BORDEAUX VIVA WINTER SCHOOL - XXXIII LIAC MEETING

29 November to 1 December | 29 Novembro a 1 Dezembro

Program

29 Novembrer | *29 de Novembro*

Reception of participant Winter School Meeting

Winter School meeting (organizers: A. Bikfalvi & J. Badaut) Presentation of students - What should be achieved in this winter school
Opening meeting : Thierry Couffinhal (VIVA action, Director) & Michel Spina (LIAC President)

CLINICAL AND EPIDEMIOLOGIC ASPECT OF VASCULAR AGING (chairmen: C. Tzourio & L. Monteiro)
Early vascular aging - P.M. NILSSON, Malmö - Sweden

Neurovascular epidemiology of aging - C. TZOURIO, Bordeaux

Cardiovascular epidemiology of aging - P. BOUTOUYRIE, Paris

Forecasted trends in disability and life expectancy - S. AHMADI-ABHARI, Liverpool - UK

Neurovascular genetic epidemiology - S. DEBETTE, Bordeaux

Vascular and thrombosis genetic epidemiology - D. TREGOUET, Paris

SELECTED ORAL PRESENTATION (Chairmen: S. Debette & J. Badaut)

• Mitochondrial function regulates vascular aging in mice - K. FOOTE, Cambridge - UK

• Structural imaging of the vascular wall - S. ALMAGRO, Reims

• Numerical assessment and comparison of pulse wave velocity methods aiming at measuring aortic stiffness - H. OBEID, Paris

• Long-term trajectories of cardiometabolic risk factors in prodromal dementia: the Three-City Study - M. WAGNER, Bordeaux

EVENING PHILOSOPHICAL CONFERENCE ANTONIO-MARIO TAMBURRO (chairman: M. Spina)
When does the vascular system age and when is there a disease? Conceptual and theoretical issues - M. LEMOINE, Tours

30 Novembrer | *30 de Novembro*

PATHOPHYSIOLOGIC FEATURES OF VASCULAR AGING (Chairmen: J.F. Arnal & M. Formato)
From physiological aging to pathological aging - J.B. MICHEL, Paris
Physiological models to study the human microcirculation - effects of hyperoxia - L. MONTEIRO-RODRIGUES, Lisbon - Portugal
Murine models for investigating vascular ageing - G. FAURY, Grenoble
New insights into vascular biology by single cell RNA sequencing - M. VANLANDEWYCK, Stockholm - Sweden
Thrombosis in aging - C. JAMES, Bordeaux
Retinal vascular imaging - C. HELMER, Bordeaux
Lunch of Winter School Meeting
Winter School meeting (organizers: A. Bikfalvi & J. Badaut) Meet the experts - Round table with experts

MATRIX STRUCTURAL AND MECHANICAL STUDIES (Chairmen: I. Brunet & P. Dufourcq)
Model of pseudoxanthoma elasticum – Vascular calcification and aging, from gene to bedside - L. MARTIN, G. LEFTHERIOTIS, Angers
Vascular aging: structure and dynamics of constitutive macromolecules and sub-fragments - V. SAMOUILLAN, Toulouse
Tubular scaffolds for applications of vascular tissue engineering - V. LA CARRUBBA, Palermo - Italy
Bioprosthetic heart valve aging deterioration and age-related benefits - M. SPINA, Padova - Italy
Elastin modification during vascular aging and pathophysiological consequences - L. DUCA, Reims

SELECTED ORAL PRESENTATION (Chairmen: M. Laffargue & D. Henrion)
• MicroRNA expressed in progenitor cells circulating in peripheral blood as prospective biomarkers of agerelated macular degeneration (AMD) in its dry and wet form – preliminary data - M. KAWA, Poland
• Molecular, conformational and thermal characterization of ventricular Remodeling in a pig model of tachycardia-induced cardiomyopathy - V. LLORENTE CORTES, Barcelona - Spain
• Valvular calcification: implication of the Semicarbazide-Sensitive Amine Oxidase (SSAO)? - N. MERCIER, Nancy

• Vascular calcification during chronic kidney disease: role of the RAGE/Cathepsin S/elastin peptides axis - A. WAHART, Reims

VIVA EVENING CONFERENCE (Chairman: T. Couffinhal)

A short history of the vessel - A. BIKFALVI, Bordeaux

1 December | *1 de Dezembro*

CELL BIOLOGY AND SIGNALING (Chairwomen: C. Duplàa & V. Llorrente Cortes)

SELECTED ORAL PRESENTATION

• Targeting Connexin40 expression or function reduces angiogenesis in the developing mouse retina - F. ALONSO, Bordeaux

• Imatinib inhibits hypoxia-induced MMP-9 overexpression and activation through impairment of LRP1 and Pyk2 phosphorylation in human coronary vascular smooth muscle cells - A. BENITEZ, Barcelona - Spain

• NRP1 supresses TGFβ-dependent ERK1/2 activation in endothelial cells - A. LAMPROPOULOU, London - UK

• The elastin receptor complex interacts with CD36 through NEU1 and regulates oxidized LDL uptake in macrophages: potential implication in the proatherogenic effects of elastin-derived peptides - P. MAURICE, Reims

• Ubiad1 promotes tumor angiogenesis by inhibiting dephosphorylation of vegfr2 in a redox dependent manner - R. SEWDUTH, Belgium

Cell senescence and telomere - E. GILSON, Nice

Estrogens as vascular anti-ageing hormones? Experimental and clinical evidences and uncertainties - J.F. ARNAL, Toulouse

Arteriolar and microcirculation aging - D. HENRION, Angers

Oxydation and inflammation in Atherosclerosis - A. LEPEDDA, Italy

Winter School Meeting (organizers: A. Bikfalvi & J. Badaut) Highlights and major topics identified by the students, group proposals (3 per group) for fictive/future projects and presentation

OPEN SESSION (Chairwomen: M.A. Renault & J. Lods)

• Endothelial cell dysfunction is a new potential therapeutic target for the treatment of critical limb ischemia - C. CARADU, Pessac

• Podosomes Mediate Basement Membrane Collagen-IV Proteolysis During Sprouting Angiogenesis - E. GENOT, Bordeaux

•How plant food bioactives may have benefits for vascular health? The case of curcumin, L.E. MONFOULET, Lille

Closing meeting: Thierry Couffinhal & Michel Spina

Table des matières

CLINICAL AND EPIDEMIOLOGIC ASPECT OF VASCULAR AGING

Early Vascular Ageing (EVA) in translation: from cell senescence to clinical implications

Peter M Nilsson, MD, PhD *1

[1] Professor of Clinical Cardiovascular Research, Lund University, Department of Clinical Sciences, Skane University Hospital, S-205 02 Malmo, Sweden
* Intervenant

Already Thomas Sydenham (1624-1689) told that *"A man is as old as his arteries"*.
The ageing process of the vasculature has increasingly been studied over the last 25 years. New data on the stiffening of arteries (*arteriosclerosis*) has been added to long-term studies on atherosclerosis. It is believed that arteriosclerosis starts early in life, based on fetal programming of the elastin content of the arterial media, as well as of the vasculature and capillaries. This will impact on the morphological changes of large elastic arteries and central hemodynamic regulation. Later on the process of atherosclerosis, as being more proximal to clinical cardiovascular events, will play an important role while the two processes will be more and more mixed and jointly increase the risk.
In the arterial wall the increasing age-dependent stiffness is characterized by a relative decrease of the elastin content of the arterial media and a relative increase of the collagen content as well as more pronounced collagen linkages. In addition there is a mechanism called mechanotransduction that will impact on the structure of the extra-cellular matrix in relation to increased tension of the arterial wall, i.e. following increased blood pressure. This will also influence vascular smooth muscle cells (VSMC) to undergo changes as the VSMC contractile phenotype is associated with an increased arterial stiffness. This can take place both in the arterial media and the adventitia [1].
In addition, some specific processes related to the adventitia may further increase stiffness. When the vascularization of the arterial wall via *vasa vasorum* is impaired, as following the administration of anti-angiogenic drugs for cancer therapy, this has been shown to increase arterial stiffness.
Furthermore, impaired autonomous nervous function is related to both increased arterial stiffness and impaired glucose metabolism. Finally, the existence of perivascular adipose tissue (PVAT) is a source of local perivascular inflammation via secretion of vasoactive cytokines. This will also influence arterial stiffness by reducing endothelial function to vasodilate.
Based on epidemiological evidence from the Malmo Diet Cancer Cohort it has been shown that increased carotid-femoral pulse wave velocity (c-f PWV) is independently associated with markers of impaired glucose metabolism [2], even adjusted for hemodynamic factors and risk factors for atherosclerosis (LDL cholesterol, smoking, renal function). This shows the close link between arterial stiffness, impaired glucose metabolism and overt type 2 diabetes – a different risk factor cluster than the more traditional cluster associated with atherosclerosis (hypertension, smoking, hyperlipidaemia).
We have also found that c-f PWV is associated with a family history of cardiometabolic disease [3], impaired cognition [4], and is predictive of type 2 diabetes [5] and all-cause mortality, even adjusted for known risk factors/markers and family history of cardiometabolic disease (abstract, ARTERY 2017, Pisa, Italy).
New drugs are being developed for vascular protection and the reduction of arterial stiffness. One promising experimental drug, currently in human Phase-1 studies, is the selective Angiotensin-II (AT2) agonist compound 21 (C21). In one animal study it was shown that C21 could reduce PWV in L-NAME induced hypertension without interfering with blood pressure [6].
In summary, early vascular ageing (EVA) has inspired research to better understand the morphological changes of the arterial wall (especially the media and adventitia) and its age-related hemodynamic changes [7]. Increased c-f PWV is a predictor not only of cardiovascular events, independent of traditional risk factors, but also of type 2 diabetes and total mortality. This shows that arterial stiffness is a marker of biological ageing in general and should be the focus for prevention and testing of new drugs for vascular protection such as C21, besides the efforts to reduce the burden of atherosclerosis, plaque formation and risk of cardiovascular events.

References
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7. Nilsson PM, Boutouyrie P, Cunha P, Kotsis V, Narkiewicz K, Parati G, Rietzschel E, Scuteri A, Laurent S. Early vascular ageing in translation: from laboratory investigations to clinical applications in cardiovascular prevention. *J Hypertens.* 2013;31:1517-26.

Forecasted trends for dementia, disability, and lifeexpectancy in England and Wales to 2040: a probabilistic Markov modelling study

Sara Ahmadi-Abhari[1] Maria Guzman Castillo[2], Piotr Bandosz[2,3], Martin J Shipley[1], Archana Singh-Manoux[1,4], Mika Kivimäki[1,5], Simon Capewell[2], Martin O'Flaherty[2], Eric J Brunner[1]
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[2] Department of Public Health and Policy, University of Liverpool, United Kingdom
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* Intervenant

Background: As life-expectancy rises, dementia and disability are expected to impose increasing societal and healthcare burden. Forecasts for prevalence of dementia and disability, and life-expectancy are vital in defining future needs and can be improved with a dynamic modelling approach that integrates calendartrends in incidence of dementia and disability with those for mortality and cardiovascular disease.
Methods: A probabilistic Markov-model labelled IMPACT-BAM (Figure 1) was developed and validated to track the progression of the population of England and Wales aged 35+ through states of cardiovascular disease, cognitive impairment, dementia, and disability through to death and forecast burden of disease and life-expectancy for the future. Data from the English Longitudinal Study of Ageing (ELSA), a representative panel study with 6 waves of data across 2002-2013, was used to inform the baseline prevalence estimates and transition probabilities in the model. Calendar trends in incidence of cardiovascular disease, dementia, and mortality rates were obtained from ELSA and data from the UK Office for National Statistics and projected to the future. Disability was defined as inability to independently perform one or more basic activities of daily living.
Results: Average Life-expectancy is estimated at 21.8 year in 2017 and projected to increase to 26.4 years by 2040 (Figure 2). The proportion of disabled to disability-free life expectancy declined over time suggesting compression of morbidity. Using IMPACT-BAM we estimated approximately 792000 (95% Uncertainty Interval 760000–823000) people are living with dementia and 3,590,000 (95% UI 3540000- 3669000) with disability in England and Wales in 2017. Despite the decline in incidence of dementia and cardiovascular disease, the numbers of people living with dementia and disability are projected to increase to 872000, 1092000, and 1205000 for dementia and 3771000, 4383000, and 4902000 for disability in 2020, 2030, and 2040 respectively. Population ageing is a main contributor as the age-standardized prevalence is declining.
Conclusion: Despite the decline in age-specific incidence of dementia and cardiovascular disease, the number of people living with dementia and disability in England and Wales is likely to increase by 52% for dementia and 37% for disability from 2017 to 2040. This increase is mainly driven by increasing lifeexpectancy.
Our projections have important implications for planning to meet future health and social care needs.
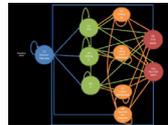
Figure 1: IMPACT-Better Ageing Model (IMPACT-
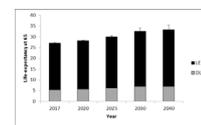
Figure 2: Average life-expectancy and disabled life-expectancy at age 65 in England and Wales estimated from IMPACT-BAM
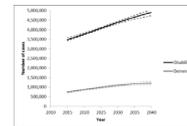
Figure 3: Projected number of people living with dementia and disability in England and Wales using IMPACT-BAM, 2015-2040. Dashed lines represent 95% uncertainty intervals.

Cardiovascular epidemiology of aging

Pierre Boutouyrie * 1

[1] Pharmacologie, Cardiologie - Hôpital Européen Georges Pompidou, Inserm U970, Equipe 7, Paris
* Intervenant

This presentation will concentrate on the arterial consequences of arterial aging, especially arterial stiffness, arterial remodeling and wave reflections. We will present the pathophysiological aspects, the different techniques available and the principal results of epidemiological surveys. We will then briefly present some directions for treating early vascular aging.

Neurovascular genetic epidemiology

Stéphanie Debette *1
[1] Inserm U1219, Bordeaux
* Intervenant

With increasing longevity, cerebrovascular disease has become the most common age-related source of disability and dependence, as well as a major determinant of dementia, representing a huge societal burden. Compared to cardiovascular disease, unraveling the genomic underpinnings of cerebrovascular disease has proven more complex due to its much more heterogeneous nature. Recent multiethnic collaborative efforts have enabled important progress in revealing genetic determinants of stroke and MRI-defined covert vascular brain injury. These findings are primarily based on common variants but preliminary results on rare and low-frequency variants are beginning to emerge. Both the "lumping approach" combining all types of strokes with large numbers and strategies focusing on highly specific subtypes of cerebrovascular disease has proven efficient, with complementary findings. Analysis of shared genetic variation with other vascular phenotypes has also provided interesting mechanistic insight. Experimentally follow-up of some of these findings is generating novel hypotheses on the biological pathways underlying stroke.

Vascular and thrombosis genetic epidemiology

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* Intervenant

During the last 30 years, the evolution of the genetic epidemiology discipline paralleled the development of high-throughput technologies. In the 80's, familial linkage analysis and candidate gene association approaches were the method of choice for identifying genetic markers associated to human complex diseases. In the last 10 years, high density arrays, followed now by next generation sequencing techniques, allowed the screening for (epi-)genetic and transcriptomic marks that influence disease susceptibility. Henceforth, it is time to interrogate other kind of molecular phenotypes, incl metabolites, proteins, through new high-throughput techniques , and to integrate their analysis with
that of (epi-)genetic data in order to disentangle in a more comprehensive way the complex architecture underlying common human diseases. This opens the era of the genomic epidemiology discipline or what could be referred to as epidemiolomics.

EVENING PHILOSOPHICAL CONFERENCE ANTONIOMARIO TAMBURRO

When does the vascular system age and when is there a disease? Conceptual and theoretical issues

Maël LEMOINE *1
1 University of Tours - Philosophy of biological and medical sciences
* Intervenant

This talk takes a very general perspective in order to question the distinction between aging and disease, in the specific case of the endothelial dysfunction. To what extent is it possible to hold the claim that the difference does not matter insofar as we treat everything we can all the same, whether it is aging or a disease? I will show that it is difficult, but possible to hold it in the face of commonly held but unscientific claims such as "atherosclerosis is aging rather than a disease because it eventually strikes everyone". Then, I will examine the hypothesis that a difference can be made locally between what resorts to aging of the arteries and what resorts to a disease of the arteries, and whether it can
matter. Eventually, I will assess the interest of general biological theories of aging to justify the distinction. In the conclusion, I will try to gather an answer to the question: does it matter to have a distinction between aging and disease of the arteries?

PATHOPHYSIOLOGIC FEATURES OF VASCULAR AGING

Physiological models to study the human microcirculation - effects of hyperoxya

Luis Monteiro-Rodrigues *1
1 Universidade Lusófona de Humanidades e Tecnologias - Lisbon, Lisboa, Portugal
* Intervenant

Models, animal or human, are crucial to look further into the mechanisms involved in a pathological or in a regulatory process to study vascular function and disease, also considering that currently, Vascular Dysfunction represents the 3rd major cause of cerebral-vascular disease. In the last years we explored various approaches to measure microcirculation in vivo, as near as possible from the normal physiological state, and identified several determinants. More recent physiological models are helping to improve the experimental conditions and contribute to accelerate knowledge in these areas.

Hyperoxia is a well known strategy in medicine, also used as a stressor to text microcirculatory regulation mechanisms. We have explored several models, human as animal using hyperoxia. More recently, focusing the improvement of neovascularization process following ischemia, we combined hyperoxia and the hindlimb ischemia, using C57/BL6 mice. In the present communication we intend to share some of our latest experience, and data, in these areas in order to better understand microcirculation physiology.

Murine models for investigating vascular ageing

Gilles Faury [*1]
[1] Laboratory HP2, Université Grenoble Alpes, France
* Intervenant

In human beings, investigating the normal or pathological ageing processes is a difficult task because of the long time course of all the implicated mechanisms, in the range of decades. The access to the elderly is also uneasy because of the laws regulating investigations in patients. In order to overcome these difficulties, numerous murine models have been designed or obtained, by genetic engineering or natural/classical selection. Regarding the investigations of normal ageing, some models allow for easier longitudinal studies because of a selected shorter lifespan, such as senescence-accelerated mice (SAM mice), whereas other models aim at the characterization of the involvement of specific target/candidate mechanisms by gene modification of invalidation, such as Klotho-/- mice. Regarding pathological ageing, or age-related pathologies, a series of models are used, mostly aiming at targeting specific actors (by invalidation, over-expression, ...), such as the mice knock-out for the Apolipoprotein E (ApoE-/-, for studies on atherosclerosis) mice or Spontaneously Hypertensive Rats rats (SHR, for studies of the mechanisms or consequences of hypertension). Some other murine models, not overtly related to the ageing, have also brought some insight into the possible modulations of or alternatives to the normal ageing processes, such as the mice heterozygous for the elastin gene (Eln+/-). However, most of these models present some undesirable side-effects which could lead to biases in the possibilities to generalize to the ageing processes that are present in "normal" animals.
The presentation will review some of the main uses of and results from several murine models widely used in the studies of (cardio-) vascular ageing.

New insights into vascular biology by single cell RNA Sequencing

Michael VANLANDEWIJCK[*1]
[1] Department of Immunology, Genetics and Pathology, Vascular Biology; Research group Christer Betsholtz, Uppsala University, Sweden
* Intervenant

Cerebrovascular disease is the third most common cause of death in developed countries, yet our understanding of the cells that make the cerebral vasculature is limited. Using large-scale analysis of vascular single cell transcriptomes, we provide molecular definitions to the principal blood vascular and vessel-associated cell types in the adult mouse brain. We uncover the transcriptional basis for the gradual phenotypic change (zonation) that occur along the arterio-venous (A-V) axis in the two major vascular cell types – endothelial and mural cells - and reveal unexpected differences between them: a seamless endothelial continuum, as opposed to a punctuated mural continuum. The latter reflects two distinct mural cell groups comprising arterial/arteriolar smooth muscle cells, and pericytes/venous smooth muscle cells, respectively. Brain pericytes are highly homogenous, but a comparison between brain and lung reveals significant pericyte organotypicity. For example, brain pericytes express a unique repertoire of molecular transporters, suggesting their active role at the blood-brain barrier. We also define a population of perivascular fibroblast-like cells that form a previously unrecognized adventitial layer in the brain vasculature. These cells are distinct from mural cells, occur on all vessel types except capillaries and provide distinct anatomical landmarks at the arteriolar-capillary and capillary-venular borders.

Summary Thrombosis in Aging

Chloé James[*1]
[1] Inserm U1034, Biology of Cardiovascular Diseases, Pessac – Bordeaux
* Intervenant

Age is a well-known risk factor for thrombosis, both in the arterial (acute myocardial infarction, stroke) and the venous (deep vein thrombosis, pulmonary embolism) system. Because ageing in humans in most cases occurs in parallel to other thrombotic risk factors (such as hypertension, obesity, cancer, diabetes), the "true" effects of ageing per se may not be easily dissected.
Recently, epidemiologic studies have suggested that aging "per se" is a thrombotic risk factor.
Based on the literature, we will expose how ageing can favour thrombosis, and especially focus on the role of the endothelium, platelets, and coagulation.

MATRIX STRUCTURAL AND MECHANICAL STUDIES

Model of pseudoxanthoma elasticum

Ludovic Martin [*1]
[1] CHU Angers
* Intervenant

Pseudoxanthoma elasticum (PXE) is a prototype of heritable ectopic mineralization. This rare, monogenic (autosomal recessive) condition presents with intriguing manifestations in the skin, retina and cardiovascular systems. Notably, all these systems are rich in elastic tissue and PXE pathological changes are closely related to elastic fibers. The causative gene of PXE is ABCC6, which encodes an ATP-binding transporter primarily expressed in the liver. PXE looks a metabolic condition with central defect in the liver and remote mineralization in the above mentioned systems. The reality might be more complicated... This talk will briefly overview the clinical presentation of PXE, review the specific pathomechanisms operative in ectopic mineralization, including the central role of inorganic pyrophosphate, and discuss whether or not PXE might be seen as an example of premature aging.

Vascular calcification and aging, from gene to bedside

Georges Leftheriotis *1
1 CHU Angers
* Intervenant

Arterial calcification is a widely demonstrated independent risk factor associated to the cardiovascular morbi-mortality. Calcification of the intimal or medial (i.e. so called mediacalcosis) layers of the arterial wall are frequently observed in acquired metabolic diseases such as chronic kidney disease or diabetes.

Since the last decade, our knowledge of the biological mechanisms underlying this degenerative process has considerably widened with the study of several rare inherited calcifying diseases. For example, the study of PXE, generalized calcification in infancy (GACI) or Arterial Calcification due to Deficiency of CD73 (ACDC) have pointed the importance of the ectonucleotidic regulation of the calcifying process. The complex role of the cell transdifferentiation has been recently evidenced in inflammatory conditions whereas atrogenic induction or protection of calcification are still under the scope of epidemiological studies. Our lecture will focus on the different facets and current concepts of the underlying complex biomineralizing mechanisms and their therapeutic perspectives.

Recommanded readings before the lecture are accessible here:

http://jasn.asnjournals.org/content/15/12/2959.full.pdf
http://ac.els-cdn.com/S147148921630008X/1-s2.0-S147148921630008X-main.pdf?_tid=5de16b18-e692-11e5-9c9f-00000aab0f01&acdnat=1457595382_5827a2f8e420f132701fcee7fc6a31b7

Vascular aging: structure and dynamics of constitutive macromolecules and sub-fragments

V. Samouillan*1, J. Dandurand1, C. Lacabanne1, M. Spina2, V. Llorente-Cortes3, B. Bochicchio4, A. Pepe4
1CIRIMAT, Université Paul Sabatier, Physique des Polymères, Toulouse
2Dipartimento di Scienze Biomediche Sperimentali, Università di PXVA, Viale Colombo, Padova, Italy.
3Institute of Biomedical Research of Barcelona - Spanish National Research Council, Barcelona, Spain.
4Dipartimento di Scienze, Universita degli Studi della Basi licata,Potenza, Italy
* Intervenant

The main constitutive macromololecules of the cardiovascular tissues, essential to the cohesion and resiliency, are also active components which can evolve with ageing.

In the first part of this talk we will show how vibrational, calorimetric and dielectric techniques can be adapted to characterize elastin and collagens. In peculiar we will see that the vibrational spectra of elastin and type I collagen allow to discriminate between their secondary structures. Using Differential Scanning Calorimetry (DSC), the thermal fingerprints of elastin and collagens evidence the strong correlation between chain architecture and mesophase organization (Fig 1).
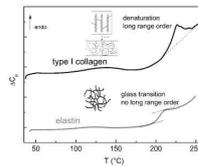

Figure 1: DSC thermograms of freeze dried collagen and elastin

Dielectric techniques reveal the distinct dynamics of these proteins, correlated to their hydrogen bonds network1. This set of data collected on pure proteins is used to help the understanding of vascular aging trough different examples.

One application deals with the use of DSC to compare the thermal signature of elastin and elastin fractions from human thoracic aortas during aging. It is shown that in the thoracic aortas from children group, proteins induce a more flexible structure in contrast with that observed in the thoracic aortas from adults group, due to the total cross-linking of elastin. In this case, there is a segregation between proteins and elastin. No significant differences can be found between the glass transition temperature of elastin in adults and old persons groups, meaning that the mechanical properties of elastin are conserved during ageing. Nevertheless, an irreversible denaturation phenomena of ☐ sheet or helical structured proteins is detected with aging, and corroborated by biochemical analyses which report an important increase of polar amino acids in the elastin-proteins fraction of elderly patients2. In this case the protein fraction would form an homogenous phase with elastin by ionic bonds.

A second application deals with the combined use of FTIR, DSC and dielectric techniques to characterize a sub fragment of elastin involved in pathological aging. Under certain pathological conditions elastin is attacked by the metalloproteinase MMP12 releasing short polypeptides able to give rise to amyloid-like fibers. Amyloid fibrils are highly ordered parallel cross ☐-sheet structures usually associated with Alzheimer's disease, Parkinson's disease, type II diabetes. Peculiar signatures of the amyloid fibers can be detected both at the localized and delocalized levels in the condensed state: DSC analysis evidences that S4 peptides are amorphous whereas S4 fibers present a long range order (Fig. 2). Their self-assembly into amyloid fibers lead to macroscopic dipole moments explaining the enhancement of these dipolar properties3 (Fig.3).
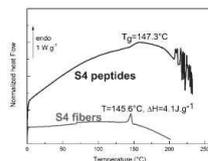
Figure 2: DSC thermograms of S4 peptides and fibers
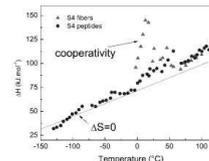
Figure 3: Fine structure analysis of S4 peptidesand fibers

In the last part, we will show how the dielectric techniques, coupled with thermal and vibrational analyses, allow to characterize vascular tissues themselves, both at the nanometric and mesoscopic levels, in the physiologic and pathologic states; this is promising to track vascular aging and also to optimize the conception of substitutive biomaterials.

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3. Samouillan V, Dandurand J, Causse N, Lacabanne C, Bochicchio B, Pepe A. Influence of the Architecture on the Molecular Mobility of Synthetic Fragments Inspired from Human Tropoelastin. *IEEE Trans Dielectr Electr Insul* 2015;**22**:1427–1433.

Tubular scaffolds for applications of vascular tissue Engineering

Vicente La Carrubba [*1]

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* Intervenant

Polymeric tubular scaffolds have been prepared through phase-separation based methods, namely Diffusion Induced Phase Separation (DIPS) and particulate leaching.

According to the DIPS protocol the scaffold, which present a vessel-like shape, can be obtained obtained by performing a phase separation process (induced by a decrease of solvent power) around a nylon fibre covered by a viscous polymeric solution. The fibre was first immersed in a PLLA/dioxane solution (dipcoating bath) at a fixed temperature. Then the fibre was slowly drawn at a constant rate from the solution and put in a second bath (DIPS bath), containing distilled water or dioxane/water at different ratios, at the same temperature, for a well-defined time interval This procedure was repeated once or twice, obtaining scaffolds characterized by a single or double layer respectively, with diameters of 2 and 4 mm.

The scaffolds with double layer are more suitable for vascular tissue engineering applications, due to the more elevated and uniform porosity and the larger mechanical strength.

According to the particulate leaching protocol, the scaffold can be obtained by immersing a fibre in a PLLA/dioxane solution containing NaCl sieved particles with a well defined size, slowly drawing the fibre at a constant rate from the solution and immersing in a second bath (DIPS bath). After drying for 24 hours, the salt particles are extracted via leaching by using sistilled water.

Also in this case single and double layer scaffolds have been prepared with diameters of 2 and 4 mm at various extraction rates. Scaffolds obtained at the extraction rate of 5 cm/min via salt leaching are more uniform with respect to the scaffolds produced via DIPS.

Cell cultures (RPMI 2650, nasal mucosa) on scaffolds produced via particulate leaching exhibited better results than the scaffolds obtained via DIPS, with a satisfactory level of adhesion and proliferation and the development of an architecture comparable to the original texture of nasal mucosa in vivo, also confirming the non-cytotoxicity of the used materials.

These preliminary results may address the use of particulate leaching towards vascular tissue engineering applications.

Bioprosthetic heart valve aging deterioration and agerelated Benefits

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Following population aging and due to congenital and acquired diseases the annual number of patients requiring heart valve replacement worldwide has been estimated to triple from approximately 290,000 in 2003, to over 850,000 by 2050 [1]. Prosthetic heart valves are broadly divided in mechanical heart valves (MHV) and bioprosthetic heart valves (BHV) while ≈55% are MHVs and ≈45% BHVs [2] not taking into account most recent transcatheter heart valves. MHVs are made of non-biological material while BHVs are either of allogenic (syn. homografts) or xenogenic origin. The last ones are treated with glutaraldehyde to prevent immune rejection. Mechanical devices have a superior durability but the recipients need permanent lifelong anticoagulant therapy. In turn biological valves exhibit excellent hemodynamic behaviour with a low risk of thromboembolic complications but show limited longevity following structural valve degeneration (SVD) particularly in patients <35 years of age. The definition of SVD has been associated with the intrinsic permanent changes of prosthetic valve leading to degeneration and/or haemodynamic dysfunction that necessitates reoperation [3]. According to an overall evaluation, 49-63% of all valve replacements with glutaraldehyde-treated BHVs [3] have been reported to be free from SVD 20 years after intervention. Otherwise the risk of structural failure is inversely related to the age of patients: it is higher in individuals <35 years of age in which up to 100% of BHVs fail within 5 years postsurgery whereas after 15 years 60-70% of older patients (>75 years) are still free of SVD [4].

In the vast majority of cases SVD is associated with leaflet calcium deposits even if in 19-25% of BHV implants reoperations occur in the absence of leaflet mineralization [5, 6] (excluding nonintrinsic abnormalities, thrombosis and infections). Therefore, considering the elevated levels of oxidized amino acids always associated with bioprosthesis dysfunction, a potentially important role has been prospected also for oxidized amino acid formation [7]. Although not fully understood the mechanism of early failure appears to be complex and multifactorial [6]. The process starts by host IgM/IgG interaction (α-Gal epitope) and macrophage deposition followed by collagen breakdown and finally calcification [8]. Otherwise calcification is believed to be driven by the chemical interaction between phospholipids, residual and oxidized aldehyde groups on one side and circulating calcium ions [4]. A procedure for the suppression of both tissue calcium binding sites and oxidation effects has been developed [9], while to avoid immune injury the use of BHV made of α-Gal free tissue (Gal knockout pigs) has been reported [10]. Moreover a novel procedure aimed to overcome comprehensively immune injury, calcification and oxidative modifications has also been recently proposed [11]. At the present only one of these procedures has been implemented in a clinical trial [9, 12].

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Elastin modification during vascular aging and pathophysiological consequences

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Cardiovascular diseases (CVD) are the leading cause of death worldwide and represent a major problem of public health. Over the years, life expectancy has considerably increased throughout the world and the prevalence of CVD is inevitably rising with the growing aging of the population. The normal process of aging is associated with progressive deterioration in structure and function of the vasculature, commonly called vascular aging. At the vascular level, extracellular matrix (ECM) aging leads to molecular alterations of long half-life proteins, such as elastin and collagen, and have critical impacts on vascular diseases. Elastin, is an insoluble and hydrophobic protein mainly produced by smooth muscle cells in the media and fibroblasts in the adventitia. As elastogenesis is restricted to fetal and infancy, mature elastin fibers remain for lifespan. Indeed, its strong reticulation makes elastin a highly stable molecule with longevity comparable with human lifespan and any proteolytic damage that does occur with age and disease is essentially irreparable. Under pathological conditions, vascular and inflammatory cells produces elastases able to degrade elastin, leading to the production of elastin derived peptides (EDPs) that actively participate through the Elastin Receptor Complex (ERC) to the development of diseases such as diabetes, atherosclerosis, thrombosis and vascular calcification. Consequently, the proactive role of the ECM suggests that the development of a prognostic value and an innovative therapy based on matrix biology has a strong potential. Finally, some modifications affecting elastin such as glycation or carbamylation could occur and possibly affect the elastin network. The works presented here will summarize and discuss these points.

VIVA EVENING CONFERENCE

History and conceptual developments in vascular biology and angiogenesis research

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Vascular biology is an important scientific domain that has gradually penetrated many medical and scientific fields. Scientists are most often focused on present problems in their daily scientific work and lack awareness regarding the evolution of their domain throughout history and of how philosophical issues are related to their research field. In this article, I provide a personal view with an attempt to conceptualize vascular development research that articulates lessons taken from history, philosophy, biology and medicine. I discuss selected aspects related to the history and the philosophy of sciences that can be extracted from the study of vascular development and how conceptual progress in this research field has been made. I will analyze paradigm shifts, cross-fertilization of different fields, technological advances and its impact on angiogenesis and discuss issues related to evolutionary biology, proximity of different molecular systems and scientific methodologies. Finally, I discuss briefly my views where the field is heading in the future.

CELL BIOLOGY AND SIGNALING

Estrogens as vascular anti-ageing hormones? Experimental and clinical evidences and uncertainties

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Aging is associated with structural and functional changes in the vasculature, including endothelial dysfunction, arterial stiffening and remodeling, impaired angiogenesis, and defective vascular repair, and with increased prevalence of atherosclerosis.
Cardiovascular risk is similar for older men and women, but lower in women during their fertile years.
In women, vascular aging seems to be accelerated during the menopause transition, particularly around the late perimenopausal period. This age- and sex related difference points to estrogen as a protective factor because menopause is marked by the loss of endogenous estrogen production.
Experimental studies have attributed part of the protective effects of estrogen to its modulatory action on vascular endothelium. Indeed, estrogen promotes endothelial-derived NO production through increased activity of endothelial NO synthase. However, inhibition of endothelial NO production does not really impair the atheroprotective actions of estrogens. In fact, numerous other cell targets can mediate the actions of estrogens, from smooth muscle cells to various immune cells… Modulations of numerous other factors, such as prostacyclin and hromboxaneA2 release, were also reported and could play a role.
In contrat to the previous epidemiological and observational evidences, the only interventional trial (Women Health Initiative) failed to report any cardiovascular benefit from estrogen replacement therapy, and revealed even an increase in CV events in the older postmenopausal women (i.e. women treated 20 years or more after menopause). This raised the "Timing Hypothesis" states that estrogen mediated vascular benefits occur only before the detrimental effects of aging are established in the vasculature, and could offer a possible « explanation» for these discrepancies. Nevertheless,a gap remains in current knowledge of cardiovascular aging mechanisms in women.
The talk will attempt to summarize clinical and experimental data on the effects of aging, estrogens, and hormone replacement therapy on vascular function of females.

Arteriolar and microcirculation aging

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Resistance arteries are the small blood vessels located upstream from capillaries. They are crucial for the delivery of blood to vital tissues at relevant flow and pressure. Disorders in resistance arteries structure and function raise capillary pressure and cause downstream organ damage such as that seen in diabetes, hypertension or kidney disease, whose frequency increases with age. Resistance arteries have a basal tone allowing a tight control of local blood flow. This basal tone results of the interaction between myogenic tone due to the activation by pressure of vascular smooth muscle contractility and flowmediated dilatation (FMD) due to the activation of endothelial cells by shear stress. A reduced FMD is the hallmark of endothelium dysfunction. FMD is altered in ageing and in both cardio-vascular and metabolic disorders. Long-term decreases or increases in blood flow induce inward or outward remodelling of resistance arteries respectively. Although flow-mediated outward remodelling is essential in post-ischemic revascularization, it is altered in ageing and this reduced responsiveness is aggravated by the main risk factors. These latter increase oxidative stress and inflammation at the tissue level leading to alter FMD and then to inward remodelling which in turn accelerates oxidative stress and inflammation. Breaking this vicious circle is a major challenge in most countries with a rise in longevity and a high level of exposure to the risk factor associated to the so-called Western way of life. Recent studies have identified the estrogens receptors has key modulators of both FMD and flow-mediated outward remodelling independently of their effect on reproduction, thus opening a new field of investigation in the search for more accurate therapeutic tools reducing vascular ageing.

Oxidation and Inflammation in Atherosclerosis

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Atherosclerosis is a chronic inflammatory condition, characterized by the accumulation of lipids and fibrous elements in the intimal layer of medium and large arteries, that could turn into an acute clinical event due to plaque ulceration and thrombosis. Indeed, plaque rupture is the predominant underlying process in the pathogenesis of acute coronary syndromes and peripheral vascular disease. Although a great deal of work has been done on it, the mechanisms underlying plaque formation and progression to advanced lesions are not yet completely known.

It is generally held that plaque instability is caused by a substantial increase in inflammatory and proteolytic activities. Furthermore, some lines of evidence suggest that unstable plaques are also characterized by pronounced oxidative environment. In situ oxidative events may determine lipid/protein metabolic fate, bioactivity, and antigenic properties. In this respect, oxidized LDL is readily internalized by macrophages through the so-called "scavenger receptor" pathway. These early modifications could initiate and/or contribute to atherogenesis, mainly when an imbalance between oxidant and antioxidant agents takes place. Although several studies report that atherosclerotic plaques contain high concentrations of some amino acid oxidation products, caused mainly by carbonylation, ROS, and RNS oxidation or thiolation, limited information are available regarding the relationship between the accumulation of markers of oxidized proteins and atherosclerosis severity. Furthermore, different oxidation-specific epitopes can be detected in blood and may reflect atherosclerosis manifestations. At present, the mechanisms underlying the formation of these by-products and the relevance for disease progression are under investigation.

Although oxidative stress has been long associated with the genesis and progression of the atherosclerotic plaque, scanty data on its *in situ* effects on protein sulfhydryl groups oxidation are available. In this respect, reduced protein sulfhydryls, which are in most cases key functional groups, represent a target for several post-translational oxidative modifications. In the last years, we investigated the entity of these oxidations, focusing on low molecular weight thiols adduction, in advanced human carotid plaques, also in relation with plaque vulnerability. By this way, we evidenced deep differences between stable and unstable atherosclerotic plaques as well as between extractable plaque proteins and plasma proteins. Furthermore, by analysing both circulating and plaque-filtered serum albumin (HSA), we evidenced that the pro-oxidant environment present in atherosclerotic tissue could modify filtered proteins also by protein-SH group oxidation, probably contributing to plaque progression. In this regard, our studies suggest, for the first time, that once filtered, albumin represents a harmful source of homocysteine and cysteinylglycine inside the plaque environment. In this respect, the contribution of GSH to the intra-plaque protein-bound LMW thiols equilibrium seems to be particularly relevant.

SELECTED ORAL PRESENTATION

MITOCHONDRIAL FUNCTION REGULATES VASCULAR AGING IN MICE

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Résumé

Mitochondrial DNA (mtDNA) damage is present in aging tissues and may promote loss of tissue function. However whether mtDNA damage or mitochondrial dysfunction is present in the aging vasculature or contributes to vascular aging is unknown. We aimed to determine the time course of functional and structural changes of normal vascular aging in mice, the effect of vessel aging on mitochondrial function, and whether decreased or increased mtDNA damage delays or promotes vascular aging respectively. Wild-type (WT) C57Bl/6 mice were studied at 8, 22, 44 and 72 wk of age by ultrasound imaging and intra-arterial blood pressure measurements using single and dual pressure catheters to provide a range of functional parameters of vascular aging. Vascular aging was detected in WT mice between 22-44wk of age, with reduced carotid arterial compliance and distensibility and increased b stiffness index (bSI), and increased aortic pulse wave velocity (PWV) (all $P < 0.05$). No additional changes were noted between 44-72wk. Aortic collagen content and elastin breaks also increased by 57% and 4-fold respectively (both $P < 0.05$) between 22-44wk. Similarly, mtDNA copy number assessed by quantitative PCR decreased significantly between 22-44wk ($P < 0.05$) and mitochondrial respiration assessed by a Seahorse flux analyser decreased by 25% ($P < 0.05$). To determine the effects of mtDNA damage, we studied vascular aging in mice that overexpressed the mitochondrial helicase Twinkle (Tw+), or with a mutation in the proof-reading ability of the mitochondrial polymerase gamma (PolG). Twinkle overexpression restored mtDNA copy number with concurrent improvement in mitochondrial respiration, and delayed all physiological parameters of vascular aging associated with decreased collagen and elastin breaks ($P < 0.05$). In contrast, PolG mice with increased mtDNA damage showed accelerated vascular aging compared to controls ($P < 0.05$). We have identified multiple, reproducible parameters of arterial aging in mice that are detected at far earlier time points than previously described; in particular, compliance, distensibility and bSI at 44wk provide the earliest discrimination. Arterial mitochondrial function reduces markedly with age, and accelerates vascular aging, whereas augmenting mitochondrial function delays aging, identifying prevention of mtDNA damage and dysfunction as a therapeutic target in aging.

Mots-Clés: vascular, aging, mitochondria

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Structural imaging of the vascular wall

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Résumé

The extracellular matrix is a complex assembly produced by our cells. This structure is crucial for the proper functioning of tissue because it provides support and mechanical anchoring. Elastin is a fibrous protein of the extracellular matrix that is synthetized before teen age. Elastin bestows its elastic properties to the arterial wall and therefore contributes to its mechanical resistance. Elastin is intrinsically auto-fluorescent and thus observable on tissue slice. As a consequence, elastin alterations such as those occurring during normal aging or chronic age-related vascular pathologies can be imaged.

The aim of our work is to image elastin inside the murine aortic wall and hereafter, to extract morphological and quantitative parameters from these images. Statistical analysis of these data could characterize the status of the system and could allow us to infer about the evolution of the vascular integrity. This preliminary work was started using murine aortic slices observed by fluorescence microscopy. Further, large aortic segments of several millimeters were imaged by X-Ray computed micro-tomography at the submicron scale.

Our very first results suggest that it is possible with a minimal set of morphological descriptors to characterize the matrix condition of an aortic section, and thus, to potentially detect the occurrence of a pathology, even at an early stage, before its clinical onset. Importantly, our first experiments with a synchrotron light source indicate that it is possible to image "large segment" of very small artery at high resolution allowing the global analysis of the ultrastructure of the matrix within the arterial wall. Using this high-resolution methodology, we observed a "weft" inside the aortic matrix structures. We think that this feature may provide mechanical resilience to the artery and that its integrity could be the sign of its good health.

Mots-Clés: Aortic wall, Elastin, Imaging, Image analysis

Numerical assessment and comparison of pulse wave velocity methods aiming at measuring aortic stiffness

Résumé

Numerical assessment and comparison of pulse wave velocity methods aiming at measuring aortic stiffness

Pulse waveform analyses have become established components of cardiovascular research. Recently several methods have been proposed as tools to measure aortic pulse wave velocity (aPWV). The carotid-femoral pulse wave velocity (cf-PWV), the current clinical gold standard method for the noninvasive assessment of aPWV, uses the carotid – to – femoral pulse pulse transit time difference (cf-PTT) and an estimated path length to derive cf-PWV. The heart-ankle PWV (ha-PWV), brachial-ankle PWV (ba-PWV) and finger-toe (ft-PWV) are also methods presuming to approximate aPWV based on time delays between physiological cardiovascular signals at two locations (‿ heart-ankle PTT, ha-PTT; ‿ brachial-ankle PTT, ba-PTT; ‿ finger-toe PTT, ft-PTT) and a path length typically derived from the subject's height. To test the validity of these methods, we used a detailed 1D arterial network model (143 arterial segments) including the foot and hand circulation. The arterial tree dimensions and properties were taken from the literature and completed with data from patient scans. We calculated PTT's with all the methods mentioned above. The calculated PTT's were compared with the aortic PTT (aPTT), which is considered as the absolute reference method in this study. The correlation between methods and aPTT was good and significant, cf-PTT ($R^2 = 0.97$; $P < 0.001$; mean difference 5 ± 2 ms), ha-PTT ($R^2 = 0.96$; $P < 0.001$; 150 ± 23 ms), ba-PTT ($R^2 = 0.96$; $P < 0.001$; 70 ± 13 ms) and ft-PTT ($R^2 = 0.95$; $P < 0.001$; 14 ± 10 ms). Consequently, good correlation was also observed for the PWV values derived with the tested methods, but absolute values differed because of different path lengths used. In conclusion, our computer model based analyses demonstrate that for PWV methods based on peripheral signals, pulse transit time differences closely correlate with the aortic transit time, supporting the use of these methods in clinical practice.

Mots-Clés: Pulse wave velocity, Pulse wave analysis

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Long-term trajectories of cardiometabolic risk factors in prodromal dementia: the Three-City Study

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Objective: To describe the trajectories of major cardiometabolic risk factors (body mass index [BMI], systolic and diastolic blood pressure [SBP, DBP], fasting blood lipids [HDL and LDL cholesterol, triglycerides] and glycemia) in the 14 years preceding dementia diagnosis, and to compare them to the trajectories of matched subjects free of dementia, using latent process mixed models.
Design: Case-control study nested in a prospective cohort.
Setting: The Three-City Study, a population-based cohort of older persons (≥65 years) enrolled between 1999 and 2001 in three French cities (Bordeaux, Dijon, and Montpellier) who underwent up to six repeated home visits with neuropsychological testing and measurements of anthropometric parameters and blood pressure, and up to three repeated blood draws for assessment of biological parameters.
Participants: 785 incident cases of dementia and with evaluation of cardiometabolic risk factors at baseline individually matched (for gender, age, education, and study center) to 3,140 controls at the time of dementia cases diagnosis.
Results: In both cases and controls, BMI, DBP and HDL declined in the 14 years preceding the matching visit, whereas SBP and glycemia increased and LDL and triglycerides remained stable over time.
Compared to controls, dementia cases had a faster decline in BMI and a lower increase in SBP ($P<.001$ and $P<0.049$ respectively for group-by-time interactions) leading to significantly lower BMI and SBP values among cases respectively 2 and 4 years before diagnosis. The other cardiometabolic risk factors (DBP, lipids and glycemia) did not evolve differently according to disease status (all P for group-by-time interactions>0.20); however cases presented lower DBP and higher fasting glycemia levels over the entire period.
Conclusion: Higher BMI, blood pressure and blood lipids were not found as strong risk factors for dementia in the older age range; on the contrary BMI and blood pressure decreased months to several years before dementia diagnosis (possibly as a consequence of changes in the prodromal phase of the disease). Blood glucose was the single cardiometabolic risk factor with constantly higher values over the 14 years before diagnosis among incident dementia cases, suggesting that elevated glycemia is among the strongest cardiometabolic risk factor for dementia in older persons.

MicroRNA expressed in progenitor cells circulating in peripheral blood as prospective biomarkers of agerelated macular degeneration (AMD) in its dry and wet form – preliminary data

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Abstract:
Objectives: Age-related macular degeneration (AMD), a worldwide public health problem, has been recognized as a common cause of visual dysfunction in elderly people. MicroRNAs (miRNAs) present in sera are characteristically altered in many pathological conditions and have been used as biomarkers for specific diseases, including AMD. Several miRNAs can be detected in bodily fluids including serum, urine and saliva, where they are released in form of exosome vesicles. However, different physical conditions, including blood oxygen tension, alter the exosome release and affect the global miRNA profile in fluids modifying their bioactivity as a consequence. In contrast, being resistant to exonucleolytic activities, intracellular miRNAs are more stable and have longer half-life than extracellular miRNA, therefore may play a role in development and progression of diseases. Due to the high abundance, stable and accessible characteristics, intracellular miRNAs could serve as putative biomarker molecule in clinically relevant samples. Thus, in our study, we analyzed selected miRNAs from peripheral blood mononuclear cells (PBMCs) in patients with dry and wet form of AMD and healthy volunteers (HV), and we investigated the intracellular miRNAs expression as potential biomarker for early diagnosis of dry or wet form of AMD.
Material and Methods: PBMCs were collected from 94 AMD patients (46 wet form and 48 dry form) and 46 HV as a source of miRNAs. Self-validated individual quantitative RT-PCR study was then performed to evaluate the expression levels of selected miRNAs. The Mann-Whitney U test was used for statistical analysis.
Results: miRNA expression analysis revealed a decreased expression of selected angiogenesis-related miRNAs in both forms of AMD, including miR-223-3p, miR-17-5p (both greatly down-regulated in dry AMD), and miR-150-5p (stronger down-regulation in wet AMD). In contrast, two angiogenic miRNAs: miR126-5p and miR126-3p were upregulated, especially in dry AMD.
Conclusion: Our results indicated that altered expression of selected miRNAs may play a role in the AMD development and progression. Further studies are required to determine causal relationships and elucidate underlying mechanisms. This work was supported by grants: STRATEGMED1/234261/2NCBR/2014 and UMO-2013/09/B/NZ7/04031
Keywords: MicroRNA, age-related macular degeneration (AMD), dry AMD, wet AMD, angiogenesis, intracellular miRNAs, disease biomarkers

Molecular, conformational and thermal characterization of ventricular Remodeling in a pig model of tachycardia-induced cardiomyopathy

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Resume
Aims: Non-ischemic dilated cardiomyopathy (NIDCM) is characterized by ventricular dilatation associated to pathological remodeling. Our aim was to compare molecular, conformational and physical characteristics of healthy and NIDCM myocardium to identify crucial changes in the evolution to pathological cardiac remodeling.
Methods and Results: Our study includes healthy pigs (control, N=7) and tachycardia-induced NIDCM pigs (DCM, N=10). Samples from right (RV) and left ventricle (LV) were frozen at -80°C. Real-time PCR showed a strong increase in collagen I and collagen **III** in both RV (2.06 and 1.38-fold) and LV (2.43 and 1.48-fold) dilated ventricles. Fourier Transform Infrared Spectroscopy (FTIR) 1st [A(1171cm-1)/A(1338cm-1)] and 2nd [A(1304cm1)/A(1338cm-1)] indicators reflect myofiber/collagen ratio while [A(1043cm-1)/A(2800cm-1- 3000cm-1)] 3rd indicator reflect carbohydrate/lipid ratio. 1st indicator was reduced in both dilated ventricles (to 43% in RV and to 37% in LV), while 2nd indicator was only reduced in dilated RV (to 37%). 3rd indicator was upregulated in both dilated ventricles, but to a higher extent in RV (2.60-fold vs 1.61-fold, P=0.049). Histological Masson's trichromic staining showed unorganized and agglomerated collagen in dilated ventricles, and a higher percentage of fibrosis in RV compared to LV (P=0.016). Both **FITR** and thin layer chromatography revealed the cancellation of the differences in cholesteryl ester and triglyceride content between RV and LV (inherent to control pigs) in DCM group. Differential Scanning Calorimetry (DSC) evidenced a strong decrease of protein denaturation temperature (a shift of 50°C in the freeze-dried state) in both dilated ventricles, associated with a significant depression of the freezable water melting point.
Conclusion: Our results point to reduced myofiber/collagen ratio, increased carbohydrate/lipid ratio and lower protein thermal stability as key alterations contributing to the deposition of unorganized and agglomerated collagen in dilated ventricles. The exacerbation of these alterations in RV underlies deeper remodeling of RV vs LV in the evolution to dilated cardiomyopathy.
Mots-Clés: Heart failure, cardiac remodeling, myofiber, collagen, FITR, DSC

Valvular calcification: implication of the Semicarbazide-Sensitive Amine Oxidase (SSAO)?

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Résumé

Introduction: Calcific aortic valve disease (CAVD) leads to aortic stenosis, a common disease associated with aging. CAVD shares similarities with the process that regulates atherosclerosis but the full mechanisms leading to CAVD are still unclear. Apart from surgical replacement of the valve, therapeutic options are limited, calling for new potential targets involved in calcifications. A relationship between serum Vascular Adhesion Protein 1 (VAP-1) and calcific aortic stenosis has been described but nothing is known on the tissular form of VAP-1. VAP-1, also called "Semicarbazide-Sensitive Amine Oxidase" (SSAO), is an enzyme that transforms primary amine into aldehyde, H2O2 and ammonia but also functions as an adhesion molecule. Interestingly, VAP-1 has been implicated in inflammatory diseases, atherosclerosis and differentiation process of several cell types. The aim of this study was to highlight the involvement of SSAO in valvular calcifications and to develop a primary culture model of interstitial cells of aortic valves (PAVICs) to study the potential molecular mechanisms involved in this disease process.
Methods: 13 Human aortic valves were either embedded into paraffin for the histological and immunolocalisation study, or snap frozen in highly, intermediated and non-calcified areas for SSAO enzyme activity assays. Porcine aortic valves are collected in the chirurgical school of Nancy to establish primary cultures of valvular interstitial cells (VICs).

Results: Our results showed a significant increase in SSAO protein expression and enzyme activity in calcified area of the human aortic valves compared to healthy area of the valves. After 14 days of cell culture, SSAO activity is also detectable in PAVICs.
Conclusion: This is the first time that tissular SSAO expression and activity has been associated with valvular calcification. However, the mechanisms need to be studied in detail.

Mots-Clés: aortic valves, calcifications, aortic valvular interstitial cells, "semicarbazide, sensitive amine oxidase", hydrogen peroxide

*Intervenant

Vascular calcification during chronic kidney disease : role of the RAGE/Cathepsin S/elastin peptides axis

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Résumé

Vascular calcification is a common feature of patients suffering from chronic kidney disease (CKD). We recently reported a role for the Receptor for Advanced Glycation End products (RAGE) in the uremic vascular calcification process. Following engagement by uremic toxins, RAGE mediates active osteodifferentiation of vascular smooth muscle cells (VSMCs) and vascular calcifications. This process is mainly found in aorta where an intense elastolysis is observed leading to the release of elastin derived peptides (EDPs). Using animal models, several reports from the literature have suggested the possible involvement of Cathepsin S in elastolysis and vascular calcification during CKD. However, the mechanisms implicated and the putative role of RAGE in Cathepsin S expression and the subsequent elastolysis have not been described.

To answer these questions, we used a mouse model of uremic vasculopathy, induced by 5 of 6 nephrectomy in the ApoE-/- or APoE-/-/RAGE-/- backgrounds. Moreover, we used primary cultures of VSMCs isolated from control or RAGE deleted animals for in vitro studies.

We found that induction of CKD increases the calcification process in the cardiac valves of ApoE-/- mice whereas ApoE-/-/RAGE-/- mice are protected. Moreover, sera and aortas analysis showed that Cathepsin S expression and elastolysis seem to be greater in ApoE-/- compared to the ApoE-/-/RAGE-/- animals. Using recombinant Cathepsin S, we found that this protease was able to directly drive insoluble elastin degradation and bioactive EDPs production as demonstrated by electron microscopy scanning and mass spectrometry analysis. Finally, we showed that an in vitro calcification process is triggered when VSMCs are incubated with inorganic phosphate. This phenomenon is increased in the presence of EDPs enhancing expression of osteoblast differentiation specific genes.

In conclusion, we report that in uremic conditions, increased Cathepsin S expression may promote elastolysis. This process generates bioactive EDPs which could accelerate osteogenic differentiation of VSMCs and apparition of vascular calcification. RAGE engagement may be involved in the initial steps leading to increased Cathepsin S production.

Mots-Clés: vascular calcification, RAGE, cathepsin S, elastin derived peptides, chronic kidney disease

Targeting Connexin40 expression or function reduces angiogenesis in the developing mouse retina

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Résumé

Introduction: Connexins constitute a large family of trans-membrane proteins that form gap junction channels enabling direct transfer of small signaling molecules from cell-to-cell. We recently found that altering Cx40 expression or function in endothelial cells impairs their ability to vascularise tumor xenografts. However, how Cx40 contributes to angiogenesis has not yet been investigated.

Methods: We used the mouse neonatal retina model and Cx40-deficient (Cx40-/-) mice to explore Cx40 expression and function in developmental angiogenesis. We used immortalized mouse brain microvascular ECs to explore the contribution of Cx40 channels on cell properties and behavior in vitro.

Results: Our data show that Cx40 is expressed in the immature vascular plexus of the developing retina. Genetic ablation of Cx40 causes retinal hypovascularization in the postnatal mouse eye, with reduced radial vessel growth and decreased capillary density as compared to WT mice. At the angiogenic front, Cx40-deficiency attenuates vessel sprouting and the mural cells recruited on the neovessels display an altered phenotype. These alterations can be attributed to disturbed endothelial cell functions as selective re-expression of Cx40 in these cells restores normal angiogenesis. In vitro, targeting Cx40 by silencing its expression or by blocking gap junction channels, decreases endothelial cell proliferation, modifies their secretome and promotes mural cell chemoattraction. Finally, a Cx40 inhibitory peptide (40Gap27) phenocopies the loss of endothelial Cx40 in vitro and in vivo.

Conclusions: Our data show that endothelial Cx40 regulates retinal vessel growth and maturation. Cx40 thus represents a novel potential target for treating pathological ocular angiogenesis.

Mots-Clés: gap junctions, retinal development, endothelium, Connexin40, angiogenesis

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*Intervenant

Imatinib inhibits hypoxia-induced MMP-9 overexpression and activation through impairment of LRP1 and Pyk2 hosphorylation in human coronary vascular smooth muscle cells

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Resume

Introduction. An imbalance in the migration of vascular smooth muscle cells (VSMCs) is associated with the progression of vascular diseases. Low-density lipoprotein receptor-related protein 1 (LRP1) is known to participate in VSMC migration through activation of metalloproteinases (MMPs) and Pyk2 in human VSMCs (hVSMCs). Hypoxia increases hVSMC migration through an upregulatory effect on LRP1-pPyk2-MMP-9 axis. Gleevec, a protein kinase inhibitor, reduces the area of the atherosclerotic plaque in diabetic apoE-K0 mice. The aim of this work was to study the effect of Gleevec in LRP1-pPyk2-MMP-9 axis and hVSMC migration.

Methodology. The hVSMCs were maintained in medium 199 supplemented with 20 % fetal bovine serum, exposed to normoxia (21% 02) or hypoxia (1% 02) and treated with Gleevec 20 p,M for 20 hours. Protein levels of p-LRP1, LRP1 (b-chain), pPyk2, total Pyk2, pERK1/2, Total ERK1/2 and β-tubulin was measured by Western blot. The formation of protein complexes was analyzed by immunoprecipitation experiments. MMP9 and MMP2 expression and activity were tested by Real time PCR and zimography, respectively. Migration studies were performed using the technique of wound repair assays after scratching a cell monolayer

Results. Real time PCR and zimography data showed that hypoxia failed to increase MMP-9 expression and activity in Gleevec-treated hVSMC. Wound repair assays showed that Gleevec prevented the increase on hVSMCs migration induced by hypoxia. Immunoprecipitation and Western blot assays revealed that the amount of LRP1/pPyk2 complexes increased under hypoxic conditions but that hey almost completely absent in Gleevedtreated hVSMC compared to control hVSMC Conclusions. Gleevec effectively prevented hypoxia-induced hVSMC migration. Therefore, Gleevec could be used to counteract the deleterious effects of hypoxia on vascular remodeling.

Mots-Cles: Imatinib, hVSMCs, LRP1, MMP9, Gleevec

NRP1 supresses TGFβ-dependent ERK1/2 activation in endothelial cells

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Vascular endothelial cells express the transmembrane receptor neuropilin 1 (NRP1) to promote vascular endothelial growth factor VEGF-A and integrin ligand-induced extracellular matrix signalling. Recently, NRP1 was shown to also repress transforming growth factor β (TGFβ) signalling to modulate endothelial cell signalling. All three pathways cooperatively regulate retinal blood vessel growth. Consistent with prior work, we also find that NRP1 represses TGFβ signalling. However, in addition to the previously proposed pathway, in which NRP1 loss de-represses canonical SMAD signalling, we find that NRP1 loss also increases endothelial TGFβ expression and noncanonical TGFβ signalling via ERK1/2 activation in both mouse and human endothelial cells. Moreover, we show that knockdown of the TGFβ receptor TGFBR2 or its co-receptor ALK1 blocks NRP1-dependent ERK1/2 activation and ERK1/2-dependent gene transcription, a pathway previously shown to accelerate cellular aging. Taken together with prior work, these findings identify NRP1 as a multifunctional regulator of endothelial cell behaviour.

The elastin receptor complex interacts with CD36 through NEU1 and regulates oxidized **LDL** uptake in macrophages: potential implication in the proatherogenic effects of elastin-derived peptides

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Resume
Vascular aging is associated with extracellular matrix (ECM) remodeling and proteolysis of elastin. Elastin is a major ECM component of the arterial wall that provides the elastic recoil properties and resilience essential for proper vascular function. Elastin degradation leads to the generation of elastin-derived peptides (EDP) that exert biological effects through the plasma membrane neuraminidase 1 (NEU1), a sialidase catalyzing the removal of terminal sialic acids from sialyloconjugates. This sialidase interacts with the elastin binding protein and the protective protein/cathepsin A forming the elastin receptor complex (ERC) at the plasma membrane. Results of our lab from the last five years have brought significant insights on the critical role played by NEU1 in signal transduction and pathophysiological effects mediated by the ERC and EDP in vascular diseases such as type 2 diabetes, atherosclerosis and thrombosis.
In this study, we developed a proteomic approach to purify and identify the interaction partners of membrane NEU1 from human macrophages differentiated from THP1 cells. Among the potential candidates, the class B scavenger receptor CD36 was identified. Interaction between NEU1 and CD36 was confirmed by co-immunoprecipitation and colocalization experiments. Using a lectin-affinity assay, we show that EDP desialylate CD36 and that CD36 desialylation by EDP is blocked by the V14 peptide, a peptide blocking the interaction between bioactive EDP and the ERC. Finally, we show that EDP increase the uptake of oxidized LDL by macrophages that is blocked by both the V14 peptide and DANA, a sialidase inhibitor.
In conclusion, these results demonstrate for the first time that the ERC interacts with CD36 through NEU1 at the plasma membrane of macrophages. Binding of EDP to the ERC modulates CD36 sialylation level and regulates oxidized LDL uptake by macrophages through NEU1. These effects could contribute to the previously reported proatherogenic role of EDP in atherosclerosis.
Mots-Cles: elastin peptides, neuraminidase, interaction partners, CD36, macrophages

UBIAD1 PROMOTES TUMOR ANGIOGENESIS BY INHIBITING DEPHOSPHORYLATION OF VEGFR2 IN A REDOX DEPENDENT MANNER

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* Intervenant

Tumor microenvironment accumulate reactive oxidative species (ROS) during tumor formation. This in important for the tumor cells to proliferate and transform but can also challenges the endothelial cells present in the tumor. That's why the tumor endothelial cells need to express antioxidants to compensate for high oxidative level and to limit cellular damage. Tumor endothelial cells are also neoangiogenic as they need to invade surrounding tissues to 'feed' the tumor. This is associated to the activation of the VEGF pathway. We are interested in an antioxidant enzyme called Ubiad1 that is located to the Golgi and important for the biosynthesis of antioxidant molecules Coq9 /10 and Vitamin K2. Ubiad1 is mutated in Schnyder crystalline corneal dystrophy (SCCD) where cholesterol accumulates in the cornea leading to blindness. Here we show that Ubiad1 SCCD endothelial cells are more angiogenic leading to formation of bigger tumors when lung carcinoma cells were implanted subcutaneously. This was associated with a higher level of activation of VEGFR2. Antioxidants Coq9/ 10 levels were also higher in Ubiad1 SCCD endothelial cells. High level of antioxidants also induce oxidative modification (glutathionylation) of the phosphatase LMW-PTP, making it inactive. This phosphatase was shown before to dephosphorylate VEGFR2 making it inactive12. Thus we propose a mechanism in which high levels of Ubiad1/ antioxidants in SCCD cells appear to promote activation of VEGFR2 by promoting oxidative modifications of LMW-PTP. This work suggests that manipulation of redox state by targeting antioxidant enzymes provides an innovative approach to block tumor angiogenesis, as inhibition of glutathionylation significantly reduced tumor growth and angiogenesis, in wild-type but not Ubiad1 SCCD mice.
Authors: C Millia, X Chen, W Lee, Elisabetta Grillo, Y Jonnson, C Aelbrecht, E Turco, C Medana, MM. Santoro

OPEN SESSION

Endothelial cell dysfunction is a new potential therapeutic target for the treatment of critical limb ischemia

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* Intervenant

Therapeutic angiogenesis for ischemic diseases including critical limb ischemia (CLI) failed to demonstrate significant clinical improvement. In the present study, we explored endothelial cell (EC) dysfunction in the pathophysiology of CLI, as a possible alternative therapeutic target. In a systematic histological analysis of CLI human biopsies, we found that CLI muscles were characterized by evident signs of myopathy, but that capillary density within the ischemic muscle was equivalent to that of a healthy skeletal muscle in control subjects. Hence, making more capillaries may not be what is necessary to improve ischemic muscle perfusion, giving a potential explanation for the failure of angiogenic therapies. However, inflammation and interstitial edema suggested the implication of EC dysfunction. We then adapted a mouse model of chronic limb ischemia recapitulating the features of human CLI to test whether ameliorating EC function may improve muscle perfusion and decrease signs of CLI. We treated mice with the Hedgehog agonist SAG to improve EC function and found that it prevented muscle inflammation and edema, improved muscle perfusion and prevented myopathy thus revealing a new alternative therapy for CLI.

Podosomes Mediate Basement Membrane Collagen-IV Proteolysis During Sprouting Angiogenesis

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Résumé

Introduction: During angiogenic sprouting, endothelial 'tip cells' emerge from existing vessels in response to VEGF-A. These cells are invasive meaning that they are capable of breaching the basement membrane that forms a sleeve around the capillary. Although it has been known for long that metalloproteases play a key role in this process, how cells handle this step have not been characterized. In the present work, we have examined whether F-actin/cortactin/P-Src-based matrix-degrading microdomains called podosomes contribute to this step.

Methods: To explore the role of podosomes in the angiogenic process, we developed a novel asymmetric 3D-assay recapitulating sprouting angiogenesis in vitro. We used the mouse neonatal retinal angiogenesis model to detect then analyse podosome formation during sprouting angiogenesis in vivo.

Results: In vitro, VEGF-A/Notch signaling regulates the formation of functional podosomes in endothelial cells in 2D as well as in tip cell-like cells in 3D. In vivo, we demonstrate that tip cells assemble podosomes during physiological retinal angiogenesis. In the retina, podosomes are also part of an interconnected network that surrounds large microvessels and impinges on the underlying basement membrane. Consistently, collagen-IV is scarce in podosome areas. Moreover, Notch inhibition exacerbates podosome formation and collagen-IV loss, indicating that Notch signaling regulates this process.

Conclusion: We propose that the localized proteolytic action of podosomes on basement membrane collagen-IV facilitates endothelial cell sprouting and anastomosis within the developing vasculature. The identification of podosomes as key components of the sprouting machinery provides another opportunity to target angiogenesis therapeutically.

Mots-Clés: cytoskeleton, actin, VEGF, A, Notch/Dll4 signaling, podosomes, tip cells, angiogenesis

*Intervenant

How plant food bioactives may have benefits for vascular health? The case of curcumin.

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Resume

Diet is recognized as an important determinant of health. During the last decade, the protective effect of fruits and vegetables on cardiovascular health has been correlated to the high content of these foods in bioactive compounds, especially polyphenols. The interest of these compounds for cardiovascular health is also supported by clinical studies reporting beneficial effects of polyphenol-rich fruit consumption on intermediate markers of cardiovascular diseases. In addition, animal and in *vitro* studies provided a number of results accounting for a positive role of polyphenols to prevent some dysfunctions associated to the development of cardiovascular diseases. Among these bioactives is curcumin. We previously showed that curcumin reduced the infiltration of immune cells into the vascular wall and prevented atherosclerosis development in mice. This study aimed to decipher the underlying mechanisms of these actions investigating the effect of curcumin on endothelial cell function. Human umbilical vein endothelial cells (HUVECs) were exposed to curcumin (0.5-1 µM) for 3 hours prior to their activation by Tumor Necrosis Factor alpha (TNF-a). Endothelial permeability, monocyte adhesion and transendothelial migration assays were conducted under static condition and shear stress that mimics blood flow. We further investigated the impact of curcumin on signaling pathways and on the expression of genes using macroarrays.

Pre-exposure of endothelial cells to curcumin reduced monocyte adhesion and their transendothelial migration in both static and shear stress conditions. Curcumin also prevented changes in both endothelial permeability and the area of HUVECs when induced by TNF-a. We showed that curcumin modulated the expression of 15 genes involved in the control of cytoskeleton and endothelial junction dynamic. Our findings demonstrate the ability of curcumin to reduce monocyte TEM through a multimodal regulation of the endothelial cell dynamics with a potential benefit on the vascular endothelial function barrier.

Mots-Clés: polyphenols, curcumin, vascular endothelial cells, integrity

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P.08 **THE ROLE OF THE BLOOD-BRAIN BARRIER IN AMYLOID-B BIOLOGY: CONTRIBUTION TO ALZHEIMER'S DISEASE.** Amaia Dominguez-Bel-loso

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Studying in vivo the influence of isometric contraction in human vascular physiology

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Résumé

The so called "muscular pump" is a major determinant of the circulatory homeostasis, in particular in the lower limb as part of the venous return mechanisms. However, the mutual relationships established between the muscle under activity and the local circulation, is not entirely characterized. So, addressing these aspects, here we develop an experimental model to evaluate the impact of the gastrocnemius activity on the microcirculation. Six healthy subjects (31 ± 9 years old) both genders (3 female and 3 male) participated in this study after informed consent. We tested the isometric plantar flection in the standing position. After standing for 5 min, subjects maintained for 1 min an isometric plantar flexion with both feet, then resuming the original position for another 5 min. Local (foot) perfusion was evaluated by photoplethysmography (PPG) and laser Doppler flowmetry (LDF). Pulse rate (PR) was obtained from PPG and the calf muscle activity followed by electromyography (EMG). Nonparametric statistics were used for phase comparisons. During the gastrocnemius activity significant perfusion changes were detected for LDF and PPG signals, rapidly returning to basal values after contraction stopped. The pulse rate did not change during the three protocol phases. Thus, this model looks suitable to further explore the the impact of muscle contraction in the human in vivo microcirculation.

Mots-Clés: gastrocnemius, microcirculation, EMG, LDF, PPG

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Exploring the murine microvascular response to hyperoxia with the wavelet transform

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Résumé

100% oxygen breathing is commonly used to dynamically assess microcirculation in vivo although its effects are not totally understood. Laser Doppler flowmetry (LDF) is a reference technique to quantify in vivo microcirculation, providing an oscillatory signal composed of several frequencies which are still poorly described in murine. Our aim was to identify these components of the murine cutaneous microcirculation Doppler signal, using the wavelet transform (WT). 16 male C57/BL6 mice (18 ± 10 weeks old) were submitted to a 100% oxygen breathing protocol under ketamine-xylazine anesthesia, consisting in three phases – 10 min room atmosphere breathing, 10 min NOB, and 10 min recovery. LDF signals were recorded on both hind paws. All procedures involving animal experimentation were ethically supervised. Nonparametric statistics were applied. Three different bilateral responses to hyperoxia were observed – a bilateral perfusion decrease (PD) was observed in 6/16 mice, a bilateral perfusion increase (PI) in 2/16 mice and a mixed response (decrease in one limb and an increase in the contralateral limb) in 8/16 mice. WT allowed the identification of several LDF frequency ranges, compatible with the cardiac (5.3-4.6 Hz), respiratory (3.8-3.2 Hz), myogenic (0.17-0.059 Hz), sympathetic (0.052-0.020 Hz), endothelial NO dependent (0.017-0.0094 Hz) and endothelial NO independent (0.0084-0.0042 Hz). The limbs that responded with PD showed a significant increase in the cardiac and myogenic activities and a significant decrease in the NO-dependent activity. The limbs that responded with PI showed a significant increase in the cardiac and myogenic activities, with no significant changes in the endothelial components. These results show the murine LDF frequency ranges, and also highlight the usefulness of the wavelet transform in characterizing the microvascular response variability to hyperoxia.

Mots-Clés: Animal model, hyperoxia, LDF, wavelet transform

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About the laser Doppler flowmetry signal oscillatory properties of a murine models of diabetes

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Résumé

Skin microcirculation is widely regarded as a representative model to study physiological and pathophysiological vascular regulation mechanisms. Laser Doppler flowmetry (LDF) signal, although its complex oscillatory origin, has been very helpful in understanding microvascular dynamics *in vivo*. Hyperoxia is also used as a stimulus that accentuates the differences in microvascular responsiveness between normal and pathological states, helping to test these dynamics. Here, we aimed to characterize the microvascular reactivity of a murine model of diabetes based on the oscillatory properties of the LDF signal. Two mice groups were subjected to a 100% oxygen breathing protocol – 8 *C57BL/KsJ-db/db* mice (24.2 $\pm$ 9.1 weeks old) and 8 age-matched controls under ketamine-xylazine anesthesia. The LDF signals were acquired at the hindpaws for 30 min (10 baseline, 10 with hyperoxia, 10 recovery) under ketamine-xylazine anesthesia. All procedures involving animal experimentation were ethically supervised. Frequency spectra of the LDF signals were created and used to determine the position of frequency components (cardiac, respiratory, myogenic, sympathetic, NO endothelial dependent and independent). The fractal and entropy profiles of the LDF components were assessed with the alpha exponent from the detrended fluctuation analysis (DFA) and the entropy profile was given by the complexity index from the multiscaled entropy analysis (MSE). Nonparametric statistics were applied. From the spectra six bands were found, consistent with the above mentioned components, already described for human. Significant differences were found between db/db and controls during hyperoxia (phase II). WT analysis revealed a significantly higher myogenic and a lower NOi activities in db/db. DFA revealed significant differences in the fractal organization of the myogenic and sympathetic LDF components between db/db and controls. MSE showed lower complexity for the myogenic and sympathetic activities in db/db. These results suggest these analysis tools are useful in distinguishing between physiological and pathophysiological microvascular profiles. In this study, our results show that the myogenic and sympathetic responses to hyperoxia are decreased in diabetes.

Mots-Clés: Diabetes, LDF, wavelet transform, DFA, MSE

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Microcirculatory response to in vivo massage in the lower limb

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Résumé

Massage is a common procedure, also used with therapeutical purposes in the lower limb to alleviate some of the symptom related with peripheral vascular disease. Its effects are still poorly understood., so we developed a protocol to characterize in vivo, the cutaneous microcirculation response measured by Laser Doppler flowmetry (LDF) and photoplethysmography (PPG), to massage. 16 young healthy subjects (19.6 ± 1.3 y.o.) both gender, were subjected to two therapeutical massage (TM) protocols, one applied in the upward direction (upM) and the other downwards (dwM), each consisting of three phases - 10 min baseline (phase I), 5 min massage (phase II) and 10 min recovery (phase III). One random lower limb was massaged (test), while the contralateral served as control. Blood flow was measured on the first and second toes of both feet with PPG and LDF, respectively. The wavelet transform (WT) analysis was applied to decompose these signals into their main activity components (cardiac, respiratory, myogenic, sympathetic, endothelial NO-dependent (NOd) and endothelial NO-independent (NOi)). Nonparametric statistics were used for phase comparisons ($p< 0.05$). During both protocols, both PPG and LDF registered a significant perfusion increase in both feet. After decomposition, significant changes in the signals' components were also detected, for both feet in all cases. For the upM protocol, both techniques recorded a significant increase in the respiratory activity for both feet. LDF also recorded a significant increase in the cardiac and a decrease in the NOd activities, while PPG recorded a significant increase in the myogenic and a decrease in the NOi activities. For the dwM protocol, both techniques recorded a significant increase in the respiratory and myogenic activities, together with a significant decrease of the NOi activity. As for LDF, a significant increase in the cardiac activity was noted, whereas for PPG a significant decrease in the NOd was noted. These results suggest that, under these conditions, massage modifies the microvascular reactivity increasing the lower limb perfusion, although different mechanisms could be implied in the two protocols, keeping in mind that LDF and PPG, although based in similar principles might register different features at different depths.

Mots-Clés: Massage, lower limb, vascular regulation, LDF, PPG

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Calculation of central blood pressure by analyzing the contour of the photoplethysmographic pulse measured at the finger with the pOpmètreR device

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Résumé

Calculation of central blood pressure by analyzing the contour of the photoplethysmographic pulse measured at the finger with the pOpmètre® device Background: Analysis of the contour of the peripheral pulse to assess arterial properties was first described in the nineteenth century. The reference technique to estimate central blood pressure (CBP), non-invasively, is by analyzing the radial pressure pulse acquired using a tonometer and then to establish a transfer function relating the radial pressure to the CBP. An alternative technique utilizes a volume pulse. This may conveniently be acquired optically from a finger (digital volume pulse obtained with the photodiode of the pOpmètre® device). Although less widely used, this technique deserves further consideration because of its simplicity and ease of use.

Objectives: The objective is to establish a transfer function estimating the central blood pressure (CBP), calibrated with a brachial pressure cuff and using the parameters obtained with the contour analysis of the photoplethysmographic pulse measured at the finger with the pOpmètre® system.

Methods: We positioned the photodiode sensor on the finger, insuring that the sensor's lens is in contact with the finger. Brachial blood pressure measurements was performed with a standard cuff. The central blood pressure values measured with Sphygmocor used as the reference values. Multiple regression analysis was done to establish the transfer functions. Pearson's correlation and Bland Altman graph were performed

Results: 69 subjects were included: 24 healthy subjects and 45 patients with essential hypertension aged 33 ± 8 years and 59 ± 17 years respectively. The correlation between the estimated central systolic pressure (CSP) and the reference one, was good and significant ($r^2 = 0.91$; p< 0.0001). A better correlation was found in terms of central diastolic pressure (CDP) ($r^2 = 0.92$; p< 0.0001). The Bland and Altman analysis, mean difference was 4 mmHg p< 0.0001 (CSP) versus 3 mmHg p< 0.0001 (CDP), the standard deviation of the difference was 5 mmHg (CSP) versus 4 mmHg (CDP), classifying the estimation as good agreement.

Conclusions: The estimation of the central blood pressure with the finger pulse qualifies as good agreement with the reference technique for the central systolic and diastolic pressure estimation.

Mots-Clés: Arterial stiffness, Central Blood Pressure

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The role of the blood-brain barrier in Amyloid-β biology: contribution to Alzheimer's disease

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Résumé

Around 80% of Alzheimer's disease (AD) cases result in cerebral amyloid angiopathy (CAA) with amyloid-β (Aβ) deposition around blood vessels. The source of Aβ however is not entirely clear. Our group have previously shown that beta-secretase 1 (BACE-1), the key enzyme cleaving amyloid precursor protein (APP), is expressed by brain endothelial cells (ECs). We therefore hypothesize that BACE-1 at the BBB might be involved in cleavage of circulating APP and or neuronal APP, thus generating Aβ locally at the BBB, reemphasizing the vascular aspect of AD. In order to investigate the BBB contribution to AD formation and progression we made use of the Thy1-SwDI mouse model, harbouring triple mutations of amyloid precursor protein APP and being described to show onset of AD symptoms by 3 months of age. We could demonstrate that the AD mice have blood-brain barrier (BBB) deficits by tracer experiments with 4KD-FITC Dextran. In order to identify vessel-related changes in AD mice during ageing we teamed up with GenXPro to perform Massive Analysis of cDNA Ends (MACE or 3 mRNA-Seq), of mouse brain microvessels (MBMVs) from AD compared to aged-matched WT mice. We are expecting to identify genes regulated in MBMVs in AD, which might shed novel light onto the BBB contribution to AD formation and progression.
Currently, we aim to further understand the role of BBB-disruption for AD formation in our mouse model and the potential benefit of re-sealing the BBB. We have shown that Wnt/β-catenin signalling contributes to BBB development and that dominant activation of endothelial β-catenin signalling re-establishes barrier properties in brain tumour vessels. Therefore, we currently test the hypothesis that dominantly active β-catenin may slow down AD progression. Preliminary data support a memory improvement in the AD mouse model upon inducible β-catenin signalling in ECs, indicated by Y-maze behavioural tests for learning and memory. Moreover, we further investigate BACE-1 at the BBB for AD. Herein we focus on the role of SIRT-1-mediated regulation of BACE-1, known to be important in ageing.
Together, our results point into a novel contribution of ECs to CAA characteristic in AD, as well as to a therapeutic potential of the canonical Wnt pathway in AD.

Mots-Clés: Alzheimer, blood, brain barrier, AD, BBB, BACE, 1

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Clinical evidence of the vascular protective effects of grapefruit flavanones in post-menopausal women and potential molecular mechanisms involved

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Résumé

Epidemiological studies reported that a high flavanone intake is associated with a reduced risk of cardiovascular diseases (Mink et al, 2007; Cassidy et al, 2012), however clinical evidence is still lacking. We carried out a cross-over RCT on 52 healthy post-menopausal women who have to consumed daily and for 6-month, 340ml of grapefruit juice (providing 212mg naringenin-glycosides) or of an iso-energetic control beverage mimicking the composition of the juice but without naringenin. The aim of this trial was to evaluate the impact of GFJ consumption (i) on vascular function (blood pressure, endothelial function and arterial stiffness) and (ii) on the gene and miRNA expression in PBMCs isolated from enrolled volunteers using microarrays.
The intervention with GFJ improved pulse wave velocity (PWV), an indicator of arterial stiffness, without affecting endothelial function (FMD) or blood pressure. The nutrigenomic study showed that the regular intake of naringenin through GFJ consumption modulated the expression of genes and miRNAs in PBMCs. Bioinformatic analysis of microarray data revealed that the differentially expressed genes and the target genes of modulated miRNAs are involved in different cellular processes, including inflammatory processes, chemotaxis, migration and cell adhesion, known to regulate interactions between vascular endothelium and circulating immune cells. The observed changes in genes and miRNAS expression profiles suggest a lower adhesion and infiltration of immune cells into the vascular wall. This

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Anthocyanins and their gut metabolites reduce monocyte adhesion and migration across TNFalpha-activated endothelial cells trough nutrigenomic mechanisms regulating endothelial permeability

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Résumé

Cardioprotective effects of dietary anthocyanins are partly attributed to their ability to maintain endothelial function. However, the underlying mechanisms of action are not fully understood. This study aimed to test the effect of anthocyanins and their gut metabolites, at physiologically-relevant conditions, on endothelial cell function and decipher the underlying molecular mechanisms of action using integrated omics approaches. Primary endothelial cells were treated with a mix of 0.1mM cyanidin-3-arabinoside, 0.1mM cyanidin-3-galactoside, 0.1mM cyanidin-3-glucoside, 0.1mM delphinidin-3-glucoside, 0.1mM peonidin-3-glucoside and 0.5mM 4-hydroxybenzaldehyde for 3h or with a mix of gut metabolites: 0.2mM protocatechuic, 2mM vanillic, 1mM ferulic and 2mM hippuric acids for 18h. Additionally, successive treatment with both mixes mimicked the anthocyanin pharmacokinetics following their dietary intake. Endothelial cell dysfunction was induced using TNFa and monocytes added to asses adhesion and transmigration. Gene and miRNA expression, signaling protein phosphorylation and molecular docking were investigated.
All mixes significantly reduced monocyte adhesion and transendothelial migration by 24.5% and 46.2% in average. Gene expression analysis using TLDA arrays showed that mixtures modulated the expression of genes involved in cell-cell adhesion, cytoskeleton organization or focal adhesion. Bioinformatic analyses were done to identify potential transcription factors involved in the observed nutrigenomic effects and signalling proteins regulating their activity. Western blot analysis showed the ability of tested compounds to modulate phosphorylation of NfkB-p65 or p38 signalling proteins. Docking identified 25 out of 63 potential cell signaling proteins to which these compounds can bind to and initiate the observed nutrigenomic effect. The mixes also affected miRNA expression. Bioinformatic analysis showed that more than half of modulated miRNAs are involved in regulation of endothelial cell permeability, contributing to the observed changes in endothelial cell function. Integration of these results revealed endothelial-protective properties of anthocyanins and their gut metabolites and deciphered new underlying multi-target and multi-layered mode of action.

Manipulable and perfusable artificial blood vessels produced by 3d vascular tissue engineering

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Today, vasculature-related improvement is a key question in clinics since cardio-vascular diseases are the first cause of premature death globally (according to World Health Organization, WHO). In addition, tissue engineering is limited in constructing functional organs essentially due to the lack of vascularization to provide nutrients. Thus, to achieve the production of thick tissues, vasculature is needed and it is crucial to develop strategies for *in vitro* blood vessel engineering.

Here, we propose a novel blood vessel engineering approach, which consist in a co-encapsulation of ECs (HUVEC), SMC, and ECM in an alginate tubular shell. With this approach, we achieved the production of artificial mature blood vessels that are 1/ manipulable, 2/ histologically relevant, 3/ with variable inner and outer diameter, 4/ perfusable, 5/ tight and 6/ contractile. Moreover, this technique circumvents the actual limitations that exist for synthesis of small diameter artificial blood vessels (<2mm).

Finally, this vascular tissue engineering approach can serve as a model for angiogenesis studies *(in vitro*, or following engraftments), cardiovascular drug testing (high-content screening in 3D tissues), tissue-engineered organoids vascularization and vascular grafts (surgical replacement of damaged tissues).

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