Wasserstoffdonatoren, Photochem. Photobiol. 4 (1965) 55–59.
[11] H. Berg, W. Jungstand, Photodynamische Wirkung auf das Ehrlich-Ascites Karzinom, Naturwissenschaften 53 (1966) 481–482.
[12] H.-E. Jacob, W. Fleck, D. Strauss, Photodynamische Wirkung und Reparatur-Inhibition photodynamisch induzierter DNA-Schäden in *Proteus mirabitis* durch das Anthracyclin–Antibioticum Violamycin B1, Z. Allg. Microbiol. 17 (1977) 569–573.
[13] K. König, V. Bockhorn, W. Dietel, H. Schubert, Photochemotherapy of animal tumors with the photosensitizer methylene blue using a krypton laser, J. Cancer Res. Clin. Oncol. 113 (1997) 301–303.
[14] V. Mantareva, M. Shopova, G. Spassova, D. Wöhrle, S. Muller, G. Jori, F. Ricchelli, Si (IV)-methoxyethylene-glycol-naphthalocyanine: synthesis and pharmacokinetic and photosensitizing properties in different tumour models, Photochem. Photobiol. 40 (1997) 258–262.
[15] G. Jori, C. Perria (Eds.), Photodynamic Therapy of Tumors and Other Diseases, Libreria Progetto Editore, Padova, 1985.
[16] H. Berg, E. Bauer, F.A. Gollmick, W. Diezel, F. Böhm, H. Meffert, N. Sönnichsen, Photodynamic hematoporphyrin therapy of psoriasis, in: G. Jori, C. Perria (Eds.), Photodynamic Therapy of Tumor and Other Diseases, Libreria Progetto Editore, Padova, 1993, pp. 338–343.
[17] J. Moser, Photodynamic Tumor Therapy, 2nd and 3rd Generation Photosensitizers, Harwood Academic Publishers, Amsterdam, 1998.
[18] T. Mang, T. Dougherty, W. Potter, D. Boyle, S. Sommes, J. Moan, Photobleaching of porphyrins used in photodynamic therapy and implications for therapy, Photochem. Photobiol. 45 (1987) 501–502.
[19] K. Oda, S. Ogura, I. Okura, Preparation of a water-soluble fluorinated zinc phthalocyanine and its effect for photodynamic therapy, Photochem. Photobiol 59 (2000) 20–25.
[20] M. Shopova, V. Mantareva, K. Krastev, D. Hadjiolov, A. Milev, K. Spirov, G. Jori, F. Ricchelli, Comparative pharmaco-kinetics and photodynamic studies with Zn(II)–phthalocyanine in hamsters bearing an induced or transplanted rhabdomyosarcoma, Photochem. Photobiol. 16 (1992) 83–89.
[21] M. Obochi, R. Boyle, J. Van Lier, Biological activities of phthalocyanines. XIII. The effects of human serum components on the in vitro uptake and photodynamic activity of Zinc–phthalocyanine, Photochem. Photobiol. 57 (1993) 242–247.
[22] G. Rodal, S. Rodal, J. Moan, K. Berg, Liposome-bound Zn(II)–phthalocyanine; mechanisms for cellular uptake and photosensitation, Photochem. Photobiol. 45 (1998) 150–159.
[23] X. Wang, I. Hönes, H. Berg, Uptake of photodynamic sensitizer by electroporated yeast cells, Bioelectrochem. Bioenerg. 47 (1998) 175–177.
[24] H. Berg, M. Lambreva, Synergism of photodynamic and electropermeation effects on cell vitality as a novel cymomoxic agent. US Patent GENE 1110/1. Berg H. et al. Membrane electroporation increases photodynamic effects, J. Electroanal. Chem. 486 (2000) 220–224.
[25] G. Kerns, E. Bauer, H. Berg, Electrostimulation of cellulase fermentation by pulsatile electromagnetically induced currents, Bioelectrochem. Bioenerg. 32 (1993) 17–22.
[26] R.A. Luben, Effects of low energy electromagnetic fields (pulsed and DC) on membrane signal transduction processes in biological systems, Health Physics 61 (1991) 15–28.
[27] B. Kula, M. Drozdz, A study on magnetic field effects on fibroblast cultures. Part 2. The evaluation of the effect of static and extremely low frequency (ELF) magnetic fields on free-radical processes in fibroblast culture, Bioelectrochem. Bioenerg. 39 (1996) 27–30.
[28] M. Mehedintu, H. Berg, Proliferation response of yeast *Saccharomyces cerevisiae* on electromagnetic field parameters, Bioelectrochem. Bioenerg. 43 (1997) 67–70.
[29] B. McLeod, A. Liboff, S. Smith, Electromagnetic gating in ion channels, J. Theoret. Biol. 158 (1992) 15–31.
[30] C. Baciu, S. Gothe, H. Berg, Electromagnetic permeabilisation of yeast cell membranes yielding enhanced photodynamic action — A synergism, Electro-Magnetobiology 20 (2001) (in press).
[31] J. Kanofsky, P. Sima, Preferential cytotoxicity for multidrug-resistant K562 cells using the combination of a photosensitizer and a cyanine dye, Photochem. Photobiol. 54 (1999) 136–144.
[32] A.A. Pilla, State of the art in electromagnetic therapeutics, in: M. Blank (Ed.), Electricity and Magnetism in Biology and Medicine, San Francisco Press, San Francisco, 1993, pp. 17–22.
[33] H.-E. Jacob, H. Oswald, Photodynamic action with Laser, Studia Biophysica (Berlin) 39 (1973) 133–139.
[34] Y. Omote, M. Hosokawa, M. Romatsomoto, T. Namieno, S. Nakagima, Y. Kubo, H. Kabagashi, Treatment of experimental tumors with a combination of a pulsing magnetic field and an antitumor drug, Jpn. J. Cancer Res. 81 (1990) 956–961.
[35] P. Pasquinelli, M. Petoini, L. Natti, S. Galimberti, M. Saviozzi, G. Malvaldi, Biological effects of PEMF: an attempt to modify cell resistance to anticancer agents, J. Environ. Pathol. Toxicol. Oncol. 12 (1993) 193–197.
[36] G. Chien, G. Cai, N. Zhao, High efficient gene transfer introduced by low-amplitude, low-frequency alternating electric field, Chin. Sci. Bull. 42 (1996) 5.
[37] Y. Rosemberg, R. Korenstein, Incorporation of macromolecules into cells an vesicles by low electric fields: induction of endocytotic-like process, Bioelectrochem. Bioenerg. 42 (1997) 275–287.
[38] D. Astumian, H. Berg, Direct electric field effects and sequential processes in biosystems, Bioelectrochem. Bioenerg. 25 (1991) 455–462.
[39] J. Costa, G. Hofmann, Malignancy treatment, US Patent 4665898, 1987.
[40] L. Pang, N. Traitcheva, G. Gothe, J.A. Camacho Gomez, H. Berg, ELF—Electromagnetic fields inhibit proliferation of human cancer cells and induce apoptosis, Electro and Magnetobiol. (2001) (in press).
[41] T. Hisamitsu, K. Narita, T. Kasahara, A. Seto, Y. Yu, K. Asano, Induction of apoptosis in human leukemic cells by magnetic fields, Jpn. J. Physiol. 47 (1997) 307–310.