interaction with DNA. Although it is one of the most efficient chemotherapeutical agents, it has serious side effects: nausea, vomiting, hair spilling, cardiotoxic effect, etc. These could be limited by conjugation of Dau to polypeptide carriers (1). Oligoarginine is a de novo designed cell penetrating peptide, capable to translocate covalently attached cargo (2). For the comparative analysis of the effect of covalent linkage and of the length of the peptide chain on antitumor and cellular uptake properties we have prepared three groups of new Dau-oligoarginine conjugates containing different number of Arg residues. In these compounds we have insererted oxime-, hydrazone- or squaric acid linkage between Dau and oligoraginine. New conjugates were characterized by mass spectrometry and RP-HPLC. The antitumor activity of the conjugates was evaluated in vitro on HL-60 human leukemia and HepG2 human hepatoma cells by MTT assay. Cellular uptake properties under different conditions (e.g. concentration) was studied by flow cytometry on two cell lines. We found that Dau-conjugates were more effective and uptake was also higher on HL-60 cells. The type of the bond in the conjugates as well as the number of Arg residues influenced markedly both cytostatic effect and cellular uptake.

These studies were supported by Medichem 2 (1/A/005/2004), Hungarian Research Fund (OTKA K-68285), Ministry of Health (ETT 459/2006) and GVOP-3.2.1-2004-04-0352/3.0

1. Hudecz, F., Reményi, J., Szabó, R., Kóczán, Gy., Mezo, G., Kovács, P., Gaál, D.: J. Mol. Recognition 16: 288-98 (2003)

2. Hudecz, F. Bánóczi, Z., Csík, G.: Medicinal Research Reviews, 25: 679-786 (2005)

POSTER SESSION

Experimental/Molecular therapeutics, pharmacogenomics 1

98 Poster

Dietary flavonoid fisetin induces a forced exit from mitosis by targeting the spindle assembly checkpoint

J. Pouwels[1], A.L. Salmela[1], A. Varis[2], P. Halonen[1], P. Toivonen[1], N. Sahlberg[1], M. Perälä[1], G. Gorbsky[3], M. Kallio[1]
[1]VTT, Medical Biotechnology, Turku, Finland; [2] Turku University, Centre for Biotechnology, Turku, Finland; [3] Oklahoma Medical Research Foundation, Molecular Cell and Developmental Biology Research Program, Oklahoma City, USA

The spindle assembly checkpoint (SAC) regulates the fidelity of cell division by ensuring that chromosome segregation is delayed until all sister kinetochore pairs have achieved stable bipolar attachments with spindle microtubules. Interference with the SAC is a promising strategy for treatment of cancer as premature SAC inactivation causes chromosome mis-segregation leading to massive aneuploidy and subsequent cell death. To discover small molecules with anti-SAC activity, we performed a high-throughput screen (HTS) for compounds that cause a forced exit from mitotic arrest induced by the microtubule destabilizing drug nocodazole. In most human cell lines with a robust SAC nocodazole treatment leads to a cell cycle arrest at mitosis during which the mitotic cells round up and become loosely attached to the substrate. Our screening strategy was based on the different cell-to-substrate attachment properties of round loosely attached mitotic and well-adhered flat interphase HeLa cells. From a library consisting of 2000 biologically active and structurally diverse compounds we identified the flavonoid fisetin (3,3',4',7-tetrahydroxy-flavone) as a strong SAC inhibitor. Time lapse microscopy of H2B-GFP expressing HeLa cells confirmed that fisetin induces escape from nocodazole arrest in a proteasome-dependent manner. We also showed that fisetin can overcome taxol (a microtubule stabilizing drug) and monastrol (an Eg5 inhibitor) induced mitotic arrests. Also non-drug treated mitotic cells underwent premature mitotic exit accompanied by cytokinetic defects upon fisetin treatment. Next we investigated how fisetin interferes with SAC signaling by studying kinetochore accumulation of key SAC proteins in the presence of the drug. We showed that fisetin causes a significant reduction in kinetochore affinity of BubR1 and Bub1 proteins, and delocalization of Aurora B kinase from the inner centromere to the chromosome arms. Furthermore, fisetin inhibited Aurora B and Cdk1 kinase activities as indicated by reduced phosphorylation of CenpA, Cdc27, and nucleolin-1, known substrates of the two kinases. We speculate that inhibition of SAC by fisetin is mediated through interference with Cdk1 and/or Aurora B function. In conclusion, utilizing our novel HTS and subsequent biochemical assays we have identified the flavonoid fisetin as a potential SAC inhibitor, which provides a mechanism of action to explain the drugs' previously reported anti-carcinogenic activity.

99 Poster

Tissue distribution and pharmacokinetics of an ATWLPPR-conjugated chlorine-type photosensitizer targeting neuropilin-1 in glioma-bearing nude mice

N. Thomas[1], L. Tirand[1], E. Chatelut[2], F. Plénat[3], C. Frochot[4], M. Dodeller[5], F. Guillemin[1], M. Barberi-Heyob[1]
[1]Centre de Recherche en Automatique de Nancy - Nancy University - CNRS, Centre Alexis Vautrin, Vandoeuvre-les-Nancy, France; [2] Institut Claudius Regaud, Laboratoire de Pharmacologie Clinique et Epidémiologique des Médicaments Anti-Cancéreux, Toulouse, France; [3] Faculté de Médecine - Nancy University, Laboratoire d'Histopathologie Expérimentale et Moléculaire, Vandoeuvre-les-Nancy, France; [4] ENSIC - Nancy university - CNRS, Département de Chimie Physique des Réactions, Nancy, France; [5] Université Paul Verlaine - Metz University, Laboratoire de Spectrométrie de Masse et de Chimie Laser, Metz, France

Destruction of the neovasculature is essential for efficient tumor eradication by photodynamic therapy (PDT). The PDT anti-vascular effect can be promoted by developing addressed photosensitizers localized preferentially to the tumor vascular compartment. A new photosensitizer conjugated to an heptapeptide [H-Ala-Thr-Trp-Leu-Pro-Pro-Arg-OH (ATWLPPR)] targeting neuropilin-1, a Vascular Endothelial Growth Factor (VEGF) co-receptor, has been synthesized. It was administered intravenously for an easier access to endothelial cells lining the vasculature in human malignant glioma-bearing nude mice. Plasma pharmacokinetic parameters were derived from plasma concentration–time data using a non-compartmental analysis and validated a relatively rapid elimination from the blood compartment with an elimination rate constant of 0.062 h^{-1} and a biological half-life of 11.0 h. The photosensitizer was mainly concentrated in organs such as liver, spleen and kidneys, which are rich in reticuloendothelial cells. In these organs, the elimination profiles of the photosensitizer were comparable, with half-lives as short as 12.2, 15.1 and 19.7 h, respectively. The peptidic moiety of the conjugated photosensitizer was degraded to various rates depending on the organ considered, most of the degradation process occurred in organs of the reticuloendothelial system. A metabolic product resulting from the enzymatic cleavage of the peptide bond between Ala and Thr was detected in plasma at all the examined time points from 2 h post-injection. The conjugated photosensitizer accumulated rapidly and at high levels in the tumor, with 2.3% of injected dose per gram of tumor tissue at 1 h after injection. Taking into account the aspecific uptake of the degradation product, the tumor levels of total photoactivable compounds might exhibit an interesting photodynamic activity. On the contrary, levels of total photoactivable compounds remained low in the skin. This study provides essential information for the choice of the time interval not to exceed to activate the photosensitizer.

100 Poster

Targeting of neuropilin-1 to improve the anti-vascular effect of photodynamic therapy in xenograft human malignant glioma

D. Bechet[1], L. Tirand[1], F. Plénat[2], N. Thomas[1], T. Bastogne[1], F. Guillemin[1], M. Barberi-Heyob[1]
[1]Centre de Recherche en Automatique de Nancy, Nancy University - CNRS, Centre Alexis Vautrin, Vandoeuvre-les-Nancy, France;
[2] Laboratoire d'Histopathologie Expérimentale et Moléculaire, Faculté de Médecine, Vandoeuvre-les-Nancy, France

The principle of photodynamic therapy (PDT) is based on the combined action of a photosensitizer (PS) localized in the tumor, light and oxygen. After light irradiation of the PS and in the presence of molecular oxygen, photo-oxydation reactions will lead to the production of reactive oxygen species, inducing a localized eradication of the tumor. However, PDT effects are mediated not only through direct killing of tumor cells but also through indirect effects, involving both initiation of an immune response and destruction of the neovasculature (anti-vascular effect).

The strategy developed in the laboratory aims to favour this anti-vascular effect by targeting tumor neovasculature. This approach was considered by coupling a PS (chlorin) to the heptapeptide ATWLPPR, targeting neuropilin-1 (NRP-1), a $VEGF_{165}$ (Vascular Endothelial Growth Factor, isoform 165) co-receptor. We previously confirmed molecular and cellular affinity for the conjugated PS and its in vitro photocytotoxicity (Tirand et al., J. Control Release, 2006). In vivo, we demonstrated that only the conjugated PS allowed a selective accumulation in endothelial cells lining tumor vessels (Thomas et al., Photochem. Photobiol. Sci., 2008). Metabolic profile and optimization of treatment conditions were performed in nude mice xenografted ectopically with U87 human malignant glioma cells (Tirand et al., Drug Metab. Dispos., 2007). The aim of this study was to validate and