Novel CCL17 chemokine neutraligands targeting atopic dermatitis

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Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by the predominant infiltration of T_H2-type cells in lesional skin. The CCL17 chemokine is clearly involved in skin inflammation, because it binds to the chemokine receptor CCR4 preferentially expressed on T_H2 cells.

Considering the role of CCR4/CCL17 axis in the development of atopic dermatitis on one hand, and the limited number of pharmacological tools to investigate their function or to correct their defects on the other hand, we aim at the identification of small compounds called "neutraligands" targeting the CCL17. These molecules bind to the chemokine not to the receptor, and neutralize its biological activity.

As we screened chemical libraries, we identified synthetic compounds inhibiting calcium responses induced by CCL17. We are carrying out the measurement of other cellular responses (cAMP assays, CCR4 internalization, chemotaxis …) induced by CCL17 and their alteration by these molecules. We also developed a screening strategy that allows to generalize the discovery of neutraligands with potential application to all other chemokines. To date, we identified potent neutralizing molecules targeting CXCL12 and CCL22.

For instance, the CXCL12 neutraligand "chalcone 4" prove useful in the reduction of inflammation in a mouse model of allergic eosinophilic airway inflammation. More generally, improving and extending the concept of neutralizing ligands as biological tools to elucidate the role of chemokines in inflammatory diseases such as atopic dermatitis, contribute to the identification of potential drug candidates.

The viral 2A peptide technology to express multiple functional proteins from a single ORF in Caenorhabditis elegans.

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Caenorhabditis elegans is a powerful in vivo model for which transgenesis is highly developed. Usually the expression of more than one protein of interest is required and it doesn't exist reliable tool to ensure their efficient concomitant and stoichiometric co-expression. Previously, many different approaches have been tried to overcome this issue (e.g. multiple transfections, multiple promoters/ORF for a unique vector and use of proteolytic cleavage sites) but none of them were efficient. Even the commonly used IRES (internal ribosomal entry sites) is notoriously nonstoichiometric and creates disproportionate transgene expression levels. In C. elegans, the SL2 trans-splicing sequence can be a way to express two products from a single promoter but the expression of multiple proteins remains uncertain. For these reasons we decided to develope the 2A viral peptides technology for C. elegans. 2As are commonly used to deliver multiple proteins from a single ORF for various vertebrate models and very recently in the drosophila. 2A are viral peptides that have the ability to be split up during translation by a "ribosomal-skip" or "STOP&GO" mechanism. From different viruses, four 2A peptides were discovered: F2A, T2A, E2A and P2A. We showed here that these four 2A peptides are working in the worm for all the cell types and all the developmental stages. Comparison of efficiency emphasized T2A, E2A and F2A. P2A, although described as the most efficient for the vertebrates, is the qualitatively less efficient for the worm. Then using 2As, we could express five different functional proteins addressed to different cell compartments (nucleus, nucleus memb., cytoplasm and cell memb.) and in the same time rescue the Y-to-PDA cell reprogramming event. To facilitate the emergence of this new technology for C. elegans we built a 2A-based toolkit for the expression of multiple proteins and also to generate 2A-tagged fosmids. 2A based vectors allow efficient and comparable expression of proteins in the worm and so constitute invaluable tool to track and visualize proteins in vivo in C. elegans. They can also be considered as a tool of choice to deliver cocktails of factors or even reconstitute sub-unit-composed proteins.

Redox-sensitive up-regulation of eNOS by purple grape juice in endothelial cells: Role of PI3-kinase/Akt, p38 MAPK, JNK, FoxO1 and FoxO3a

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The vascular protective effect of grape-derived polyphenols has been attributable, in part, to their direct action on blood vessels by stimulating the endothelial formation of nitric oxide (NO). The aim of the present study was to determine whether Concord grape juice (CGJ), which contains high levels of polyphenols, stimulates the expression of endothelial NO synthase (eNOS) in porcine coronary artery endothelial cells and, if so, to determine the signaling pathway involved. CGJ dose- and time-dependently increased eNOS mRNA and protein levels and this effect is associated with an increased formation of NO in endothelial cells. The stimulatory effect of CGJ on eNOS mRNA is not associated with an increased eNOS mRNA stability and inhibited by antioxidants such as MnTMPyP, PEG-catalase, and catalase, and by wortmannin (an inhibitor of PI3-kinase), SB 203580 (an inhibitor of p38 MAPK), and SP 600125 (an inhibitor of JNK). Moreover, CGJ induced the formation of reactive oxygen species (ROS) in endothelial cells and this effect is inhibited by MnTMPyP, PEG-catalase, and catalase. The CGJ-induced the phosphorylation of p38 MAPK and JNK kinases is abolished by MnTMPyP. CGJ induced phosphorylation of transcription factors FoxO1 and FoxO3a, which regulate negatively eNOS expression, and this effect is prevented by MnTMPyP, PEG-catalase, wortmannin, SB203580 and SP600125. Moreover, chromatin immunoprecipitation assay indicated that the FoxO3a protein is associated with the eNOS promoter in control cells and that CGJ induced its dissociation. Thus, the present study indicates that CGJ up-regulates the expression of eNOS mRNA and protein leading to an increased formation of NO in endothelial cells. The stimulatory effect of CGJ is a redox-sensitive event involving PI3-kinase/Akt, p38 MAPK and JNK pathways, and the inactivation of the FoxO transcription factors, FoxO1 and FoxO3a, thereby preventing their repression of the eNOS gene.

Medium-chain triglycerides used in parenteral nutrition are able to induce endothelial dysfunction in isolated porcine coronary arteries

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Lipid emulsions are commonly used in parenteral human nutrition, where they provide energy intake with essential fatty acids such as triglycerides. Recent studies indicate that elevation of circulating free fatty acid levels can impair endothelial function. The endothelium plays a key role in the control of vascular homeostasis mainly by releasing potent vasodilators such as nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF). Moreover, the endothelium controls vascular permeability and prevents thrombotic events. Thus, the aim of the present study was to assess the effect of different lipid emulsions for parenteral nutrition on the endothelial function of isolated coronary arteries and to determine the underlying mechanism.

Vascular reactivity was assessed in organ chambers using porcine coronary artery rings. Rings with or without endothelium were pre-incubated for 30 min with either Lipidem®, Medialipid® or Intralipid® (1 or 2 % v/v) before the evaluation of the endothelial function in response to bradykinin.

The incubation of porcine coronary artery rings with either Lipidem® or Medialipid® (medium-chain triglycerides), but not with Intralipid® (long-chain triglycerides), induced a significant reduction of the endothelium-dependent relaxation to bradykinin. In contrast, Lipidem® did not affect endothelium-independent relaxation to sodium nitroprusside, a NO donor. Moreover, Lipidem® reduced endothelium-dependent relaxations by affecting both the NO and the EDHF components induced by bradykinin. In addition, the endothelial dysfunction induced by Lipidem® was significantly improved by indomethacin, a COX inhibitor, and by antioxidants such as N-acetylcysteine, catalase and superoxide dismutase.

The present study indicates that lipid emulsions containing medium-chain triglycerides (Lipidem® or Medialipid®), but not long-chain triglycerides (Intralipid®), induce endothelial dysfunction in a porcine coronary artery. Moreover, the Lipidem®-induced endothelial dysfunction involves reduced NO- and EDHF-mediated relaxations, as well as increased vascular oxidative stress and formation of COX-derived vasoconstrictor prostanoids.

Microparticles as bioeffectors during septic shock: pharmacological modulation of microparticle-mediated vascular response in a rat model

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Objective: To study mechanisms by which circulating microparticles (MPs) are involved in vascular dysfunction during septic shock and to establish the contribution of a pharmacological modulation by recombinant human activated protein C (aPC).

Design: Prospective, randomized, controlled experimental study with repeated measurements.

Setting: Investigational animal laboratory.

Subjects: MPs were isolated from sham or septic rats obtained by cecal ligation and puncture (CLP), resuscitated and treated or not by aPC at 33 μg/kg/h. Sixty healthy recipient rats were randomly allocated to four groups and inoculated with MPs isolated from either sham or septic rats.

Interventions: Healthy recipients were infused with identical amounts of MPs and heart rate, mean arterial pressure (MAP) and carotid artery blood flow were recorded during 4 hours. MPs and organs were harvested for further analysis at the end of the record.

Measurements and Main Results: (a) Circulating MP level and phenotype are altered in septic rats with enhanced contribution of leukocyte-derived MPs that was diminished in rats treated by aPC. (b) Treatment of septic rats with aPC significantly decreases norepinephrine necessary to achieve the MAP goal (100 mmHg). (c) In healthy recipients, MPs isolated from septic rats decrease MAP (-35 mmHg). Conversely, MPs from aPC treated septic rats increase MAP (+16 mmHg) and MP-mediated effects are related to a significant increase in MP thromboxane content. (d) MPs modulate vascular inflammation and blunt arterial activation of NF-κB, pIκB-α, COX-2 and iNOS after anticoagulant treatment. (e) Inoculation of MPs from aPC-treated septic rats pharmacologically modulates the phenotype of circulating microparticles in recipient rats, with increased platelet and endothelial MPs.

Conclusions: Our study evidenced increased circulating procoagulant MPs during septic shock. aPC treatment is responsible for MP modifications and MPs behave as cellular effectors conveying the anti-inflammatory message resulting in hemodynamic effects.

Key words: Microparticles; sepsis; shock; anticoagulant; endothelial dysfunction; inflammation; activated protein C.

Depth-dependent photolabeling of transmembrane proteins. A proof of concept toward virus/host cell interaction study.

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Understanding the way envelope proteins of a pathogenic virus interact with membranes of host cells during infection is essential to better characterize the infection process and try to inhibit it at an early stage. Envelope proteins are involved in particular in fusion, a critical step of the viral infection that leads to the delivery of the viral genetic material into the cytoplasm of the host cell. Two structural elements, common to all known fusion proteins, play a key-role in fusion: the fusion peptide and the transmembrane domain.[1] The fusion peptide is a short hydrophobic sequence present either at its N-terminus or internal to the fusion protein. Mapping these key-hydrophobic regions in viral fusion proteins is therefore crucial to understand the structure/function relationships during the fusion process.

We recently proposed a strategy to delineate the hydrophobic regions of viral fusion proteins in the membrane, through hydrophobic covalent photoaffinity labeling.[2] This approach relies on the use of a collection of new lipid molecules containing both a photoactivable benzophenone group and a fluorescent probe for the detection of adducts of the reaction.[3] To monitor peptide/lipid interactions and represent viral fusion, the probes were manipulated in lipid bilayers.

We precedently focused on the preparation of the building blocks intented for the lipidic probes assembly.[4] Fatty acyl benzophenones (FABPs) were prepared long enough to allow the insertion of the probe into a lipid bilayer. They were capable of reacting with hydrophobic amino-acids present in its neighborhood.

Here the position of the benzophenone double ring within the fatty chain is demonstrated very relevant. We report a depth-dependent exploration of three transmembrane proteins, two models and a viral one and give some preliminary results on a putative peptide identified by this method.

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Implication of mu and delta opioid receptors in mouse experimental colitis-induced inflammation and pain

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Background and Aims. Controlling abdominal pain in patients suffering from inflammatory bowel diseases (IBD) is an important unmet medical need. Mu opioid receptor (MOR) activation appears to protect from IBD, as MOR knockout (KO) mice were susceptible to trinitrobenzene sulfonic acid (TNBS)-induced colitis. Here we evaluate whether mu and delta opioid receptors (DOR), and particularly receptors on peripheral sensory neurons, can constitute therapeutic targets for visceral inflammatory pain by comparing opioid receptor KO mice to control mice in the dextran sodium sulfate (DSS)-induced colitis model.

Methods. To investigate the role of mu and delta opioid receptors in the control of colitis-induced inflammation and pain, we compared mice with a global knockout, or a conditional deletion of MOR or DOR in primary nociceptive neurons (Nav1.8 neurons) to control mice with floxed mu or delta receptor genes (2). Male and female animals, aged 14 to 20 weeks, of mixed genetic background (C57Bl6/J-129SvPas) were used. Protocols were approved by Com'Eth (N° 2012-038). Mice were administered 3% DSS in drinking water (colitis groups) or water (controls) for 5 days. Visceral pain was recorded using visceromotor responses (VMR) to colorectal distention (CRD). On day 1, electromyography electrodes were implanted into abdominal muscle under anesthesia. On day 5, VMR to CRD (successive distensions of 15, 30, 45 and 60mmHg pressures) was recorded. After sacrifice, the animals' disease activity indices (DAI) were assessed including body weight loss, diarrhea, blood in stools and macroscopic inflammation criteria: edema, erythrema, length and thickness of the colon.

Results. Among DSS 3% colitis groups, DAI of global MOR-deficient mice (n=8) was significantly higher than that of control floxed mice (n=13), p=0.045, whereas no difference was found for delta receptor KO mice (n=10). These findings for MOR deletion are in accordance with a previous study with the TNBS colitis model . There was a significant effect of gender on VMR in colitis groups. The effects of global or conditional MOR or DOR KO on visceral pain using CRD are being investigated.

Conclusions. We have shown that global body mu rather than delta receptor activity control colon inflammation in the DSS colitis model. DSS mice altogether display more sensitivity to CRD pain measurement than control mice (n=40/group, p=0.049), with an influence of gender. Experimental groups will be completed to delineate the implication of local nociceptive and global opioid-mediated mechanisms involved in colon inflammatory processes and visceral pain in the DSS colitis model.

Effets des microparticules monocytaires et d'origine pancréatique exocrine sur la fonction et le devenir des cellules à insuline dans le contexte de l'infection du diabète de la mucoviscidose

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Introduction: Les microparticules (MPs), effecteurs cellulaires, sont des fragments de membrane plasmique émis par les cellules stressées. Elles sont des marqueurs pathogènes de désordres thrombotiques et exposent souvent du facteur tissulaire (FT). La mucoviscidose, résultant d'une mutation du gène CFTR, s'accompagne d'un diabète particulier associant une insulinorésistance et défaut d'insulinosécrétion. Objectifs: définir le rôle des MPs dans l'évolution du diabète de la mucoviscidose.

Hypothèse: L'infection chronique des patients favorise la génération de MPs d'origine monocytaire et pancréatique exocrine contribuant à la dysfonction des cellules à insuline.

Matériel et méthodes: Les cellules à insuline RINm-5F (n=5) de rat sont traitées 20h par 10nM de MPs isolées de monocytes (THP-1) ou de cellules pancréatiques exocrines porteuses de CFTRΔF508 (CFPAC-1) et stimulées par le LPS (0,2-15µg/ml). L'activité métabolique est mesurée par rouge neutre, l'insuline secrétée par ELISA, les MPs totales par test prothrombinase et leur contenu en FT quantifié. Le transfert des MPs aux RINm-5F est évalué grâce à la sonde lipidique PKH26.

Résultats: Le LPS (0,2µg/ml) inhibe significativement l'activité métabolique des RIN-m5F (-17,8%;p<0,001), la sécrétion d'insuline restant inchangée. Par contre, les MPs émises par les CFPAC-1 en réponse au LPS portent 13 fois plus de FT que les MPs exocrines normales, et augmentent l'activité métabolique des RIN-m5F (+10,32%;p<0,01). Le transfert des MPs exocrines est observé dans 55,86% des RIN-m5F et diminue de 50% (p<0,01) l'insuline secrétée. Les MPs monocytaires réduisent l'activité des RIN-m5F (-14,28%;p<0,01) mais l'augmentent dans les cellules exocrines (+86,67%;p<0,01). Les MPs monocytaires inhibent l'apoptose des cellules exocrines induite par le LPS et restaurent l'activité métabolique (+100%;p<0.01).

Conclusion: Les données suggèrent qu'en situation d'infection chronique, le LPS n'altère pas directement la fonction des cellules β et que les MPs exocrines et monocytaires ont des effets délétères sur la fonction des cellules à insuline.

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Animal models of Centronuclear Myopathy and therapeutic rescue using Adeno-Associated Virus (AAV).

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Centronuclear myopathies (CNM) are debilitating rare diseases. No curative treatments exist and the molecular mechanisms leading to CNM are not well understood. CNM are characterized by hypotonia, and small rounded fibres with central nuclei. Several forms have been documented: an autosomal recessive form due to BIN1 mutations (ARCNM), a dominant form with DNM2 mutations (ADCNM), and a severe X-linked form with MTM1 mutations (XLMTM or XLCNM). The aim of this research is to better understand the role of these proteins in healthy muscle, the pathological mechanisms underlying CNM, and identify potential new therapies. ARCNM-We are characterizing BIN1 constitutive (CMV) and muscle-specific (HSA) knockout (KO) mice. Both the CMV and HSA KO died perinatally, due to a primary defect in skeletal muscle. This is the first characterization of an animal mouse model for ARCNM. ADCNM-We exogenously expressed wildtype (WT) or R465W DNM2, the most common ADCNM mutation, in adult WT mice by intramuscular AAV injections. Expression of R465W DNM2 induced a CNM phenotype and reduced muscle force, suggesting that ADCNM arises from increased DNM2 function. XLMTM-We characterized the murine MTM1 KO model that reproduces the histopathological findings from human patients. Re-expression of phosphatase-inactive MTM1 mutants with AAV improved most phenotypes, to a similar extent compared to WT MTM1. However, the aberrant shape of triads, membrane structures underlying excitation-contraction coupling were not fully rescued.

These findings support a phosphatase-independent role for MTM1 in skeletal muscle organization, while the phosphoinositides substrates of MTM1 are implicated in membrane curvature at the triad. This project has improved our understanding of BIN1/DNM2/MTM1 function in muscle and the pathological mechanisms leading to CNM, and should suggest novel rescuing approaches.

In vivo pharmacokinetics (PK) and blood-brain barrier passage (BBB) available in your Techmed^{ILL} platform

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In complement of early ADME parameters determination, the Techmed^{ILL} platform provides determination of early stage pharmacokinetic (PK) and blood-brain barrier (BBB) data. During evaluation of new chemical entities, PK and/or BBB are a prerequisite for successful animal pharmacology and toxicology studies. It is recognized that quantitative measures of drug exposure are crucial for complete interpretation of preclinical efficacy studies. Moreover, PK data can also help in the design or species selection of preclinical toxicology studies.

Each study is customizable according to client's demand. The quality management of the Techmed^{ILL} platform has been recognized by international ISO9001 certification. Studies are conducted according to in-house standard Operating Procedures, and with the approval of the local ethics committee that regulates animal research at the University of Strasbourg (CREMEAS). Blood and/or tissue (e.g. brain) samples are obtained from test animals following dose administration (IV, PO, IP, SC, IN). Parallel sampling or when it's possible, serial sampling, are available.

Samples are analyzed using a UHPLC coupled to a triple quadripole mass spectrometer (LC-MS/MS).

The data are used to generate concentration *versus* time curves, and allow the determination of the essential PK parameters.

Microparticles (MPs) convey CD14 from monocytes to leucocytes and amplify lipopolysaccharide (LPS)-induced apoptosis in cellular crosstalk models

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LPS induces septic inflammation cascade. It interacts with its specific receptor CD14 on monocytes and triggers signaling through TLR4 complex. Cell response is characterized by the upregulation of AP-1 and NF-κB. MPs shed from LPS-stimulated monocytes are known vectors of TF and imbedded IL-1β, disseminating procoagulant and inflammatory potentials. We investigated the proapoptotic signal conveyed by monocyte MPs after LPS stimulation and the role of TLR4 pathway in MP-mediated cellular crosstalk models. MPs isolated from LPS- treated monocyte THP-1 cells were applied to THP-1 or CEM $CD4^{+}$T cells. Apoptosis was assessed by hypodiploid DNA, MPs quantified by prothrombinase assay. Target cell membranes were examined by fluorescent labeling and soluble CD14 (sCD14) measured by ELISA. In crosstalk experiments, naïve THP-1 or CEM were submitted to MPs for 4 h (5 and 20 nM PS, respectively), washed and challenged overnight by LPS.

In the supernatant of LPS-treated THP-1, MP levels (0.98±0.09 vs. 0.74±0.05 nM PS/7.5 10^{5} cells) were correlated to apoptosis (1.79±0.47% *vs.* 0.46±0.17%; $p<0.05$). MPs bore CD14, and were able to restore its expression by CD14 antibody pretreated THP-1. After LPS challenge, THP-1 apoptosis raised up to 4.33±0.87% ($p<0.05$). MPs alone did not induce apoptosis, excluding MP mediated LPS transfer. Deprived MPs supernatants had no effect, excluding sCD14 and IL-1β as inducers. Moreover, neither recombinant IL-1β supplementation nor modulation of its synthesis by the ICE GYKI-66114 inhibitor altered the response. LPS induced apoptosis was reduced by added sCD14 (0.1-9 µg/mL) without membrane CD14 expression, suggesting LPS deprivation effect. MPs were able to transfer CD14 to CEM cells by fluorescent labeling, and promoted LPS induced apoptosis (0.86±0.11 vs. 0.34±0.02%, $p<0.05$). In sepsis, MPs may contribute to apoptosis amplification loops through the transfer of CD14/TLR4.

Involvement of RF-amide peptides in the modulation of nociception and analgesic effects of opiates.

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Mammalian RF-amide peptides are encoded by five different genes and act through five different G protein-coupled receptors. Neuropeptides SF and VF (NPSF and NPVF, also called RFRP1 and RFRP3) have been proposed to represent endogenous peptides for NPFF1 receptor, neuropeptides AF and FF (NPAF and NPFF) for NPFF2 receptor, and Prolactin releasing peptide (PrRP), Kisspeptin (or metastin) and 26RFa for GPR10, GPR54 and GPR103, respectively.

However, results from several studies suggest that the selectivity of these peptides for their receptors is low and that expression patterns of these receptors and their corresponding ligands only partially overlap. In this study, we took advantage of the cloning of the five human RF-amide receptors to systematically examine their affinity for and their activation by all human RF-amide peptides. Binding experiments performed with membrane from CHO cells expressing these receptors indicated that GPR10, GPR54 and GPR103 display a remarkably good affinity and selectivity for their endogenous ligands. Conversely, we observed a high affinity of NPFF1 and NPFF2 receptors for all RF-amide peptides. Moreover, GTPgammaS and cAMP assays performed with cells expressing these receptors indicated that almost all RF-amide peptides efficiently activate NPFF1 and NPFF2 receptors. As NPFF has been shown previously to induce hyperalgesia and/or to prevent the analgesic effect of morphine, we further performed a systematic analysis of the hyperalgesic and anti-morphine analgesic effect of mammalian RF-amide peptides in mice.

Our results show that all RF-amide peptides induced hyperalgesia and/or prevented morphine analgesia following icv administration. Moreover, these effects were prevented by administration of RF9, a compound that blocks NPFF1 and NPFF2 receptors but not GPR10, 54 and 103. These results point to NPFF receptors as essential targets for the hyperalgesic and/or anti-morphine analgesic effect of exogenously administered RF-amide peptides. Taken together our data suggest that NPFF receptors could be endogenously activated not only by their known endogenous ligands (NPSF, NPVF, NPAF, NPFF) but also by other RF-amide peptides PrRP, Kisspeptin and 26RFa.

We provide here novel in vitro and in vivo evidence that all RF-amide peptides display hyperalgesic and/or anti-morphine analgesic effects and that two receptors of the family (NPFF1 and NPFF2 receptors) are critically involved in these effects.

Immunotargeting Proliferating Cell Nuclear Antigen in living cancer cells

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Cancer drug resistance to chemotherapeutic treatment is a persistent clinical problem. Amongst several mechanisms involved, it has been described that translesion synthesis (TLS) of damaged DNA could contribute to acquired tumor chemoresistance[1]. Although Proliferating Cell Nuclear Antigen (PCNA) represents an essential protein in the DNA duplication processes, its modification by ubiquitination is a limiting step for TLS activation. Monoubiquitinated PCNA triggers the switch between replicative and several specialized DNA polymerases that are capable to elongate damaged DNA. Because PCNA plays an essential role for cell growth and may act as a key-factor contributing to chemoresistance, we have develop several strategies to isolate biomolecules issued from the immune system to test whether they can, when delivered or expressed intracellularly, inhibit TLS thereby rendering the cells more sensitive to genotoxic agents. We present the selection and the characterization of antibodies and recombinant antibody fragments in the single-chain Fv (scFv) format binding specifically to PCNA and usable in an intracellular context of cancer cells.

The human PCNA protein was expressed in BL21 *E. coli* cells and purified by immobilized metal affinity chromatography and gel filtration. After mice immunization, we have isolated within several hybridomas secreting anti-PCNA antibodies a clone (2H3) that produces an antibody binding exclusively to native PCNA in fixed cultured cells. Moreover, this antibody is able to bind endogenous PCNA after transduction in living cells (HeLa, U2OS, MEL501) by electroporation, a new method set up in our laboratory for the efficient intracellular delivery of proteins. In parallel, we have selected by phage-display anti-PCNA scFv from a semi-synthetic library[2]. These antibody fragments were produced and purified following the similar protocol than recombinant human PCNA. Selected scFvs immunoprecipitate PCNA from cell extracts and permit to detect endogenous PCNA in fixed HeLa cells. One particular anti-PCNA scFv (P40 clone) also binds specifically to endogenous PCNA after transfection of living cells.

Altogether, we show that scFvs and monoclonal antibodies can be used to track endogenous nuclear proteins under physiological conditions after transfection or electroporation. Whether they inhibit precise PCNA functions following co-treatment of the cells with genotoxic agents is in progress.

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Common and specific cellular defects leading to different forms of centronuclear myopathies

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Centronuclear myopathies (CNM) are congenital muscle diseases characterized by severe muscle weakness, myofiber atrophy and abnormal nuclear centralization. Several CNM forms have been described mainly attributable to mutations in the MTM1, BIN1, DNM2 genes as well as the RYR1 and MTMR14 genes. The exact molecular mechanism causing these pathologies is still unknown. In this study, using isolated muscle fibers from two mouse models KO for *Mtm1* and *Bin1* respectively, we aim to identify changes in the muscle structural organization that could potentially contribute to disease progression. Cytoskeletal and triad markers have been assessed by protein immunofluorescence and confocal microscopy. *Mtm1* KO muscle fibers from 5 week old mice display differential microtubule organization compared to control as well as BIN1 mislocalisation. *Bin1* KO muscle fibers from newborn mice display normal SR positioning and similar microtubule organization compared to control however, DHPRa and MTM1 appear to be mislocalised in the KO mouse. Our data suggests that MTM1 and BIN1 possibly depend on each other for their correct localization on the triad. Additionally, changes in the microtubule cytoskeleton in *Mtm1* KO mice can potentially affect intracellular transport, organelle positioning and correct skeletal fiber structure. Altered DHPRa positioning on the triad in *Bin1* KO mice could affect the process of excitation-contraction in muscle due to defective calcium handling at the triad.

Altogether, such study should identify common and specific pathways altered in different forms of CNM that can be targeted for further pre-clinical trials.

Oxyluciferin and its synthetic derivatives: Photophysical properties and their application for fluorescence imaging microscopy in living cells.

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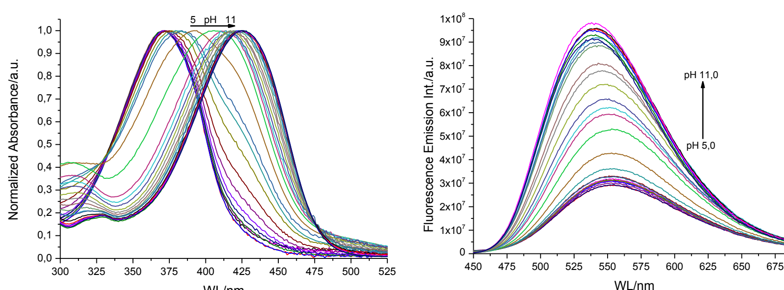
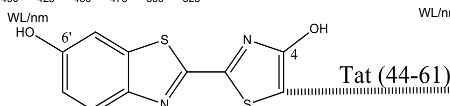
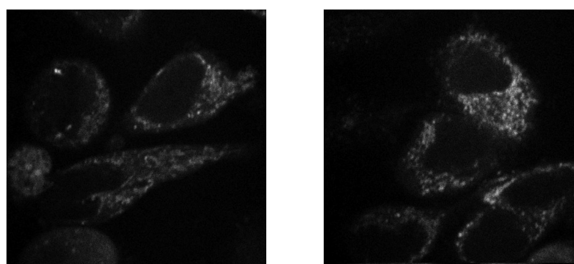
Applications of in-vivo bioluminescence imaging, employing excited state bioluminescence of bacteria/fungai/invertebrates draws enormous attention for researchers around the globe in the past decade. Its immense potentials for application to novel ultrasensitive bioanalytical techniques amplify further growth of this frontier research field. This amazing naturally occurring phenomenon, commonly observed in firefly, involves luciferin/luciferase complex formation which results in a fluorescence emission from the chemically excited Oxyluciferin (OxLuc) that displays high quantum yield. However the chemical equilibrium involved in this natural phenomenon is poorly understood so far. Hypothetically six intermediate chemical species of OxLuc may be implicated in this phenomenon. To unravel the underlying photophysical mechanisms, we used a variety of techniques: steady state and time resolved spectroscopy with several structurally modified derivatives of OxLuc in different chemical environments. In a next step, an OxLuc derivative was used as a fluorescent label to visualize a HIV protein (Tat) within living cells.







Search for V1A ligand by high throughput screening using HRTF and calcium flux measurements

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The fully equipped robotized "High Throughput Screening Platform" of the UMS 3286 PCBIS, Strasbourg, is able to perform screenings at high throughput scale. The PCBIS has expertise with many diverse biological models used in the search for new drugs. These models range from in vitro enzymatic tests to cell based assays.

We present here an example of screening realized with cell based assays. This screening aims to find new ligands for vasopressin V1A receptor. This work was realized in two steps. At first we used a HTRF (Homogeneous Time Resolved Fluorescence) receptor ligand binding assay in order to detect compounds able to bind to the V1A receptor. Then we used a functional test (intracellular calcium flux measurement) to characterize the agonist or antagonist activity of the hits.

Miniaturization and automation allowed us to test 6508 molecules of synthetic or natural origin from Strasbourg Chemical Library and Prestwick Chemical Library©. From HTRF screening we found 121 molecules. 37 of them showed antagonist activity at 10 µM. We chose 6 molecules and validated them with dose-response curves using functional assay.

TechMed^{ILL} - Drug technologies platform

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Search of new potentially therapeutic agents is now easier thanks to recent advancements in genomics, proteomics, medicinal chemistry and especially high-throughput screening methodologies. From the large number of hits generated by these technologies, researchers need to select and optimise the most promising compounds.

One of the major step of this drug discovery process is the study of ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity). The determination of these properties at an early stage of development is essential to save time and money.

TechMed^{ILL} is a technologic platform, located in ESBS, which provides services in the area of ADMET. The platform is established since 2008 and works with academic laboratories and biotech companies. The portfolio of assays includes all aspects of the ADMET properties of small drug molecules.

Quality management of the TechMed^{ILL} platform has been recognized by the international ISO 9001 certification.

Liraglutide regulates apoptosis and insulin secretion by insulin secreting Rin-m5f cells, in response to microparticles, implication for islet transplantation

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Introduction : Islets graft is followed by the early inflammatory response IBMIR (Instant Blood Mediated Inflammatory Reaction) characterized by drastic cytokine secretion. Microparticles (MPs) are plasma membrane fragments shed from stressed cells that act as cellular effectors. Recently, anti-apoptotic effects of incretinomimetics and their interest for islet protection was suggested.

Objective : Evaluate the effect of Liraglutide on apoptosis and β cell dysfunction, mediated by MPs, in a model of oxidative and inflammatory cell stress.

Material and Methods : Rat β cells, Rin-m5f, were exposed to an oxidative (100µM H_2O_2) and a cytokinic (50U/ml IL-1β, 1000U/ml TNF-α) stress and treated by Liraglutide (1µM). MPs generated by oxidative (MP_{ox}) and cytokinic (MP_{cyt}) stress were isolated and applied to Rin-m5f for 24h. Apoptosis was assessed by hypodiploid DNA quantification and insulin secretion mesured by ELISA (n=9).

Results : Following oxidative stress, apoptosis was increased (3% vs 18%, $p<0,001$), MPs generation was doubled ($p<0,001$) and insulin secretion reduced (61%, $p=0,01$). Cytokinic stress promoted similar responses, except for apoptosis remaining unchanged . MP_{ox} were deleterious for Rin-m5f as shown by doubled apoptosis ($p=0,006$) and symmetrical 50% decrease in insulin secretion ($p=0,01$). Insulin secretion was only observed by MP_{cyt} (21%, $p=0,05$).

Liraglutide protected from oxidative stress induced apoptosis (10% vs 18%, $p=0,01$). Insulin secretion was enhanced (oxidative stress : +55%, cytokinic stress : +25%) and MP shedding reduced (respectively : -25%, $p=0,006$; -18%, $p=0,01$). Liraglutide also protected β cell function in the presence of MP_{ox} and MP_{cyt} as assessed by insulin secretion.

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Conclusion : Liraglutide shows a beneficial effect on β cell by decreasing apoptosis and MP shedding. Additionally Liraglutide protects from MP cellular deleterious effects. Our data bring new hints on liraglutide cytoprotective effect in islet transplantation.

This work has received the financial support of Novo Nordisk.

Investigation by high resolution microscopy of the internalization of the neuropeptide Y bound to its Y_1 receptor

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The human neuropeptide Y (NPY) modulates numerous physiological processes, including regulation of cardiovascular and renal functions, intestinal motility, memory, anxiety, seizure, feeding, circadian rhythms and nociception. The NPY G-protein coupled receptor (GPCR) Y_1 is thought to mediate most of the physiological and physiopathological actions of NPY. Thus, the study of the mechanisms involved in the regulation of the Y_1 receptors should contribute to a better understanding of the mechanism and functions of NPY. Previous fluorescence studies have shown that activated GPCR Y_1 rapidly internalize through clathrin-coated pits, and subsequently recycle from early and recycling endosomes [1]. Furthermore, the C-terminal cytoplasmic tail of the Y_1 GPCR was found to play a role for NPY induced internalization [2,3]. In an effort to characterize the NPY intracellular pathway, the Y1 receptor was tagged with eGFP, while NPY was fluorescently labeled at its N-terminus by Cy5. This probe was selected for performing stochastic optical reconstruction microscopy (STORM), when it is used together with an oxygen scavenger, and enables imaging of small objects with a lateral resolution well below the diffraction limit.

Our images present an overlap between the TIRF images of the eGFP-labeled Y1 receptors and the STORM images of Cy5-NPY, confirming that NPY peptides bind to the receptors. Further studies revealed an intracellular distribution of small fluorescent spots, whose size are consistent with that of endosomes. The intracellular distribution and spot sizes were found to change with time confirming previous spectroscopic sudies[1,2,3].

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Lung function and early abnormality of glucose tolerance (GT) in cystic fibrosis (CF) patients

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Diabetes is a frequent complication of CF and occurs in about 50% of patients older than 30 years (1). Its presence is correlated with the decline of lung function (2). Early diagnosis is based on the oral glucose tolerance test (OGTT) which could lack sensitivity in some of these patients. Continuous glucose monitoring (CGM) could be a useful tool for evaluating early abnormalities of GT in CF patients (3).

In a single-center, observational, prospective, cross sectional study, we compared nutritional status, lung function and antibiotic courses of CF patients depending on the presence or absence of abnormal GT in OGTT or in CGM. Known diabetics were excluded. From June 2008 to July 2012, 68 patients were included: male 46%, mean age 25 years (12-57). 51 patients (75%) had a normal OGTT (group 1), 11 (16%) were glucose intolerant and 6 (9%) had diabetes (group 2). In group 2, FEV was lower (80,8%±23,1 Vs. 61,6%±23,1; p=0.01), FVC lower (94,5%±17,5 Vs. 77,5%±20,5; p=0.002), HbA1c higher (p=0.005), while the number of antibiotic courses was increased (0,86±1,11 Vs. 1,71±1,31; p=0.01). Using CGM we defined 2 groups: maximum glucose < 2g/L (Group A, n=32) or ≥ 2g/L (Group B, n=20). In group B, HbA1c was higher (p=0.01), FEV lower (81,9%±22,9 Vs. 66,5±25,8; p=0.03). Among patients with normal OGTT (n=38), those with a pathological CGM (n=12, 31.6%) had FEV (87,4%±17,1 Vs. 68,2%±25,6; p=0.01) and FVC (99,3%±3,4 Vs. 86,1%±19,4; p=0.02) significantly lower compared to patients with normal CGM (n=26).

Thus, an early abnormality of GT in CF patients is associated with greater impairment of lung function. CGM seems an interesting tool to diagnose early abnormalities of glucose tolerance in CF patients.

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Polydiacetylenes Self-Assemblies : A (Nano)World of Possibilities

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Under the control of weak interactions such as van der Waals forces, hydrogen bonding and/or hydrophobic interactions, amphiphiles are known to exhibit a broad spectrum of self assembling structures in aqueous solutions.

The study of new anionic, neutral or cationic diacetylenic amphiphiles has shown some various and very different self-assembled structures, including micelles, ribbons, nanorings, laths and nanotubes.

Role of RPA phosphorylation in Translesion Synthesis.

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The genome of the cell is constantly subjected to exogenous and endogenous damages, which create lesions on the DNA. Despite the existence of efficient DNA repair mechanims, some unrepaired lesions remain on the DNA during replication and block the progression of replicative DNA polymerases. Stalled DNA replication forks may collapse into structures that cause a Double Strand Break (DSB), thereby increasing genomic instability. TransLesion Synthesis (TLS) allows the completion of replication by the use of specialized DNA polymerases which are able to synthesize nucleotides opposite to the lesions. In response to genotoxic agents, PCNA (Proliferating Cell Nuclear Antigen) the processivity factor of DNA polymerases, is mono-ubiquitinated by the E2-E3 complex composed of Rad6 and Rad18. This modification is required for the switch of the DNA polymerases (replicative versus translesional) occuring during TLS. The molecular events that regulate the post-translational modification of PCNA, still remain elusive.

Replication Protein A (RPA) is a heterotrimeric protein complex that binds specifically to single-stranded DNA (ssDNA). It is composed of three subunits, RPA70, RPA32, and RPA14. DNA lesion cause an exposure of extended stretches of ssDNA in the vicinity of stalled replication forks. A model in which RPA plays an important role in the DNA damage tolerance pathway has been proposed. Indeed, the RPA complex acts as a signal not only for the initiation of the checkpoint reponse, but also for the recruitment of the Rad6/Rad18 complex, which subsequently mono-ubiquitnates PCNA thereby activating translesion synthesis. In reponse to genotoxic agents, RPA is phosphorylated within the N-terminal 33 residues (on serine 4, 8, 33 and threonine 21) of RPA32 by the phosphatidylinositol 3-kinase-like kinase (PIKK)-family members. The role of this post-translational modifications is still unknown.

The aim of this project is to study the interaction of Rad18 with RPA and to analyze the influence of RPA phosphorylation on PCNA mono-ubiquitination, in the context of translesion synthesis. We use an *in vitro* replication assay in which the switch of the DNA polymerases, in a human cell-free extract, is coupled to PCNA mono-ubiquitination (Schmutz et al., 2007). We have shown that in this assay RPA 32 is phosphorylated during DNA synthesis, by the DNA Dependent Protein Kinase (DNA-PK). The influence of RPA phosphorylation on PCNA mono-ubiquitination is analyzed *in vitro* in extracts from DNA-PK knock out cells and *in vivo* in the same cells treated with various genotoxic agents.

Realization of this project will help to clarify the molecular mechanisms underlying the translesion synthesis pathway and its regulation.

Synthetic flavone derivatives as potential new antimalarial agents

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Malaria is a burden that concerns almost half of the world's population. It's a parasitic disease due to a protozoan from the genus *Plasmodium* that is transmitted from an infected man to a safe subject by a female *Anopheles* mosquito. In 2010 malaria has affected 219 millions of people and caused approximately 660.000 deaths, mostly among African children. Since 2000, mortality rates have fallen by more than 25%, indicating that the WHO Global Malaria Program is efficient[1]. Nevertheless, the emergence of resistance to artemisinin, a reference drug for the treatment of malaria in multidrug-resistant area, could reverse the trend of decreasing mortality[2]. Under these circumstances, there is an urgent need of discovering new treatments associated with the exploration of novel targets.

For this purpose, we are developing new synthetic antimalarial agents with an original structure inspired by nature. The isolation of an active biflavonoid from *Campnosperma panamense* (IC_{50} = 450 nM, *Plasmodium falciparum* strain K1), which present an interesting selectivity index (SI = 130, L6 cells)[3] led us to the development of simplified synthetic analogs. Structure Activity Relationship (SAR) study is still in progress, but several active compounds have already been synthesized. Nevertheless, the most active compounds *in vitro* are poorly bioavailable and were inactive *in vivo* on the murine model *Plasmodium berghei* ANKA. One compound, MR70, is less active than others (IC_{50} = 1.9 μM, *Plasmodium falciparum* strain K1) but present an interesting bioavailability that could potentially induce an *in vivo* activity (plasma level of 8.1 μM two hours after an intraperitoneal injection of 100 mg/kg). Indeed, in preliminary studies conducted *in vivo* on mice infected intraperitoneally by an *inoculum* of 3.10^7 parasites (*P. berghei* ANKA), MR70 enabled to reverse the parasitemia of 50% of infected mice when administered intraperitoneally one hour after parasites inoculation. However, data collected *in vivo* for this compound were questionable and need to be confirmed.

Within this context, the SAR study should be pursued in order to increase the antimalarial activity and the bioavailability so as to obtain an active molecule *in vivo*. Once an active molecule will be satisfactory, we will try to elucidate its mechanism of action, with the hope of discovering a new target.

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A neutraligand of the CXCL12 chemokine attenuates the fibroproliferation in obliterative bronchiolitis after murine heterotopic tracheal transplantation

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Introduction. Obliterative bronchiolitis (OB) occurs during chronic allograft rejection of lung transplantation. OB is characterized by obstruction of the small airways with inflammatory cell infiltrate and fibrosis.

The chemokine CXCL12 and its receptor CXCR4 are implicated in the chemotaxis of hematopoietic cells, lymphocytes and fibrocytes and are expressed after human lung transplantation. Our laboratory developed a neutraligand – chalcone 4, which neutralizes the activity of CXCL12 (Hachet-Haas et al, JBC 2008; Gasparik et al ACS Med Chem Lett 2012; Daubeuf, Hachet-Haas et al, JBC 2013).

Aim. The aim of this study was to evaluate the effect of chalcone 4 compared to the CXCR4 antagonist AMD3100 using the common heterotopic tracheal transplantation model of OB in mice.

Methods. Balb/c recipient mice (iso- and allografts) were treated by IP injection with chalcone 4 (350**µ**mol/kg/day) or its solvent carboxymethyl-cellulose 2%, or with AMD3100 (10mg/kg/day) or its solvent (physiological solution) for 21 days. Transplanted tracheas (n=8 for isografts and n=16 for allografts) were harvested, paraffin-included and stained with hematoxylin-eosin. The surface of fibroproliferative tissue occluding the tracheal lumen was measured with the CellSence Dimension software.

Results. Chalcone 4 significantly inhibited the tracheal fibroproliferative obstruction in allografts at D21: 74.8±8.5% obstruction was quantified in chalcone 4-treated group vs 95.6±2.7% for the solvent-treated group ($p<0.05$). Additionally, in AMD3100-treated group, we show 68.6±8% fibroproliferative obstruction vs 94.8±2,3% in the solvent-treated group ($p<0.05$). No effect of the compounds was observed in isografts, where no fibroproliferative obstruction is occurring at D21.

Conclusion. We showed that neutralization of the CXCL12-CXCR4 axis is associated with inhibition of the fibroproliferative phase of OB in our murine model.

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Carrier-free gene silencing with cationic siRNAs

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Small interfering RNA mediated gene silencing has become a drug development paradigm. But, siRNA-mediated gene silencing requires intracellular delivery of the nucleic acid. A carrierless molecular approach that follows the same cell entry route as cationic supramolecular complexes was developed, yet should avoid the extracellular barriers encountered by nanoparticles. Cationic oligospermine−oligonucleotide conjugates (ZNAs, *i.e.* Zip Nucleic Acids) were synthesized stepwise on an oligonucleotide synthesizer using a DMT-spermine phosphoramidite derivative. They were shown to enter cells, provided their formal charge ratio N/P was >1.5. Cationic siRNAs that fulfilled this condition were shown to achieve selective inhibition of luciferase gene expression in constitutively luciferase-expressing cells.

Subsequently, similar specific results were observed with cationic siRNAs targeting the endogeneous Lamin gene of HeLa cells.

PCBIS: an academic drug discovery platform with industrial standards

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High Throughput Screening (HTS) is the technology which best facilitates the search of new molecules with the potential of becoming the drugs of tomorrow. Until recently this expensive technology was only available in pharmaceutical companies.

Starting 13 years ago, the "Plate-forme de Chimie Biologique Integrative de Strasbourg" (PCBIS) developed the expertise in this field in order to be able to offer this technology in an academic context. One of our main goals is to offer our expertise to laboratories aiming to find new drugs to cure rare and/or neglected diseases. Our commitment to quality drove us to set up a quality management system granted by ISO 9001 certification.

The PCBIS's expertise and equipment necessary for new drug discovery is now proposed to academic laboratories, start-ups and industries interested in a fast paced approach to screening.

We also propose to train people and give an access to our technologies to interested laboratories.

We will show some of the tools that PCBIS can propose to the scientific community.

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Crataegus special extract WS® 1442 prevents hypertension and renal injury in DOCA-salt-induced hypertension in rats

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Several studies indicate that regular intake of polyphenol-rich sources could protect the cardiovascular system, at least in part, by improving the endothelial function (1). The *Crataegus* special extract WS®1442 (WS®1442) is an extract from hawthorn species (*Crataegus oxyacantha*/*Crataegus monogyna*) that induces endothelium-dependent NO- and EDHF-mediated relaxations in coronary artery rings via the activation of the redox-sensitive Src/PI3-kinase/Akt-dependent pathway (2,3). The aim of the present study was to determine whether chronic intake of WS®1442 is able to prevent the development of hypertension and renal damage in deoxycorticosterone acetate (DOCA)-salt induced hypertension.

Male Wistar rats (50) were divided into five groups: control group receiving normal diet, WS®1442 group receiving 300 mg/kg/day diet, DOCA-salt group, and DOCA-salt groups receiving WS®1442 at 100 or 300 mg/kg/day. Rats in the DOCA groups received weekly subcutaneous injection of DOCA (50 mg/kg) for 12 weeks and had free access to water containing 1% NaCl. WS®1442 was administered in the diet at doses of 100 or 300 mg/kg/day. Systolic blood pressure (SBP) was measured by tail-cuff plethysmography twice weekly for 12 weeks. Vascular reactivity was assessed in mesenteric artery rings suspended in organ chambers, oxidative stress by dihydroethidine staining, proteins expression by immunofluorescence staining and the severity of kidney injury by the method of Gomori.

The WS®1442 diet (100 mg/kg/day and 300mg/kg/day) significantly prevented the DOCA-salt-induced increase in SBP in non-nephrectomized rats. In mesenteric artery rings with endothelium, the acetylcholine-induced NO- and endothelium-derived hyperpolarizing factor-mediated relaxations were similar in the control group, the DOCA-salt group and the WS®1442-treated groups. DOCA-salt treatment resulted in substantial damage to kidneys in comparison to the control group as indicated by glomerular and tubulointerstitial lesions, which were prevented by the chronic intake of either 100 or 300 mg/kg/day of WS®1442. In addition, the WS®1442 diet prevented the increase of oxidative stress in the kidney induced by DOCA-salt. These findings indicate that WS®1442 diet prevents DOCA-salt induced hypertension and renal injury possibly by preventing oxidative stress.

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Androgen actions in neurons and gender bias in autism and intellectual disability

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Autism (ASD) and Intellectual Disability (ID) are two common neurodevelopmental disorders with comorbidity and genetic overlap. Another common feature of these two diseases is the existence of a gender bias, very strong for ASD (4 males for 1 female) and notable for the ID (1.4 males: 1 female). Although a large number of genes on the X chromosome are involved in ID and some in ASD, rare and fully penetrant mutations in these genes, if they participate to this male excess, cannot entirely explain it. This sex bias remains unsolved and thus we decided to examine one hypothesis that could explain in part the increased susceptibility of males to neurodevelopmental diseases: the role of prenatal exposure to androgens during male brain development.

Several observations suggest the involvement of prenatal androgens in ASD, such as the correlation between prenatal testosterone and traits associated with ASD (works of Baron-Cohen's team) or differential expression of genes involved in the steroid synthesis in ASD individuals. It is known that AR is expressed by various cells in the brain and in particular by neurons, and that androgens exert a neuroprotective activity and modulate synaptic density in different brain regions, but little is known about AR target genes in neuronal cells. We are currently investigating the issue of how the effect of androgens in the brain could play a role in the observed sex bias in ASD or ID by analyzing their regulation of gene expression in human neurons.

In order to do that, we are analyzing the effect of androgens in neuronal cells, neuronal precursors (NSC) and neurons differentiated from embryonic stem cells (ES), which express AR, by transcriptomic analysis and ChIPseq approach. We are searching if some overlaps exist between the genes/pathways regulated by androgens in neuronal cells and those involved in ASD/ID. In parallel, we are investigating the non-genomic effects of androgens in neurons, searching for AR co-regulators or for androgen effects on synaptogenic function. Finally, to better understand the role of AR in brain development during the two critical early periods of androgen exposition, we will complete the analysis by looking at the expression in fetal and neonatal human brain, by ISH and/or immunohistochemistry.

This study will generate important knowledge on genes and pathways regulated by androgens during human brain development. We hope that it will shed light on molecular mechanisms underlying the sex difference in the susceptibility to neurodevelopmental disorders.

Blackcurrant juice prevents endothelial dysfunction in the mesenteric artery of cirrhotic rats with hepatopulmonary syndrome

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Chronic liver diseases with portal hypertension are characterized by a progressive vasodilatation (with endothelial dysfunction) which is especially observed in the splanchnic and pulmonary beds. The latter is referred as to the hepatopulmonary syndrome (HPS). It has been suggested that one of the physiopathologic mechanisms leading to the general vasodilatation and especially to the HPS is related to bacterial translocation which has been shown to improve in CBDL rats by changing the intestine microbiota. As polyphenol-rich products have been showed to improve endothelial function *in vivo*, the present study has evaluated the effect of ingestion of a polyphenol-rich Blackcurrant juice (PRBJ) on the endothelial function in a rat model of HPS (CBDL), and, if so, to determine the underlying mechanism.

Male Wistar rats (8 rats per group) received either control drinking water or a dose of 60 mg/kg of polyphenol-rich Blackcurrant juice for 7 weeks. After 3 weeks, the rats underwent surgery with either the ligations and resections of the common bile duct (CBDL rats) or sham surgery (sham rats), and were followed for 4 weeks. Reactivity of mesenteric artery rings was assessed in organ chambers. The expression levels of proteins in the vascular wall of mesenteric artery and/or aorta were assessed by immunohistochemistry. The vascular formation of reactive oxygen species (ROS) was evaluated using dihydroethidine. Plasma levels of pro-inflammatory cytokines including TNF-α, IL-1α, MCP-1 and IL-4 were evaluated by flow cytometry using a 5plex-FlowCytomix kit.

Both the NO- and the EDHF-mediated relaxations to acetylcholine were significantly reduced in CBDL rats compared to sham rats, whereas relaxations to sodium nitroprusside (an exogenous donor of NO) and to levcromakalim (an ATP-sensitive K^+ channel opener) were similar. Impaired EDHF-mediated relaxations were associated with reduced vascular expression of Cx37 and SK_{Ca} and increased expression of eNOS. In aortic sections we found increased NADPH oxidase subunits, iNOS, COX_2 and increased vascular formation of ROS and peroxynitrites. The deleterious effect of CBDL on EDHF-mediated relaxations was significantly prevented by PRBJ through up-regulation of vascular expression of Cx37 and SK_{Ca} and down-expression of eNOS. PRBJ treatment also reduced vascular oxidative stress in aorta. Finally the plasma levels of pro-inflammatory cytokines were improved.

Taken altogether, these results indicate that PRBJ ingestion prevented the blunted EDHF-mediated relaxation and the increased vascular oxidative stress in the mesenteric artery of CBDL rats suggesting the contribution of endotoxemia and inflammation in the endothelial dysfunction.

Original liquid and solid phase synthesis of new non natural amino acid derivatives

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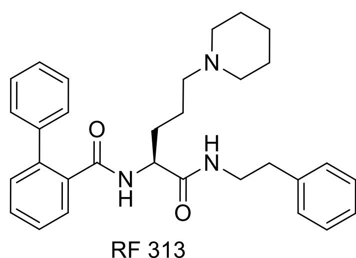
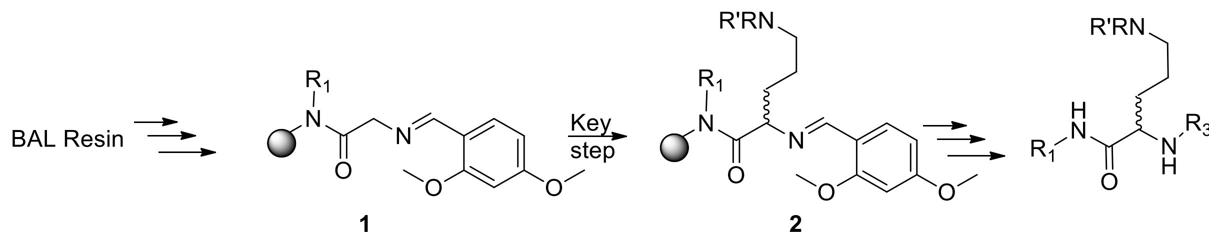
The non natural ornithine derivative RF313 is the first orally active NPFF antagonist capable of reversing opioid induced hyperalgesia at low dose in rodents.



Starting from Fmoc-L-Glu(OtBu)-OH, we developed an efficient liquid phase synthesis allowing to obtain RF313 in a gram scale. However, this pathway is not convergent and not adapted to quickly perform a structure activity relationship analysis of RF313.

Consequently we developed an innovative solid phase synthesis leading to racemic derivative of RF313. The key step of this synthesis implies a selective α-alkylation of the supported glycine **1** to obtain **2**. This new procedure led us to reduce noteworthy the time of synthesis and can also be carried out in parallel.



New perspectives in targeted prostate cancer therapy: XRP44X and Elk3

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Objectives

Tumour therapy is associated with considerable toxicity, presumably because of their broad effects on microtubules. This toxicity could be reduced if more specific agents could be developed, that target key cancer related functions. We have uncovered a particularly interesting pathway, which appears to connect microtubules with molecules and functions that are important for prostate cancer: Ets factors and bone metastasis.

Elk3 is one of the Ets factors that is activated in prostate cancer. It is involved in the growth factor-Ras signalling pathway, angiogenesis, cell movement and the response to hypoxia. A cell based screen for small molecule inhibitors of Elk3 activation by the Ras oncogene led to the identification of XRP44X, that turned out to be a microtubule directed agent. Our objectives are to characterise the activity of XRP44X in pre-clinical models of cancer, in order to identify important microtubule related pathways to target for tumour therapy.

Methodology

We have studied the activities of XRP44X using metastatic cell lines (PC-3M-Pro4, C6, LL2), ex-vivo models of microvessel sprouting (aorta), and mouse models of cancer [prostate cancer progression (TRAMP), and metastasis to the bone (cardiac injection of PC3) and lung (xenografts of LL2 and C6)]. The genes and pathways that mediate the activities of XRP44X were investigated using arrays of cancer related genes, immunohistochemistry and shRNA mediated knockdown in cells.

Results

In vitro, XRP44X inhibits FGF-2 activation of the Erk-1/2 pathway, microvessel sprouting from aorta in organ cultures, cell growth through G2/M arrest. It also affects the tubulin and actin cytoskeletons. These effects are specific for a sub-class of tubulin poisons, which includes combretastatin A4, but not docetaxel, vincristine, or nocodazole. Inhibition of expression of the original target for XRP44X, Elk3, in PC3M-pro4-Luc2, has effects on cell properties relevant for cancer and metastasis.

In vivo, XRP44X inhibits the growth of tumours and metastases formed by LLC1 and C6 cells in nude mice. XRP44X strongly modulates the expression of genes involved in EMT and bone metastasis. XRP44X also inhibits prostate cancer cell metastasis to the bone, following intra-cardiac injection of PC3M-pro4-Luc. Furthermore, formation of micro-metastases is inhibited, as shown using second-generation PC3 derivatives (PC3M-pro4-Luc2). Treatment from 16-29 weeks of TRAMP mice with XRP44X slows tumour progression without any overt effects on body weight or organ morphology, suggesting that this molecule could be effective in preventative protocols.

Conclusion

XRP44X has interesting effects on tumour formation and metastasis in pre-clinical models and appears to be an interesting tool to identify specific mechanisms by which tubulin directed molecules

Revealing the role of the HIV-1 nucleocapsid protein on reverse transcriptase pause sites during plus-strand DNA synthesis of HIV-1

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During HIV-1 reverse transcription, the single strand genomic RNA is converted into a proviral double strand DNA through the joint activities of the reverse transcriptase (RT) and the nucleocapsid protein (NCp7). A complex interplay between RT and NCp7 is required to optimize reverse transcription efficiency and fidelity.

RT is an enzyme endowed with RNA- and DNA-dependent polymerase activity, as well as with RNAse-H endonuclease activity allowing notably priming, initiation and extension of primer/template duplexes *in vitro*. The RT-directed synthesis of the (-) and (+) strands of the viral DNA is not monotonic. Strong pause sites where RT is thought to stall have been identified. These pausing sites occur when RT encounters particular sequences and/or stably secondary structures of the template, as for instance stem-loops. A direct consequence of the pause sites is the low RT polymerization efficiency as observed with long DNA products *in vitro*. Moreover, these pause sites can lead to premature termination of polymerization and formation of non-infectious virus, underlining the critical role of accessary proteins assisting RT. In the role of accessary protein, the efficiency and fidelity of reverse transcription is notably enhanced by NCp7. In this work, we investigated the effect on RT's binding and orientation on nucleic acid substrates that include pause sites by using state of art fluorescence techniques.

Our preliminary data indicated the nature of RT orientation and its binding parameters on two different pausing sites and non-pausing sites during plus-strand synthesis. We documented here, the presence of two different RT pausing mechanisms where RT can either dissociate from the nucleic acid extremity supposed to be elongated or stall while remaining bound due to a change in its orientation.

Keywords: Nucleocapsid protein, reverse Transcriptase, pause sites, single molecule spectroscopy, FRET

Site-selective Characterization of the NCp7 Interactions with its Molecular Targets

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Due to its high conservation and critical functions in HIV-1, the nucleocapsid protein NCp7 appears as a target of choice for developing new drugs. In order to further understand the specific role of NCp7, and thus, inhibit its key functions, the characterization of its interactions with different targets such as nucleic acids, lipids and proteins in solution and in the cellular context is strongly demanded.

To reach this objective, we synthesized NCp7, internally labeled with a 3-hydroxychromone-based amino acid analog [1], characterized by an environment-sensitive multichannel response. This probe shows a strong response to the polarity, H-bonds and electric fields, so that the ratio of its emission at two wavelengths could be used to sensitively monitor environmental changes [2]. This allowed us to site-selectively monitor at the labeled NCp7 position, the interaction of NCp7 with its molecular targets by steady-state fluorescence spectroscopy. Moreover, we performed time-resolved experiments to check whether specific lifetime signatures are associated with the interaction to the different biomolecules. In a second step, we micro-injected the labeled peptide in cells, and by using FLIM (fluorescence lifetime imaging microscopy), we analyzed the intracellular distribution of the interactions of NCp7 with its molecular targets.

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Gene delivery with polycationic fullerene hexakis-adducts

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C_{60} is a unique three dimensional scaffold in chemistry, that permits to obtain structure with biological properties[1]. To date few examples of C_{60} derivates are able to gene deliver[2]. Based on the methodology developped in our laboratory[3], it was possible to create polycationic fullerene hexakis-adducts with remarkable gene delivery capabilites.

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Role of Fragile X Mental Retardation Protein in neuronal mRNA metabolism

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Fragile X syndrome is the first cause of inherited intellectual disability and is due to the absence of FMRP protein. FMRP is an RNA binding protein, which in neurons is proposed to regulate the RNA metabolism of a subset of specific mRNAs important for dendritic spine maturation and synaptic plasticity. Several mRNAs are considered as validated target of FMRP, but which mRNAs are the most affected by FMRP absence and how FMRP regulates the expression of its mRNA targets remains controversial.

We have previously shown that FMRP specifically binds to G-quadruplex RNA structures in vitro and these structures are over-represented in the 3'UTR of dendritically localized mRNAs where they serve as dendritic localization signal in the two FMRP targets: PSD95 and CamkIIa. To determine whether FMRP mostly and specifically binds to mRNAs bearing G-quadruplexes, we measured by Cross-Linking-ImmunoPrecipitation and qRT-PCR the interaction of FMRP to 23 best known dendritically localized mRNAs in *Fmr1* Wt and KO primary cortical neurons: best FMRP bound mRNAs all contain a G-quadruplexe consensus in their 3'UTR. The microarray analysis on these neuron extracts revealed a list of 314 cortical mRNAs. Remarkably, one mRNA is found predominantly bound by FMRP, suggesting the existence of a main target. The best-bound mRNAs show a significant enrichment of G-quadruplex consensus in their 3'UTR.

Development of a Caco-2 permeability assay

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The Caco-2 permeability assay is designed to model the transport events of a compound through the intestinal epithelium barrier in the organism. This method utilizes a polarized Caco-2 cell layer grown on a supportive membrane surface that separates two compartments. The flux of the tested compound is determined by applying the compound to either the apical or the basolateral side of the cell layer and measuring the redistribution of the compound between the two compartments. The transport ratio is determined by applying bidirectional measurements (apical to basolateral and basolateral to apical).

PCBIS offers a 24-well permeability assay using LC-MS/MS for the analysis of samples.

Customization is possible (compound concentration, time points, format…), contact us to learn more about how Caco-2 study can be used in your applications.

New cell-based assays for 5-HT receptor investigations using Tag-lite® technology

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Cisbio Bioassays and its collaborators, the University of Strasbourg and the Institute of Functional genomics, have performed the development of a new range of innovative tools to investigate the membrane receptors such as the GPCRs and ligand-gated ion channels, using the Tag-Lite® technology. Tag-Lite® is a unique and non isotopic integrated method combining the use of suicide enzymes as tags fused to targets of interest with Homogeneous Time Resolved Fluorescence (HTRF®) as detection readout. HTRF® reagents could be easily miniaturized while maintaining their accuracy and reproducibility.

We performed engineering of several plasmids encoding for SNAP-Tag or HALO-Tag fused to all members of the serotonin receptors which have been successfully expressed at the cell surface and we validated their functional properties. Previously, we succeeded the development of specific fluorescent ligands coupled to a HTRF® compatible fluorophore to investigate 5HT1A, 5HT1B and 5HT4 receptors (Kd 76nM, 12nM and 25nM). Recently we succeeded the preliminary studies about new specific fluorescent ligands coupled to a HTRF® compatible fluorophore to address 5HT2B, 5HT2C, 5HT5A, 5HT6, 5HT7 and 5HT3A receptors. These ligands were validated on cells using several well-described compounds as reference.

Development of a microfluidic device for time-lapse imaging and analysis of cellular dynamics in the nematode *C. elegans*

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In multicellular organisms such as the nematode *Caenhorabditis elegans*, developmental studies depend on the ability to image animal at the cell and subcellular level. In asymmetric cellular division (ACD), one cell gives rise to two different cells: This is a basic mechanism to generate cellular diversity, which is used in a large variety of developmental contexts.

Here, the lab has established the worm *Caenorhabditis elegans* as a powerful model to study the impact of ACD on cell potential : we study the ACD of a differenciated rectal cell (named K) that will generate the K.a and K.p daughter cells. Kp will subsequently give rise to the neuron DVB. This event takes place during larval development, in the tail, the funnel-shaped body part of the nematode. We have developed rectal and neuronal markers to follow K division and DVB formation. This model is a model of choice to decipher the poorly understood molecular mecanisms that direct AC.

The aim of this project is to develop a new tool to allow high resolution imaging of K division in real time and the visualization of the segregation of the molecular components involved in this ACD. Here, we describe the development of a microfluidic device as a tool for anaesthetic free rapid immobilization, and high-resolution fluorescent imaging of live nematodes *C. elegans*. The use of a temp-dependant gel-solid transition hydrogel (PF127) allows the suppression of wriggling movements[1], while the physiological development is conserved. This tool will allow us to investigate, at the single cell level, the dynamic subcellular localization of molecules involved in ACD and further screen for mutants affecting these cellular events.

Vasorelaxant effects of *Phyllanthus amarus* extracts on porcine coronary artery rings

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In recent years, developing countries, in particular those in sub-Saharan Africa, have observed a pronounced increase in the prevalence of arterial hypertension (HTA), which is associated with an increased cardiovascular mortality [1,2]. In Ivory Coast, *Phyllanthus amarus* (Euphorbiaceae family) is traditionally used to treat arterial hypertension as a decoction of the whole plant. Previous studies have reported that an aqueous extract of *Phyllanthus amarus* induces hypotensive effects associated with inotrope and chronotrope positive effects in normotensive rabbits [3]. The aim of the present study was to evaluate the vasorelaxant effect of an aqueous extract of *Phyllanthus amarus* (AEPA) and of its different fractions using porcine coronary artery rings, and, if so, to characterize the underlying mechanism.

The AEPA was obtained after decoction of the aerial part of the plant and lyophilisation. The ethanolic fraction (EF) was obtained by decantation of AEPA in 70 % aqueous ethanol. The AEPA was submitted to fractionation by liquid/liquid extraction with solvents of increasing hydrophobicity, giving successively cyclohexane (CHF), chloroform (CF), ethyl acetate (EAF), n-butanol (BF) fractions and finally an aqueous residue (AR). Fraction-induced relaxations were assessed using porcine coronary artery rings suspended in organs chambers.

The aqueous extract (AEPA) and the ethanolic fraction (EF) induced similar endothelium-dependent relaxations. Comparative study of its fractions indicated that the relative order of potency is CF > EAF > CHF > BF > EF > AEPA >>> AR. The most potent fraction, CF, induced endothelium-independent relaxations in rings. Moreover, the CF fraction significantly potentiated relaxations induced by forskolin (an activator of adenylyl cyclase) and isoproterenol (an agonist of beta-adrenergic receptors) suggesting the involvement of the cyclic AMP relaxing pathway.

Altogether, these findings indicate that *Phyllanthus amarus* extract induces endothelium-dependent relaxations. Moreover, the chloroformic fraction seems to contain the most active compounds for inducing vasorelaxation by acting directly at the vascular smooth muscle. Therefore, we will further evaluate the CF fraction by a phytochemical analysis for the determination of the active compounds, and characterize the sequence of events leading to vasorelaxation.

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