Ber. nat.-med. Ver. Salzburg	Band 11	S. 49 - 59	Salzburg 1996

PHOTODYNAMIC DIAGNOSIS AND THERAPY OF CANCER: IN-VITRO RESEARCH WITH CLINICAL RELATION

PHOTODYNAMISCHE TUMORDIAGNOSE UND -THERAPIE: IN-VITRO-FORSCHUNG MIT KLINISCHEM BEZUG

BARBARA KRAMMER, KARL ÜBERRIEGLER, ALEXANDER HUBMER, DAGMAR FIEDLER, GERLINDE SCHNITZHOFER, THOMAS VERWANGER, ISABELLA REITER, ELFRIEDE BANIEGHBAL AND FRIEDERIKE BLUM

Institute of Physics and Biophysics, University of Salzburg, Hellbrunnerstr. 34, 5020 Salzburg, Austria

Kurzfassung

Die Photodynamische Tumortherapie setzt sich aus einer wirksamen Kombination von Chemo- und Strahlentherapie zusammen, wobei jede der Komponenten (Photosensibilisator und Rotlicht) für sich nicht toxisch ist. In-vitro-Versuche zu dieser Therapie werden von unserer Forschungsgruppe meist mittels Zellinien von Fibroblasten durchgeführt. Schnelle Effizienztestmethoden und Zellkulturtests ermöglichen ein Screening von verschiedenen Sensibilisatoren (z.B. Porphyrinen) und Testprotokollen, wie die Kombination von photodynamischer Therapie mit Strahlentherapie und Hyperthermie, und der Effekt von Radioprotektoren auf photodynamische Prozesse. Grundlagenforschung soll einerseits Wechselwirkungen photodynamischer Prozesse mit Zell- und der Kern-Membran, dem Ca^{2+}-Metabolismus, der Expression von Onkogenen und der Mutagenität von Sensibilisatoren klären, andererseits die Aufnahme- und Bildungskinetiken von externen und endogenen Porphyrinen, die Bildung von Photoprodukten, die intrazelluläre Porphyrinverteilung und die intrazellulären Targets für photoinduzierten Schaden untersuchen. Ein Überblick über die auf diesem Gebiet gewonnenen Ergebnisse wird hier präsentiert.

Keywords: Photosensitizer, tumor therapy, human skin fibroblasts, radiotherapy, hyperthermia, mutagenicity

Abstract

The photodynamic tumor therapy represents an effective combination of chemo- and radiation therapy with both single components (photosensitizer and red light) in a non-toxic form. In-vitro experiments concerning this therapy are carried out by our research group using mainly fibroblast cell strains. Fast efficiency test procedures and cell culture tests help to screen different sensitizers (e.g. porphyrins) and test protocols, such as the combination of photodynamic tumor therapy with radiotherapy and hyperthermia or the effect of radioprotectors on photodynamic processes. Basic research tries to clarify, on the one side, interactions of photodynamic processes with cellular and nuclear membranes, the Ca^{++}-metabolism, expression of oncogenes and the mutagenicity of the sensitizers, and on the other side, uptake- and formation kinetics of external and endogenous porphyrins, photoproduct formation, intracellular porphyrin distribution and targets for photodamage. An overview about the results obtained in this field will be presented here.

1 Introduction

The photodynamic diagnosis (PDD) and therapy (PDT) of cancer is based on the following processes: 1) Photosensitizers can be excited by visible light and desactivate either by light emission (fluorescence) or by energy or electron transfer to other molecules. By this, reactive intermediates (e.g. oxygen) are formed, which damage other substances such as cell components. 2) Porphyrins as photosensitizers are selectively taken up by or generated in, and/or retained in tumor tissue as compared to healthy tissue. 3) Visible low-energy (laser) light has to be transported via fiber optics to the tumor site, thereby inducing either red-fluorescence (diagnosis) or cytotoxic reactions of the sensitizer, with that killing the tumor cells (therapy). Beside the mainly used sensitizer hematoporphyrin derivative (HPD) several new sensitizers such as phthalocyanines, chlorins or endogenous protoporphyrin IX are under investigation. Clinically, the dye is applied to the patient (i.v., i.m., orally, i.p, etc.) and activated at the tumor site via fiber optic with red light for therapy 2 - 3 days later, when an optimal sensitizer accumulation ratio between tumor/normal tissue is reached. The cytotoxic desactivation of the sensitizer involves the formation of radical oxygen intermediates at/in intracellular targets (mainly cellular membranes and mitochondria) (Gomer 1991, Unsöld and Jocham 1988).

Several thousend patients have been treated worldwide successfully with PDT. Side-effects are minimal as compared to other therapies with mainly skin sensitivity for up to a month.

Basic mechanisms and interactions can be investigated best on the cellular level, where also simple and rapid efficiency testing of different sensitizers and protocols can be carried out. Our research group therefore uses mainly fibroblast cell strains.

For clinical practice, the combination of PDT with radiotherapy (Schnitzhofer and Krammer in press) and hyperthermia (Krammer 1996) and the effect of radioprotectors (Krammer 1995) on photodynamic processes was investigated. Photodynamic interaction with cellular and nuclear membranes (Krammer-Reubel 1992, Krammer et al. 1993), Ca^{++}-metabolism (Hubmer et al. 1996), mutagenicity of the sensitizers (Fiedler et al. 1994 and in press), uptake- and formation kinetics of porphyrins (Krammer and Überriegler in press, Hubmer et al. 1994), intracellular porphyrin distribution and photoproduct formation (Krammer and Überriegler in press) and intracellular targets for photodamage (Überriegler et al. 1995) were studied. An overview about clinically related in-vitro results will be presented here. PDT-induced expression of human oncogenes, the role of macrophages and cytokines in PDT, apoptotic cell kill and intracellular metabolic mapping is presently under investigation.

2 Material and Methods

2.1 Cells and Cell Culture Conditions

Human skin fibroblasts were established from child preputium tissue (LKA Salzburg, Kinderchirurgie) in our laboratory. WI38 (human lung fibroblasts), the transformed strain WI38VA13, V79 hamster fibroblasts and the skin fibroblasts were cultivated in BME (basal medium Eagle) supplemented with hepes-buffer (20mM), 10% FCS (fetal calf serum), L-glutamine (2mM), non-essential amino-acids, penicillin/streptomycin (50 IU/ml, resp. 50 µg/ml) and incubated in a CO_2 incubator. Cell culture supplies were obtained from Flow Laboratories. For incubation with a sensitizer, medium without serum was used. Before and after sensitizer incubation, cells were always washed twice with phosphate buffered solution (PBS). Hepatocytes from Female Fischer 344 rats were grown in minimum essential medium (MEM) with Earle's salts and non-essential amino acids and gentamicin (50 µg/ml) obtained from Grand Island Biological Supply Company via "Life Technologies", Vienna, Austria.

2.2 Photosensitizers

Photosan 3® (PS3), a HPD (hematoporphyrin derivative), similar to Photofrin 2®,HPD, enriched with polymers (HPD-P), HPD, enriched with monomers (HPD-M). All three substances were obtained from Seehof-Laboratories, Wesselburenerkoog, Germany. Additional sensitizers were: Photofrin 2® (PF2; American Cyanamid Company, New York) and HPD (Organic Chemistry, Academy of Science, Sofia, Bulgaria; tetrasulfonated chloraluminium phthalocyanine ($AlPcS_4$) and tetrasulfonated tetraphenylporphine ($TPPS_4$), both obtained from Porphyrin Products, Logan, Utah, USA. 5-aminolevulinic acid (ALA) was obtained from Sigma.

2.3 Photoprotectors

For the membrane integrity test, following photoprotectors were solved in medium without serum: Vitamin C, vitamin E, reduced glutathione with Se (Na-Selenit: 0.4 ng/ml in the medium), foetal calf serum and histidine.

2.4 Chemicals

Ringer-solutions with 10^{-10}, 10^{-3}, 10^{-2} M, Mn for fluorescence quenching, Collagenase, EGF, insulin, staining solution were supplied by Sigma, Fluo-3 by Molecular Probes.

2.5 Radiation source

Co^{60}-γ -source with an activity of 220 Ci and dose rate at the cells 2700 R/h (Gammatron, Siemens).

2.6 Membrane integrity testing

A modified trypan blue exclusion test was used at fibroblast monolayers in the way previously described (Krammer-Reubel and Hofmann 1991), where the "critical" fluence to induce loss of the membrane integrity 2 - 20 h after sensitizer incubation was used as a comparable parameter. A HeNe-laser (wavelength 632 nm; power density: 300 mW/ cm^2) served as irradiation source for HPD, related compounds and $TPPS_4$, and a 670 nm diode laser (power density: 180 mW/ cm^2) for $AlPcS_4$. Using this test, relative sensitizer efficiency at cell membranes can be determined by comparing the applied fluences.

2.7 Oxygen consumption measurements

The test set-up was previously described (Krammer 1990). The measurement of oxygen partial pressure was carried out at 37° C by the reduction at membrane coated polarographic Clark-type electrodes, in closed but light transparent chambers. The resulting signal was amplified by a microsensor, displayed in % oxygen and converted into nmol/ml·min. The decrease of molecular oxygen in the chamber can be taken as a measure of the photodynamic efficiency, because molecular oxygen is transfomed by sensitizers and suitable (chemical) targets under illumination to reactive oxygen species and singlet oxygen and further to oxidized products. Irradiation was carried out by a halogen lamp with a red filter (80% transmittance above 600 nm; power density: 27 mW/cm^2).

2.8 Hyperthermic treatment

After control measurements, the fibroblasts were incubated at 45° C for 30 min and 2 h. Combined treatment: 1) 30 min hyperthermic treatment at 45° C was followed 1 hour later by Ps3 incubation with 2, 5 and 10 µg/ml. 2) Ps3 incubation with 2, 5 and 10 µg/ml was followed immediately by hyperthermia for 30 min.

2.9 Transmembrane potential measurements

Intracellular measurements were carried out by using KCl-filled (0.3 M) glass-microelectrodes with tip diameters of less than 0.5 µm and resistances of at least 10 MegaOhms. The potential was measured differentially between an intracellular voltage recording electrode and an extracellular Ag/AgCl reference electrode. A speed- and distance- controlled piezo-manipulator (Sci.Trad.Prod., BRD) was used for penetration of the cells. Measurements were taken from about 30 cells per petri dish, summarized in mean and standard deviation. Statistical comparison between the data points was carried out using the u-test for non-matched pairs.

2.10 Low light imaging with a CCD camera and fluorescence microscope

Fluorescence digital imaging microscopy was performed using an Olympus epifluorescence microscope and an air-cooled slow scan CCD camera (Variocam PCO Computer Optics). Quantitative analysis of the images was performed using the Sigma-Scan software (Jandel Scientific). A perfusion chamber was constructed for the permanent flow of culture medium and additives.

2.11 Mutagenicity test

Mutagenicity test was carried out by investigation of chromosomal aberrations and by a micronucleus assay.

2.12 Additional intruments used

FACS: Fluorescence activated cell sorter and analyzer (FACSTAR PLUS, Becton Dickinson)
PCR-setup (Robocycler Gadient 40, Fa. Chemomedica)
Confocal laser fluorescence microscope (Bio-Rad/MRC 600 system, eqipped with a Leitz inverted microscope and an argon ion laser.
Absorption (U-2000, Hitachi) and *fluorescence spectrophotometer* (F-2000, Hitachi).

3. Results and Conclusion

3.1 Combination of PDT with radiotherapy (Schnitzhofer and Krammer in press)

The effect of photodynamic mechanisms, using Photosan 3 as sensitizer, combined with γ-irradiation on the colony forming ability of CHO fibroblasts was studied. Only the combination of the lowest doses used (5 μg/ml PS3, 100 mJ/cm^2 red light fluence, 1 Gy γ-radiation) shows a more than additive effect, possibly due to inhibition of repair mechanisms. Combinations of higher doses lead to a greater survival than could be expected on the basis of additive effects of PDT and γ-irradiation alone. This could be due to adaptation processes. The results lead to the conclusion that the combination of both therapies can be more efficient for the patient than single treatments.

3.2 Combination of PDT with hyperthermia (Krammer 1996)

Photodynamic action with Photosan 3 in combination with hyperthermic treatment was investigated using transmembrane resting potential (TMRP) measurements of human skin fibroblasts. This method is relevant for the detection of damage to the plasma membrane, which is expected to be the main cellular target in both therapies.
While PDT (2 - 10 μg/ml) alone induces a concentration dependent decrease of the transmembrane resting potential of fibroblasts, hyperthemia (0.5 and 2h at 45°C) leads finally after fluctuations to a moderate increase of the TMRP. The combination of both treatments in both orderes (PDT first or last) shows additive effects with a strong decrease ending in plateaus. One exception resulting in the highest damage by lack of any final plateau-effect was a treatment with 30 min hyperthermia, followed by PDT with 10 μg/ml Photosan 3. It can be concluded that the suitable combination of both therapies could enhance the cell damage and possibly increase tumor cell kill.

3.3 The effect of radioprotectors on photodynamic processes (Krammer 1995)

In vitro investigations by two sensitization efficiency test systems, one using human skin fibroblasts, were carried out taking as photosensitizers HPD, PS3, PF2, $AlPcS_4$ and $TPPS_4$, and as photoprotectors the antioxidants vitamin E, C and glutathione, histidine and fetal calf serum. Only the serum showed satisfying results in quenching singlet oxygen in vitro and in cell membranes. This could be due to binding of the sensitizers to the amino acids histidine and tryptophan in the serum proteins. Reduction of the post-irradiation skin sensitivity by vitamins should therefore be tested in vivo.

3.4 Transmembrane resting potential (TMRP) measurements of normal and transformed human fibroblasts following photodynamic laser therapy (Krammer-Reubel 1992)

Similar depolarisation kinetics at the cell membrane were found for normal and transformed fibroblasts: PS3 concentrations of 3.3 - 100 µg/ml for normal and 10 - 100 µg/ml for transformed cells caused statistically significant depolarisation of the mean TMRP values. The potentials decreased from an average of about -9 mV to about - 2mV within 18 - 30 min after the onset of treatment. This was followed by a loss of the cell viability some hours later. The decrease was faster for higher sensitizer concentrations. A correlation of the drug dose with the depolarisation time for the decrease of the TMRP to 75% of the control value showed a negative correlation, with a faster decrease for transformed fibroblasts compared to non-transformed fibroblasts. Transformed cells seems to be more sensitive in the high concentration range, and more resistant in the low range than their non-transformed counterparts. Because ion balance at the membrane does not break down immediately after lethal photodynamic treatment, a gradual leakage of K^+-ions can be assumed by oxidative induction of leakiness of the permeability barrier for small molecules.

3.5 Photodynamic effects on the nuclear envelope of human skin fibroblasts (Krammer et al. 1993)

The results obtained by confocal laser fluorescence microscopy and TMRP measurements show staining of the nuclear envelope after short incubation times with the photosensitizers PS3, PF2 and HPD (in mainly monomeric form). Maximum staining was found at the center of the nuclear envelope. The sequence of fluorescence intensity was HPD > PF2 > PS3. After lethal treatment with PS3 and $AlPcS_4$, the transmembrane potential of the nuclear membrane decreased from -20 mV to about -10 mV with reference to the plasma membrane potential. Although the nucleus itself seems not to be photodynamically affected, all intranuclear structures in contact with the nuclear envelope can transfer photodynamic damage into the nucleus, to the DNA.

3.6 Role of calcium in photodynamically induced cell damage of human fibroblasts (Hubmer et al. 1996)

Photodynamically induced changes in the cytoplasmatic free calcium concentration ($[Ca^{2+}]_i$) and its role in cell damage were investigated in human skin fibroblasts using confocal laser scanning microscopy and the Ca^{++}-indicator dye fluo-3. Fluorescence and absorption spectrophotometry measurements indicate that the photosensitizer $AlPcS_4$ binds to the plasma membrane and only after irradiation is able to enter the cells, causing massive morphological alterations. Upon irradiation of sensitizer-treated cells, the intracellular Ca^{2+} concentration increased relative to

the amount of light and the extracellular Ca^{2+} concentration. The increase in $[Ca^{2+}]_i$ was substantially reduced in the absence of $[Ca^{2+}]_e$ Cell damage or death after photodyanmic treatment was prevented and delayed towards higher fluence by increasing $[Ca^{2+}]_i$ at high $[Ca^{2+}]_e$ and was accelerated at low $[Ca^{2+}]_e$. Application of Ca^{2+} channel blockers, such as Co^{++}, Cd^{++} or verapamil could not prevent the increase of $[Ca^{2+}]_i$ Our results indicate that the activation of the photosensitizer, $AlPcS_4$, causes an influx of Ca^{2+}, which protects cells from photodamage. At low $[Ca^{2+}]_e$ and high fluence values, release of Ca^{2+} from internal stores probably as a protective measure occurs in order to increase the $[Ca^{2+}]_i$

Fluorescence spectrophotometric measurements of $[Ca^{2+}]_i$ using the dual-wavelengths excitation of Fura 2-AM (340/ 380 nm) to indicate the binding state of Fura to cytosolic free Ca^{2+}, showed that human fibroblasts, incubated with $AlPcS_4$ in different concentrations and incubation times, increase their $[Ca^{2+}]_i$ moderatly within 1 10 min after irradiation with red light. Within a consecutive 5 min-period of dark incubation, the $[Ca^{2+}]_i$ decreases again.

3.7 Mutagenicity of Photosan 3 and ALA induced protoporphyrin IX (Fiedler et al. 1994 and 1996)

In the first investigation cultures of rat hepatocytes were treated with the photosensitizer Photosan 3 and examined for possible mutagenic effects in the absence of light. Hepatocytes are particularly useful in genotoxicity studies because of their capacity to metabolize a large variety of promutagenic/procarcinogenic compounds. The cytogenetic endpoints determined were chromosomal aberrations and the induction of micronuclei.

Three hours of incubation with Photosan 3 induced significantly elevated levels of chromosomal aberrations and micronuclei already at a concentration of 0.1 µg/ml compared to the controls. A concentration of 100 µg/ml PS3 appeared to be cytotoxic: no mitotic figures could be detected.

The second study was designed to investigate the mutagenic potential of 5-aminolevulinic acid (ALA) and/or the endogenously produced photosensitizer protoporphyrin IX (PpIX). ALA is a precursor of PpIX in the biosynthetic pathway for heme. The presence of exogenous ALA bypasses the feedback control and may induce the accumulation of PpIX. Since heme-containing enzymes are essential for energy metabolism, every nucleated cell in the body must have at least a minimal capacity to synthesize PpIX.

PDT leads to oxidative damage including the formation of genotoxic membrane degradation products via lipid peroxidation. Additionally, it was demonstrated that ALA itself can form the reactive oxygen species $O_2°$, H_2O_2 and OH° by autoxidation, suggesting that it could potentially induce DNA damage.

Therefore cultures of rat hepatocytes which have been demonstrated to be very sensitive indicators for genotoxic effects induced by the lipid peroxidation product 4-hydroxynonenal and analogous aldehydes, were examined for possible mutagenic

effects after treatment with ALA in the absence of light. The cytogenetic endpoints determined were again chromosomal aberrations and the induction of micronuclei. Compared to the controls, significantly elevated levels of chromosomal aberrations and micronuclei were observed at concentrations ≥ 1 µg/ml. Chromosomal aberrations and micronuclei were found to increase up to a concentration of 100 µg/ml ALA. While micronuclei are decreasing at higher concentrations, chromosomal aberrations remain at the same level. The kinetics of PpIX-formation after induction with 100 and 1000 µg/ml ALA appears to be the same for both concentrations, eventually suggesting that at least the induction of chromosomal aberrations may be due to PpIX.
Although the mutagenicity of PS3 and PpIX shows a significant increase already at low agent concentrations, it is still reduced as compared to other tumor therapies.

3.8 Formation kinetics of endogenous protoporphyrin IX in human fibroblasts (Krammer and Überriegler in press)

The influence of pH-value, incubation time and concentration of ALA on the PpIX formation of human skin fibroblasts was investigated by fluorescence spectrophotometry. The PpIX formation increases 1) with the pH-value of ALA, 2) with the ALA incubation time in a moderate sigmoidal manner, 3) with the ALA concentration up to 700 µg/ml. Other parameters such as cell washing procedures, have no influence on the PpIX production. ALA has to be applied in a concentration 30 times higher than external Pp IX and Photosan 3 in order to produce the same cytotoxic damage.
PpIX bleaching and the generation of a photoproduct at 646 nm was found, which could be a chlorin type.
Additional information about intracellular PpIX formation kinetics and its topographical correlation to cell structures was gained by a Low-Light-Imaging System. A few minutes after the onset of incubation with ALA, PpIX generation is observed in the mitochondria, followed by relocalization in the cytoplasm and the nuclear membrane. The intracellular targets differ from that after treatment with external porphyrins, which are mainly nuclear and cellular membranes first and the whole cytoplasm and even the nucleus later.

3.9 Photosensitizer uptake and retention in different cell lines (Hubmer et al. 1994)

For investigation of the heterogenity of different cell lines, which reflects the condition in the tumor, human skin fibroblasts, hepatocytes and endothelial cells were incubated with the sensitizer Photosan 3 up to 24 hours. Measurements of the cell suspension and the supernatant were carried out by fluorescence spectrophotometry. Results show different fluorescence levels and uptake kinetics for the different cell lines. A general aspect is that all cell lines take up most of the

sensitizer within some minutes, which is followed by a period of missing or reduced uptake.

3.10 Intracellular targets of photodamage (Überriegler et al. 1995)

The aim of the present study was to investigate the onset of destructive changes in living cocultivated Wi38 and VA13-transformed WI38 human fibroblasts following ALA incubation, PpIX production and subsequent irradiation by white halogen light with a dose of 2.2 kJ/m^2 Specific fluorescence markers such as 3,3'-dihexyloxacarbocyanine-iodide for endoplasmatic reticulum staining and dihydrorhodamine for intact mitochondria mapping combined with a low light imaging system are a versatile and sensitive tool to examine the photoinduced destruction of organelles in living cells, while artefacts are minimized. Mitochondria as primary targets of PpIX undergo a condensation under irradiation and are finally destroyed. Photodynamic treatment induces further a significant decomposition of endoplasmatic reticulum, although PpIX localization could not be determined. Initial destabilization and vesiculation of ER is followed by a porous network with large cisternae (indicating the breakdown of cell integrity and cell/nucleus membrane damage). Normal cocultivated lung fibroblasts showed a delay in destruction compared to the transformed WI38-VA13 cells. The observed decomposition pattern resembles the morphological pattern of apoptosis.

3.11 Topics of present and future investigations are:

1) photodynamically induced expression of human (proto)oncogenes, such as jun, myc, fos, K-ras and N-ras by quantitative RT-PCR,
2) stimulatory or damaging effects of PDT on the immune system, specially on macrophages and cytokines,
3) the mode of PDT induced cell kill: apoptosis versus necrosis, and
4) intracellular mapping of the metabolites ATP, glucose, lactate, NAD(P)H and calcium after photodynamic activation via chemoluminiscence by a Single Photon Counting set-up.

4. References

FIEDLER, D., B. KRAMMER and P. ECKL (1994): Cyto-and genotoxic potential of the photosensitizer Photosan 3 in the absence of light. - J Photochem.Photobiol. 22: 241-246.

FIEDLER, D., P. ECKL and B. KRAMMER (1996): Does delta-aminolevulinic acid induce genotoxic effects?. - J Photochem.Photobiol. 33,1: 39-45

GOMER, CH.J. (1991): Preclinical examination of first and second generation photosensitizers used in photodynamic therapy. - Photochem. Photobiol. 54, 6: 1093-1107.

HUBMER, A., F. BLUM, D. FIEDLER, and B. KRAMMER (1994): Fluorescence measurements of photosensitizer uptake and retention in different cell lines. - Dt. Zschr. f. Onkologie 26/6: 117-119.

HUBMER, A., A. HERMANN, K. ÜBERRIEGLER and B. KRAMMER (1996): Role of calcium in photodynamically induced cell damage of human fibroblasts.- Photochem. Photobiol. 64,1: 211-215

KRAMMER, B.(1990): A fast test system for the effect of in-vitro parameters on sensitizeing efficiency in photodynamic laser (tumor) therapy. - Onkologie 6, 13: 462-464.
KRAMMER-REUBEL, B. and W. HOFMANN (1991): Concentration and fluence dependence of the disturbance of the membrane integrity of human fibroblasts by the photodynamic action of photosan III. - Photochem. Photobiol. 10: 111-118.
KRAMMER-REUBEL, B. (1992): Transmembrane potential measurements of normal and transformed human fibroblasts following photodynamic lasertherapy. - Bioelectrochem. Bioenergetics 27: 19-22.
KRAMMER, B., A. HUBMER and A. HERMANN (1993): Photodynamic effects on the nuclear envelope of human skin fibroblasts. - J Photochem.Photobiol. 17: 109-114.
KRAMMER, B. (1995): The effect of photoprotectors on photodynamic processes in vitro. - Dtsch. Zschr. Onkol. 27, 3: 76-80.
KRAMMER, B. (1996): Investigation of the combined effect of photodynamic tumor therapy and hyperthermia by transmembrane resting potential measurements. - Bioelectrochem. Bioenergetics 39: 109-113.
KRAMMER B. and K. ÜBERRIEGLER (in press): In vitro investigation of ALA induced protoporphyrin IX. - Photochem. Photobiol.
SCHNITZHOFER, G. and B. KRAMMER (in press): Combination of photodynamic treatment and radiotherapy - Cancer Letters.
ÜBERRIEGLER, K., E. BANIEGHBAL and B. KRAMMER (1995): Subcellular damage kinetics within co-cultivated WI38 and VA13-transformed WI38 human fibroblasts following 5-aminolevulinic acid induced protoporphyrin IX formation. - Photochem. Photobiol. 62, 6: 1052-1057.
UNSÖLD, E. and D. JOCHAM (1988): Grundlagen photodynamischer Laser-Therapie-Verfahren. Der Chirurg 59: 75-80.

Acknowledgements: Project supported by a grant from the FWF, Austria, Nr.: P08921-MED and Nr.: P10798-MED

Korrespondenzadresse:

B. Krammer
Institut für Physik und Biophysik
Universität Salzburg
Hellbrunnerstr. 34
5020 Salzburg
Tel.: 0662/8044/5703, FAX: 5704
e-mail: Barbara.Krammer@sbg.ac.at