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Cancer therapy: Binary modes

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In binary tumor therapy the concomitant application of two non toxic agents is selectively synergized in-situ to provide highly efficient antitumor effects otherwise unattainable by either one alone.

Photodynamic Therapy (PDT) of Solid Tumors with Pd-Bacteriopheophorbide (TOOKAD) and light (In collaboration with Prof. Avigdor Scherz, Department of Plant Sciences).

Photodynamic therapy (PDT) is a novel mode of cancer therapy in which drug action is locally controlled by light. In PDT, a nontoxic pigment (sensitizer) is photosensitized in-situ by non-hazardous light, to generate cytotoxic Reactive Oxygen Species (ROS) that cause cell death and necrosis of tumor components, with minimal damage to the surrounding tissue. Pd-Bachteriopheophorbide (TOOKAD) is a PDT agent synthesized in our laboratories which eradicates tumors by destruction of their blood supply (Scherz et al., 1999; Brandis et al., 2001). TOOKAD has been selected by us and STEBA BIOTECH NV as the lead compound for PDT in clinical trials that will be started by the end of the year 2001.

Research objectives: The major part of our research is focused on resolving basic aspects of the response of tumor cells and tissues to PDT with special attention on the response of tumor blood vessels to ROS generation by light-activated bacteriochlorophyll derivatives. Pharmacokinetic studies of TOOKAD are carried out in animals. The respective PDT protocols for treatment and evaluation of response to PDT are being developed in preclinical studies. PDT is also studied in cell cultures for evaluation of therapeutic parameters. The response of cultured cells to sublethal levels of ROS is being examined in the context of their possible involvement in normal physiology.

Research achievements: PDT of human prostate cancer (PC) tumors was examined in human small cell carcinoma of the prostate (SCCP) the most malignant variant of PC, using the WISH PC2 xenograft model. Three implantation sites (subcutaneous, orthotopic or intraosseus) representing various disease characteristics that enabled the evaluation of the response parameters in human tissue were used. Subcutaneous tumors showed complete cure within 28-40 days, reaching an overall long-term cure rate of 73% 90 days after PDT, as histologically confirmed. Good response was also confirmed histologically in the orthotopic model. Intratibial bone lesions responded by complete cure within 14-24 days after treatment as histologically documented, with complete tumor elimination in 50% of the treated animals 70-90 days after PDT. To the best of our knowledge this is the first report of successful PDT of tumor bone lesions (Koudinova et al., 2001) (study in collaboration with J. Pinthus and Z. Eshhar, Dept. of Chemical Immunology).

PDT of melanotic M2R melanoma tumors in mice showed significant curative response enabled by the near infrared absorption of TOOKAD, a characteristic not shared with other PDT drugs. Blood vessels were injured and became congested with thrombi; necrotic areas and hemorrhages were apparent from 1 to 48h post PDT. Necrosis developed from 16 to 48h after PDT and healing began with tissue granulation by 6 to 10 days post PDT with complete wound healing by 30 days after PDT. Specific immunostaining for lipid peroxidation, using anti-4-hydroxy-2-nonenal (HNE) was chosen to demonstrate the photodamage induced by PDT. HNE is a major aldehyde formed following peroxidation of n6-poly unsaturated fatty acids. Connective tissue staining was negative suggesting selective PDT of the tumor stroma (Brandis et al., 2001).

PDT of remote metastases was also studied. Local control of C6 glioma xenografts in the mouse foot by PDT was up to 64% whereas in surgically treated mice, local tumor control was absolute. However the number of metastases in the groin and in the lungs were up to 10-fold lower following PDT with overall cure rates of 36% and 6% (8 weeks) for PDT and surgery respectively. The basis for this observation is being examined (Schreiber et al., 2001).

Phototoxicity of TOOKAD in cultured cells: A few highlights of the results are listed: Phototoxixity of TOOKAD was found to depend on ambient oxygen concentrations in a manner similar to that reported in the literature for the cytotoxic effects of ionizing radiation. TOOKAD transport into the cells is inhibited at 4°C but the phototoxic effect can be mediated by extracellular

sensitizer. The response of mulidrug resistant (MDR) cells to PDT with TOOKAD was found to be lower than of the parent wild type cells. The basis of this observation is currently under investigation (in collaboration with M. Liscovitch, The Dept. of Biological Regulation). Photocytotoxicity was demonstrated, under real time conditions (using NMR spectroscopy of 31P-ATP) as rapid light dependent decline in intracellular ATP. (See also Scherz et al., page 94).

Effects of ROS on cellular processes

Research Objective: To study the role of oxygen free radicals in normal physiology.

Research achievements: Controlled photogeneration of ROS was microscopically visualized in cultured cells (using ROS specific fluorescent probes) and permitted the study of the response of particular enzymes and processes to sublethal doses. By using TOOKAD + light we affected the activity of MAP kinases and phospholipases in melanoma tumor cells and established their differential response in defined subcellular compartments. ROS induced Ca2+ transients were illustrated in endothelial cells and the basis of this observation is being investigated. The ability to control ROS production by this photoswitch, provides a novel approach to the study of the general effects of ROS on cells, their role in PDT and in particular their potential role in cellular signaling.

Boron Neutron capture therapy (BNCT): pharmacology and noninvasive imaging of ^{10}B-agents for cancer therapy (In collaboration with Dr. Peter Bendel, Department of Chemical Services).

BNCT is an experimental cancer treatment in which a neutron beam is used for irradiating ^{10}B-labeled substances, which accumulated in the tumor, to locally release cytotoxic alpha particles.

Research Objective: (i) To investigate the chemistry and the pharmacology of candidate boron delivery agents in tumor models, (ii) to develop boronated substances for clinical use and (iii) to develop non-invasive *in-vivo* detection methods based on Magnetic Resonance Spectroscopy (MRS) and imaging (MRI).

Research achievements: We have obtained the first *in-vivo* images of ^{10}B-enriched borocaptate sodium (BSH) in tumor-bearing mice, using ^{10}B MRI. This method was also implemented and tested on a human MRI scanner and could pave the way for noninvasive, on-line quantitative mapping of the drug in patients who are about to undergo neutron irradiation thereby overcoming a serious deficiency in the way BNCT is applied today. We also confirmed that the borocaptate dimer (BSSB) has a much longer retention time in malignant cells than the monomer BSH, using on-line NMR monitoring of the drugs uptake and washout in cultured superfused cells (Bendel et all 2001, Elhanati et al 2001).

Selected Publication:

Scherz A., Salomon Y., Scheer H. and Brandis, A., (1999) International PCT Patent Application No. PCT/IL99/00673, Palladium-substituted bacteriochlorophyll derivatives and use thereof. International PCT Patent Application No. PCT/IL99/00673,

Brandis, A., Mazor, O., Gross, S., Koudinova, N., Hammi, R., Kammhuber, N., Rosenbach-Belkin, V., Greenwald, M., Bondon, A, Simonneaux, G., Scheer, H., Salomon, Y. and Scherz (2001) Palladium-Bacteriochlorophyll derivatives: Synthesis and Phototoxicity (submitted)

Koudinova, N., Pinthus, J.H., Brandis, A., Brenner, O., Bendel, P., Ramon J., Eshhar, Z., Scherz, A, Salomon, Y. (2001) Tookad based photodynamic therapy: successful *in vivo* treatment of human prostatic small cell carcinoma xenografts (Submitted).

Schreiber S., Gross S., Brandis A., Harmelin A., Rosenbach-Belkin, V., Scherz A., and Salomon Y. (2001) Local photodynamic therapy (pdt) of rat C6 glioma xenografts with pd-bacteriopheophorbide leads to decreased metastases and increased animal cure compared to surgery. Int. J. Cancer. (in press).

Bendel, P., Koudinova, N., and Salomon Y. (2001) *In-vivo* imaging of the neutron capture therapy agent BSH in mice using ^{10}B MRI. Magn. Reson .Med. 46, 13-17.

Elhanati, G., Salomon, Y. Bendel, P. (2001) Significant differences in the retention of the borocaptate monomer (BSH) and dimer (BSSB) in malignant cells. Cancer Lett. 172, 127-132

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