Hotel "Gli Dei" - Pozzuoli 28/29 Ottobre 2010

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tenth workshop on

pharmacobiometallics

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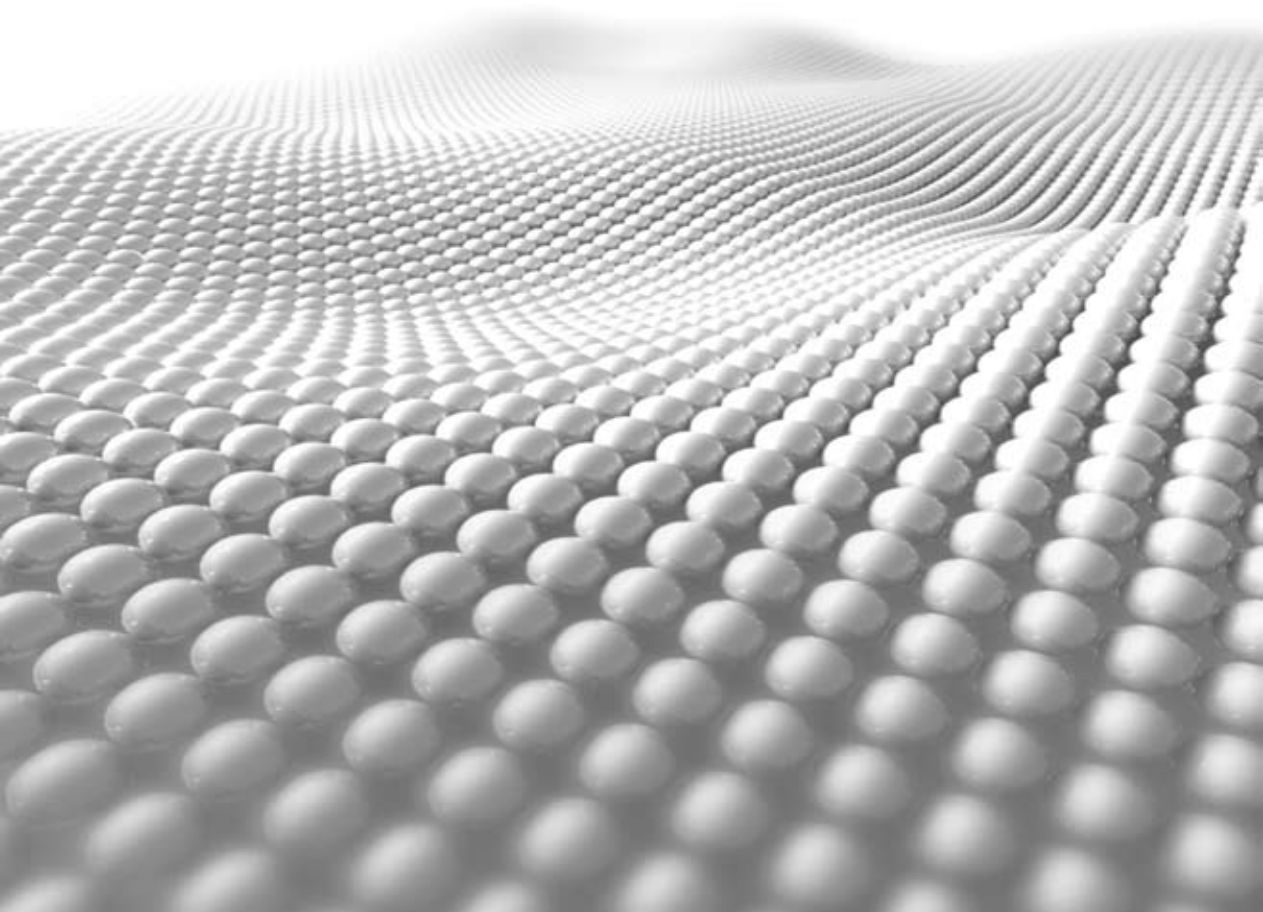

Hotel “GLI DEI” – Pozzuoli, Napoli
29-30 Ottobre, 2010

Tenth Workshop on

pharmacobiometallics

Organizzato da:

CIRPeB – Università di Napoli “Federico II”

per il

Consorzio Interuniversitario di Ricerca in Chimica dei Metalli nei Sistemi Biologici

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Plenary Lecture

Biochemical bases of selectivity in the protein-uranium interaction

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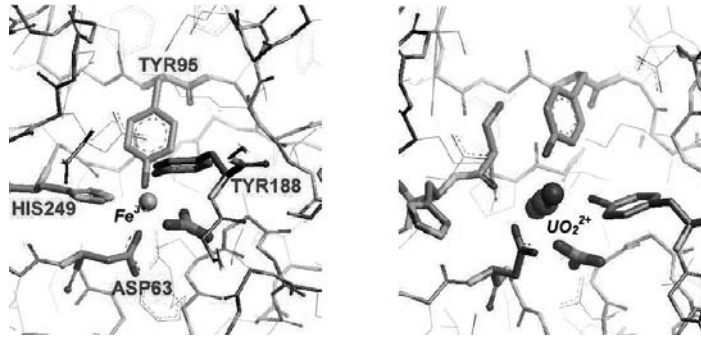
Uranium is a widespread metal cation in the environment due to its natural abundance and to its large use in both civilian and military industries. Owing to the low specific activity and long half-lives of its major radioisotopes, natural uranium is considered more as a chemical toxicant than as a radiotoxicant. The major form in biological media is the uranyl cation (UO_2^{2+}). Uranium absorbed via inhalation or ingestion rapidly enters the bloodstream and forms ionic complexes with a large variety of biological ligands. The current toxicokinetic model for uranium (ICRP 69) proposes a simplistic model with 50% bound to bicarbonate anion, 30% to transferrin, and 20% to the surface of erythrocytes. It roughly fits with the bio-distribution pattern observed at high dose but fails to explain its distribution at low dose and does not enable the design of an efficient strategy for decorporation.

A large study has been launched to revisit the biochemical features of uranium-protein interaction. Several proteins, from peptides [1], transferring [2], C-reactive protein [3], to antibodies [4, 5] have been characterized. The structural, thermodynamic, and kinetic results indicate that this binding is very selective *in vitro*. However, the biological data largely demonstrate the pleiotropic behaviour of uranium. The biological mechanism has been approached in both lung and kidney epithelial cells ; candidate biomarkers have been identified [6, 7] but pertinent target proteins responsible for the toxic effects are yet sought. Dedicated methods are currently being designed in this perspective [8-10].



A comparative modelling of uranyl in the metal binding site of transferrin. The model fits with most of the constraints identified from the survey of PDB ; i.e. 5 ligands in the equatorial plane and carboxylate, carbonyl, phenolate, or carbonate as preferred donors.

References

1. Jeanson et al. New J Chem (2009) 33, 976.
2. Vidaud et al. Biochemistry (2007) 46, 2215.
3. Pible O, Vidaud C, Plantevin S, Pellequer J.-L, Quéméneur E. Protein Science (2010) in press
4. Odorico et al. Biophys J (2007) 93, 645.
5. Reisser-Rubrecht et al. Chem Res Toxicol (2008) 21, 349.
6. Prat O, Bérenguer F, Steinmetz G, Ruat S, Sage N, Quéméneur E. Toxicol. In Vitro (2010) 24, 160.
7. Malard V, Gaillard JC, Bérenguer F, Sage N, Quéméneur E. BBA - Proteins and Proteomics (2009) 1794, 882.
8. Bou Maroun E, Hagège A, Basset C, Quéméneur E, Vidaud C, Asfari Z. Supramol Chem (2009) 21, 585.
9. Basset C, Dedieu A, Guérin P, Quéméneur E, Meyer D, Vidaud C. J Chromatogr A (2008) 1185, 233.
10. Dedieu A, Berenguer F, Basset C, Prat O, Quéméneur E, Pible O, Vidaud C. J. Chromatogr. A (2009) 1216, 5365.

Biomineralizzazione e Biocristallografia

Controlled release of platinum complexes from biomimetic hydroxyapatite nanocrystals

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The aim of this work is the study of the role of biomimetic hydroxyapatite (HA) nanocrystals as bone substitutes and describes their use as bone-targeted drug delivery devices for *in situ* treatment of bone tumours upon local implantation. The adsorption and release kinetics of bis-{ethylenediamineplatinum(II)}-2-amino-1-hydroxyethane-1,1-diyl-bisphosphonate (A) and bis-{ethylenediamineplatinum(II)}medronate (B) on four kinds of HA nanocrystals having different dimensions, morphologies, crystallinity degrees, and surface areas have been investigated.

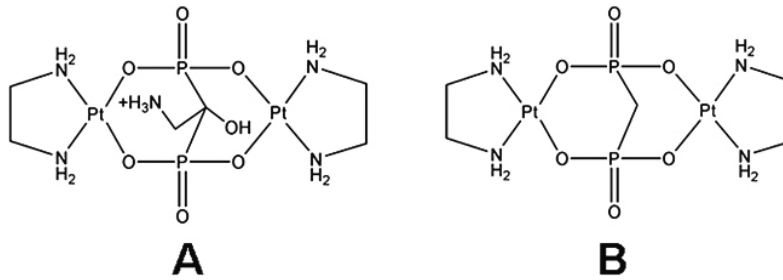

Figure 1. Sketches of bis-{ethylenediamineplatinum(II)}-2-amino-1-hydroxyethane-1,1-diyl-bisphosphonate (A) and bis-{ethylenediamineplatinum(II)}medronate (B).

The chemical properties of the two Pt complexes appreciably affect not only the affinity towards the different HAs, but also their release. [1,2] The cytotoxicity of the Pt complexes released from the HAs were tested against human cervix carcinoma cells and, interestingly, were found to be more cytotoxic than the precursor complexes A and B. The results reported in this study demonstrate that HA nanocrystals and antitumour drugs can be conjugated in such a way to yield a smart bone filler delivery system, which is capable to act as bone substitute and at the same time as platinum drug releasing agent with the final goal of locally inhibiting the tumour re-growth and reducing the systemic toxicity. Moreover, these results suggest the possibility of using the chemico-physical differences of HA nanocrystals, in order to strongly tailor the Pt complex release kinetics.

References

1. M. Iafisco, B. Palazzo, M. Marchetti, N. Margiotta, R. Ostuni, G. Natile, M. Morpurgo, V. Gandin, C. Marzano, N. Roveri J. Mater. Chem. (2009), 19, 8385.
2. B. Palazzo, M. Iafisco, M. Laforgia, N. Margiotta, G. Natile, C. L. Bianchi, D. Walsh, S. Mann, N. Roveri Adv. Funct. Mater. (2007), 17, 2180.

FT-IR microscopy study of electrospun nanostructured fibers of collagen-hydroxyapatite on titanium

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Titanium and its alloys are currently the mainly used materials for biomedical and dental implants due to their good mechanical properties, high corrosion resistance, and excellent biocompatibility[1,2]. Among the different materials used for the surface modification of Ti substrates, the best way could be the application of a coating to replace the extracellular matrix (ECM) of bone, basically constituted of hydroxyapatite (HA) nanocrystals and collagen fibers, that, for their natural origin and biocompatibility, are particularly suitable material for tissue-engineering. In this work, we present a biomimetic bone-like composite made of self assembled collagen reconstituted fibers and nano-crystal hydroxyapatite (HA) produced by the electrospinning technique[3] and we describe the contribute of FTIR microscopy to highlight structural interactions between the two components in the topographic distribution on titanium plates. To extrapolate the representative spectrum of electrospun coatings, using both point-point and multivariate analysis, we have analyzed two different samples: electrospun collagen (A) and collagen/HA composite (B). Figure 1 shows the scanning electron microscopy (SEM) images and energy dispersive X-rays (EDAX) analysis of electrospun pure collagen (A) and collagen-hydroxyapatite (B) coatings. Comparing the spectra of the two samples, the presence of the Ca and P signals of HA in the mineralized coating is evident.

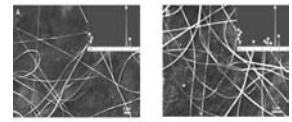

Figure 1: SEM images of electrospun pure collagen (A) and collagen-hydroxyapatite (B) coatings. The insets are the relative energy dispersive X-rays (EDAX) analysis.

Figure 2A shows the average representative FT-IR spectrum of the collagen (sample A) where tipical band is present: 1640 cm^{-1} (Amide I). The collagen/HA composite (sample B) FT-IR spectrum (Figure 2B) shows the bands of collagen as well as the bands of HA. The analysis of the 1230/1040 cm^{-1} band ratio (collagen N-C=O bending and C-N stretching / hydroxyapatite PO_4 bending) has been performed to evaluate the interaction between the two components and the results have been confirmed by multivariate analysis (HCA and PCA not shown).

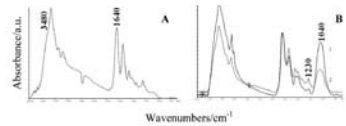

Figure 2 : representative spectra of electrospun collagen (A) and collagen/HA composite (B) in the region 4000-700 cm^{-1}.

References

1. V. Srimaneepong, T. Yoneyama, E. Kobayashi, H. Doi, T. Hanawa, Dent. Mat. **2008**;24 839-845.
2. M. P. Prabhakaran , J.Venugopal, S. Ramakrishna, Acta Biomat **2009**;5:2884–2893
3. I. Foltran, E. Foresti, B. Parma, P. Sabatino, N. Roveri, Macromolecular Symposia **2008;**269:111-118.

Biosensori e Biostrumentazione

A peptide derived from Herpes Simplex Virus type-1 glycoprotein H: membrane translocation and applications to the delivery of quantum dots

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Cell membranes are impermeable to most molecules not actively imported by living cells, including practically all macromolecules and even small molecules whose physiochemical properties prevent passive membrane diffusion.

However, over the past decades, we have seen the development of increasingly sophisticated methodology for intracellular drug delivery. Cell-penetrating peptides (CPPs), representing different families of short peptides believed to enter cells by penetrating cell membranes, have attracted a great deal of interest in the hope of enhancing gene therapy, vaccine development, and drug delivery. Therefore, it is fundamental to identify novel molecules which use different internalization mechanisms. We previously reported the identification of a membrane-perturbing domain in the protein gH of Herpes simplex virus type 1[1,2]. The peptide gH625 interacts with biological membranes, contributing to the merging of the viral envelope and the cellular membrane. When gH625 is in helical conformation, the polar residues concentrate on one face of the helix, giving an amphiphilic character common to fusion peptides of most fusion glycoproteins of enveloped viruses; moreover, gH625 is very effective in inducing lipid mixing of model membranes.

Here, we report on a novel peptide molecule (gH625) ability to traverse the membrane bilayer and to transport a cargo into the cytoplasm. We used as cargo molecule quantum dots (QDs) that are almost unable to traverse the membrane bilayer on their own[3].

References

1. S. Galdiero, A. Falanga, M. Vitiello, H. Browne, C. Pedone, M. Galdiero J. Biol. Chem. (2005), 280, 28632.
2. S. Galdiero, A. Falanga, M. Vitiello, L. Raiola, L. Russo, C. Pedone, C. Isernia, M. Galdiero J. Biol. Chem. (2010), 285, 17123
3. A. Falanga, M. Vitiello, M. Cantisani, R. Tarallo, D. Guarnieri, E. Mignogna, P. Netti, C. Pedone, M.Galdiero, S. Galdiero Nano Letters (2010), submitted.

The interaction between platinum(II) complexes and DNA evaluated by means of an electrochemical biosensor

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The interaction between metal complexes and DNA is an important aspect in pharmacology. In recent years many important technological strides have been made in the development of new techniques to monitor biorecognition and biointeraction on solid devices. The interaction between DNA and drugs can cause chemical and conformational modifications and, thus, variation of the electrochemical properties of nucleobases. The propensity for a given compound to interact with DNA is measured as a function of the decrease of guanine oxidation signal, S%, on a DNA electrochemical biosensor.
Covalent binding at N7 of guanine and intercalation are the main events that can be detected by this kind of biosensor. In this setting, the interaction between a series of Pt-complexes with general formula $[PtCl_n(NH_3)_{4-n}]^{(2-n)}$ (n = 0-4, for n = 2, *cis*- and *trans*- isomers, respectively), and double-stranded DNA, using an electrochemical DNA-biosensor was studied as a semi-quantitative model of the analogous interaction occurring in the biological environment.

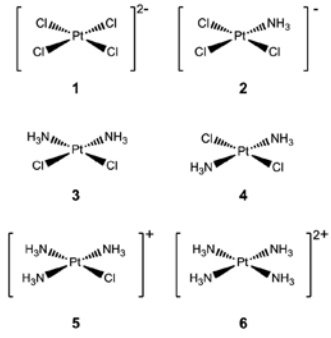

Figure 1. Sketch of the platinum complexes under investigation

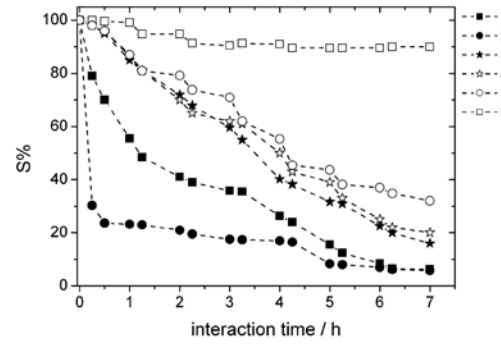

Figure 2. S% vs. interaction time for 0.25 mM Pt-complexes and ds-DNA in 0.25 M PB / 5 mM NaCl (pH = 7.4) solutions

References

1. M. Mascini, G. Bagni, M. L. Di Pietro, M. Ravera, S. Baracco, D. Osella, BioMetals (2006), 19, 409.
2. M. Ravera, G. Bagni, M. Mascini, J. C. Dabrowiak, D. Osella, J. Inorg. Biochem. (2007), 101, 1023.
3. M. Ravera, G. Bagni, M. Mascini, D. Osella, Bioinorganic Chemistry and Applications (2007) Article ID 91078, 11 pages.

Aggregating Cu(II) metalloporphyrin as chiroptical antenna system

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Biomolecular structural motifs play a central role in their functioning. In the case of proteins, for example, misfolding or unfolding of the native structure can have major physiological implications, especially for neurodegenerative diseases.[1] Considerable effort has been focused recently on supramolecular interactions in the search for new molecular probes capable of reporting on specific conformations of nucleic acids and proteins.[2] Porphyrins are appealing candidates because of their rich spectroscopic features which are markedly affected by self-aggregation and by the specific microenvironment.[3] Cationic achiral porphyrins can bind to negative biopolymers, such as poly-L-glutamate or DNA, leading to adducts which exhibit induced circular dichroism (ICD) signals in the wavelength region where the bound chromophores absorb light.[4] The biopolymer behaves as a template on which the porphyrins form organized supramolecular assemblies stabilized by an interplay of electrostatic, hydrogen bonding and dispersive forces.
Here we report on the chiroptical properties of an aggregating cationic metalloporphyrin – *trans*-bis(N-methylpyridinium-4-yl)diphenylporphirinato copper(II) (t-CuPagg) – on poly-glutamate, as a model scaffold. We demonstrate that this porphyrin shows great promise as a structural probe of protein conformation. The interaction between t-CuPagg and poly-L-Glu has been studied through the combined use of UV/vis extinction, circular dichroism (CD), and Resonance Light Scattering (RLS) techniques. The dependence of the nature of the porphyrin/protein interaction on protein concentration, ionic strength and pH has been investigated, with particular focus on the effect exerted by the pH induced transition between poly-L-Glu α-helix and random coil conformations.[5] Furthermore, the effect of the order of addition of the reactants is explored, showing the change in the response of the sensor when preformed t-CuPagg aggregates are added to the biopolymer.

References

1. S. B. Prusiner Proc. Natl. Acad. Sci. U. S. A. (1998), 95, 13363.
2. M. Balaz, A. E. Holmes, M. Benedetti, P. C. Rodriguez, N. Berova, K. Nakanishi, G. Proni J. Am. Chem. Soc. (2005), 127, 4172. R. F. Pasternack Chirality (2003), 15, 329.
3. R. F. Pasternack, E. J. Gibbs in Metal Ions in Biological Systems (1996), 367. A. D'Urso, A. Mammana, M. Balaz, A. E. Holmes, N. Berova, R. Lauceri, R. Purrello J. Am. Chem. Soc. (2009), 131, 2046.
4. R. F. Pasternack, A. Giannetto, P. Pagano, E. J. Gibbs J. Am. Chem. Soc. (1991), 113, 7799. X. F. Huang, K. Nakanishi, N. Berova Chirality (2000), 12, 237.
5. G. De Luca, A. Romeo, L. Monsù Scolaro, R. F. Pasternack Chem.Commun. (2010), 46, 386.

Diagnostici innovativi in
oncologia e malattie cardiovascolari

Neuroglobin-prion protein interaction: a new perspective or just a sight?

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Neuroglobin (Ngb) and cellular prion protein (PrP^C) are structural and functional intriguing proteins sharing a role in neuroprotection, possibly by reactive oxygen species detoxification in neurons and activating protective pathways. Ngb is predominantly observed in the cytosol of neurons and retina, shows the typical globin fold, reversibly binds O_2 and can act as a NO scavenger[1,2]. Ngb structural peculiarities are the low sequence similarity with vertebrate myoglobin and hemoglobin, hexacoordination of Fe^{2+} and an unusual sliding of the heme into a preformed cavity upon CO binding. PrP^C is an ubiquitous glycoprotein highly expressed in the nervous system. It consists of a glycine-rich N-terminal unfolded region, and a C-terminal globular domain usually anchored to cell surface. However, others prion protein topologies reside in the cytosol or partially cross the endoplasmic reticulum. PrP^C seems necessary for neuronal copper metabolism, mainly through N-terminus histidine coordination in the so called octarepeat region[3] The amino-terminal domain of prion plays a key role in PrP^C misfolding and aggregates accumulation associated with transmissible spongiform encephalopathies (TSE). Recently, it was demonstrated retinal ganglion cell layer co-expression and localization of Ngb and PrP^C and it has been shown that PrP^C aggregates rapidly in the presence of Ngb via the PrP^C N-terminus, with no influence on PrPC structure or Ngb ligand affinity, whereas PrPC does not aggregate in the presence of myoglobin (Mb), suggesting that Ngb could scavenge cytosolic prion molecules through electrostatic complementarity between PrP^C N-terminus and Ngb[4]. Ngb appears as the first partner of prion protein that retains its functionality *in vivo* notwithstanding of oligomerization. In this study, we have evaluated Ngb-PrP^C association because the occurrence of a strong and specific binding could lead to a better approach to TSE therapy. Prion-Ngb (or Mb) interactions were measured in vitro by Surface Plasmon Resonance (SPR) technique, using Biacore X100 instrument. Recombinant bovine PrP^C, in the range of concentration from 840 to 52.5 nM, was tested versus purified His-tagged neuroglobin (Murine) or myoglobin (Horse Hearth) immobilized on Nitrilotriacetate (NTA) or carboxymethyldextran (CM5) chips, respectively. Kinetic parameters of Neuroglobin-prion interaction were estimated according to 1:1 model using Biacore X100 Evaluation Software. Although many PrP^C interacting proteins are recognized in the cell, there are scarce data on their quantitative binding to prion. At best of our knowledge, submicromolar equilibrium dissociation constant value for PrP^C-Ngb binding reported appears the best result among the cytosolic PrP^C partners. Furthermore, experimental results here reported, which display an unequivocal binding interaction between PrP^C and Ngb with no detectable complex formation with Mb, corroborate the previous indication of charges complementarity between the basic PrP^C N-terminus, probably the lysine residues before and after the octarepeat region, and the negatively charged Ngb residues.

Acknolewdgement

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References

1. Burmester, T. and Hankeln, T. *J. Exp. Biol.* **2009**, 212, 1423
2. Brunori, M et al. Proc. Natl. Acad. Sci. USA, **2005**, 102 8483
3. Prusiner, S.B. *Proc. Natl. Acad. Sci. USA* **1998**, 95, 13363
4. Lechauve, C. et al. *J. Mol. Biol.* **2009**, 388, 968

Smart Gd(III)-loaded liposomes as enzyme responsive MRI probes

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When Magnetic Resonance is the imaging modality of choice, it is necessary to design highly sensitive systems in order to overcome the relatively low sensitivity of such an imaging modality. One way to solve this problem is the use of nanosystems, such as liposomes, able to deliver a large number of imaging units to a given biological target. There is a great interest to design agents whose MRI response is sensitive to the presence of a specific enzyme.

We have envisaged several approaches to design enzyme-responsive agents based on the use of Gd(III)-loaded liposomes:

i) Liposomes exposing a peptide acting as a MMP substrate and encapsulating a clinically approved Gd(III) agent were prepared and tested *in vitro* by measuring the relaxivity over time in the presence of collagenase. We demonstrate that the enzyme triggers the release of the imaging probe through a destabilization of the liposome membrane, thus causing a relaxivity enhancement. Preliminary *in vivo* kinetic experiments following the intratumor injection of the lipopeptide-based liposome in a melanoma mouse model indicated a rapid washout of the imaging probe from the tumor. This finding is consistent with a release of the MRI probe in the extracellular tumor fraction, where MMPs accumulates. Suitable control liposomes do not show any relaxivity enhancement in the presence of the enzyme *in vitro*, whereas in vivo they showed a different kinetic that suggests a cellular uptake of the nanovesicles. As further goal, an anti-tumoral drug will be co-encapsulated with the MRI probe, allowing supervise the tumoral therapy.

ii) The interaction of anionic liposomes and protamine yields to supramolecular aggregates with low relaxivity. The action of the serine protease trypsin causes the digestion of protamine and the consequent de-assembly of the aggregates that results in a relaxivity enhancement. An illustrative example of the possible use of this responsive agent consists of the entrapment of the aggregates in alginate vesicles that have been used as gaskets for in vivo transplantation of cells. In such a way, the enhancement in T1 contrast may act as a sensor of the protease activity in the biological environment in which the cells are located.

Summarizing, we have prepared molecular probes that work as an enzymatic substrate in a particular microenvironment and able to enhance the MRI contrast as a function of enzymatic activity.

Multicontrast proton MRI agents: an useful approach to assess the *in vivo* intratumor trafficking of paramagnetically labeled liposomes.

Walter Dastrù[1], Daniela Delli Castelli[1], Enzo Terreno[1], Evelina, Cittadino[1], Elena Torres[1], Francesco Mainini[1], Michela Spadaro[2], Silvio Aime[1]

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Many nanocarriers have been explored in drug delivery, and liposomes have been often used mostly for their ability to load both hydrophilic and lipophilic molecules. In spite of the long history, extensive development, and proven therapeutic efficacy, the in vivo behaviour of such nanovesicles is still poorly understood. An improved knowledge is expected to result in better formulations not only for drug delivery but also for imaging-guided therapy, which is a new emerging field of theragnosis (therapy and diagnosis). Loading the vesicles with paramagnetic metal complexes resulted in new classes of MRI contrast agents that are under intense development, because they can overcome the intrinsic low sensitivity of the technique. In this contribution, we report a novel approach to obtain indirect evidence of in vivo intratumor trafficking of paramagnetic liposomes through monitoring the time evolution of different contrast modalities (T_1, T_2 and chemical exchange saturation transfer (CEST)). The study utilized two types of liposomes loaded with either [Gd-HPDO3A] complex (acting as T_1 and T_2 agent) or $[Tm\text{-}DOTMA]^-$ (acting as T_2 and CEST agent).

The bases for the approach used are: (i) the maximum of T_1 contrast enhancement occurs when the nanovesicles lose their integrity to release their content; (ii) the maximum T_2 contrast occurs when a contrast agent is concentrated, i.e., when the nanovesicles are intact or slightly diluted in intracellular organelles, and (iii) the maximum CEST contrast is observed when intact liposomes are free in the extracellular fluids. The data was analyzed through a kinetic model in which the overall process was analyzed using five consecutive kinetic steps corresponding to recruiting and cellular uptake, degradation or disassembling of liposomes, cytosolic release of imaging probes, cellular efflux of the probes, and washout of the probe from the tumor region to the blood stream. The model was validated by using liposomes known to have different stabilities.

Ultrasounds triggered release from paramagnetic liposomes for the development of MRI-guided drug delivery protocols.

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The non invasive in vivo visualization of drug release triggered by externally applied ultrasounds (US) is an emerging topic in molecular imaging field. Most of the reported studies were performed using nano- or micro-bubbles in which the presence of the gas-filled core makes cavitation (and US detection) possible[1]. However, the use of imaging modalities with an improved spatial resolution and wider diagnostic/ therapeutic potential like MRI would be very useful.

Liposomes, nanovesicles endowed with an aqueous core, are extensively used as drug delivery nanocarriers and are also under intense scrutiny in the field of MRI contrast agents where they represent one of the most promising nanoplatform for designing highly-sensitive probes[2].

The most straightforward approach to design liposomal MRI probes whose image contrast can report about the US-induced cavitation of the nanocarrier is the encapsulation of a large amount (100-200 mM) of a clinically approved Gd(III) agent in the vesicle. The ability of the encapsulated agent to generate a good T_1 contrast is strongly limited by the water permeability of the vesicle membrane. Upon the probe release, the T_1 "quenching" is removed and a contrast enhancement can be observed.

In this contribution, we demonstrate that low frequency US (20 kHz) can induce a probe release much more efficiently than using high frequency waves (3 MHz). The evaluation of the probe release, monitored in vitro at 0.5 T on a fixed frequency relaxometer, was performed at different US frequencies, power, application times, liposome size and chemical composition of the membrane, thus allowing the development of a model to predict the efficiency of the release mechanism. The in vitro work was followed by an *ex vivo* and *in vivo* (on a xenografted B16 melanoma mouse model) MRI study that successfully demonstrated the potential of this approach to visualize the probe release following a local US application.

References

1. Gao Z. et al. Ultrasonics, **2008**, *48,* 4, 260-270
2. Delli Castelli D. et al. *Coord. Chem. Rev.* **2008**, *252,*: 21-22 , 2424-2443

Nanoparticles for targeted cancer therapy

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Nanomedicine is an emerging field of biomedical research and is defined as the application of nanotechnology to achieve breakthroughs in healthcare. It exploits the improved and often novel physical, chemical and biological properties of materials at the nanometer scale.[1] The conjugation of nanoparticles with antibodies combines the properties of the nanoparticles themselves with the specific and selective recognition ability of the antibodies to antigens. Also, the improvement in the cellular uptake as well as the major intracellular stability may be of the major advantages of using antibody conjugated nanoparticles. In this frame we have realized a firm attachment of immunogammaglobulins (IgG) at the solid surfaces of hydroxyapatite nanoparticles (HA-NP; range 30-80 nm). Synthetic HA-NP are very interesting biomaterials not only in replacing hard tissues, but also for drug delivery, having low toxicity and high hydrophilicity due to the fact that they are the inorganic mineral of hard tissues in vertebrates.[2]

In a first series of experiments human IgG were coupled to these particles in different conditions (different pH, IgG concentration, incubation time). At pH 7.4 for definite amounts of HA-NP the curve dose-response was found to be saturable and the coupling stable. An IgG molecule is composed of four polypeptide chains, two heavy and two light chains, S-S linked to give a "Y-shaped" conformation, consisting of two identical Fab domain at the N-terminus, responsible for binding the antigen, and an Fc domain. Because the antigen-binding sites are located at the Fab N-terminus, it is desired the IgG molecules to be adsorbed onto the adsorbent surface through an anchoring Fc. To evaluate this condition FT-IR analysis on the HA-NP-IgG conjugates were performed, revealing weak bands at 3300 e 3077 cm^{-1}, which are typical of NH stretching, whereas the 1398 cm^{-1} peak, distinctive of C=O bound, is lacking. Therefore, Fab portion (NH_3^+ groups) seems to be exposed, hypothesizing a binding between HA and the Fc domain (COO^- group). On the basis of these preliminary data, two monoclonal antibodies developed in the laboratory and directed against two tumour associated markers expressed at the cell surface[3,4] were then coupled to HA-NP. Their immunochemical performance was analyzed. This study may be useful to develop new chemotherapeutic strategies based on immunological tools. Functionalization can act as a strategy to fine-tune the bioactivity of HA-NP, as a targeted delivery system for drugs with controlled release properties.

References

1. S. Pietronave, M. Iafisco, D. Locarno, L. Rimondini, M. Prat J. Appl. Biomater. Biomech. (2009), 7, 77
2. M. Iafisco, P. Sabatino, I. G. Lesci, M. Prat, L. Rimondini, N. Roveri Colloids Surf. B: Biointerf. (2010), 81, 274
3. M. Prat, I. Morra, G. Bussolati and P.M. Comoglio Cancer Res. (1985), 45, 5799
4. M. Prat, R. P. Narsimhan, T. Crepaldi, M.R. Nicotra, P.G. Natali and P.M. Comoglio Int. J. Cancer (1991), 49, 323.

Gd-loaded liposomes as MRI probes for the intratumor visualization of pH triggered drug release

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The development of nanometric drug carriers able to release their bioactive content through the action of specific triggering stimuli is an emerging topic in medical field.[1]
In addition to their use in therapy, nanoparticles have been also widely investigated in diagnosis, especially in MRI field, as highly sensitive probes.[2] Among the nanoparticles that have been considered so far, liposomes occupy certainly a leading role being successfully used since a long time in pharmaceutical field as drug-delivery systems. The aim of this work was the preparation of suitably formulated liposomes that can release the entrapped material (imaging reporter + drug) as a consequence of a mildly pH reduction. [3] In addition to report about drug delivery, there is a growing interest to design systems able to visualize the release process. Through the encapsulation of a Gd-complex into a liposome, the T_1 contrast can be strongly reduced owing to the compartmentalization of the paramagnetic agent, and therefore the release of the probe from the nanocarriers leads to a detectable contrast enhancement.
A novel formulation of pH sensitive liposomes based on POPE (palmitoyl-oleyl-phosphatidylcholine) and THS (α-tocopherol-hemisuccinate) was developed. The probe release is due to the aggregation and membrane destabilization of the vesicles following the protonation of THS moiety. The pH induced release from such vesicles of hydrophilic Gd(III) complexes with different size and relaxivities was tested in vitro and in vivo after intratumor injection on a xenografted B16 melanoma mouse model. It was found that the release kinetic is related to the size of the encapsulated agent. Furthermore, since the tumor accumulation after i.v. injection requires a prolonged blood circulation, stealth pH sensitive liposomes where PEG chains are linked to the vesicles through a degradable disulfide bond were prepared and tested.

References

1. S. Ganta, H. Devalapally, A. Shahiwala, M. Amiji J. Control. Release (2008), 126, 187.
2. S. Aime, D. Delli Castelli, S. Geninatti Crich, E. Gianolio, E. Terreno Acc. Chem. Res. (2009), 42, 822.
3. S. Simoes, J. Nuno Moreira, C. Fonseca, D. Nejat, M.C. Pedroso de Lima Adv. Drug Delivery Rev. (2004), 56, 947.

Ioni metallici nelle patologie degenerative croniche

NMR characterization of the products of reaction between artemisinin and Fe(II) species

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Artemisinin is a sesquiterpene lactone containing an endoperoxide bond, showing marked antimalarial activity against chloroquine resistant *Plasmodium falciparum*[1]. This intra-erythrocytic parasite digests hemoglobin as amminoacid source for proteins, producing high levels of ferrous ion as a by-product, mostly in the form of heme. To avoid the accumulation of heme and the potential toxicity of iron(II)-heme, the *Plasmodium* polymerizes this redox-active species into hemozoin, a non toxic microcrystalline polymer of iron(III). The capability of artemisinin to bind heme prevents its polymerization in hemozoin and, as a consequence, the adduct artemisinin-heme becomes toxic for *Plasmodium*[2]. Although the exact parasiticidal mechanism of artemisinin has not been fully understood, the presence of the endoperoxide bridge, necessary for its pharmacological activity, suggests the involvement of free radicals and/or other reactive intermediates. Moreover, some studies show a role of non-hemic iron in the mechanism of artemisinin activation[3]. Therefore, the aim of our work has been to assess the role of ferrous ion in antimalaric action of artemisinin by multinuclear and multidimensional NMR spectroscopy.

^{1}H and ^{13}C NMR and 2D ^{1}H-^{13}C NMR HSQC spectra were performed to study the artemisinin reactivity with simple ferrous chemicals, iron(II)-D gluconate (D-Gluconic acid iron(II) salt dihydrate) and iron chloride ($FeCl_2$), as a source of reactive iron(II) in aqueous solution, and to characterize the reaction products.

Reaction of artemisinin with iron(II) always involved an intramolecular rearrangement, following the formation of carbon centered radicals, according to two main pathways: 3-α-hydroxydeoxyartemisinin, a stable alcohol obtained by rearrangement of an epoxide, and 4,7-dimethyl-8-oxodecahydrofuro[3,2-i] isochromen-10-yl acetate, a tetrahydrofuran ring containing product, obtained by contraction of the peroxydic ring[4].

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References

1. 1. K. Borstnik, I.H. Paik, G.H. Posner Medicinal Chemistry (2002), 2, 573–583.
2. 2. A. Robert, F. Benoit-Vical, B. Meunier Coordination Chemistry Reviews (2005), 249, 1927–1936.
3. 3. N. Sibmooh, R. Udomsangpetch, A. KiJJoa, U. Chantharaksri, S. Mankhetkorn Chem. Pharm. Bull. (2001), 49, 12, 1541-1546.
4. 4. A.J. Lin, D.L. Klayman, J.M. Hoch J. Org. Chem. (1985), 50, 4504-4508.

Copper(I) and copper(II) inhibit Aβ peptides proteolysis by insulin-degrading enzyme differently: implications for metallostasis alteration in Alzheimer's disease

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Accumulation of neurotoxic amyloid-β peptide (Aβ) and alteration of metal homeostasis (metallostasis) in the brain are two main factors that have been very often associated with neurodegenerative diseases such as Alzheimer's disease (AD). Aβ is constantly produced from the APP precursor and immediately catabolized under normal conditions, whereas dysmetabolism of Aβ and/or metal ions seems to lead to a pathological deposition. Although Insulin-degrading enzyme (IDE) is the main metalloprotease involved in Aβ degradation in the brain being up-regulated in some areas of AD brains, the role of IDE for the onset and development of AD is far from being understood. Moreover, the biomolecular mechanisms involved in the recognition and interaction between IDE and its substrates are still obscure. In spite of the important role of metals (such as copper, aluminum and zinc), which has brought to propose a "metal hypothesis of AD", a targeted study of the effect of metallostasis on IDE activity has never been carried out. In this work, we have investigated the role that various metal ions (i.e., Cu^{2+}, Cu^{+}, Zn^{2+}, Ag^{+} and Al^{3+}) play in modulating the interaction between IDE and two Aβ peptide fragments, namely $A\beta_{1-16}$ and $A\beta_{16-28}$. It was therefore possible to identify the direct effect that such metal ions have on IDE structure and enzymatic activity without interferences caused by metal-induced substrate modifications. Mass spectrometry and kinetic studies revealed that, among all the metal ions tested, only Cu^{2+}, Cu^{+} and Ag^{+} have an inhibitory effect on IDE activity. Moreover, the inhibition of copper(II) is reversed by adding zinc(II), while the monovalent cations affect the enzyme activity irreversibly. The molecular basis of their action on the enzyme is also discussed on the basis of computational investigations.

Copper complexes of a peptide fragment within the N-terminal repeat region in opossum PrP protein

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It is well known that PrP^C has sites of great affinity towards copper(II) ions and that it may be involved in the copper metabolism.[1] PrPs of eutherian mammals contain five octa- or nona-repeats (PH/QGGG/T(G) WGQ). The tammar wallaby PrP has four nona- or deca-repeats (PQGGGTNWGQ, PHPGGSSWGQ, PHAGGSNWGQ, PHGGSNWGQ), and there are five deca-repeats in the Brazilian opossum PrP (PQGGGTNWGQ, PHAGGSNWGQ, PRPGGSNWGQ and duplicated PHPGGSNWGQ).[2] A spectroscopic (UV-Vis, CD and EPR), thermodynamic and voltammetric study has been carried out on the copper(II) complexes with the Ac-PHPGGSNWGQ-NH_2 polypeptide, which contains the apparently dominant 10-residue marsupial repeat, in order to find differences between the structural features of the copper complexes with the eight-residue motif of mammals (PHGGGWGQ).[3] Both potentiometric and spectroscopic data suggest the formation at neutral pH of a highly distorted [Cu(L)H_{-2}] complex species, the stereochemistry of which is ascribable to a square base pyramid. At pH values higher than the neutrality, the coordination sphere of the copper complex in this species changes its stereochemistry towards a pseudo-octahedron, as suggested by the change in the copper parallel hyperfine coupling constant of the EPR spectra.

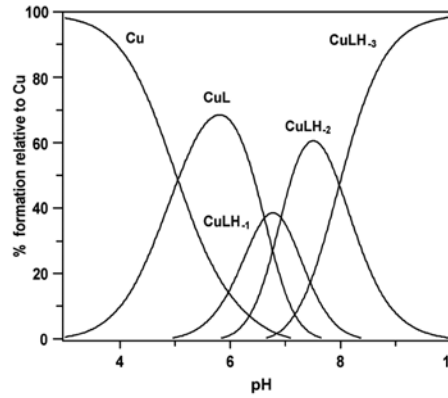

Figure 1. Species distribution diagrams for the Copper(II) complexes with Ac-PHPGGSNWGQ-NH_2 (L).

References

1. J. Stockel, J. Safar, A.C. Wallace, F.E. Cohen, S.B. Prusiner, Biochemistry (1998), 37, 7185.
2. M. Premzl, M. Delbridge, J.E. Gready, P. Wilson, M. Johnson, J. Davis, E. Kuczek, J.A. Marshall Graves, Gene (2005), 349, 121
3. R.P. Bonomo, V. Cucinotta, A. Giuffrida, G. Impellizzeri, A. Magrì, G. Pappalardo, E. Rizzarelli, A.M. Santoro, G. Tabbì and L.I. Vagliasindi, Dalton Trans. (2005), 150.

Copper(II) interaction with the human angiogenin protein

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Human angiogenin (hAng) is a 14 KDa single polypeptide chain that belongs to the pancreatic ribonuclease superfamily.[1] Ang is normally present in the plasma of healthy individuals, but its expression raises in the presence of several types of cancers.[1] It is a potent angiogenic factor and its presence is required for cell proliferation induced by other angiogenic agents such as VEGF. Although the precise molecular mechanism by which Ang induces the formation of new blood vessels has not been determined, three distinct sites seem to be necessary for the angiogenic activity: a catalytic site for RNase activity involving residues H13, K40 and H114, a putative cell binding site encompassing residues 60-68 (KNGNPHREN) and nuclear translocation site 31-35 (RRRGL).[1] Recently, it has been reported that angiogenin play a role in motor neuron physiology and loss-of-function mutations in ANG coding region have been found in patient with amyotrophic lateral sclerosis (ALS).[2]

Interestingly, the Ang proliferative activity as a result of binding affinity between Ang and endothelial cells, is largely increased in the presence of copper ions. We showed that the a peptide encompassing the Ang putative endothelial cells binding site is able to bind one copper(II) ion and the predominant complex species at physiological pH is a five-coordinated complex in a square pyramidal arrangement.[3] It is well known that copper(II) is a strong angiogenic signal in vivo, but its specific molecular mechanism and the targets of its activity remain unclear. Recently, it has been demonstrated the extracellular translocation of the cellular copper during angiogenesis processes in vitro, suggesting the metal binding to an extracellular protein involved in angiogenesis, such as Ang.[4]

It has been showed that Ang binds 2.4 copper ions per molecule but so far, there are not detailed molecular studies on copper(II) interaction with Ang.

In this context, we report the copper(II) interaction with the entire protein by means of NMR, EPR, UV-vis, CD techniques. In order to better understand the coordination mode, different Ang peptide fragments were synthesized and their copper(II) complexes characterized.

References

1. B.L. Vallee, J.F. Riordan Cell. Mol. life sci. (1997), 53, 803.
2. S. Li, G.F. Hu Int. J. Biochem. Mol. Biol. (2010), 1, 26.
3. D. La Mendola, A. Magrì, L.I. Vagliasindi, Ö. Hansson, R.P. Bonomo, E. Rizzarelli Dalton Trans. (2010), in press, doi 10.1039/c0dt00732c.
4. L. Finney, S. Mandava, L. Ursos, W. Zhang, D. Rodi, S. Vogt, D. Legnini, J. Maser, F. Ikpatt, O.I. Olopade, D. Glesne Proc. Natl. Acad. Sci. USA (2007), 104, 2247.

Tuning the affinity of ubiquitin for membranes: implications for neurodegenerative disorders

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Ubiquitin (Ub) is a small and highly conserved protein in all eukaryotic cells. It plays a major role in the proteasome and the lysosome pathways, wherein polyubiquitination serves as a signal for *intracellular* protein degradation. Failure to eliminate misfolded proteins leads to formation of *amyloid* aggregates enriched with Ub, that are a hallmark of most neurodegenerative disorders including Parkinson's, Alzheimer's, amyotrophic lateral sclerosis, and prion diseases.[1,2] Amyloid fibril formation appears to be preceded by structural changes in native proteins leading to *sticky,* partially misfolded intermediates. Although these species are considered to be the primary toxic species, very little is known about their conformational features.

Figure 1. Amyloid-like E16V deposits on a quartz surface (left) and on anionic phospholipid bilayers (right)

We produced a Ub mutant (E16V), specifically designed to neutralize a negative charge at one edge strand. In water and at physiologic temperature (37°C), E16V is soluble and folded. In contrast, in water/trifluoroethanol (80:20, v/v) E16V undergoes a time-dependent increase in β-sheet content which drives the protein to interact with the hydrophobicity probe 1-anilinonaphthalene-8-sulfonic acid and to form amyloid-like deposits on a glass surface. When E16V is added to anionic (but not to switterionic) phospholipid liposomes, it forms β-rich oligomers able to penetrate the phospholipid bilayer and to react with the A11 antibody, which specifically recognizes toxic amyloid oligomers.[3] The finding that E16V forms amyloid-like oligomers able to affect membrane integrity prospects the possibility of using this mutant as a model system for elucidating the intricate mechanisms of neurodegenerative diseases. Moreover this study emphasizes the importance of subtle surface modifications in tuning the interaction of proteins with membranes of defined lipid composition.

References

1. Ciechanover A.; Brundin P. Neuron (2003), 40, 427.
2. Arnesano F.; Scintilla S.; Calò V.; Bonfrate E.; Ingrosso C.; Losacco M.; Pellegrino T.; Rizzarelli E.; Natile G. PLoS One (2009), e7052.
3. Kayed R.; Head E.; Thompson JL; McIntire TM; Milton SC; Cotman CW; Glabe CG. Science. (2003), 300,486.

Metalloproteine come catalizzatori biologici

Allosteric modulation of heme-albumin reactivity

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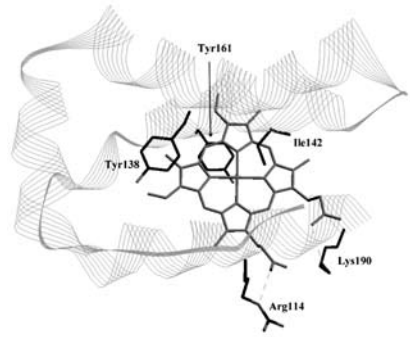
Human serum albumin (HSA), the most abundant protein in plasma, is characterized by an extraordinary ligand binding capacity providing a depot and carrier for many endogenous and exogenous compounds[1]. HSA is able to bind up to seven equivalents of long chain fatty acids (FAs) at multiple binding sites (labeled FA1 to FA7) with different affinity. The FA1 pocket has evolved to bind the heme with a high affinity, so that HSA participates physiologically to heme scavenging [1]. In turn, heme endows HSA with globin-like reactivity and spectroscopic properties. Remarkably, both ferric heme (heme-Fe(III)) binding to HSA and human serum heme-albumin (HSA-heme) reactivity are modulated allosterically.[1-7] The site-directed mutagenesis of pivotal amino acid residues for HSA-heme complex formation (Ile142, Tyr161, and Leu185) and for ligand binding to Sudlow's site I (Arg218, and Arg410) is on going. Moreover, to elucidate the relationship between Sudlow's sites I and II and the FA1 cleft, the truncated HSA isoform (Asp1-Glu382) will be expressed. Present results strongly suggest that HSA-heme may display transient catalytic properties in vivo.



HSA-heme binding geometry

References

1. P. Ascenzi, M. Fasano Biophys. Chem. (2010), 148, 16-22.
2. P. Ascenzi et al. Biochem. Biophys. Res. Commun. (2005), 334,481-486.
3. A. Bocedi et al. FEBS J. (2005), 272, 6287-6296.
4. G. Fanali et al. FEBS J. (2007), 274, 4491-4502.
5. P. Ascenzi et al. Biochem. Biophys. Res. Commun. (2008), 369, 686-691.
6. P. Ascenzi et al. J. Biol. Chem. (2009), 284, 31006-31017.
7. P. Ascenzi et al. IUBMB Life (2010), in press.

Resonance raman spectroscopy of bacterial truncated hemoglobins

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In bacteria, three classes of "Hb-like proteins" have been identified: flavo-Hbs, monomeric Hbs and 2/2 globins (also named truncated Hbs or trHbs). TrHbs are characterized by the absence of the A-helix and the presence of an extended loop substituting for most of the F-helix. On the basis of the amino-acid sequence, trHbs have been classified into three groups. The crystal structures of members of group II trHbs (HbO) revealed that their active site is characterized by the invariant Fe-histidine covalent link on the proximal side and by multiple combinations of different amino acid side chains within the heme distal pocket, which provides a highly polar distal environment. The distal amino acid residues play an important role in regulating ligand binding, however, their function is not yet fully understood. In order to highlight the structural properties of these proteins, Hb from thermophilic actinobacterium T. fusca and from the cold-adapted Antarctic marine eubacterium P. haloplanktis TAC125 have been studied by resonance Raman, electronic absorption and EPR spectroscopies in their ferric and ferrous states and in the presence of different ligands. Interestingly, both proteins have a high affinity for hydrogen sulfide which is thought to have a possible physiological significance[1]. Both proteins contain the polar distal triad TrpG8/TyrB10/TyrCD1. To single out the contribution of each of these three key distal amino acids, single, double, and triple Phe mutants have been obtained at each position for T. fusca HbO. The two proteins are quite different. In particular, while ferric T. fusca HbO at neutral pH contains a water molecule bound to the heme iron, P. haloplanktis TAC125 HbO, in addition to the aquo hexacoordinated high-spin form, shows multiple hexacoordinated low-spin forms, where either TyrCD1 or TyrB10 can likely coordinate the iron. This is the first example in which both TyrCD1 and TyrB10 are proposed to be the residues alternatively involved in heme hexacoordination by endogenous ligands. The results highlight the unique features of the ferric state among 2/2 Hbs of Group II. The high protein structural flexibility is suggested to derive from the ability of cold-adapted organisms to survive at permanently low temperatures. In fact, to perform their physiological functions at adequate rates in freezing habitats, these organisms have overcome constraints imposed by the cold environment through biochemical and physiological adaptations.

References

1. F. P. Nicoletti, A. Comandini, A. Bonamore, L. Boechi, F. Boubeta, A. Feis, G. Smulevich, A. Boffi, Biochemistry (2010), 49, 2269.

De novo designed $Cu^{+/2+}$ metalloproteins as models for copper nitrite reductase

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De novo protein design provides an attractive approach to model the active sites of metalloproteins. Synthetic constructs which precisely mimic the first coordination sphere of a known metalloenzyme site can be developed. Moreover, the chemistry of these systems can be probed in aqueous solution over a large pH range rather than in organic solvents such as acetonitrile. We present here the study of a copper(I/II) metallopeptide belonging to the **TRI** peptide family. In aqueous solution these peptides form α-helices which aggregate into three stranded coiled coils. With the aim to model the N_3 site of Cu Type 2 in Nitrite Reductase (NiR), we have designed the peptide **TRI** L23H which form a helix bundle in water, where the $(His)_3$ site in a hydrophobic pocket is capable to coordinate the copper ion.

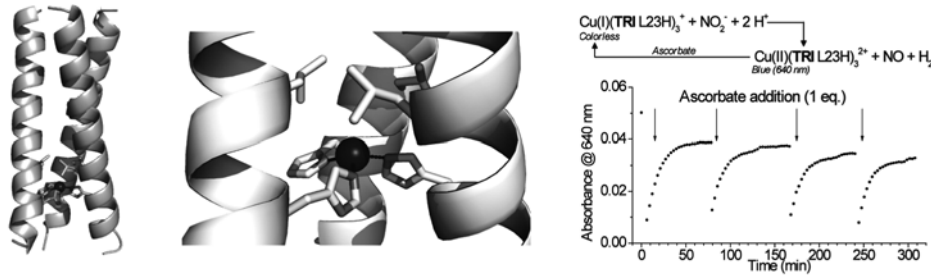

Figure 1. Model of Cu(I)(**TRI** L23H)$_3^+$ (left), of its active site (center), and NiR activity test (Cu^{2+} visible absorption, right).

Both Cu(I) and Cu(II)(**TRI** L23H)$_3$ metalloproteins have been characterized in aqueous solution and for both oxidation states at pH 7.4 the copper ion is $(His)_3$ coordinated. Actually, both the stretching frequency of the CO bound to Cu(I), and the visible and EPR features of the Cu(II) metallopeptide are very similar to those of Cu T2 in wtNiR.

The NiR activity has been evaluated in aqueous solution. The Cu(I)(**TRI** L23H)$_3^+$ metallopeptide is capable to reduce nitrite to NO at pH 6 with formation of the Cu(II) peptide. Upon addition of ascorbate, further nitrite can be reduced. The NiR activity showed an intriguing pH dependence (active at pH < 6.5, inactive at pH 7.4) which can be associated to a change in the coordination sphere of Cu in the active site. Relevant models connected with this behaviour will be discussed.

References

1. A.F.A. Peacock, O. Iranzo, V.L. Pecoraro Dalton Trans. (2009), 2271

Biological X-ray Absorption Spectroscopy (BioXAS): an overview on the strategies employed to improve the structural characteristics of coupled binuclear copper sites

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The access to the structure of the metal site, which often corresponds to the catalytic site in enzymes, plays an important role in understanding catalytic processes and to probe biological functions in greater detail. In spite of the complexity of the biological systems and of the high dilution of the active species, the research approach of the x-ray absorption spectroscopy (XAS) technique has demonstrated capabilities to solve structural problems on the characterization of their metal binding domains. Presently the XAS approach can be considered an ideal method for biological experiments.

The technical improvements in the synchrotron radiation facilities and the progresses of the theory have increased the quality of the XAS measurements and their analyses. Now the k-range of EXAFS signal is extended, the EXAFS programs take into account physical phenomena like multiple scattering (MS) processes, and the fitting of the XANES region is a reality. The only limitation of protein studies with the XAS approach, in comparison with x-ray diffraction (XRD) and nuclear magnetic resonance (NMR) is that XAS is a local structural method, giving a structural description of the metal site and not of the whole protein. However there are various advantages: the protein can be in solution, there are no limits on protein size and metal sites are described at atomic resolution [1]. Thus a XAS study can be complementary to NMR and XRD experiments, but can become the only structural approach.

For hemocyanins (Hcs) the XAS approach allows the derivation of fundamental structural information. Due to difficult crystallization and high molecular weight, both XRD and NMR are not suited for the structural investigation of these proteins, which are also EPR silent in native and derivative forms. The quantitative analysis of the XAS spectrum is usually a complex problem that becomes very difficult for a binuclear site with local structural contributions due to the two different absorbing metal atoms.

An overview of the logical and theoretical paths followed [2] will be shown. It will outline the strategies employed, the role of the MS calculations, both to refine the EXAFS modulation of absorption spectra, where the metal-metal contribution overlaps with the Cu-His signals, and to extract quantitative structural information from the XANES region in order to refine the fine structure of the coupled binuclear copper cores of Hcs.

References

1. S. S. Hasnain, K. O. Hodgson, J. Synchrotrron Radiation (1999), 6, 852.
2. E. Borghi et al., Biophys. J. (2002), 82, 3254; E. Borghi, P.L. Solari, J. Inorg. Biochem. (2003), 95, 103; E. Borghi, P. L. Solari, Micron (2004), 35, 81; E. Borghi, P. L. Solari, J. Synchrotron Radiat. (2005), 12, 102; E. Borghi, L. Casella, Phys. Chem. Chem. Phys. (2010), 12, 1525.

Synthesis and reactivity of copper-amyloid-β peptide (1-16) complex

Ciregna D. *et al.*

Oxidative stress plays a key role in Alzheimer's disease (AD). In addition, the abnormally high CuII ion concentration present in senile plaques has provoked a substantial interest in the relationship between the amyloid peptide (Aβ) found within plaques and redox-active copper ions.
In the reduced form, the Cu^{I} ion binds only to His13 and His14 of peptide Aβ in
a linear, two-coordinate structure. The reactivity of the Aβ peptide, as most of the oxidative damage associated with this toxic peptide is attributed to reactions with dioxygen or hydrogen peroxide of the Cu^{I} complex. However, considerable confusion arises from the fact that free Aβ acts as an antioxidant in vitro, at least up to nanomolar concentration and, according to recent reports, even when complexed with Cu or Fe ions Aβ exhibits antioxidant activity and may thus be neuroprotective. To unravel the intricate pattern of reactivities of Cu-Aβ complexes in such a complex frame it is important to unequivocally assess their reactivity against each individual reactive species. Herein we report that the Cu^{II} complex with the soluble Aβ(1-16) peptide fragment of sequence DAEFR5HDSGY10EVHHN15K exhibits efficient superoxide dismutase (SOD) activity but, contrary to what was described in an earlier report, has no phenol monooxygenase (tyrosinase-like) activity.

Nuovi farmaci in oncologia

Pyrazolate gold(I) derivatives. Preliminary biological evaluation of their anticancer properties

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Pyrazolate gold(I) complexes is a well known class of gold(I) complexes readily obtained by reacting pyrazolates and Lgold(I)chloride precursors. Whether L in the gold precursor complex is dimethylsulphide, tetrahydrothiophene, triphenylarsine ligands dinuclear[1], trinuclear or polynuclear derivatives[2] can be obtained. On the other hand when the gold(I)chloride precursor contains a softer ligand such as phosphane the displacement is more difficult and mononuclear derivatives can be directly obtained. Mononuclear gold(I) derivative of diaryl or dialkyllpyrazolate and N-alkylimidazolate are reported in literature and they show peculiar features in the field of emission properties and material science.[3] However, their low solubility in polar solvents make difficult to considerate these compounds for biological targets.

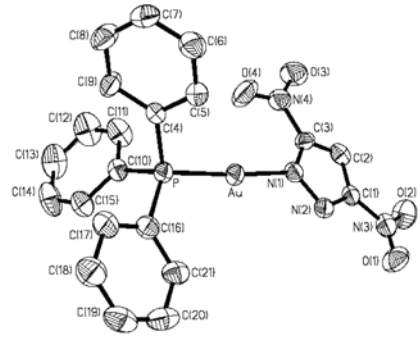

Figure 1. ORTEP structure for compound [3,5-dinitropyrazolategold(I)tris(phenyl)phosphane]

Accordingly to the introduction of more polar substituents in the azoles a better water solubility can be achieved making possible their antitumoral evaluation. A series of mononuclear gold(I) pyrazolate and imidazolate complexes have been evaluated for their in vitro antitumor activity against a panel of human cancer cells some of which suitably selected for their resistance to cisplatin or belonging to Multi-Drug Resistance (MDR) phenotype. Since gold complexes have been validated as efficient inhibitors of the selenoprotein thioredoxin reductase (TrxR)[4], the most promising derivatives were also tested for the ability to inhibit cytosolic and mitochondrial TrxR. The inactivation of the closely related oxidoreductases glutathione reductase (GR) and glutathione peroxidase (GPx) was also examined.

References

1. G. Yang, J. R. Martinèz, R. G. Raptis, Inorg. Chim. Acta. (2009), 362,1546.
2. H. H. Murray, R. G. Raptis, J. P. Fackler, Jr. Inorg. Chem. (1988), 27, 26
3. A. A. Mohamed, T. Grant, R. J. Staples, J. P. Fackler, Jr. Inorg. Chim. Acta, (2004), 354, 1761.
4. C.Marzano, V.Gandin, A.Folda, G.Scutari, A.Bindoli, M.P.Rigobello, Free Radic. Biol. Med. (2007), 42, 872.

New insights on the biological activity of [Ni(*S*-tcitr)$_2$]

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The metal complex [Ni(*S*-tcitr)$_2$] (S-tcitr=S-citronellalthiosemicarbazonate) shows interesting antiproliferative characteristics.[1] It has been assessed that it enters the cell and induces G_2M cell cycle arrest, p53 independent-intrinsic-apoptosis by down-regulation of Bcl-2, mitochondrial membrane potential loss and caspase activation. A DNA damaging action was observed by the alkaline Comet Assay. The use of specific glycosilases to detect oxidative damage showed that DNA damage is not preferentially due to oxidative species. Further analyses on ROS and TBARS induction were conducted on U937 cell line and support this hypothesis.

We hypothesize that the genotoxic insult by itself or an excessive or altered repair of the DNA damage could produce the observed cell killing, due to the fact that [Ni(*S*-tcitr)$_2$] treatment did not induce gene mutation or chromosomal damage. New assays on damage recovery support this action mechanism.

To better understand the interaction between this molecule and cellular pathways, we use a genomic phenotyping approach based on yeast (*S. cerevisiae*) as a model system which shows a sensitivity against [Ni(*S*-tcitr)$_2$] toxic effects comparable to human leukemic cells. The whole BY4742 haploid strain deletion collection from the *S. cerevisiae* Genome Deletion project, which includes 4826 single gene deletion mutants in non essential genes, has been screened at cytotoxic concentrations. This collection includes deletants in non essential genes, involved in biological processes, that are the main targets of antitumor drugs in human cells.[2-4] The analysis of the drug cytotoxic effect on the complete collection identifies only two mutants able to grow at drug cytotoxic concentrations. These two deletants are unable to produce proteins involved in the ribosomal assembly. Bioinformatics studies showed that the relative genes are conserved in humans.

The cell subpanel previously used, derived from the "US National Cancer Institute 60 human tumor cell line anticancer drug screen",[5,6] has been extended to better identify the histotype sensitivity and the assays showed an interesting cytotoxic activity of [Ni(*S*-tcitr)$_2$] not only on hematopoietic but also on NCS cell lines at very low concentrations.

References

1. Buschini A, Pinelli S, Pellacani C, Giordani F, Ferrari M, Bisceglie F, Giannetto M, Pelosi G, Tarasconi P. J. Inorg Biochem. (2009) 103, 666.
2. Winzeler EA, Shoemaker DD, Astromoff A,et al. Science (1999) 285, 901.
3. Giaever G, Chu AM, Ni L. Nature (2002) 418, 387.
4. Simon JA, Bedalov A. Nat. Rev. Cancer (2004) 4, 481.
5. Shoemaker RH. Nat Rev Cancer (2006) 6, 813.
6. Paull KD, Hamel E, Malspeis L. Prediction of Biochemical Mechanism of Action from the In Vitro Antitumor Screen of the National Cancer Institute. http://www.dtp.nci.nih.gov/docs/compare/compare_intro.html

Exploring the biochemical mechanisms of cytotoxic gold compounds: proteomic studies on A2780 platinum sensitive and resistant cells

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Very little is known concerning the molecular mechanisms underlying the pharmacological effects of gold(III)-based antitumor metallodrugs. Notably, gold(III) compounds seem to exert their effects through mechanisms distinct from those of the classical anticancer platinum(II) complexes. We have shown that a group of structurally diverse gold compounds are highly cytotoxic toward a panel of 36 human tumour cell lines through a variety of biochemical mechanisms[(1)]. A classic proteomic approach is exploited here to gain deeper insight into those mechanisms. The first investigation is focused on Auoxo6, a novel binuclear gold(III) complex and Auranofin, a clinically established gold(I) antiarthritic drug. First, the 72-h cytotoxicity profiles of Auoxo6 and Auranofin were determined against A2780 human ovarian carcinoma cells (cis-platin sensitive and resistant). Subsequently, protein extraction from gold-treated A2780 cells (cis-platin sensitive and resistant) and 2D gel electrophoresis separation were carried out according to established procedures. Notably, both metallodrugs caused relatively modest changes in protein expression in A2780 sensitive cells in comparison with controls, as only 11 out of approximately 1,300 monitored spots showed appreciable quantitative changes. Very remarkably, six altered proteins were in common between the two treatments. Overall, the results of this proteomic study point out that the mode of action of Auoxo6 is strictly related to that of Auranofin and that the induced changes in protein expression are limited and selective[(2)]. Nowadays the success of platinum-based chemotherapy is limited by the pre-existence or development of cancer cell resistance. To better understand the molecular mechanisms of drugs resistance we used the previews proteomic criteria to study the proteome of gold-treated A2780 resistant cells. Doing so it's possible to find proteomic differences in the two cellular types independently of the treatment and to know how different they respond to the gold treatments. We are now analysing the results of these experiments.

References

1. S.Nobili, E. Mini, I. Landini, C. Gabbiani, A. Casini, L. Messori Med. Res. Rev. (2010), May;30(3):550-80.
2. F. Magherini, A. Modesti, L. Bini, M. Puglia, I. Landini, S. Nobili, E. Mini, M.A Cinellu., C. Gabbiani, L. Messori J. Biol. Inorg. Chem. (2010), May;15(4):573-82.

Latest insights into the anticancer activity of gold(III)-dithiocarbamato complexes

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During the last decade, we have been developing some gold(III)-dithiocarbamato derivatives showing outstanding *in vitro* antitumor properties toward several human tumor cell lines and no cross-resistance with the reference anticancer drug cisplatin.[1,2] The rationale of our research work was to combine the well-known antitumor properties of the gold(III) metal center with the potential chemoprotective function of dithiocarbamates, in order to provide protection against the severe side-effects (in particular, renal toxicity) generally recognized for the clinically-established platinum drugs.[3] In this regard, we here report on the latest *in vivo* results, including both the anticancer activity on xenografts[4,5] and the toxicological profile[6] of some selected gold(III)-dithiocarbamato derivatives, whose relevance confirms the effectiveness of our designing strategy.

Although their mechanism of action has not been fully elucidated yet, proteasome has been recognized as a major *in vitro* and *in vivo* target,[7] as well as the selenoenzyme thioredoxin reductase.[5,8] In this regard, We have investigated the interaction of a representative gold(III)-dithiocarbamato complex with selected molecules, such as 1-methylimidazole, *N*-acetyl-L-cysteine and dimethylsulfide, which may be regarded as reliable models of suitable target biomolecules (*i.e.* histidine residues, glutathione and cysteine-rich proteins, and methionine residues, respectively). Somewhat unexpected and unprecedented results obtained by means of detailed mono- and multinuclear NMR spectroscopy studies are reported and discussed, and possible reaction pathways described.

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References

1. L. Ronconi, D. Fregona J. Dalton Trans. (2009), 10670.
2. L. Ronconi, D. Aldinucci, Q.P. Dou, D. Fregona Anti-Cancer Agents Med. Chem. (2010), 10, 283.
3. L. Kelland Nat. Rev. Cancer (2007), 7, 573.
4. V. Milacic, D. Chen, L. Ronconi, K.R. Landis-Piwowar, D. Fregona, Q.P. Dou Cancer Res. (2006), 66, 10478.
5. L. Cattaruzza, D. Fregona, M. Mongiat, L. Ronconi, A. Fassina, A. Colombatti, D. Aldinucci Int. J. Cancer (2010), DOI: 10.1002/ijc.25311.
6. C. Marzano, L. Ronconi, F. Chiara, M.C. Giron, I. Faustinelli, P. Cristofori, A. Trevisan, D. Fregona Int. J. Cancer (2010), DOI: 10.1002/ijc.25684.
7. X. Zhang, M. Frezza, V. Milacic, L. Ronconi, Y. Fan, C. Bi, D. Fregona, Q.P. Dou J. Cell. Biochem. (2010), 109, 162.
8. D. Saggioro, M.P. Rigobello, L. Paloschi, A. Folda, S.A. Moggach, S. Parsons, L. Ronconi, D. Fregona, A. Bindoli Chem. Biol. (2007), 14, 1128.

Polymerases and RNA incorporation of free *N7*-platinated purines

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The main cellular targets of the antitumor drug *cisplatin* are DNA and RNA, where platinum is able to form covalent bonds with the *N7* of purines.[1] We hypothesized that under physiological conditions, the incorporation of platinated purines, during the nucleic acid synthesis, could compete with direct DNA/RNA platination. Previously we were able to demonstrate that platinated DNA can be formed by incorporation of platinated purines by DNA polymerases.[2]

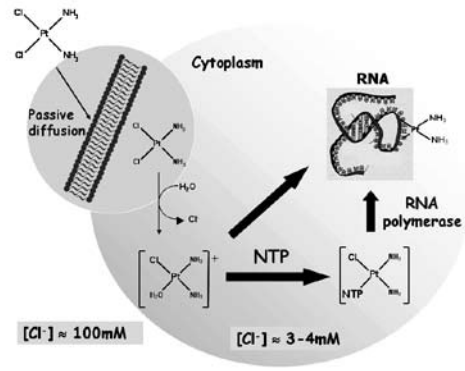

Figure 1. In the scheme are represented both: the mechanism of direct RNA platination by cisplatin aquated species and the newly proposed mechanism of RNA platination mediated by platinated nucleotides and RNA polymerases.

We evaluated the possibility that metallated purines could be inserted into RNA during the RNA transcription process. The model complex [Pt(**dien**)(*N7*-GTP)], **1** (**dien** = diethylenetriamine; GTP = 5'-guanosine triphosphate), unable to form chelates with DNA/RNA was tested for incorporation into RNA by *in vitro* transcription of the luciferase SP6 control plasmid with SP6 RNA polymerase.
The insertion of platinated purines, into newly synthesized RNA was shown by both agarose gel electrophoresis and ICP-AES Pt quantitative analysis of synthesized RNA. Gel fragments, corresponding to the observed bands in the lanes containing variable **1**/GTP ratios, were analyzed for Pt, confirming the **1** incorporation. A platinum level strictly related to the increasing **1**/GTP ratio was observed in correspondence of the RNA bands.

References

1. S. J. Lippard, Chem. Rev., (1999), 99, 2498 and references therein.
2. M. Benedetti, C. Ducani, D. Migoni, D. Antonucci, V. M. Vecchio, A. Ciccarese, A. Romano, T. Verri, G. Ciccarella, F. P. Fanizzi, Angew. Chem. Int. Ed., (2008), 47, 507; M. Benedetti, C. Ducani, D. Migoni, D. Antonucci, V. M. Vecchio, A. Romano, T. Verri, F. P. Fanizzi, (2009). In: A. BONETTI et al. "Platinum and Other Heavy Metal Compounds in Cancer Chemotherapy" pp. 125-132, Humana Press.

Biological studies on a new class of gold complexes with pyridinyl-benzoimidazole

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The antitumor properties of gold compounds are attracting much interest because of their high *in vitro* and *in vivo* activity against a series of tumor cell lines[1], with a cytotoxic mechanism that probably involves thioredoxin reductase inhibition[2].
A number of mononuclear and dinuclear gold(I,III) complexes with the ligand pyridinyl-2-benzoimidazole (PbNH) have been prepared and structurally characterized.

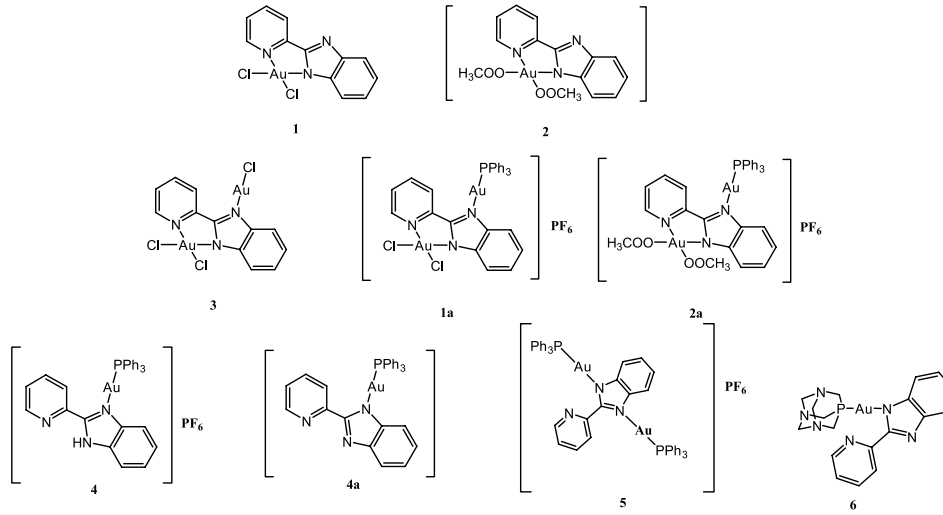

Figure 1.

The solution behaviour of these compounds was evaluated over 24 h in phosphate buffer pH 7.4. All these compounds were tested *in vitro* against a human ovarian carcinoma (A2780) cell line, sensitive and resistant to cisplatin, revealing high cytotoxicity towards both lines. Reactivity with proteins (Cytochrome C and Lysozyme) was studied by ESI MS and UV-visible spectrometry.
Methods and results of this study will be presented.

References

1. S. Nobili, E. Mini, I. Landini, C. Gabbiani, A. Casini, L. Messori, Med. Res. Rev., 2010, 30, 3, 550-580.
2. A. Pratesi, C. Gabbiani, M. Ginanneschi, L. Messori, Chem. Commun. (2010), 46, 7001-7003.

[Pt(**dien**)(*N7*-**G**)] transport by the mitochondrial deoxynucleotide carrier: possible role in the cytotoxicity of platinated purines

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Cisplatin is one of the most widely used anticancer drugs. The main cellular target of *cisplatin* is DNA, where platinum is able to form covalent bonds with purines. We have recently demonstrated that platinated DNA can be formed in vitro by *Taq* DNA polymerase promoted incorporation of a model platinated purine, i.e. [Pt(**dien**)(*N7*-G)], **1**, **dien** = diethylenetriamine, **G** = 5'-dGTP.[1]

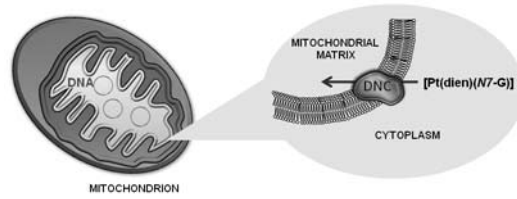

Figure 1. Transport of [Pt(**dien**)(*N7*-**G**)], **1**, in the mitochondria catalyzed by the deoxynucleotide carrier, **DNC**.

As the cell death resulting from treatment with platinum drugs seems to be caused also by mitochondrial DNA damage[2] we examined if platinated purines are transported in these organelles. We first studied their import into proteoliposomes by the model fruit fly DmTpc1p, the ortholog of the human mitochondrial deoxynucleotide carrier SLC25A19. NMR and ICP-AES Pt data have shown that the recombinant *E. coli* over-expressed DmTpc1p, reconstituted into phospholipid vesicles, is able to transport **1** across the liposomal membranes with high affinity. In a second experiment, we were able to demonstrate also that the *N7*-platinated deoxyguanosine triphosphate, **1**, can be transported, by the human mitochondrial deoxynucleotide carrier, **DNC**,[3] in intact and respiring mitochondria isolated from rat liver. Finally, if our ongoing studies will provide evidence of insertion of platinated deoxyguanosine triphosphate in the mitochondrial DNA, it will be possible to develop a new generation of anticancer deoxynucleoside triphosphate analogues, with a specific cellular target.

References

1. M. Benedetti, C. Ducani, D. Migoni, D. Antonucci, V. M. Vecchio, A. Ciccarese, A. Romano, T. Verri, G. Ciccarella, F. P. Fanizzi Angew. Chem. Int. Ed. (2008), 47, 507; M. Benedetti, C. Ducani, D. Migoni, D. Antonucci, V. M. Vecchio, A. Romano, T. Verri, F. P. Fanizzi, 2009. In: BONETTI A. et al. "Platinum and Other Heavy Metal Compounds in Cancer Chemotherapy" pp. 125-132 Humana Press.
2. A. Rebillard, D. Lagadic-Gossmann, M.T. Dimanche-Boitrel Curr. Med. Chem. (2008), 15, 2656.
3. D. Iacopetta, C. Carrisi, G. De Filippis, V. M. Calcagnile, A. R. Cappello, A. Chimento, R. Curcio, A. Santoro, A. Vozza, V. Dolce, F. Palmieri, L. Capobianco FEBS J. (2010) 277, 1172; V. Dolce, G. Fiermonte, M.J. Runswick, F. Palmieri, J.E. Walker PNAS (2001), 98, 2284.

Valproate enhances cisplatin-based chemotherapy in malignant mesothelioma

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Malignant pleural mesothelioma (MPM) is a lethal tumor which is caused by previous asbestos exposure. Combination chemotherapy is currently the treatment option associated with acceptable response rates. However, management of patients is controversial and optimal standard chemotherapy regimens are needed. An exaustive meta-analysis of clinical trials published between 1965 and 2001[1] indicated cisplatin-based polychemotherapy as the most active protocol for MPM treatment. Since then, combinations of platinum derivatives with new agents have considerably broadened the spectrum of therapeutic options. A recent in vitro study showed a synergistic effect of gemcitabine pre-treatment with cisplatin against MPM cells lines.[2] The action of the DNA-damaging (cisplatin) and the antimetabolite (gemcitabine) drug could be potentiated by combination with hystone deacetylases complex (HDAC) inhibitors, as valproate.[3] Indeed, HDAC inhibitors are known to enhance the chemosensitivity of cancer cells.[4] Thus, we investigated the pharmacological effects of gemcitabine followed by the combination of cisplatin and valproate. The combinations were tested on human mesothelioma cell lines BR95, MM98, and its cisplatin-resistant sub-line MM98R. Pharmacological interactions were evaluated using the combination index (CI) and the dose reduction index (DRI).[5] We first studied valproate interactions without pre-treatment. Simultaneous exposure of gemcitabine and valproate was synergistic in BR95 (epitelioid) and MM98 (sarcomatous), while additivity was found for MM98RCP (cell line made resistant to cisplatin). The association between cisplatin and valproate showed synergism in BR95 and an additive effect in MM98. Gemcitabine pre-treatment of cisplatin/valproate schedule exerted a modest enhancement of the overall antitumor activity. However, this schedule was characterized by a significant DRI for cisplatin, that can ameliorate its side-effects without lower the efficacy of such a therapeutic treatment. Furthermore, 3D cell cultures (spheroids) were employed as more reliable tumor model, since they better mimic the tumor tissue behaviour.[6]

References

1. T. Berghmans, M. Paesmans, Y Lalami, I. Louviaux, S. Luce, C. Mascaux, AP Meert Lung cancer. (2002), 38.
2. I. Zanellato, CD Boidi,G.Lingua, S.Orecchia, E.Monti, D.Osella Cancer Chemother. Pharmacol. (2010).
3. F. Vandermeers, P. Hubert, P Delvenne,C. Mascaux, B. Grigoriu, A. Burny Clin. Cancer Res (2009), 35, 8.
4. J. Purrucker, A. Fricke, M. Fang Ong, C. Rube J. Oncology reports (2010), 23.
5. TC. Chou, P. Talalay Adv. Enzyme Regulation (1984), 22.
6. F. Hirschhaeuser, H. Menne, C. Dittfeld, J West J. Biotechnol (2010), 148.

$[Au_2(phen^{2Me})_2(\mu\text{-}O)_2](PF_6)_2$, a novel dinuclear gold(III) complex showing excellent antiproliferative properties

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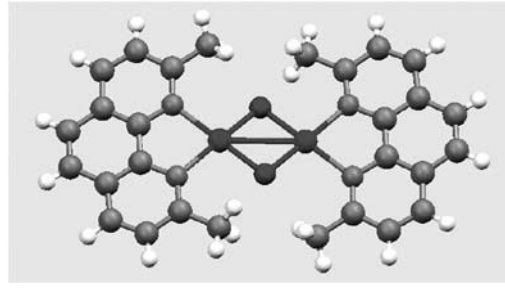
A novel dioxo-bridged dinuclear gold(III) complex with two 2,9 dimethylphenanthroline ligands was synthesized and thoroughly characterized [1]. Its crystal structure was solved and its solution behavior assessed. Remarkably, this compound revealed excellent antiproliferative properties in vitro against a wide panel of 36 cancer cell lines, combining a high cytotoxic potency to a pronounced tumor selectivity. Most likely, these properties arise from an innovative mode of action (probably involving HDACs inhibition), as suggested by COMPARE analysis. In turn, ESI MS studies provided valuable insight into its molecular mechanisms of activation and of interaction with protein targets. Gold(III) reduction, dioxo bridge disruption, coordinative gold(I) binding to the protein and concomitant release of the phenanthroline ligand were proposed to occur upon interaction with protein. In view of the obtained results this novel gold(III) compound qualifies itself as an optimal candidate for further pharmacological testing.



References

1. M.A. Cinellu, L. Maiore, M. Manassero, A. Casini, M. Arca, H.-H. Fiebig, G. Kelter, E. Michelucci, G. Pieraccini, C. Gabbiani, L. Messori, ACS Med. Chem. Lett. (2010), *in press*

Synthesis of new pivaloamidine Pt(II) and pivaloamidinato Pt(III) complexes

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A relevant class of biologically active Pt(II)-based drugs[1] has been prepared from the Pt(II) precursors *cis* and *trans*-$[PtCl_2(N{\equiv}CR)_2]$ (R=Me or Ph) by taking advantage of their ability to undergo nucleophilic addition of ammonia or methylamine to the coordinated nitriles to afford the amidine derivatives *cis* and *trans*-$[PtCl_2\{HN{=}C(R)NHR'\}_2]$ (R=Me or Ph, R'=H or Me).[2] Unfortunately, in the case of reaction with ammonia, its strong tendency to coordinate to the metal center promotes the formation of the cationic species $[PtCl(NH_3)\{HN{=}C(R)NH_2\}_2]Cl$. In this work, new pivaloamidine complexes, *cis* and *trans*-$[PtCl_2\{HN{=}C(Bu^t)NH_2\}_2]$ and *cis* and *trans*-$[PtCl_2(NH_3)\{HN{=}C(Bu^t)NH_2\}]$, have been prepared and a new synthetic procedure has developed with the aim of avoiding the formation of the cationic species. The pivaloamidine Pt(II) complexes have been fully characterized and their citotoxic activity against 8 human cancer cell lines, one of these choosen for its cisplatin resistance, has been tested. Moreover the complex *cis*-$[PtCl_2\{HN{=}C(Bu^t)NH_2\}_2]$ has been crystallized and analyzed by X-Ray diffraction. In the crystal, the complex forms dimeric aggregates, and this appears to be responsible for easy oxidation by oxygen to a Pt(III) dimer.

Thus, the acetamidine platinum(II) complex *trans*-$[PtCl_2\{HN{=}C(Me)NH_2\}_2]$ is oxidized by oxigen dissolved in water to the dinuclear platinum(III) specie with bridging acetamidinato ligand $[Pt^{III}_2Cl_4\{HN{=}C(Me)NH\}_2\{HN{=}C(Me)NH_2\}_2]$.[3] We have adopted a similar procedure for the oxydation of *trans*-$[PtCl_2(NH_3)\{HN{=}C(Bu^t)NH_2\}]$ to the corresponding Pt^{III} species $[Pt^{III}_2Cl_4\{HN{=}C(Bu^t)NH\}_2(NH_3)_2]$. The new dinuclear platinum(III) complex with two bridging pivaloamidinato ligands has been fully characterized by elemental analysis, ESI-MS and NMR spectroscopy.

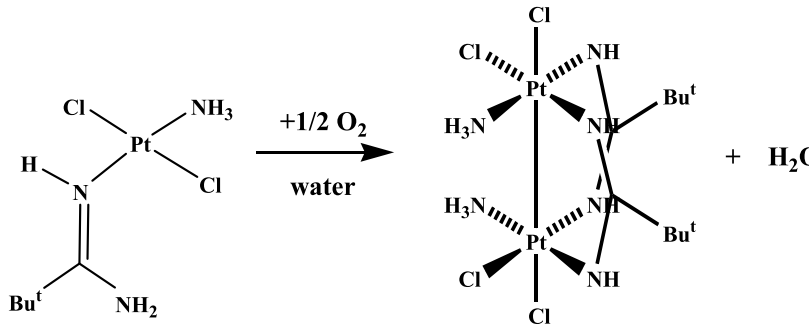

Figure 1. Oxidation by air of the ammine-pivaloamidine-platinum(II) complex.

References

1. Marzano, C.; Sbovata, S.; Bettio, F.; Michelin, R.; Kiss, T.; Venzo, A.; Bertani, R.; *Journal of Biological Inorganic Chemistry*. **2007**, *12*, 477-493.
2. Intini F.P.; Pellicani R.Z.; Boccarelli A.; Sasanelli R.; Coluccia M. and Natile G. *Eur. J. Inorg. Chem*. **2008**, 4555-4561
3. Cornacchia D.; Pellicani R. Z.; Intini F.P.; Pacifico C., and Natile G. **2009**, *48*, 10800-10810.

Bu2Sn(N-acetyl-L-cysteinate) antitumor activity on HepG2 cells

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Many drugs, currently used in anticancer therapies, act by activating cytotoxic death by apoptosis. This death mechanism is often accompanied by mitochondrial events (intrinsic apoptosis) or the activation of death receptors (extrinsic pathway).[1] It is interesting to observe that cancer cells preserve themselves by apoptosis because express high levels of survival factors which induce resistance to the drugs. Thus, the goal of anticancer research is the design of drugs capable of inducing apoptosis in tumor cytotypes by downregulating survival factor expression while non-tumor cells, which do not have high level of these factors, are not affected by the drugs. Recently, we have synthesised and characterized a class of R_2Sn(N-acetyl-L-cysteinate) complexes, including Bu_2Sn(N-acetyl-L-cysteinate) (= NAC2).[2] Biological investigations showed that NAC2 induced a marked antiproliferative effect on a large collection of tumor cells derived from different cancer types, including lung, colon, melanoma, renal, ovarian, brain, and leukemia. Moreover, the novel derivative was very efficacious in HepG2 cells, an *in vitro* model of hepatocellular carcinoma, one of the most common and highly lethal diseases worldwide, while it was almost inactive towards Chang liver cells, an immortalized non tumor hepatic cell line used as control. NAC2, while maintaining a significant cytotoxicity in tumor cells, was less toxic with respect to the parent Bu_2SnCl_2 suggesting that the conjugation of N-acetylcysteine to organotin(IV) modulated the strong cytotoxic activity of the parent. The study on death mechanism evidenced that the reduction in cell viability exerted by NAC2 was mainly due to induction of apoptosis, as demonstrated by the increase in the percentage of HepG2 cells confined in subG0/G1 phase of cell cycle and the cleavage of caspase-8 and caspase-3. Apoptosis was accompanied by the induction of oxidative stress and the involvement of mitochondrial events. Finally in NAC2-treated HepG2 cells we observed a marked reduction in the level of some survival factors such as phospho-AKT and survivin,[3-4] while the levels of phospho-PAR-4(Thr163), a pro-apoptotic factor which is a negative regulator of AKT,[5] were clearly increased.

References

1. O. Pellerito, G. Calvaruso, P. Portanova, A. De Blasio, A. Santulli, R. Vento, G. Tesoriere, M. Giuliano Mol. Pharmacol. (2010), 77, 854.
2. L. Pellerito, C. Prinzivalli, G. Casella, T. Fiore, O. Pellerito, M. Giuliano, M. Scopelliti, C. Pellerito J. Inorg. Biochem. (2010), 104, 750.
3. E. Tokunaga, E. Oki, A. Egashira, N. Sadanaga, M. Morita, Y. Kakeji, Y. Maehara Curr. Cancer Drug Targets (2008), 8, 27.
4. M.J. Duffy, N. O'Donovan, D.J. Brennan, W.M. Gallagher, B.M. Ryan Cancer Lett. (2007), 249, 49.
5. J.W. Lee, K.F. Lee, H.Y. Hsu, L.P. Hsu, W.L. Shih, Y.C. Chu, W.T. Hsiao, P.F. Liu Cancer Lett. (2007), 257 , 252.

Targeting of Pt(II)- and Pt(IV)-complexes to TSPO

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There are some severe side effects that limit the use of the antitumoral drug cisplatin and, to overcome some of these problems, it is possible to use the "drug targeting and delivery" strategy in wich cisplatin is associated to organ- or receptor-specific carriers.[1] We are pursuing this approach[2,3] by focussing on the over-expression of the peripheral benzodiazepine receptor (PBR), recently renamed "translocator protein 18 kDa (TSPO)", occurring in many tumour types, such as brain, glial, hepatic, and mammary tumours, breast carcinoma, and ovarian and colorectal cancers.[4] Recently two square-planar Pt(II) compounds carrying a TSPO-specific ligand have been tested for receptor binding ability and for cytotoxicity against human and rat glioma cells and A2780 human ovarian carcinoma cells sensitive and resistant to cisplatin. Their ability to induce apoptosis and to accumulate into cells, has also been studied[2,3]. We are now investigating the formation of Pt(IV) complexes with axial TSPO-specific moieties. These Pt(IV) derivatives could overcome some drawbacks typical of Pt(II) antitumoral drugs, such as the rapid deactivation by platinophiles,[5][6] together with a change in activity consequent to the modification of the square-planar coordination shell. Unlike Pt(II) species, Pt(IV) complexes act as prodrugs therefore, often targeting to specific tumours overexpressing TSPO and entering the cell, they can undergo reduction with release of the active Pt(II) species and two equivalents of the axial ligands wich are, themselves, cytotoxic. We are testing two different synthetic pathways: 1) condensation of the axial OH's of a Pt(IV) complex with a carboxylic function of the TSPO ligand, 2) condensation of axial succinato ligands of a Pt(IV) complex with the aminic function of a TSPO ligand.

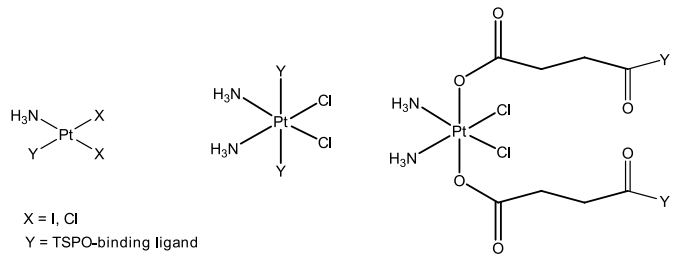

Figure 1. General structures of Pt(II) and Pt(IV) complexes with a TSPO–specific ligand

Acknowledgments

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References

1. E. Gabano et al., Curr. Med. Chem. (2009), 16, 4544-4580
2. N. Margiotta et al., J. Med. Chem. (2007), 50, 1019-1027
3. N. Margiotta, et al., J. Med. Chem., (2010), 53, 5144-5154
4. A.M. Scarf, J. Med. Chem. (2009), 52, 581-592
5. K. R. Barnes, A. Kutilokov, S. J. Lippard, Chemistry & Biology, (2004), 11, 557-564
6. M.R. Reithofer, B. K. Keppler,et al., J. Med. Chem., (2007), 50, 6692-6699

Interactions of water-soluble porphyrins with DNA

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DNA is a polymorphic polymer that can adopt a variety of secondary structures ranging from the canonical right-handed B form to the left-handed Z conformation.[1] Porphyrins have been widely studied as structural reporter but have been also investigated as anti-cancer drugs. Formation of porphyrin-DNA complexes (which is believed to be an important step leading to antitumor activity) is known to be facilitated by the electrostatic attractions between the periphery of cationic porphyrins and the anionic phosphate backbone. However, also the interaction of anionic porphyrins with DNA has been shown to be feasible. In this communication I will shortly overview our past and present studies concerning the interactions of cationic and anionic porphyrins with DNA both as chiroptical reporters of DNA structures and as possible model systems for logic gates.[2-6]

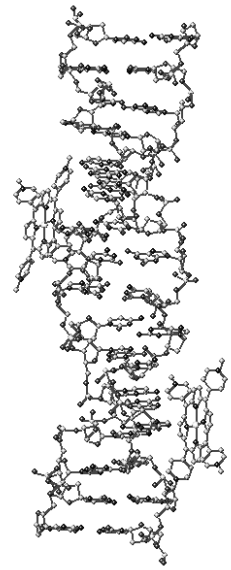

Figure 1. Proposed structure for a Zinc(II) porphyrin - Z-DNA complex

References

1. P. Belmont, J. F. Constant, M. Demeunyck, Chem. Soc. Rev. (2001), 30, 70.
2. A. D'Urso, A. Mammana, M. Balaz, A. Holmes, N. Berova, R. Lauceri, R. Purrello, J.Am.Chem. Soc.(2009), 131, 2046.
3. M. Balaz, M. De Napoli, A. Holmes, A. Mammana, K. NakanishiN. Berova, R. Purrello, Angew. Chem. Int. Ed. (2005), 44, 4006.
4. R. Lauceri, R. Purrello, S. Shetty, M.G. Vicente, J.Am.Chem. Soc. (2001), 123, 5835.
5. R. Lauceri, T. Campagna, A. Contino, R. Purrello, Angew. Chem. Int. Ed.(1996), 35(2), 215.
6. C. Bustamante, S. Gurrieri, R.F. Pasternack, R. Purrello, E. Rizzarelli, Biopolymers (1994), 34, 1099.

The behavior of hydrophilic copper(I) complexes in solution. Effects on the speciation of $[Cu(P)_4]^+$-type compounds ongoing from millimolar to micromolar levels

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It is generally believed that copper(I) is the permeating form of the metal, and that delivery of Cu(I) from the bloodstream to the cell membrane is managed by methionine-rich sequences of the N-terminal extracellular domain of hCtr1[1]. Intrigued by the oxidation state of the metal operating in physiological processes, we have been searching for the preparation of water soluble copper(I) complexes stable in physiological media, a synthesis which has been elusive for decades. We have employed hydrophilic phosphines (e.g. P1 = tris(hydroxymethyl)phosphine, P2 = 1,3,5-triaza-7-phosphaadamantane) for the synthesis of homolepticphosphino copper(I) complexes[2]. The resulting 'CuP_4'-type complexes displayed stability to metal disproportionation in aqueous media and proved to be easy to handle during in vitro and in vivo tests. 'CuP_4'-type complexes retain the tetrahedral stereochemistry in the solid state (X-ray crystal structure) and in the solution state at 10^{-2}-10^{-3} M (^{31}P and ^{63}Cu NMR), but they display detectable lability at micromolar concentrations, as assessed by ESI-MS measurements[3] and confirmed by speciation studies. Inverse correlation between the stability and the cytotoxic activity of 'CuP_4'-type complexes has been established, indicating that the more favored is the displacement of the ligand(s) from the $[CuP_4]^+$ parent complex, the more favored is in turn the possibility for the copper ion to directly interact with biological substrates including pharmacological targets related to cancer proliferation. The monocationic$[Cu(P1)_4]^+$ complex (CP1) was found to strongly reduce human cancer cell viability with potency over 40-fold higher than that of the reference anticancer metallodrug cisplatin. CP1 showed high selectivity against tumor cells compared with non-tumor cells and overcame cisplatin, oxaliplatin and MDR resistances. CP1 acted by inducing a programmed non-apoptotic cell death termed paraptosis coherent with the inhibition of proteasome 26S and the induction of Unfolded Protein Response. Moreover, distinctive 'CuP_4'-type complexes, by virtue of their lability in biological media and mild toxicity versus normal cells, may be used as suitable Cu(I) donor agents in the treatment of disorders associated with copper-deficiency.

References

1. EB Maryon, SA Molloy, AM Zimnicka, JH Kaplan. BioMetals, (2007), 20, 355.
2. F Tisato, C Marzano, M Porchia, M Pellei, C Santini. Med. Res. Rev. (2010) 30, 708.
3. F Tisato, F Refosco, M Porchia, M Tegoni, V Gandin, C Marzano, M Pellei, G Papini, L Lucato, R Seraglia, P Traldi. Rapid Commun. Mass. Spectrom. (2010) 24, 1610.

A new class of *trans*-amine-amidine-Pt(II) cationic complexes: influence of chemical structure and solvent on in vitro and in vivo tumor cell proliferation

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In recent years, a great variety of *trans*-platinum complexes have been shown to possess significant in vitro and in vivo antitumor activity similar to or even higher than that of cisplatin[1]. The introduction of cyclic aliphatic amines, in particular cyclohexylamine, in the platinum coordination sphere, may be of interest because of their steric hindrance and flexibility, resembling nonplanar heterocyclic amine ligands such as 4-picoline, piperidine, or piperazine[2]. The cyclohexylamine ligand has been recently reported in a new class of potential anticancer drugs of the type *trans*-[(NHC)PtI_2(amine)][3]. Furthermore, the presence of the cyclohexyl ring also characterizes the series of platinum complexes that are based on the 1,2-diaminocyclohexane (DACH) carrier ligand, among whom Oxaliplatin and Ormaplatin, have entered clinical trials[4]. From this perspective, we report the synthesis, characterization, and biological activity of a new class of cationic *trans*-Pt(II) complexes of the type *trans*-[Pt(amine)$_2$(amidine)$_2$][Cl]$_2$ that are derived from the reaction of *trans*-[$PtCl_2(NCCH_3)_2$] with cyclopropylamine, cyclopentylamine, and cyclohexylamine[5]. The solution behaviour and biological activity have been studied in different solvents (DMSO, water, polyethylene glycol (PEG 400), and polyethylene glycol dimethyl ether (PEG-DME 500)). The biological activity was strongly influenced by the cycloaliphatic amine ring size, with *trans*-[$Pt(NH_2{\ulcorner}CH(CH_2)_4{\urcorner}CH_2)_2\{N(H){=}C(CH_3)N(H){\ulcorner}CH(CH_2)_4{\urcorner}CH_2\}_2]^{2+}[Cl^-]_2$ (**3**) being the most active compound. Complex **3** overcame both cisplatin and MDR resistance, inducing cancer cell death through p53-mediated apoptosis. Alkaline single-cell gel electrophoresis indicated a direct DNA damage, reasonably attributable to DNA adducts which can evolve to produce disruptive and non-repairable lesions on DNA, thus leading to the drug-induced programmed cell death. Preliminary in vivo studies on C57BL mice bearing Lewis lung carcinoma highlighted that complex **3** promoted a significant and dose-dependent tumor growth inhibition without adverse side effects.

References

1. M. Coluccia, G. Natile. Anti-Cancer Agents Med. Chem. (2007) 7, 111.
2. Y. Najajreh, T. Peleg-Shulman,; Moshel, N. Farrell, D. Gibson. J. Biol. Inorg. Chem. (2003) 8, 167.
3. M. Skander, P. Retailleau, B. Bourri, L. Schio, P. Mailliet, A.N. Marinetti. J. Med. Chem. (2010) 53, 2146.
4. P.O'Dwyer, J.P.Stevenson, S.W. Johnson, In Platinum Based Drugs in Cancer Therapy; Kelland, L. R.; Farrell, N. P., Eds.; Humana Press, Totowa, NJ, 2000; pp 231− 249.
5. C. Marzano, S. Mazzega Sbovata, V. Gandin, D. Colavito, E. Del Giudice, R.A. Michelin, A. Venzo, R. Seraglia, F. Benetollo, M. Schiavon, R. Bertani. J. Med. Chem. (2010), 53, 6210.

Ligands effect on the *in vitro* cytotoxic activity of mixed polyazolylborato phosphino-Cu(I) complexes

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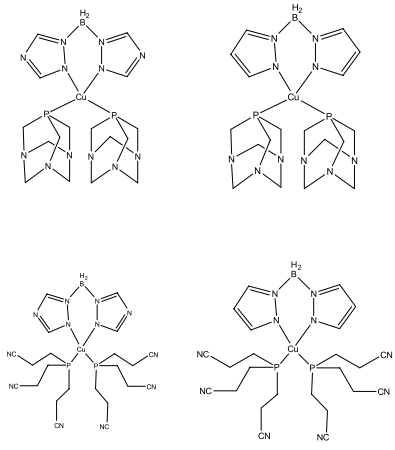
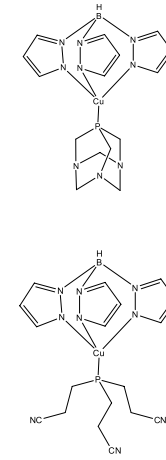
In the last three decades, the research of metal complexes, other than platinum derivatives, as antitumor agents have spread over a large number of metals and ligands. Despite the huge effort devoted to this field and the thousands of tested compounds, no new drugs have entered the clinical use yet. Among the considered metals, copper, an endogenous element potentially less toxic than non-essential metals such as platinum, has gained a growing interest and many classes of copper (I/II) complexes have been extensively investigated as antitumor metallodrugs. In the last years we have reported the synthesis of homoleptic phosphinocopper(I)complexes and of heteroleptic copper(I) compounds comprising both phosphine and scorpionate ligands. They showed a remarkable in vitro cytotoxic activity also on cisplatin sensitive and resistant cell lines suggesting a DNA-independent mechanism of action. Here we present some new copper(I) complexes containing monodentate phosphines and polyazolylborate ligands with the aim of finding a correlation between their in vitro cytotoxic activity and the different hydrophilicity, hapticity and steric hindrance of the ligands.



"2+1+1" Cu(I) complexes



"3+1" Cu(I) complexes

Proteomic and metallomic strategies in the study of anticancer metallodrugs

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Since the discovery of cisplatin, metal compounds have been intensely investigated as potential anticancer agents. A deeper understanding of their mode of action, still rather obscure, might turn crucial for the design and the obtainment of new and better anticancer agents. Due to the extreme complexity of the biological systems, it is now widely accepted that innovative and information-rich methods are absolutely needed for these studies. Recently, both proteomic and metallomic strategies have been successfully implemented in the elucidation of specific mechanistic features of anticancer metallodrugs within an innovative "Systems Biology" perspective. The advantages and limitations of these approaches will be described. A further extension of those studies and a closer integration of proteomic and metallomic strategies and technologies might realistically lead to rapid and significant advancements in the mechanistic knowledge of anticancer metallodrugs.

Preliminary studies on inhibition of DHFR by a class of phosphine gold(I) compounds

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The mechanism of action of antirheumatic or antiproliferative gold complexes has been under question for a long time. Concerning their antirheumatic properties, several enzymes such as cylclooxigenases, cysteine proteases and mammalian thioredoxin reductase (TrxR) have been considered as possible targets of gold based complexes, and the suppression of the activities of these enzymes as well as others has been reported.[1] Phosphine gold(I) complexes with pyrazole or imidazole as co-ligands have been found to possess antimicrobial activities.[2] Nevertheless, so far there are no studies about the inhibition by gold compounds of an enzyme strictly related to cell proliferation as dihydrofolate reductase (DHFR) (EC 1.5.1.3).[3]

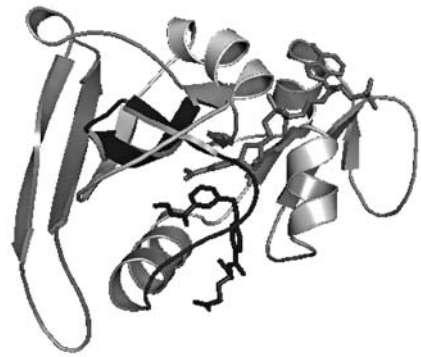

Figure 1. Ribbon diagram of *E. coli* DHFR bound to the substrate (dihydrofolate) in blue, and the cofactor (NADPH) in red.

In this work we have considered a class of phosphine gold(I) complexes with deactivated pyrazolate or imidazolate as co-ligands, whose citotoxic activities have been already established and studied, as inhibitors of DHFR. The aim of this work was to investigate whether the cytotoxic effects of this class of gold(I) compounds might be a consequence of a direct interaction with DHFR. Enzymatic inhibition has been tested through spectrophotometric assays, and the thermodynamic dissociation constants have been measured by fluorescence quenching analysis.

Our results demonstrated that the phosphine gold(I) complexes behave as effective inhibitors of DHFR, with inhibition constants in the nanomolar range. Furthermore it has been attempted to relate the inhibitory activity to the lipophilic/hydrophilic balance by logP determination of the gold(I) compounds. From this preliminary study it can be elicited that the phosphine gold(I) complexes act as antimicrobial agents and can be promising candidates for chemotherapy .

References

1. I. Ott, Coord. Chem. Rev., 2008, doi:10.1016/j.ccr.2009.02.019.
2. K. Nomiya, R. Noguchi, K. Ohsawa, K. Tsuda, M. Oda, J. Inorg. Biochem., 78 (2000), 363.
3. J.R. Schnell, H. Jane Dyson, P.E. Wright. Annu. Rev. Biophys. Struct. 2004, 33: 119-140

Metal complexes of tetrakis-2,3-[5,6-di(2-thienyl) pyrazino]porphyrazine: synthesis, characterization and photochemical properties

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Pyrazinoporphyrazine macrocycles carrying peripherally pyridine rings and having formula [Py_8TPyzPzM] were previously reported.[1,2] The presence of the pyridine rings strongly influences the electronic structure of the macrocycles which can be classified as electron-deficient macrocycles due to the electron-withdrawing effect of the peripherally attached heterocycles.

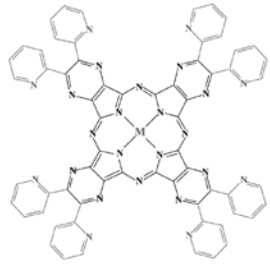

Figure 1A. [Py_8TPyzPzM]

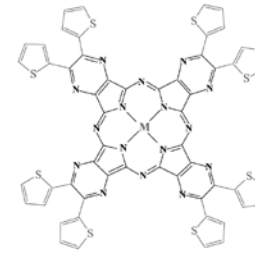

Figure 1B.[Th_8TPyzPzM]

A parallel investigation has recently been started on pyrazinoporphyrazine analogs bearing externally 2-thienyl rings, ie. the tetrakis-2,3-[5,6-di(2-thienyl)pirazino]porphyrazine, [Th_8TPyzPzH_2] and its metal derivatives [Th_8TPyzPzM] (M = $Mg^{II}(H_2O)$, Zn^{II}, Co^{II}, Cu^{II}). The type of acceptor/donor properties of the external 2-thienyl rings has been studied and the ability of the macrocycles to act as photosensitizers in photodynamic therapy (PDT) has also been investigated. Besides, the presence of sulfur containing heterocyclic rings is a new element of interest worth to be explored as to their ability to coordinate metal centers. In this respect, a preliminary work has been carried out on the related precursor 2,3-dicyano-5,6-di(2-thienyl)-1,4-pirazine, [$(CN)_2Th_2$Pyz] shown to be able to fix $PdCl_2$ units, this resulting in the formation of the bispalladate [$(CN)_2Th_2$Pyz$(PdCl_2)_2$]. Some of the results just on these materials will be briefly presented and discussed.

References

1. a) M.P. Donzello, Z. Ou, F. Monacelli, G. Ricciardi, C. Rizzoli, C. Ercolani, K.M. Kadish Inorg. Chem (2004), 43, 8626; b) M.P Donzello, Z. Ou, D. Dini, M. Meneghetti, C. Ercolani, K.M. Kadish Inorg. Chem. (2004), 43, 8637.
2. a) M.P Donzello. E. Viola, C. Xiaohui, L. Mannina, C. Rizzoli, G. Ricciardi, C. Ercolani, K.M. Kadish, A. Rosa Inorg. Chem. (2008), 47, 3903; b)E. Viola, M.P. Donzello, S. Ciattini, G. Portalone, C. Ercolani Eur. J. Inorg. Chem. (2009), 1600.

From phoshines to water soluble carbenes: synthesis and biological investigation of 11th group metal complexes

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Complexes of group 11 metals featured by aromatic tertiary phosphines or diphosphines were investigated as antitumour agents. Structure activity relationship for diphosphine and phosphinopyridyl metal complexes suggested that the selectivity for cancer cells over normal cells could be tuned by adjusting their hydrophilic/lipophilic balance. In this research field our group investigated the antitumour activity of three series of $[M(L)_4]^+$ compounds (M = Cu(I), Ag(I) and Au(I); L = tris(hydroxymethyl)phosphine (thp), 1,3,5-triaza-7-phosphaadamantane (PTA) or tris(hydroxypropyl)phosphine (thpp): cytotoxicity data confirmed that it depends either on the type of coordinated phosphine and on the nature of the metal.[1] Thp, PTA and thpp ligands coordinate the soft M(I) ion producing homoleptic tetrahedral compounds of variable hydrophilicity. The best results in terms of *in vitro* antitumour activity were achieved with metal-thp species and, among the coinage metal complexes, copper derivatives were found to be the most efficient drugs. In addition mixed-compounds were prepared by combining hydrophilic N_2-scorpionate ligands with lipophilic 'CuP_2' unsaturated moieties (P_2 = bidentate phosphines or two monodentate phosphines) trying to enhance the hydrophilic character of the overall phosphine-scorpionate copper assembly compatible with physiological media. With the same purpose water soluble N-heterocyclic carbene ligands (NHCs) have been synthesized and used to obtain 11th group metal complexes. Carbenes are better σ-donor ligands if compared to phosphines and the related Au(I), Ag(I) and Cu(I) complexes could have higher stability and better cytotoxicity activity. Good stability in water could prevent ligand dissociation and unwanted dismutation. In view of the strategy to modify transition metal complexes by attaching phase tags, which infer the desired solubility properties, sulfonate- and carboxylate-functionalized *N*-heterocyclic mono-, bis- and tris-carbene ligands have been synthesized. The related water soluble silver complexes have proven themselves to be very adept at transferring to a variety of other metals such as gold(I) and copper(I). Now the work is in progress to investigate the biological activity of the coinage metals NHC complexes.

References

1. C. Santini, M. Pellei, G. Papini, B. Morresi, R. Galassi, S. Ricci, F. Tisato, M. Porchia, V. Gandin, C. Marzano J. Biol. Inorg. Chem. (2010) submitted.

The antitumor cobalt-alkyne complex Co-ASS derived from acetylsalicylic acid is active on malignant mesothelioma

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[(Prop-2-ynyl)-2-acetoxybenzoate]dicobalthexacarbonyl (Co-ASS), a metallo-carbonyl derivative of the nonsteroidal anti-inflammatory drug aspirin (ASS), showed high cytotoxic activity against various tumor cell lines [1][2].The overall Co-ASS mechanism of action has been firmly ascribed to a strong inhibition of cyclooxygenase-1 and 2 (COX-1 and COX-2)[3]. These enzymes are a promising pharmacological target for the management of malignant mesothelioma (MM), which still remains the most lethal pleural, peritoneal and pericardial cancer [4]. Overexpression of cyclooxygenase-2 (COX-2) often occurs in MM[5]. COX-2 inhibitors revealed a specific MM-antiproliferative activity both *in vitro* [6] and *in vivo* [7]. This effect was recently shown to be ascribed to the impairment of infiltrating myeloid-derived suppressor cells (MDSC) that co-localise with COX-2 expression in those areas where tumour growth takes place [8].

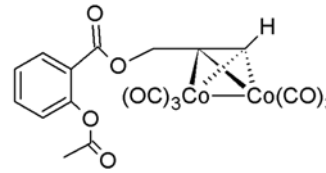

Figure 1. Co-ASS sketch view

Thus, we tested Co-ASS on mesothelioma cell lines, having both epithelioid and sarcomatoid phenotype and also on a cisplatin-resistant cell line. IC_{50} values were similar to those of cisplatin, and significantly lower in the case of the resistant cell line. We then aimed to investigate Co-ASS mechanism of action on MM.

References

1. I.Ott, B.Kircher, R. Gust, J. Inorg. Biochem (2004), 98
2. I. Ott, & R. Gust, Biometals (2005), 18
3. I. Ott, K. Schmidt, B. Kircher, P. Schumacher, T. Wiglenda, R. Gust, J. Med. Chem. (2005), 48.
4. M. Ray, H.L. Kindler, Chest (2009) 136, 3.
5. S.L. O'Kane, L. Cawkwell, A. Campbell, M. J. Lind, Eur. J. Cancer. (2005), 41, 11.
6. A. Marrogi, H.I. Pass, M. Khan, L.J. Metheny-Barlow, C.C. Harris, B. I. Gerwin, Cancer Res. (2000), 60, 14.
7. A. Catalano, L. Graciotti, L. Rinaldi, G. Raffaelli, S. Rodilossi, P.G. Betta, W. Gianni, S. Amoroso, A. Procopio, Int. J. Cancer (2004), 109, 3.
8. J.D. Veltman, M. E. Lambers, M. van Nimwegen, R.W. Hendriks, H.C Hoogsteden, J.G. Aerts, J. P. Hegmans, BMC Cancer (2010) 10, 464.

Electrochemical properties of copper complexes of oxicam group Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

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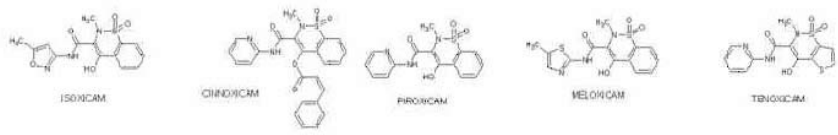
The oxicam group of non steroidal anti-inflammatory drugs (NSAIDs) have emerged as highly effective class of drugs against various arthritic conditions and post-operative inflammation. Experimental evidences highlight that copper(II) complexes with NSAIDs as ligands improve the pharmaceutical activity of the drugs themselves and reduce their undesired toxicity in human and veterinary medicine.[1] Here we present an electrochemical characterization of the complexes $[Cu(HPir)_2(H_2O)_2]$, $[Cu(HMel)_2(H_2O)_2]$, $[Cu(HTen)_2(H_2O)_2]$ and $[Cu(MBT)_2(PPA)_2]$ (HPir, HMel and HTen are the oxicam monoanions of molecules reported in Scheme 1; MBT {3-(methoxycarbonyl)-2-methyl-2*H*-1,2-benzothiazin-4-olate-1,1-dioxide; PPA = 3-phenyl-N-pyridin-2-ylacrylamide} is obtained by reaction of copper(II)-acetate with cinnoxicam) as contribution to the knowledge of their anti-inflammatory mechanism of action.[1,2]



Schema 1

Herein, we present the electrochemical and spectroelectrochemical behaviour of the mentioned Cu(II) complexes also in comparison with that of their free ligands. As a typical example, Figure 1 illustrates the redox activity of $[Cu(HTen)_2(H_2O)_2]$ in DMSO solution. It displays one ligand-centred oxidation and three successive reductions. The first process, $E_p \approx -0.5$ V is copper centred, whereas the successive processes are ligand-centred. The redox behaviour of all the complexes will be discussed.

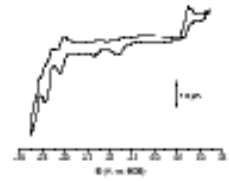


Figure 1. Cyclic voltammogram of $[Cu(HTen)_2(H_2O)_2]$

References

1. R. Cini, G. Tamasi, S. Defazio, M. Hursthouse, J. Inorg. Biochem., (2007), 101, 1140-1152 and references therein.
2. M.R. Moya-Hernández, A. Mederos, S. Domínguez, A. Orlandini, C.A. Ghilardi, F. Cecconi, E. González-Vergara, J.Inorg. Biochem.,(2003), 95, 131-140.

Supramolecular aggregates containing platinum complexes and labelled with Octreotide peptide

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Platinum based drugs are currently used in the treatment of a large number of solid tumors; anyway they induce several side effects.[1] One strategy to reduce toxicity and to prevent inactivation of the chemiotherapic drugs, is based on the encapsulation of the cytotoxic drug in liposomes. Moreover, a liposomal formulation may overcome platinum resistance by delivering a high dose of the administered drug at the tumor site. Stabilized PEGylated ("stealth") liposomal formulations of cisplatin have been developed in last decade. [2] These preparations exhibited an extended circulation time, increased anti-tumor efficacy and reduced toxicity compared to the free drug. In last years we developed peptide containing mixed aggregates able to deliver contrast agents and drugs to tumor cells overexpressing peptide receptors. The objective of present work is to formulate new aggregates able to vehicle selectively new platinum amphiphilic complexes to cancer cells. The selected bioactive peptide chosen as delivery tool is octreotide, it is a somatostatin analog able to bound somatostatin receptors subtypes (SSTR2 and SSTR 5) overexpressed in a wide number of tumoural pathologies. The new peptide derivative supramolecular aggregates are obtained by combining together two amphiphilic molecules: a first monomer bearing the hydrophilic octreotide peptide and as lipophilic moiety two eighteen carbon aliphatic chains, and a second monomer containing the same hydrophobic moiety bound to a lysine bearing on N^{α} and N^{ε} amino functions an N-etilglycine platinum complex and a PEG 1500 chain which increases the hydrophilicity and circulation time.
Supramolecular aggregates are obtained by mixing the two monomers in aqueous Hepes buffer at pH 7.4. The aggregates are characterized by dynamic light scattering (DLS) in order to determine the size and by SANS to obtain information about their shape. In *vitro* biological assays are currently in progress.

References

1. B. Lippert Cisplatin. Chemistry and Biochemistry of a Leading Anticancer Drug, Wiley–VCH, Weinheim, **1999**.
2. M. S. Newman, G.T. Colbern, P. K. Working, C. Engbers, M. A. Amantea Cancer Chemother.Pharmacol. (1999), 43, 1.

Cytotoxic properties of a new class of amidine complexes of Pt(II) stabilized by PPh_3 phosphine

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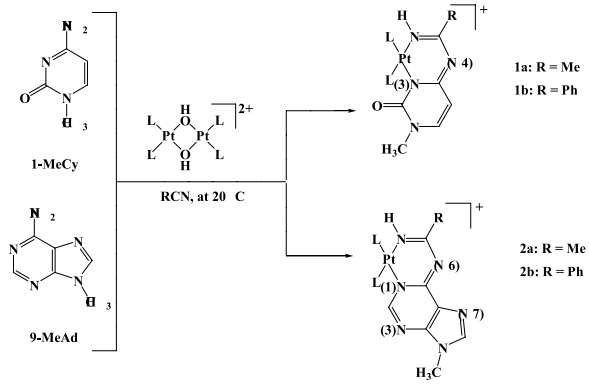
The deprotonation of the model nucleobases 1-methycytosine (1-meCy) and 9-methyladenine (9-MeAd) obtained by reacting the hydroxo complex *cis*-$[L_2Pt(\mu\text{-}OH)]_2(NO_3)_2$ (L= PPh_3) in solution of nitriles (CH_3CN, PhCN) affords the amidine complexes shown in the scheme.

The antitumor activity of the compounds **1a,b**, **2a,b** as well as that of the amidine ligands $[H_2N{=}C(Ph)\{1\text{-}MeCy(-H)\}]NO_3$ (3b), $[H_2N{=}C(Ph)\{9\text{-}MeAd(-H)\}]NO_3$ (**4b**) and the neutral derivatives $H_2N{=}C(Ph)\{1\text{-}MeCy(-2H)\}$ (**5b**), $H_2N{=}C(Ph)\{9\text{-}MeAd(-2H)\}$ (**6b**) has been investigated via MTT test against a panel of human cancer cell lines.

The IC_{50} (μM) ± S.D. values are collected in the Table below.



Compound	A375	MCF-7	A431	2008	C13*	LoVo
1a	6.93±1.31	24.14±2.25	13.52±1.54	7.70±1.16	12.64±1.90	8.54±2.11
1b	2.27±1.00	4.58±1.13	2.87±0.79	3.47±1.02	3.36±1.12	2.89±1.25
2a	16.34±1.00	24.67±1.90	22.11±0.94	20.23±3.01	24.41±1.98	19.54±1.38
2b	19.11±1.75	31.15±2.62	23.47±1.28	23.89±1.63	24.73±2.17	10.47±1.28
3b	87.56±3.44	86.35±8.17	58.36±3.66	98.32±4.78	85.33±5.37	98.93±2.79
4b	74.60±2.41	55.40±3.17	39.21±2.84	78.38±2.11	80.63±2.33	67.42±3.37
5b	>100	>100	>100	>100	>100	>100
6b	>100	>100	>100	>100	>100	>100
Cisplatin	2.23±0.94	8.60±1.24	1.65±0.51	3.23±1.11	29.85±2.94	7.13±2.10

Antitumour activity *via* MTT test. IC_{50} values have been evaluated by probit analysis ($p < 0.05$, test of ^{2}). Cells have been treated for 72 h with increasing concentration of tested compounds. Resistant factor (RF) is defined as IC_{50} resistant/parent line.

References

1. Montagner, D.; Bandoli, G.; Zangrando, E.; Longato, B. Inorg. Chem. 2006, 45, 1805.
2. Montagner, D.; Venzo, A.; Zangrando, E.; Longato, B. Inorg. Chem. 2010, 49, 2103.

Radiofarmaci nella diagnostica e terapia tumorale

Tc-99m-labelling of haematopoietic progenitor cells for the detection of intestinal neuropathies

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Cell-based therapy by transplantation of progenitor cells has emerged as a promising development for rescuing neuropathy, but non-invasive imaging approaches are required to monitor the fate of transplanted cells.[1] Our research group has recently set up an animal model of a persistent herpes simplex virus type 1 (HSV-1) infection in the enteric nervous system (ENS) which determines intestinal neuropathy.[2] The use of multipotent stem cell lines seems to be a valuable therapeutic approach for ENS disorders characterized by the loss of critical neural subpopulations. Our animal model of HSV-1-induced intestinal neuropathy seems to be a valuable tool for testing the beneficial effects of stem cell-based therapy.[3] Radiopharmaceuticals are used in molecular imaging as non-invasive tools for diagnosis and monitoring disease progression. This study aimed to label bone marrow stem cells (BMSCs) with Tc-99m and its subsequent in vivo and ex vivo detection in our animal model. Donor BMSCs were obtained from femurs of male C57Bl/6 mice (body weight 22±3 g). The collected stem cells were cultivated for 4 days and then adjusted to a final concentration of 3–1x10^6/100 µl PBS. Immunophenotyping of BMSCs cultures was performed using FITC-labelled anti-mouse CD117 and analyzed by flow cytometry. BMSCs were then labelled by incubation with (0.1 – 12 MBq) of lipophylic ^{99m}Tc-ECD complex for 30 min at 37°C and labelling efficiency, radioactivity retention, cellular viability were assessed. Preliminary results showed that radiolabeling of BMSCs with ^{99m}Tc-ECD is feasible but has moderate labelling efficiency (≈65 %) and limit stability to indicate that integrity of BMSCs is possibly affected. Based on the fact that lipophilicity is fundamental to increase the labelling, an alternative method to label BMSCs was developed using $^{99m}Tc(CO)_3(SS\text{-}R)(PPh_3)$ complexes, able to change their lipophilicity by mean of modification of dithiocarbamate bifunctional ligand backbone. Optimization of labelling procedures using methyldithiocarmamate[5] or the dithiocarbamate of isonipecotic acid[6] in the synthesis of the tricarbonyl [2+1] technetium complexes are in progress.

References

1. J. Gao, JE. Dennis, et al. Cells Tissues Organs (2001), 169, 12.
2. P. Brun, M.C. Giron, et al. Gastroenterology (2010), 138, 1790.
3. G. Martino, S. Pluchino, et al. Nat Rev Neurosci. (2006), 7, 395.
4. L. Meléndez-Alafort, A. Nadali, et al. Nucl Med Biol. (2009), 36, 57.
5. M. Riondato, D. Camporese, et al. Eur J Inorg Chem. (2005), 20, 4048.
6. A. Carrer, J. Suades, et al. J Labelled Comp Rad. (2009), 52, S466.

^{99m}Tc(N)-DBODC(5) from cardiology to oncology: in-vitro studies.

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^{99m}Tc(N)-DBODC(5) is a new lipophilic cationic mixed compound currently under clinical investigation as potential myocardial imaging agent [1]. The findings that this complex, analogously to ^{99m}Tc-Sestamibi, accumulates in mitochondrial structures through a mechanism mediated by the negative mitochondrial membrane potential, and that its rapid efflux from non-target tissues seems correlated to the Pgp/MRP transport functions opens the possibility to extend the clinical applications of this complex to tumor imaging and to non invasive multidrug resistance studies [2]. Standing on this ground, the kinetic of uptake at 4 and 37 °C of ^{99m}Tc(N)-DBODC(5) was evaluated in vitro in suitable human cancer cell lines (MCF7, A2780, 2008), and in the corresponding sub-lines (MCF7/Adr$^+$, A2780/ Adr$^+$, C13*), before and after MDR-modulator treatment (Vrp, CsA, BSO, MK571), using ^{99m}Tc-Sestamibi as reference.
Generally, in terms of cellular uptake, the different lipophilicity and solubility of the two compounds affect their permeability and relative affinity for the PgP/MDR transporters determining differences in their initial accumulations. The complexes displayed a similar accumulation profile. The uptake value of each tracer rapidly increased during the first 30 min and reached a plateau at 30 min which was maintained for the subsequent 2 hours. At 4 °C the percentage of non specific uptake was 2-3%. After the addition of CCCP, more then 70% of accumulated cell activity were released indicating a selective accumulation of both tracers in mitochondria. A reduction of the net cell uptake between drug sensitive cells and MDR tumor cells was observed for both ^{99m}Tc(N)-DBODC(5) and ^{99m}Tc-Sestamibi. After MDR-modulator treatment an high increase of % cell uptake of the two radiotracer was observed. Results from this study clearly indicated that: ì) the uptake of both ^{99m}Tc(N)-DBODC(5) and ^{99m}Tc-Sestamibi was correlated to metabolic activity of the cells. In fact, low temperature inhibited the cellular uptake of both ^{99m}Tc-tracers indicating that there was no binding to the cell membranes or to the hydrophobic regions of the cell membrane protein. ìì) The cellular accumulation is correlated to the level of Pgp/MRP expression, in fact an enhancement of uptake in resistant cells was observed after treatment with a MDR inhibitor/modulator, indicating the selective blockade of Pgp/MPR prevents efflux of the tracers. ìiì) In evaluating and comparing the usefulness of these two tracers it seems that ^{99m}Tc(N)-DBODC(5) may have advantage over ^{99m}Tc-Sestamibi in assessing MRP-mediated drug resistance. This study give a preliminary indication on the applicability of ^{99m}Tc(N)-DBODC(5) to tumour imaging and to the detection of MDR-mediate drug resistance in human cancer.

References

1. Cittanti, C. et al. *J. Nucl. Med.* 2008, **49,** 1299–1304;
2. Bolzati, C., et al. *J. Nucl. Med.* 2008, **49,** 1336–1344.

Indice dei titoli

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