Acta Médica Portuguesa – Student iMed Conference 8.0 Lisbon 2016 Fundação Astrazeneca

Online Abstract Book
Fibrosis and morphometric characterization of right atrial tissue in atrial fibrillation following aortic valve replacement surgery

M FRAGÃO-MARQUES, C SOUSA-MENDES, AF LEITE-MOREIRA, I FALCÃO PIRES

1Department of Physiology and Cardiothoracic Surgery, Faculty of Medicine, University of Porto, Porto, Portugal
2Department of Cardiothoracic Surgery, Hospital São João, Porto, Portugal

PURPOSE: Atrial Fibrillation (AF) is the most common persistent arrhythmia with adverse clinical outcomes. [1] Furthermore, AF is the most common complication after cardiac surgery (postoperative atrial fibrillation, PoAF), recurring in about 40% of patients. Recent studies report an increase in the time spent in the intensive care unit (ICU), in-hospital stay, stroke incidence and 30 day and long-term mortality. [2] EADs (early afterdepolarizations) and DADs (delayed afterdepolarizations) significantly contribute to AF pathogenesis [3] and structural and electrical remodeling is present in most forms of AF. [4] Atrial fibrosis is key in structural remodeling and it alters cardiomyocyte electrical coupling due to misplacing and changing the structure of connexins, thus inducing a fragmented electrical conduction. [5] Several other mechanisms are present in the pathophysiology of the arrhythmia, including autonomic, genetic and anatomic. These holistic changes in the atrium induce contractile dysfunction which contributes to the enlargement observed in echocardiographic studies and sometimes persistent after cardioversion to sinus rhythm. [3,6]

METHODS: Right atrial tissue samples from aortic stenosis patients submitted to isolated aortic valve replacement were collected and subdivided in two groups according to PoAF occurrence. Haematoxylin and eosin stain was used to measure cardiomyocyte dimensions (60 cells per patient). Fibrosis was quantified by Sirius Red and Masson’s trichrome stains (eight fields per patient).
RESULTS: There were no significant differences between groups regarding age and gender. All patients had a preserved ejection fraction (>55%) and severe aortic stenosis. Cardiomyocytes from PoAF patients had significantly increased dimensions but decreased nucleus to cytoplasm ratio. Both nuclear and cytoplasmic changes contributed to the decreased ratio, but only the increased cytoplasm had a linear correlation with cell dimensions. There were no significant differences in fibrosis between the two groups.

CONCLUSION: Atrial cardiomyocytes of PoAF patients have morphological alterations when compared to control individuals, although they have no differences in interstitial fibrosis.

REFERENCES


Stability evaluation of chitosan/dextran sulfate nanoparticles

JF PONTES, A GRENHA

1 CBMR – Centre for Biomedical Research Drug Delivery Laboratory, University of Algarve, Campus de Gambelas, Faro, 8005-139, Portugal
2 CCMAR – Centre of Marine Sciences, University of Algarve, Campus de Gambelas, Faro, 8005-139, Portugal

Nanoparticles are, nowadays, one of the vehicles with the strongest potential in drug delivery applications. Apart from carrying the drug molecules (1), these vehicles also comprise a protective environment from the sometimes aggressive conditions of the organism (pH, enzyme content) thus improving bioavailability. However, due to the small size, nanocarriers present physical and chemical instability that may lead to particle aggregation and drug leakage, respectively, limiting their applications (2). The following study was conducted on the subject of nanoparticle stability, where the cryoprotectant capacity of two sugars – glucose and sucrose – was evaluated. The influence of the process of freeze-drying and the effect of cryoprotectants on nanoparticle’s size and zeta potential were evaluated, before and after the process. Three assays were conducted: i) chitosan/dextran sulfate (CS/DS) nanoparticles were produced by polyelectrolyte complexation (initial characteristics: (250.3 ± 47.4) nm and (-45.9 ± 4.5) mV for glucose and (290.6 ± 4.5) nm and (-43.6 ± 4.3) mV for sucrose) and then adjusted to obtain suspensions of 1 mg/mL and 2 mg/mL. After that, glucose or sucrose at 5% or 10% (w/w) were added to the dispersions before freezing, and nanoparticles were characterised for size and zeta potential before and immediately after freeze-drying, as depicted in Figure 1.1; ii) CS/DS nanoparticles were produced, adjusted to 1 mg/mL and 2 mg/mL (no cryoprotectants added) and stored at 4 ºC for 113 days; iii) CS/DS nanoparticles were produced, then adjusted to obtain suspensions of 1 mg/mL and 2 mg/mL, to which glucose or sucrose were added at 5% or 10% (w/w). After freeze-drying, nanoparticles were stored in a desiccator at room temperature. Nanocarriers were characterized before the process of freeze-drying and then every 15 days until day 90, after the necessary reconstitution. In the first approach of freeze-drying, it was possible to conclude that glucose has a better cryoprotectant effect than sucrose, as shown by a ratio of size comparison (before and after freeze-drying) close to 1 (Figure 1.2), independently of the nanoparticle and cryoprotectant concentration used. Regarding zeta potential, the effect was the opposite, thus sucrose providing a better protection, but the alteration produced in presence of glucose was devoid of physiological relevance (maximum changes around 9 mV, data not shown). The second assay (nanoparticles stored at 4 ºC), evaluated the behavior of nanoparticles upon storage as aqueous suspension. A decrease of nanoparticle’s size in both concentrations tested was detected by day 50. It was also detected, in some samples, the presence of a sediment that would explain the aggregation of nanocarriers at some point. On the third assay, where freeze-dried nanoparticles were stored at room temperature and then
reconstituted at each 15 days during three months, it was possible to verify that sucrose has a better cryoprotectant capacity than glucose on the long-term. During the 90 days, sucrose at 5% (w/w) and at 10% were able to maintain nanoparticle size ratio at a maximum value of 1.0 ± 0.3 and 1.2 ± 0.7, respectively, for 1 mg/mL of nanoparticle’s suspension. In turn, when glucose was used, an increase on size and zeta potential of CS/DS nanoparticles was detected by day 60, which was coincident with the observation of the same type of sediment seen in the previous experiment, which possibly results from aggregation. In conclusion, considering the need of a long-term stability, sucrose was deemed more effective to achieve a better cryoprotectant effect.

REFERENCES


Synergistic effect of the homologous PB1–NA gene constellation in Influenza A virus reassortants: Evaluation and characterization of reassortant influenza variant viruses crossresistant to influenza Neuraminidase Inhibitors.

J LOURO1,2, H REBELO DE ANDRADE1,2

1Host–Pathogen Interaction Unit, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av.Gama Pinto 1649–003 Lisboa, Portugal.

Corresponding Author: h.rebelo.andrade@insa.min-saude.pt

Previous work: Team research areas include: genetic and evolutionary analysis of influenza virus, role of gene reassortment in the genetic evolution, virulence determinants and antiviral drug resistance. From 2012 to 2014 I took part in the project: “Evaluation and characterization of the emergence of resistance to influenza antiviral drugs in the context of acute respiratory infection”, regarding the genotypic and phenotypic characterization of influenza virus A(H1N1)pdm09 and A(H7N9). The work plan has focused on the phylogenetic and mutational analysis of influenza viral strains circulating in Portugal from 2009–2013. Based on the international collaboration of the team, it was possible to develop complementary experimental work in the Respiratory Virus Unit at Public Health England, U.K. During this period I have developed experimental work for the optimization of a non–infectious transfection system to express non–human influenza virus proteins and further characterize the profile of a N9 neuraminidase (NA) from a zoonotic influenza A(H7N9) virus. Recently, I have been involved in the project: “Neuraminidase Inhibitors: new antivirals and new brands” at INSARJ. This study aimed to compare available cost–efficient alternative reagents for NA inhibition assay (NIA) and to assess the phenotypic susceptibility profiles of influenza virus from different (sub)types to both the classic (oseltamivir and zanamivir); and the new (laninamivir and peramivir) NA inhibitors (NAIs). Since 2015 I have been working on the project: “Functional compatibility of the replication complex as a determinant of virulence in influenza virus” [1][2]. In this context, we found functional compatibility when PB1 is homologous to antigenic proteins NA and hemagglutinin (HA), which is suggested by an increasing in viral fitness. The presence of PB1 homologous to HA and NA in the A(H1N1)pdm09 vaccine seed prototype prototypes in the backbone of A/Puerto Rico/08/34 [PR8: A(H1N1)pdm09–HA,NA,PB1] resulted in statistically significant virus growth improvement, when compared to the 6:2 classical seed prototypes [PR8: A(H1N1)pdm09–HA,NA] [2]. Also, we have identified mutations –R386K, I517V and L298I– in the PB1 protein of A(H1N1)pdm09, that may have contributed to an enhanced compatibility between PB1 and HA. L298I has already been associated with
increased pathogenicity in mice [3]. Our current line of research explores the phenotypic outcome of creating seed viruses bearing PBI homologous to HA and NA, in the kinetics of viral growth and antigen yield. Additionally, we continuously analyze and characterize the phenotypic and genotypic NAIs susceptibility profiles of influenza viruses circulating in Portugal, using the in-house MUNANA-based IC50 (concentration of NAI required to inhibit 50% of the virus NA activity) fluorescence assay, provided by PHE [4]. Influenza antivirals are an important line of defense in case of a newly emerging influenza virus strain, and particularly in immunocompromised and elderly patients [5]. In this context, the emergence and spread of influenza A viruses, with diminished susceptibility to NAIs, increase the need to understand the impact of specific mutations on evolution and viral fitness of drug-resistant virus.

INTRODUCTION: The neuraminidase (NA) inhibitors (NAIs) oseltamivir phosphate (Tamiflu®) and zanamivir(Relenza®) are currently the only effective antiviral drugs available in Europe for the management of influenza - they have been approved for use in many countries since 1999/2000 [6]. Each one presents known limitations in scope, effectiveness and emergence of resistance strains [7]. The emergence in 2007/2008 of an oseltamivir-resistant seasonal A(H1N1) variant (carrying the H275Y NA mutation) and the detection of sporadic cases of this variant among A(H1N1)pdm09 viruses in Australia (2011) and Sapporo, Japan and USA (2013–2014) have demonstrated the potential for drug-resistant influenza viruses to arise and spread globally within the community in the absence of drug-selective pressure [8][9]. Additional NA mutations detected in A(H1N1)pdm09 viruses may have potentially compensated for the expected fitness deficits and reveal why H275Y mutants were able to emerge and become widespread [7]. I223R and S247N NA mutations of H275Y mutant A(H1N1)pdm09 viruses have been reported to have a synergistic effect with the H275Y substitution on the reduction of NA inhibitor susceptibility, prompting the concern that these variants may have acquired cross-resistance to other NAIs [9][10]. Recent studies reporting variants harboring mutations conferring cross-resistance to approved NAIs [10] expose the potential for emergence and spread of resistant viruses and reinforce the demand for a close evaluation of virus susceptibility to NAIs. Albeit various mutations in the NA conferring reduced susceptibility to NAIs have been identified, the amino acid substitutions that confer cross-resistance with undiminished viral fitness have not been comprehensively characterized and thus remain poorly understood [10].

OBJECTIVE: This project aims to elucidate the functional impact of specific mutation induced changes in the NA and PBI genes on the viral fitness and cross-resistance profile of A(H1N1)pdm09 viruses.

METHODS: A genomic library of the parental viruses A/Puerto Rico/8/1934(H1N1) (PR8) and A/Portugal/82/2009 (pdm09) has been previously constructed in a bidirectional plasmid vector [2] and is available for study. The prototype reassortant PR8.(A(H1N1)pdm09-HA,NA,PBI has been generated in vitro by plasmid-based reverse genetics and are also available for this study. Site-directed mutagenesis using
appropriate primers and a commercial mutagenesis kit will be used to induce mutations in NA and PB1 genes and the mutated viral reassortants will be generated by reverse genetics. The plasmid containing the NA gene will be used for the introduction of single (H275Y) and double (H275Y/S247N) mutations; the plasmid containing the PB1 gene will be used for the introduction of L298I mutation. The resulting plasmids: pNAH275Y, pPB1L298I, pNAH275Y/S247N and pNAH275Y/PB1L298I will be evaluated for their specific impact on viral replicative kinetics and on the NA enzymatic properties of the generated PR8:(A(H1N1)pdm09-HA-NA-PB1 reassortants. Growth kinetics will be tested from 12 to 60h post-infection by Hemagglutination titer (HA) and Tissue Culture Infectious Dose (TCID50). NA activity and NA inhibition assay will be determined by an in-house fluorescence MUNANA-based assay [4] in independent assays at different time points post-infection.

EXPECTED RESULTS: By constructing reverse genetics reassortants bearing different genetic features (individual or a group of specific mutations) involving the NA and PB1 genes, we expect to appraise their impact on viral phenotype, regarding the viral replication kinetics and susceptibility profile to NAIs. Additionally, the results obtained in this study may contribute to a better clarification on the phenotype–genotype relationship. Elucidating evolutionary trends in the genes encoding influenza virus internal proteins and profiling of influenza A viruses to antiviral drugs are essential to assess the risk of influenza virulence and antiviral effectiveness, contributing significantly to future public health strategies.

REFERENCES

Alternative splicing during neuronal development

M ASCENSÃO FERREIRA1,2, N BARBOSA-MORAIS1

1Instituto de Medicina Molecular – Faculdade de Medicina da Universidade de Lisboa
2Instituto Superior Técnico, Universidade de Lisboa

There is growing evidence that alternative splicing misregulation plays a role in neurodevelopmental disorders. Irimia and colleagues showed (1) that a set of neuronalspecific, highly conserved microexons is disregulated in autism spectrum disorder. Also, Traunmüller’s work showed (2) that synaptic interactions influencing mouse behavior are modulated by neurexin alternative splicing. Li’s work in a mouse model of Rett syndrome recently revealed (3) the misregulation of hundreds of alternative splicing events caused by the loss of function of MeCP2 protein, which acts as a transcription regulator and interacts with splicing modulators. Moreover, both biochemical and bioinformatics studies have demonstrated that one RNA-binding protein (RBP) controls many targets in vivo, contributing to RNA complexity and ultimately to the biological complexity of higher organisms. (4) Recent studies using crosslinking immunoprecipitation sequencing (CLIP-seq) support the dependency of splicing outcomes on RBPs’ binding sites: the inclusion or exclusion of an exon is dependent on whether its splicing regulator binds upstream or downstream. (5) In order to unveil the normal profile of splicing during neurodevelopment, both in human and mouse, we have been using public (6, 7) neurogenesis RNA-sequencing datasets and transcriptomic data from a mouse neuronal marker reporter cell line of neural progenitor cells (46C). The splicing quantification is achieved using percent-spliced-in (PSI) values, obtained from reads aligning to splice junctions as the ratio between number of reads supporting the inclusion of the exon over the total number of both inclusive and exclusive reads. Preliminary principal component analysis showed an alternative splicing signature during neuronal development which is responsible for a clear separation of cells/tissues within neurodevelopmental stages, consistent with the temporal direction of differentiation (figure 1). Moreover, we have already spotted a number of novel alternative splicing events in which exons are progressively more included or excluded during neuronal differentiation, suggesting a highly tuned mechanism of regulation (figure 2). Therefore, we feel encouraged to further explore not only the general alternative splicing signature of neurodevelopment in human and mouse, but also the regulation mechanisms underlying its specific pattern, since it is now evident that alternative splicing provides an extra layer of transcriptomic specificity in biological processes beyond gene expression.
REFERENCES

Diabetes progression differently affects endothelial function in the aorta and pulmonary artery

IB MARTINS1, J PRIETO-LLORET1, BF MELO1, JF SACRAMENTO1, SV CONDE1

1CEDOC, NOVA Medical School, Faculdade Ciências Médicas, NOVA University, Lisbon, Portugal

Diabetes is associated with a higher prevalence of cardiovascular disease, and recently it was shown as a risk factor in the development of pulmonary arterial hypertension (PAH). It is well known that diabetes is associated with vascular dysfunction and an elevation in the systemic pressure, being the endothelial function in aorta compromised in diabetic rats (1, 2). However, it is not consensual that PAH is caused by endothelial dysfunction (3, 4, 5). Herein we have investigated the effect of different stages of type 2 diabetes progression on the contractility machinery and endothelial function in the pulmonary and systemic circulation. The experiments were performed in male Wistar rats of 8 week old. Three pathological animal models have been used: the high-sucrose animal model (HSu), obtained by submitting rats to 35% sucrose in drinking water for 4 weeks, the high-fat (HF) animal model, which is obtained by submitting animals to 3 weeks of 60% lipid-rich diet, and the high-fat/high-sucrose (HFHSu) animal model, in which animals are submitted to a combined diet of 60% lipid-rich diet and 35% sucrose in drinking water during 14 weeks. All the pathological animal models have been compared with age-matched controls. At the end of the diet period, rats were anesthetized with pentobarbitone (60 mg/kg, ip.) and the pulmonary and aortic arteries were removed and dissected. Contractility was then evaluated by small vessels myography in response to increasing doses of prostaglandin F2α (PGF2α), and expressed as % of the contractile response against 80mM of external K+ (KPSS). Endothelial function was evaluated by monitoring the relaxation effect of acetylcholine over the contraction induced by PGF2α. NO levels in aortic and pulmonary arterial trees were measured. PGF2α produced a dose-dependent increase in arterial contractility in aortic arteries in all animal groups. Pulmonary artery contractility to PGF2α was significantly enhanced in HF animals, while it was diminished in the HFHSu animals. Endothelial function was compromised in the aorta in the HF and HFHSu animals, as the dose-response relaxation curve to ACh was significantly decreased in relation to control animals, while in the pulmonary artery was only affected in HFHSu animals. In conclusion, endothelial function in the pulmonary artery was affected in HFHSu animals but not in prediabetes animal models, while systemic endothelial function was affected since the beginning of diabetes progression. This suggests that pulmonary artery is more resistant to diabetes-induced endothelial dysfunction than systemic arteries.
REFERENCES


Pulmonary arterial hypertension (PAH) is characterized by remodelling of the pulmonary vasculature, with progressively increasing pulmonary vascular resistance and imposing pressure overload to the right ventricle (RV). Although initially the RV adapts compensatorily, heart failure (HF) and premature death will eventually occur (1). PAH’s pathogenesis is poorly understood but endothelial dysfunction (2), inflammation (3), neurohumoral activation (4), inhibition of apoptosis and increased vascular smooth muscle cell proliferation (VSMC) (5) were shown to play a role in pulmonary remodelling. Only a few molecular pathways targeting the pulmonary vascular endothelial dysfunction and vasoconstriction were found to be therapeutically significant. Despite some improvements, the great majority of patients remain symptomatic, with a mortality rate of 20 to 40% three years after diagnosis (6). Any further advance in the comprehension of the pathophysiology and treatment of PAH is urgent.

Polyphenols, which are one of the non-alcoholic component of some alcoholic beverages like beer, were shown to provide several benefits in pre-clinical models of cardiovascular diseases and cancer (7, 8). For instance, polyphenol-compound xanthohumol (XN) was shown to have anti-oxidant, anti-inflammatory, anti-proliferative and pro-apoptotic effects, and, as such, as been used as anti-tumor treatment in several studies (9). Similarly, pulmonary VSMC of PAH patients display a “quasi-malignant” phenotype granted by the expression of anti-apoptotic and pro-survival genes, resulting in dysregulated cellular proliferation and the characteristic vascular hypertrophy described before (10). Given XN’s “anti-malignant” effects, XN administration could be of value in PAH. Furthermore, XN appears to exert anti-estrogenic effects, dulling the pro-estrogenic environment that has been described to be on the basis of PAH development (11).

AIM: To test if the ingestion of xanthohumol enriched-beverage could modulate the natural history of PAH in a pre-clinical model.

METHODS: Adult Wistar Rats (180–200g) were subcutaneously injected with monocrotaline (to induce PAH; MCT group) or with the equivalent volume of NaCl solution (control or NaCl group). Each group was then submitted to a diet based on xanthohumol-enriched beer (MCT+XN, NaCl+XN) or a diet drinking 5% ethanol (MCT+EtOH, NaCl+EtOH). At day 25–27 following MCT or NaCl injection, all animals were subjected to VO2max
test and invasive hemodynamic evaluation. After sacrifice, right ventricle (RV) and lung samples were collected for morphometric and histological analysis.

RESULTS AND DISCUSSION: XN administration (MCT+XN) improved survival and prevented the disease-related functional deterioration, as measured by VO2max (p < 0.05 vs MCT+EtOH). Animals treated with XN presented a reduction in RV end-systolic pressure, and therefore decreased pressures on the pulmonary vasculature (p < 0.01 vs MCT+EtOH). This was paralleled by a decrease in medial pulmonary artery hypertrophy (p<0.05 vs MCT+EtOH), suggesting that XN was effective in preventing pulmonary remodelling. Moreover, XN supplementation also attenuated right ventricle remodelling as shown by the prevention of hypertrophy and fibrosis (p<0.05 vs MCT+EtOH).

CONCLUSION: Our results suggests that xanthohumol supplementation in PAH could have dual beneficial effects by preventing pulmonary vascular remodeling and myocardial hypertrophy.

REFERENCES
K+ Channels in Neurons from rat Dorsal Root Ganglia reveal new therapeutic leads for Inflammatory and Neuropathic Chronic Pain

B SZWARC1,2, J SERRÃO2, MA ROBERTO1, M SOUSA1, PA LIMA1,3

1Lab. Fisiologia - NOVA Medical School, Univ. Nova de Lisboa, Lisboa, Portugal.
2Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal.
3Sea4Us – Biotecnologia e Recursos Marinhos, Lda., Sagres, Portugal.
4Hospital S. José-Centro Hospitalar de Lisboa Central EPE, Lisboa, Portugal.

INTRODUCTION: Chronic pain affects 21% of the human population and, to date, treatments are only partially effective [1]. In this context, nociceptive fibers that are responsible for the transmission of pain stimuli are in a higher state of hyperexcitability. Such neural excitation degree is controlled by sets of ion channels. In a naïve situation neural excitation is proportional to the strength of the external mechanical stimulation. However, in chronic pain this relationship is disrupted, resulting in deregulated peripheral excitability [2]. Of all sensory fibers, small diameter dorsal root ganglia (DRG) neurons are found to have an augmented activity in chronic pain patients[3], which may be caused by abnormal firing patterns modulated by voltage-gated potassium (Kv) channels. Our goal is to elucidate the shared and specific mechanisms of neuropathic and inflammatory chronic pain in rat models by studying the biophysics of Kv currents/channels in small diameter DRG neurons. Like this, we aim to identify which channels are relevant and how should we affect them in new therapeutic strategies.

METHODS: Animal care and experimental studies with Wistar rats were in accordance with Directive 2013/63/EU. To study neuropathic pain, a chronic constriction injury (CCI) model was performed by making 4 loose ligations in the right sciatic nerve of the animals (120g–140g rats) (n=5) and the model was maintained throughout 4 weeks[4]. For the inflammatory pain model, an intra-articular injection of complete Freund’s adjuvant (CFA, 100μL) was applied in the right knee (120–140g rats)(n=5) and the model was maintained throughout 2 weeks[5]. Four naïve animals were used as controls for each model. Mechanical sensibility was scored based on von Frey monofilament stimulations as experimental paradigm [6]. After 2 or 4 weeks of injury, rats were euthanized by decapitation after anaesthesia with pentobarbital. L4–L6 DRGs were removed and enzymatically dissociated to isolated single cells, which were plated and incubated overnight. Whole-cell voltage-clamp was used to record Kv currents within 24h of dissociation.

RESULTS AND CONCLUSIONS: Maximal mechanical nociceptive scores validated both chronic pain models. Whole-cell voltage-clamp results demonstrate that neuropathic pain alters the biophysical properties of the Kv currents. Specifically, data revealed that the peak of the K+ current density is smaller in CCI and CFA DRG neurons when compared to Naïve. Concomitantly, the CFA pain model showed a depolarized shift in the
activation curve. Moreover, regarding the voltage dependence of inactivation, the CCI pain model showed a depolarized inactivation curve shift. Most revealing, analysis of the voltage activated K+ currents showed clear differences in the nature of the current-components: a fast, A-type current noticeable on Naïve neurons, disappears in neurons from both pain models; a slowly inactivating current remains observable in all the three models. Altogether, results account for the increase of neuroexcitability which is associated with the exacerbation of excitation that is not proportional to the strength of the external stimulation. Importantly, they point out for the identification of K+ current components that are differentially expressed in the chronic pain models. This information, together with protein expression assays that are being conducted, will further dictate the molecular nature of the K+ current-component, thus revealing new therapy leads.

REFERENCES

Identifying the role of Tau protein in molecular mechanisms of post-anaesthesia cognitive deficits

P. Gomes¹,², H. Leite-Almeida¹,², I. Sotiropoulos¹,²

¹Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Campus de Gualtar, 4710-057, Braga, Portugal
²ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, 4710-057, Portugal

General anaesthetics (GA) are widely used due to their multiple clinical applications. However, clinical studies report that postoperative patients often experience cognitive deficits such as short-term memory loss and learning disabilities¹. Similarly, animal studies show that repeated exposure to anaesthetics that are commonly use in clinical practise, e.g. Ketamine, an antagonist of NMDA receptor, lead to memory impairment; yet, the underlying mechanisms remain unknown. Accumulating evidence suggests the cytoskeletal Tau protein and its hyperphosphorylation as an essential mediator of neuronal and synaptic malfunction underlying memory impairment in Alzheimer’s disease² as hyperphosphorylated Tau is causally related to microtubule instability as well as synaptic loss³ and plasticity⁴. Moreover, recent work for our team demonstrates that Tau is necessary for hippocampal atrophy and cognitive deficits induced by chronic stress linking synaptic Tau with NMDA receptor excitotoxicity⁵. As the above findings highlight Tau as a key protein in neuronal atrophy/malfunction and related cognitive deficits and based on the dual cytoskeletal and synaptic role of Tau, the current project aims to monitor the potential role of Tau and its hyperphosphorylation in the structural/functional deficits of the brain after anaesthetic exposure.

For that purpose, we use 6-7 months old male B6 mice lacking Tau protein (Tau-KO) and their wildtype (WT) littermates that were subjected to a 2 month-long set of 6 injections of saline (control) or ketamine providing behavioural, neurostructural, imaging and molecular analysis one week after the last injection. Using a battery of tests for assessment of cognition and mood, we found that repeated exposure to Ketamine induced deficits of short-term memory in WT, but not Tau-KO, animals suggesting an essential role for Tau in ketamine-induced cognitive deficits. In contrast, administration of Ketamine reduced anxiety levels in both WT and Tau-KO animals indicating the selective role of Tau in the mediation of cognitive deficits induced by repeated exposure to ketamine. 3D neurostructural analysis demonstrates that Ketamine administration induced neuronal atrophy in CA1 hippocampal neurons and hypertrophy in dentate gyrus (DG) granular cells of WT animals. In line with absence of ketamine-driven cognitive deficits in Tau-KOs, neuronal structure of Tau-KOs hippocampus were not affected by ketamine. Our current molecular analysis shows that repeated exposure to ketamine triggers Tau hyperphosphorylation and missorting to synaptic compartments of WTs hippocampal neurons enhancing synaptic signalling related to Fyn and NMDA receptors.
Our findings, so far, suggest that Tau protein is essential for the neuroplastic deficits of hippocampal neurons as well as the related memory deficits caused by repeated exposure to Ketamine pointing towards a strong Tau-NMDAR interaction in neuronal deficits of ketamine. This project provides novel insights of the molecular underpinnings of neuronal and memory deficits caused by prolonged use of a commonly used anaesthetic, Ketamine which recently is suggested to have beneficial effect against depressive pathology.

REFERENCES

3. Kimura et al., 2007 EMBO reports
Local Anaesthetics as a new therapeutic approach in Oral Squamous Cell Carcinoma – An In Vitro Study

A RIBEIRO1,2, C DOMINGUES1,3, S NEVES1,3, P MATAFOME1,4, R SEIÇA1,4, A BERNARDINO2, A SARMENTO-RIBEIRO1,2,3,4, M DOURADO1,3

1Faculty of Medicine, University of Coimbra, Portugal
2University Hospital Centre of Coimbra, Portugal
3Center of Investigation on Environment Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, Portugal
4Center for Neuroscience and Cell Biology (CNC)-Institute for Biomedical Imaging and Life Sciences (IBILI), University of Coimbra, Portugal

Oral squamous cell carcinoma (OSCC) represents the most frequent malignant neoplasia that affects the oral cavity, accounting for more than 90% of cases. The ethology of OSCC is multifactorial and involves intrinsic and extrinsic factors, namely tobacco and alcohol. Besides the new and advanced therapeutic strategies, patients with OSCC show poor survival rates.

Local anaesthetics are usually used to control pain in patients with head and neck tumours, but recent reports have been shown that also can inhibit cancer cell proliferation, invasion and migration.

In this work, we proposed to evaluate the potential therapeutic efficacy of the local anaesthetics, Lidocaine and Mepivacaine, in in situ and metastatic OSCC cell lines, alone and in combination with conventional chemotherapeutic drugs (Cisplatin, 5-Fluorouracil) and studied the underlying mechanisms, namely their role in cancer invasion and metastization.

For these proposes, two OSCC cell lines were maintained in culture, the HSC-3 (metastatic) and BICR-10 (in situ) cells, in absence and in presence of different concentrations of Lidocaine or Mepivacaine in monotherapy (daily or single dose administration) or in association with conventional chemotherapeutic drugs (Cisplatin or 5-Fluorouracil). Cell viability was assessed by the rezasurin assay and cell death by optical microscopy (May-Grunwald-Giemsa staining) and flow cytometry using the Annexin V/Propidium Iodide double staining. The influence of these compounds in cell cycle (Propidium Iodide incorporation), mitochondrial membrane potential (JC-1 probe), caspases (Apostat kit), reactive oxygen species production (hydrogen peroxide and superoxide anion were evaluated by 2,7-Dichlorofluorescein and Dihidroetidium, respectively) and in the level of the antioxidant defence reduced glutathione (using Mercury Orange) were performed by flow cytometry. Cell adhesion was evaluated by measure E-cadherin expression, by flow cytometry, and β1-Integrin and β-Catenin expression, by western blot. Cell migration evaluation was realized by measuring basal expression of matrix metalloproteinases 2 and 9 by flow cytometry, and their proteolytic activity by zymography assay, and, posteriorly, performing the wound healing assay. Results were statistically analysed.
Our study showed that local anaesthetics in monotherapy and in combination with conventional chemotherapy inhibited cell proliferation and migration, and induced cell death mainly by later apoptosis/necrosis in both cell lines in a dose, time and administration schedule and cell type dependent manner, being the HSC-3 the most sensitive relatively to BICR-10. At 48 hours, the IC50 of both local anaesthetics, in HSC-3 cells, was reached at 4.5 mM, while in BICR-10 cells the IC50 was reached with 5.5 mM of Lidocaine and 10 mM of Mepivacaine. These results may be related with the increased in caspases and superoxide anion levels and decreased mitochondrial membrane potential, suggesting the involvement of mitochondria in cell death. The high sensitivity of HSC-3 cells to local anaesthetics may be related with the lowest reduced glutathione levels when compared to BICR-10 cells. The pre-G1 peak observed in cell cycle analysis with Lidocaine and Mepivacaine at IC50 doses confirms apoptosis. The association of both local anaesthetics with chemotherapeutic drugs induced an anti-proliferative effect with blockade of cell cycle, predominantly in S phase. Cell adhesion was not affected significantly by local anaesthetics, although we observed a tendency to increase E-cadherin expression levels. Local anaesthetics and its association with Cisplatin and 5-Fluorouracil also inhibits cell migration as we observed a decrease in matrix metalloproteinases proteolytic activity (more prominent in metastatic cell line), and a gap in wound healing assay after treatment comparing with the non-treated condition after 24 hours of scratch. As conclusion, our in vitro results showed that Lidocaine and Mepivacaine alone or in combination with conventional chemotherapeutic treatment may constitute a new complementary therapeutic approach in OSCC, namely in metastatic cancer.

REFERENCES
Natural killer cell expression of CCR4 chemokine receptor and its ligand CCL5 (rantes) are significantly down-regulated in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors

INTRODUCTION: Chronic Myeloid Leukemia (CML) is a cancer of white blood cells associated with a characteristic chromosomal translocation that causes the BCR-ABL fusion gene, resulting in aberrant tyrosine kinase activity. Tyrosine kinase inhibitors (TKIs) constitute the first line of treatment in CML. Due to Imatinib (first generation TKI) resistance observed in certain patients, second and third generation TKIs have more recently been developed. Dasatinib is a second generation TKI that presents a more potent inhibition of the BCR-ABL protein as well as inhibition of other tyrosine kinases. Natural Killer (NK) cells, which are dysfunctional in CML, are thought to benefit from TKIs’ action. Dasatinib has been shown to increase NK cell cytotoxic activity.[1] However, the mechanisms by which TKIs like Dasatinib influence NK cells are still unknown.

PREVIOUS RESULTS: A previous study [2] has found that CCR4 chemokine receptor expression and its ligand CCL5 are significantly down-regulated in NK cells of CML patients. CCR4 is essential for the activation of NK cytotoxicity.[3] AIMS: In order to clarify the mechanism by which Dasatinib acts on NK cells, the present project aims to analyse Dasatinib’s influence on NK cells’ CCR4 expression in CML patients.

METHODS: Three NK cell sources of samples will be used: NK cells from control (healthy) patients, NK cells from CML patients and a NK92 cell line, the first two being obtained by cell sorting. Using different cell cultures, each of these different NK cell sources will be treated in vitro with Dasatinib (2nd generation TKI), Imatinib (1st generation TKI) and IFN-α (biological agent). A fourth cell culture for each NK cell group will be made using only culture medium for control purposes. After incubation, the CCR4 expression profile of NK cells will be analysed using Flow Cytometry and NK cytotoxic activity will be evaluated through Target cell Visualization Assay (TCVA).
EXPECTED RESULTS: It is expected that NK cells from CML patients treated with Dasatinib will show higher CCR4 expression levels as well as cytotoxicity activity when compared with CML NK cells treated with Imatinib or IFN-α. Also, NK cells from CML patients will be expected to present lower CCR4 expression levels and poorer cytotoxicity activity than NK cells from other groups.

PROJECT IMPACT: This project will help in elucidating Dasatinib’s mode of action in CML patients’ NK cells, that are themselves fundamental anti-tumor agents of the human immune system. By uncovering this mechanism, it will be possible to develop and improve therapeutic agents in order to potentiate NK cell function, as in using Dasatinib in conjunction with other pharmaceuticals. In the long-term, this line of research might allow for improved life quality and expectancy of Chronic Myeloid Leukemia patients, the effective treatment of which is not yet attainable by most.

REFERENCES

2. Sá-Martã E, Almeida JS, Couceiro P, Alves V, Růžičková L, Freitas-Tavares P, Santos-Rosa M, - Rodrigues-Santos P. Natural killer cell expression of CCR4 chemokine receptor and its ligand CCL5 (RANTES) are significantly down-regulated in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors. [manuscript in preparation]