**Carbohydrate-Porphyrin Conjugates with Two-Photon Absorption Properties as Potential Photosensitizing Agents for Photodynamic Therapy**

**Summary**

We report the synthesis of a series of conjugated zinc porphyrin oligomers designed as photodynamic therapy agents. These compounds exhibit exceptionally high two-photon absorption cross-sections, redshifted linear absorption spectra, and high singlet oxygen quantum yields, making them ideal for one-photon- and two-photon-excited photodynamic therapy. These products are substituted by three α-mannose units on each chromophore with the aim to target tumor cells with over-expressing lectin-type membrane receptors.

**Meso-tetraphenyl porphyrin derivatives: The effect of structural modifications on binding to DMPC liposomes and albumin**

**Summary**

Three series of glycoconjugated and hydroxylated derivatives of 5,10,15,20-meso-tetraphenyl porphyrin (TPP) were studied in order to evaluate the effect of a porphyrin structure on its binding to dimyristoylphosphatidylcholine (DMPC) liposomes and to human serum albumin (HSA). The studied derivatives have been developed as potent photosensitizers for photodynamic therapy (PDT) of cancers. Steady state and time resolved fluorescence emission spectroscopy, Stern-Volmer quenching and fluorescence anisotropy were used for this evaluation. The lipophilicity of the compounds has been deduced from their retention time in reverse phase liquid chromatography. The results demonstrated that the more polar glycoconjugated compounds presented limited aggregation in aqueous media and very rapid binding kinetics to DMPC liposomes and HSA. Derivatives having intermediate or high hydrophobicity showed extensive auto-association in aqueous media and as a consequence slow association kinetics. The strength of porphyrin binding to DMPC liposomes also depended on their lipophilicity and was lower for the polar glycoconjugated analogues. The highest affinity for liposomes was observed for hydroxylated derivatives with intermediate lipophilicity. In contrast, the highest binding constant for albumin was observed for a polar tetra-glycoconjugated analogue. The depth of penetration into the phospholipid bilayer did not appear to be directly related to the global hydrophobicity of the compounds, but depended more on the number of apolar, non-substituted phenyl groups grafted to a tetrapyrrolic macrocycle. Furthermore, liposome-albumin competition studies revealed that the porphyrins were always mainly partitioned into the phospholipid bilayer.
Markhaba Tukenova, Catherine Guibout, Mike Hawkins, Eric Quiniou, Abdeddahir Mousannif, Hélène Pacquement, David Winter, André Bridier, Dimitri Lefkopoulos, Odile Oberlin, Ibrahima Diallo, Florent de Vathaire (2010 Jul 22)

Radiation therapy and late mortality from second sarcoma, carcinoma, and hematological malignancies after a solid cancer in childhood.


Summary

**PURPOSE:**
To compare patterns of long-term deaths due to secondary carcinomas, sarcomas, and hematological malignancies occurring after childhood cancer in a cohort of patients followed over a median of 28 years.

**METHODS AND MATERIALS:**
The study included 4,230 patients treated at eight institutions, who were at least 5-year survivors of a first cancer, representing 105,670 person-years of observation. Complete clinical, chemotherapeutic, and radiotherapeutic data were recorded, and the integral radiation dose was estimated for 2,701 of the 2,948 patients who had received radiotherapy. The integral dose was estimated for the volume inside the beam edges. The causes of death obtained from death certificates were validated.

**RESULTS:**
In total, 134 events were due to second malignant neoplasm(s) (SMN). We found that the standardized mortality ratio decreased with increasing follow-up for second carcinomas and sarcomas, whereas the absolute excess risk (AER) increased for a second carcinoma but decreased for second sarcomas. There was no clear variation in SMN and AER for hematological malignancies. We found a significant dose-response relationship between the radiation dose received and the mortality rate due to a second sarcoma and carcinoma. The risk of death due to carcinoma and sarcoma as SMN was 5.2-fold and 12.5-fold higher, respectively, in patients who had received a radiation dose exceeding 150 joules.

**CONCLUSIONS:**
Among patients who had received radiotherapy, only those having received the highest integral radiation dose actually had a higher risk of dying of a second carcinoma or sarcoma.

Year of publication 2010


2D and 3D radial multi-gradient-echo DCE MRI in murine tumor models with dynamic R*2-corrected R1 mapping

*Magnetic resonance in medicine* : 64 : 313-318 : DOI : 10.1002/mrm.22396

Summary

Dynamic contrast-enhanced MRI is extensively studied to define and evaluate biomarkers for early assessment of vasculature-targeting therapies. In this study, two-dimensional and
three-dimensional radial multi-gradient-echo techniques for dynamic $R^*(2)$-corrected $R(1)$
mapping based on the spoiled gradient recalled signal equation were implemented and
validated at 4.7 T. The techniques were evaluated on phantoms and on a respiratory motion
animated tumor model. $R(1)$ measurements were validated with respect to a standard
inversion-recovery spin-echo sequence in a four-compartment phantom covering a range of
relaxation rates typically found in tumor tissue. In the range of [0.4, 3] sec$^{-1}$, $R(1)$
differences were less than 10\% for both two-dimensional and three-dimensional
experiments. A dynamic contrast-enhanced MRI pilot study was performed on a colorectal
tumor model subcutaneously implanted in mice at the abdominal level. Low motion
sensitivity of radial acquisition allowed image recording without respiratory triggering. Three-
dimensional $K(\text{trans})$ maps and significantly different mean $K(\text{trans})$ values were obtained for
two contrast agents with different molecular weights. The radial multi-gradient-echo
approach should be most useful for preclinical experimental conditions where the tissue of
interest experiences physiologic motion, like spontaneous extracerebral tumors developed
by transgenic mice, and where dynamic contrast-enhanced MRI is performed with high-
relaxivity contrast agents.

Paul Guichard, Denis Chrétien, Sergio Marco, Anne-Marie Tassin (2010 Mar 27)
Procentriole assembly revealed by cryo-electron tomography.
The EMBO journal : 1565-72 : DOI : 10.1038/emboj.2010.45

Summary

Centrosomes are cellular organelles that have a major role in the spatial organisation of the
microtubule network. The centrosome is comprised of two centrioles that duplicate only once
during the cell cycle, generating a procentriole from each mature centriole. Despite the
essential roles of centrosomes, the detailed structural mechanisms involved in centriole
duplication remain largely unknown. Here, we describe human procentriole assembly using
cryo-electron tomography. In centrosomes, isolated from human lymphoblasts, we observed
that each one of the nine microtubule triplets grows independently around a periodic central
structure. The proximal end of the A-microtubule is capped by a conical structure and the B-
and C-microtubules elongate bidirectionally from its wall. These observations suggest that
the gamma tubulin ring complex (gamma-TuRC) has a fundamental role in procentriole
formation by nucleating the A-microtubule that acts as a template for B-microtubule
elongation that, in turn, supports C-microtubule growth. This study provides new insights into
the initial structural events involved in procentriole assembly and establishes the basis for
determining the molecular mechanisms of centriole duplication on the nanometric scale.

ALLARD Aurore, HADDY Nadia, LE DELEY Marie-Cécile, RUBINO Carole, LASSALLE Mathilde,
SAMSALDIN Ahthar, QUINIOU Eric, CHOMPRET Agnès, LEFKOPOULOS Dimitri, DIALLO Ibrahima,
DE VATHAIRE Florent (2010 Mar 10)
Role of radiation dose in the risk of secondary leukemia after a solid tumor in
International journal of radiation oncology, biology, physics : 78 : 1474-1482 : DOI :
Summary

Purpose
The purpose of this study was to estimate the risk of secondary leukemia as a function of radiation dose, taking into account heterogeneous radiation dose distribution.

Methods and Materials
We analyzed a case–control study that investigated the risk of secondary leukemia and myelodysplasia after a solid tumor in childhood; it included 61 patients with leukemia matched with 196 controls. Complete clinical, chemotherapy, and radiotherapy histories were recorded for each patient in the study. Average radiation dose to each of seven bone marrow components for each patient was incorporated into the models, and corresponding risks were summed up. Conditional maximum likelihood methods were used to estimate risk parameters.

Results
Whatever the model, we failed to evidence a role for the radiation dose to active bone marrow in the risk of later leukemia, myelodysplasia, or myeloproliferative syndrome, when adjusting for epipodophyllotoxin and anthracycline doses. This result was confirmed when fitting models that included total dose of radiation delivered during radiotherapy, when fitting models taking into account dose per fraction, and when restricting the analysis to acute myeloid leukemia.

Conclusions
In contrast to results found in similar studies that included children treated before the use of epipodophyllotoxins, this study failed to show a role for radiotherapy in the risk of secondary leukemia after childhood cancer in children treated between 1980 and 1999. This discrepancy was probably due to a competitive mechanism between these two carcinogens.

Markhaba Tukenova, Ibrahima Diallo, Mike Hawkins, Catherine Guibout, Eric Quiniou, Hélène Pacquement, Frederic Dhermain, Akhtar Shamsaldin, Odile Oberlin, Florent de Vathaire (2010 Mar 5)

Long-term mortality from second malignant neoplasms in 5-year survivors of solid childhood tumors: temporal pattern of risk according to type of treatment.
Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology : 19 : 707-715 : DOI : 10.1158/1055-9965.EPI-09-1156

Summary

BACKGROUND:
The temporal pattern in mortality from late second malignant neoplasms in solid childhood
cancer survivors, according to the type of treatment, has not been investigated in detail.

METHODS:
We studied 4,230 5-year survivors of solid childhood cancer diagnosed between 1942 and 1986 in France and the United Kingdom. Complete clinical, chemotherapy, and radiotherapy data were recorded and the integral radiation dose was estimated for 2,701 of the 2,948 patients who had received radiotherapy.

RESULTS:
After a median follow-up of 28 years, 134 fatal events were due to second malignancies, compared with the 13.3 expected from the general France-UK population rates. The standardized mortality ratio was of a similar magnitude after radiotherapy alone and chemotherapy alone and higher after both treatments. The standardized mortality ratio decreased with follow-up, whereas the absolute excess risk increased significantly over a period of at least 25 years after the first cancer. This temporal pattern was similar after chemotherapy alone, radiotherapy alone, or both treatments. We observed a similar long-term temporal pattern among survivors who had died of a second malignant neoplasm of the gastrointestinal tract and breast. Survivors who had received a higher integral radiation dose during radiotherapy were at a particularly high risk, as well as those who had received alkylating agents and epipodophyllotoxins.

CONCLUSIONS:
Five-year survivors of childhood cancer run a high long-term mortality risk for all types of second malignant neoplasms whatever the treatment received and require careful long-term screening well beyond 25 years after the diagnosis.

Jayachandran Gopalakrishnan, Paul Guichard, Andrew H Smith, Heinz Schwarz, David A Agard, Sergio Marco, Tomer Avidor-Reiss (2010 Jan 20)

**Self-assembling SAS-6 multimer is a core centriole building block.**
The *Journal of biological chemistry*: 8759-70 : DOI: [10.1074/jbc.M109.092627](https://doi.org/10.1074/jbc.M109.092627)

**Summary**

Centrioles are conserved microtubule-based organelles with 9-fold symmetry that are essential for cilia and mitotic spindle formation. A conserved structure at the onset of centriole assembly is a “cartwheel” with 9-fold radial symmetry and a central tubule in its core. It remains unclear how the cartwheel is formed. The conserved centriole protein, SAS-6, is a cartwheel component that functions early in centriole formation. Here, combining biochemistry and electron microscopy, we characterize SAS-6 and show that it self-assembles into stable tetramers, which serve as building blocks for the central tubule. These results suggest that SAS-6 self-assembly may be an initial step in the formation of the cartwheel that provides the 9-fold symmetry. Electron microscopy of centrosomes identified 25-nm central tubules with repeating subunits and show that SAS-6 concentrates at the core of the cartwheel. Recombinant and native SAS-6 self-oligomerizes into tetramers with approximately 6-nm subunits, and these tetramers are components of the centrosome,
suggesting that tetramers are the building blocks of the central tubule. This is further supported by the observation that elevated levels of SAS-6 in Drosophila cells resulted in higher order structures resembling central tubule morphology. Finally, in the presence of embryonic extract, SAS-6 tetramers assembled into high density complexes, providing a starting point for the eventual in vitro reconstruction of centrioles.

Maillard P., Lupu M., Thomas C.D., Mispelter J. (2010 Jan 1) 
**Towards a new treatment of retinoblastoma?**

**Summary**

Photodynamic therapy (PDT) is a recent approach for the treatment of small cancerous tumours, on-surface or accessible by endoscopy in which a dye (usually a tetrapyrrolic macrocycle) absorbs light and generates cytotoxic reactive oxygen species leading to cellular damage. Retinoblastoma (Rb) is a rare intraocular tumour of childhood. All the multifocal forms are hereditary and constitute a syndrome of genetic predisposition in the cancer. The current treatments with etoposide or carboplatine expose the patient to the late risk of second cancer. The use of PDT as cancer therapy is particularly attractive due to the use of few mutagenic and non-toxic photosensitizers (PS) prior light excitation and to the localized tumour illumination. The photoefficiency towards Rb of a glycoconjugated porphyrin is discussed and compared with the results obtained with a second-generation photosensitizer, the Foscan. Some in vivo results on an animal model of Rb are presented by a point of view of photoefficiency, biodistribution, pharmacokinetic and longitudinal follow-up of the PDT effect using a new non-invasive method of magnetic resonance imaging of real-time. Photodynamic treatments in association with non-invasive sodium imaging open ways for new treatment tailoring or treatment individualization of retinoblastoma in clinic.[PMID: 20569775]

**Effect of cholesterol and sugar on the penetration of glycodendrimeric phenylporphyrins into biomimetic models of retinoblastoma cells membranes**

**Summary**

Photodynamic therapy (PDT) is considered one efficient treatment against retinoblastoma. The specificity of a photosensitizer and its penetration into cancerous cells are crucial for achieving tumor necrosis. The selection of photosensitizers such as porphyrin derivatives by tumor cells thus depends to a large extent on their ability to interact with the biological membrane. In this work, we have studied by surface pressure measurements and fluorescence spectroscopy the interaction between three newly synthesized dendrimeric phenylporphyrins and monolayers or liposomes with increasing cholesterol content.
mimicking the retinoblastoma cell membrane. The morphology of phospholipid-cholesterol-porphyrin mixed monolayers was also analyzed by Brewster angle microscopy. The results showed that the increase in cholesterol content in the model membranes had almost no effect on the effective penetration of the drugs into the lipid layers. Conversely, the chemical structure of the glycodendrimeric phenylporphyrins and the presence of sugar moieties especially appeared to play a crucial role. Although the non-glycoconjugated phenylporphyrin penetrated to a greater extent than glycodendrimeric ones into the liposome membrane, this could be achieved at a high lipid/porphyrin ratio only. Glycodendrimeric porphyrins exhibited improved surface properties compared to the non-glycoconjugated derivative and could penetrate into lipid layers even at low lipid/porphyrin ratios and high surface pressures. Our work highlights the role in the passive diffusion of porphyrins into biomimetic cancer cell membranes, of complex interactions among the lipid molecules, the sugar moieties, and the hydrophobic macrocycle of the porphyrins.[PMID: 20527940]

Year of publication 2009

Nebraska Zambrano, Paul P Guichard, Yanzhen Bi, Bastien Cayrol, Sergio Marco, Véronique Arluison (2009 Jul 3)

**Involvement of HFq protein in the post-transcriptional regulation of E. coli bacterial cytoskeleton and cell division proteins.**

*Cell cycle (Georgetown, Tex.)* : 2470-2

**Summary**


**Simultaneous two-voxel localized (1)H-observed (13)C-edited spectroscopy for in vivo MRS on rat brain at 9.4T: Application to the investigation of excitotoxic lesions**

*Journal of magnetic resonance (San Diego, Calif.)* : 198 : 94-104 : DOI: 10.1016/j.jmr.2009.01.023

**Summary**

(13)C spectroscopy combined with the injection of (13)C-labeled substrates is a powerful method for the study of brain metabolism in vivo. Since highly localized measurements are required in a heterogeneous organ such as the brain, it is of interest to augment the sensitivity of (13)C spectroscopy by proton acquisition. Furthermore, as focal cerebral lesions are often encountered in animal models of disorders in which the two brain hemispheres are compared, we wished to develop a bi-voxel localized sequence for the simultaneous bilateral investigation of rat brain metabolism, with no need for external additional references. Two sequences were developed at 9.4T: a bi-voxel (1)H-(13)C STEAM-POCE (Proton Observed Carbon Edited) sequence and a bi-voxel (1)H-(13)C PRESS-POCE adiabatically decoupled.
sequence with Hadamard encoding. Hadamard encoding allows both voxels to be recorded simultaneously, with the same acquisition time as that required for a single voxel. The method was validated in a biological investigation into the neuronal damage and the effect on the Tri Carboxylic Acid cycle in localized excitotoxic lesions. Following an excitotoxic quinolinate-induced localized lesion in the rat cortex and the infusion of U-(13)C glucose, two (1)H-((13)C) spectra of distinct (4x4x4mm(3)) voxels, one centred on the injured hemisphere and the other on the contralateral hemisphere, were recorded simultaneously. Two (1)H bi-voxel spectra were also recorded and showed a significant decrease in N-acetyl aspartate, and an accumulation of lactate in the ipsilateral hemisphere. The (1)H-((13)C) spectra could be recorded dynamically as a function of time, and showed a fall in the glutamate/glutamine ratio and the presence of a stable glutamine pool, with a permanent increase of lactate in the ipsilateral hemisphere. This bi-voxel (1)H-((13)C) method can be used to investigate simultaneously both brain hemispheres, and to perform dynamic studies. We report here the neuronal damage and the effect on the Tri Carboxylic Acid cycle in localized excitotoxic lesions.

Ibrahima Diallo, Nadia Haddy, Elisabeth Adjadj, Akhtar Samand, Eric Quiniou, Jean Chavaudra, Iannis Alziar, Nathalie Perret, Sylvie Guérin, Dimitri Lefkopoulos, Florent de Vathaire (2009 Apr 24)

Frequency distribution of second solid cancer locations in relation to the irradiated volume among 115 patients treated for childhood cancer.

Summary

PURPOSE:
To provide better estimates of the frequency distribution of second malignant neoplasm (SMN) sites in relation to previous irradiated volumes, and better estimates of the doses delivered to these sites during radiotherapy (RT) of the first malignant neoplasm (FMN).

METHODS AND MATERIALS:
The study focused on 115 patients who developed a solid SMN among a cohort of 4581 individuals. The homemade software package Dos_EG was used to estimate the radiation doses delivered to SMN sites during RT of the FMN. Three-dimensional geometry was used to evaluate the distances between the irradiated volume, for RT delivered to each FMN, and the site of the subsequent SMN.

RESULTS:
The spatial distribution of SMN relative to the irradiated volumes in our cohort was as follows: 12% in the central area of the irradiated volume, which corresponds to the planning target volume (PTV), 66% in the beam-bordering region (i.e., the area surrounding the PTV), and 22% in regions located more than 5 cm from the irradiated volume. At the SMN site, all dose levels ranging from almost zero to >75 Gy were represented. A peak SMN frequency of approximately 31% was identified in volumes that received <2.5 Gy.
CONCLUSION:
A greater volume of tissues receives low or intermediate doses in regions bordering the irradiated volume with modern multiple-beam RT arrangements. These results should be considered for risk-benefit evaluations of RT.

Liliane Mouawad, Adriana Isvoran, Eric Quiniou, Constantin T Craescu (2009 Jan 22)
**What determines the degree of compactness of a calcium-binding protein?**

**Summary**

The EF-hand calcium-binding proteins may exist either in an extended or a compact conformation. This conformation is sometimes correlated with the function of the calcium-binding protein. For those proteins whose structure and function are known, calcium sensors are usually extended and calcium buffers compact; hence, there is interest in predicting the form of the protein starting from its sequence. In the present study, we used two different procedures: one that already exists in the literature, the sosuidumbbell algorithm, mainly based on the charges of the two EF-hand domains, and the other comprising a novel procedure that is based on linker average hydrophilicity. The linker consists of the residues that connect the domains. The two procedures were tested on 17 known-structure calcium-binding proteins and then applied to 59 unknown-structure centrins. The sosuidumbbell algorithm yielded the correct conformations for only 15 of the known-structure proteins and predicted that all centrins should be in a closed form. The linker average hydrophilicity procedure discriminated well between all the extended and non-extended forms of the known-structure calcium-binding proteins, and its prediction concerning centrins reflected well their phylogenetic classification. The linker average hydrophilicity criterion is a simple and powerful means to discriminate between extended and non-extended forms of calcium-binding proteins. What is remarkable is that only a few residues that constitute the linker (between 2 and 20 in our tested sample of proteins) are responsible for the form of the calcium-binding protein, showing that this form is mainly governed by short-range interactions.

**23Na MRI longitudinal follow-up of PDT in a xenograft model of human retinoblastoma**

**Summary**

BACKGROUND Photodynamic therapy is an established cancer treatment in which a photosensitizing agent is activated by exposure to light thus generating cytotoxic reactive oxygen species that cause cellular damage. METHODS A new photosensitizer synthesized at Curie Institute was used to treat retinoblastoma xenografts in mice, a glycoconjugated meso
substituted porphyrin derivative, that showed some retinoblastoma cell affinity. The longitudinal follow-up of the tumors was carried out by (23)Na MRI (without adding exogenous contrast agents) to map the extracellular compartment and to characterize cell packing. Two regimens were followed to target either blood vessels alone or blood vessels and cancer cells simultaneously. RESULTS AND CONCLUSIONS Only the protocol targeting both cancer cells and blood vessels effectively induces cellular death, confirmed by histology at the end of the experiment. Sodium MRI evidences a huge change in the cellular density of tumors only 24h after a double targeting (vascular and cellular) PDT treatment. We suggest that this change was possibly due to a bystander effect that can be promoted by the intercellular signaling favored by the high cellular density of retinoblastoma. These results indicate that non-invasive (23)Na imaging (which detects the tumor response to treatment from very early stages) in association with non-mutagenic therapies represents an effective option for tailored and individualized clinical treatments.