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mTOR pathway activation has already been observed in thyroid cancer, but the biological consequences regarding tumor behavior and patient prognosis remain poorly explored. We aimed to evaluate mTOR pathway associations with clinicopathological and molecular features and prognosis, through the characterization of pmTOR and pS6 expression (as readouts of the pathway). This was a retrospective observational study. We studied 191 papillary thyroid carcinomas (PTC). A total of 118 patients treated and followed in the university hospital were included. Mean follow-up (±SD) was 8±6.7 years. Predictive value of pmTOR expression for distant metastasization and association with aggressiveness. pmTOR expression was significantly associated with distant metastases (P=0.05) and persistence of disease (P=0.05). Cases with higher pmTOR expression had a significantly lower sodium iodide symporter expression (r(44) =-0.3; P=0.03) and were submitted to more 131I treatments (r(102)=0.2; P=0.02) and higher cumulative doses of radioactive iodine (r(100)=0.3; P=0.01). Positive pmTOR expression showed to be an independent risk factor for distant metastases (OR=18.2; 95% confidence interval 2.1-157.9; P=0.01). On the other hand, pS6 was significantly associated with absence of extrathyroidal extension (P=0.001), well defined tumor margins (P=0.05) and wild type BRAF status (P=0.01). There was no correlation between the expression of pmTOR and pS6 (r(140)=0.1; P=0.3). pmTOR expression is an indicator of aggressive metastatic PTC, being possibly implicated in refractoriness to therapy, while pS6 expression is associated with less aggressive pathological features. Further studies are needed in order to understand better the biological consequences of the mTOR pathway activation in thyroid cancer behavior, namely the contribution of other pmTOR downstream effectors.
UNRAVELLING OPIOID-INDUCED HYPERALGESIA MECHANISMS IN THE BRAIN

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Abstract

Opiates represent the most commonly used drugs for managing moderate-to-severe pain. Chronic opioid treatment is associated with the development of opioid-induced hyperalgesia (OIH). OIH is characterized by hypersensitivity to non-noxious or noxious stimuli during sustained opiate administration but its molecular mechanisms are not fully understood. Increased pain facilitation is thought as one of the underlying mechanism. Here we studied the involvement of the dorsal reticular nucleus (DRt), an area involved in pain facilitation. We studied the effects of chronic morphine infusion in naïve and neuropathic animals (spared nerve injury-SNI-model) and evaluated the involvement of the DRt in OIH by pharmacological inactivation of the DRt and by knocking down the expression of the µ-opioid receptor (MOR) at the DRt. Naïve and neuropathic male Wistar rats were implanted subcutaneously with osmotic pumps for morphine or saline infusion. Pain behavior was tested 2, 4 and 7 days later. The pharmacological inactivation of the DRt was achieved by the local injection of lidocaine. MOR knock down was accomplished by a lentiviral vector stereotaxically injected at the DRt. Pain behavior was assessed before and 7 days after surgeries. MOR expression was evaluated by immunohistochemistry. Chronic morphine induced hyperalgesia both in naïve and SNI animals which was fully reversed by lidocaine. MOR expression was significantly higher in the morphine group both in naïve and SNI animals. MOR knock-down prevented the development of OIH in the morphine group and induced hyperalgesia in the saline group both in naïve and SNI animals. Our results indicate that chronic morphine exposure induces OIH in naïve and neuropathic animals and, that the DRt is involved in the mediation of OIH, likely through MOR activation whose effects appear to switch from inhibitory to facilitatory upon chronic morphine treatment.
Anal squamous intraepithelial lesions are well-recognised precancerous lesions of epidermoid anal cancer. This is a new area of research and information regarding epidemiology, pathophysiology and treatment are lacking. Men who have sex with men, human immunodeficiency virus infected individuals, women with a history of lower genital tract neoplasia and renal transplant patients are high-risk groups for anal cancer and should submitted to screening. There is no information regarding the risk of anal squamous intraepithelial lesions in other immunsuppressed populations. This is important to define the need of prevention (like vaccination) and the need of screening in other populations. Immune response and genetic factors are fundamental in reducing the risk of these human papillomavirus (HPV) related lesions. The role of genetic polymorphisms and microRNAs although have been implicated on cervical cancer and cervical intraepithelial lesions but there are no studies in anal carcinogenesis. AIM1: To evaluate the prevalence of anal squamous intraepithelial lesions in possible high-risk populations, namely liver transplant recipients and patients underdoings immunosuppressant therapy (Inflammatory bowel disease patients). AIM2: To evaluate the pharmacogenomic profile, microRNAs and HPV16 variants in patients with anal squamous intraepithelial lesions. AIM 1-Prospective case-controls studies, involving liver transplant recipients and other immunsuppressed patients that will be compared with a healthy control group. All patients will be submitted to anal cytology and anal HPV testing. Patients with anal cytology abnormal results will be submitted to high-resolution anoscopy with biopsies of any suspicious lesions. AIM 2- For pharmacogenomics profiles and circulating microRNAs: prospective cross-sectional case-control study comparing patients with histological confirmed anal squamous intraepithelial lesions and healthy controls. Peripheral blood samples will be
collected and DNA extraction and genotyping will be made. For HPV16 variants and microRNAs in the anal tissue: cross-sectional case-control study comparing low-grade and high-grade anal squamous intraepithelial lesions.

**DEVELOPMENT OF A NOVEL WEB-BASED ANATOMY STUDY PLATFORM – VIMU**

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**Abstract**
The role of technology in contemporary medicine is acknowledged. Technology changed the way that human body is seen and approached, interfering with the traditional concept of anatomy. In this context, the teaching and learning processes in anatomy rely increasingly in new tools, as is the case of e-learning and e-assessment. Following the development of “Virtual Quiz”, a tool build with the objective to improve identification of anatomical structures, we proceeded with reformulation of this project by creating of a new web-based application which functions as a study manager towards the improvement of students’ cognitive competences in anatomy in undergraduate medical course. However, the evolution of the medical knowledge requires the continuous updating of competencies in long life learning. In order to fulfill this requirement, we adapted the study manager with the objective to use this tool for the teaching/learning processes of recertification/post-graduation of medical doctors (namely General Practitioners) in the field of Anatomy. The study manager software allows students to practice their capacity to identify anatomical structures in x-ray, TC and MRI films, as well as images of sectional anatomy (“Virtual Quiz”), as well training their neuroanatomical
correlations and clinical features in the format of multiple choice question examination (“Clinical Vignettes”). Using this software it is possible to evaluate the users’ learning progress, which is useful to implement pedagogical actions in regard of the best knowledge acquisition. This work aims to present this software and discuss its use as a tool for teaching/learning in medical recertification/post-graduation and in continuous education. Keywords: Anatomy, web-based application, e-learning, recertification.

RELATIONSHIP BETWEEN ANGIOGENESIS AND METABOLISM IN DIABETES

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Abstract

Diabetes Mellitus is a chronic disorders with an increasing incidence worldwide, rendering it a major public health problem. According to World Health Organization (WHO), diabetes prevalence reached 9% in adults in 2014. And the estimate of WHO for 2030 indicates that Diabetes will be the seventh main cause of death. Although there are several types of diabetes, the more frequent ones are type 1 Diabetes, characterized by decreased levels of insulin secreted by the pancreas, and type 2 Diabetes, which is associated with insulin resistance. Chronic hyperglycaemia, a feature of diabetes, causes various structural and metabolic derangements, playing a crucial role in a wide variety of complications throughout the organism, including macrovascular (often leading to coronary artery disease, peripheral artery disease, and stroke) and microvascular ones (associated with retinopathy and nephropathy. The dysfunction of vascular endothelium is an important factor in the pathogenesis of
these complications. Interestingly, the same diabetic patients may develop increasing vascular structures in some organs (e.g. retina, kidney) and impairment of this process in other organs (e.g. heart, skin). Therefore, metabolism in each specific organ is likely to influence the vascular behavior, within the endothelial cells. The aim of this study is evaluate the mechanisms by which metabolic changes characteristic of diabetes affect vascular complications in distinct organs, especially in those where the angiogenic paradox is established. An animal model (C57Bl/6 mice) of type 1 Diabetes is being established by administering streptozocin, either in the presence and absence of insulin injection; age and sex-matched controls will be used; endothelial cells will be isolated from kidney and heart by cell sorting (FACS); Angiogenic or metabolic markers will be evaluated in these endothelial cells by microarray assay. After identifying the most relevant markers in each organ, functional studies will be performed in endothelial cell cultures by pharmacological or siRNA inhibition for confirmation. The purpose of this presentation is to discuss the established procedure and to present the preliminary findings observed (glycemia, mice weight, number of endothelial cells obtained per organ). The identification of this crosstalk between metabolism and angiogenesis in diabetic heart and kidney endothelium may open novel therapeutic strategies against these vascular complications that cause high morbidity and mortality rates among diabetic patients.

IMPLICATIONS OF MITOCHONDRIAL DYSFUNCTION IN HEART FAILURE WITH PRESERVED EJECTION FRACTION

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Abstract

Heart failure with preserved ejection fraction (HFpEF) represents 50% of patients with heart failure and its prevalence is associated with metabolic syndrome (MetS). The mechanisms underlying cardiovascular disease in MetS are complex and include, among others, metabolic and mitochondrial alterations, which culminate with increased oxidative stress. Thus, with this work we intend to evaluate mitochondrial function and oxidative stress in an animal model of HFpEF triggered by MetS. Lean ZSF1 (ZSF1Ln, n = 7) and obese ZSF1 (ZSF1Ob, n = 7) rats were submitted an effort testing to determine the maximal O2 consumption (VO2max) and echocardiographically evaluated, at 20-25 weeks. Left ventricular (LV) samples were collected for mitochondrial functional studies, protein expression by immunoblotting and for in situ detection of myocardial oxidative stress by immunofluorescence. ZSF1Ob rats have a lower tolerance to VO2max effort and anaerobic threshold, despite the increased cardiac output. Echocardiographically, the ZSF1Ob group showed a LV mass increase, which was accompanied by preserved ejection fraction and LV diastolic dysfunction. Mitochondrial functional studies showed that ZSF1Ob group presents significant dysfunction of complex 1 and a tendency of the complex 2, alterations in the permeability of the mitochondrial pore and an increase in the time for the repolarization of the membrane potential, results that confirm the mitochondrial deterioration. The protein expression studies revealed a significant increase in the expression of NADPH oxidase 2 and a significant decrease in NADPH oxidase 4 in ZSF1Ob rats, enzymes responsible for regulating the formation of reactive oxygen species (ROS). In situ detection of superoxide anion was significantly higher in ZSF1Ob rats, which proves the formation of ROS. Additionally, ZSF1Ob group presented a decrease in eNOS phosphorylation, impairing the production of nitric oxide. Mitochondrial dysfunction induced by oxidative stress in rats with MetS is an important pathophysiological mechanism in HFpEF.
MODULATION OF CARDIAC STRUCTURE BY EPICARDIAL ADIPOSE TISSUE

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Abstract
Diastolic heart failure (DHF) is an important cause of cardiovascular mortality. Several risk factors, like obesity, are associated with its development. In obesity, due to adipocyte hypertrophy and dysfunction, there is an increased secretion of proinflammatory adipokines. These adipokines produced by epicardial adipose tissue (EAT) can act in a paracrine manner directly on the myocardium. We aim to characterize the profile of EAT under conditions of DHF and to evaluate their possible changes in cardiac structure. EAT of 20-weeks-old lean and obese ZSF1 rats was collected for: 1) separation of proteins to mass spectrometry (MS) identification, 2) adipokines’ expression, 3) adipocytes fibrosis and cross-sectional area assessment, 4) for 24h DMEM-incubation to obtain conditioned medium (CM). Organotypic-cultures were prepared from 7-day-old Wistar-Kyoto cardiac explants and incubated for 24h with the CM previously obtained from both groups. After incubation, cross-section area of cardiomyocytes and fibrosis were evaluated. In EAT of the obese ZSF1, MS-results presents decreased levels of 3-ketoacyl-CoA thiolase protein enzyme as a compensatory mechanism in order to inhibit fatty acid oxidation and increase lumican and collagen-alpha-1(I)proteins suggesting a link between inflammation caused by obesity and increases of adipose tissue extracellular matrix. The histological and molecular studies revealed hypertrophy of adipocytes in obese animals (1505±80.01μm2 vs.7595±265.5μm2,p).
GAMETE DONATION, CITIZENSHIP AND BIONETWORKING IN PUBLIC BANKS

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Abstract
Studies in the field of gamete donation have focused mostly on the dichotomy between the resource-poor countries/conditions of the donors and the richer Western countries’ recipients and hosting research projects. Furthermore, most independent studies focus on only one group of donors for a specific aim: reproduction or research. Finally, there is a shortage of original empirical research that crosses the perspectives of donors, recipients and health professionals. This study aims to understand how social, cultural, and economic characteristics intertwine with the health experiences, knowledge and identities of those involved in gamete donation, by taking a bionetworking approach to assess policies (regulations, policy-making, standard setting), provision (donor/recipients selection, patient-centered care), and production (infrastructures, platforms) linked to gamete donation. This study will employ a mixed-methods approach, relying on: 1) documentary analysis (legislation, guidelines, protocols, informed consent sheets, websites of fertility clinics); 2) lab ethnography in the Portuguese public gametes bank; 3) questionnaires, and 4) semi-structured interviews with donors and recipients attending the Portuguese public bank of gametes during a 12-month period, and with practitioners and embryologists responsible for Portuguese fertility centres offering heterologous cycles. This project will produce relevant data to tackle two main societal challenges: 1) the promotion of excellence in public health services, a key goal of the Portuguese Smart Specialization Strategy 2014-2020; 2) sustainable patient-centered care and inclusive and innovative societies, topical themes set out in the European Work Programme for the H2020. Its innovativeness relies on: crossing the views of donors, recipients and health professionals; exploring gamete donation both for purposes of reproduction and...
research; generating knowledge to prevent and deal with obstacles to safe, just and ethical collaborations between fertility units engaged in the flows of eggs and sperm.

MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL PROFILE OF HEREDITARY DIFFUSE GASTRIC CANCER

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Abstract

Hereditary Diffuse Gastric Cancer (HDGC) encompasses a spectrum of precursor and invasive lesions. We aimed to characterize the morphology of early and advanced HDGCs and investigate the relationship between morphology and biomarkers of cell-adhesion, proliferation, anoikis, epithelial-mesenchymal-transition and cancer cell stemness. Twenty-one lesions from 17 HDGC patients encompassing 12 intramucosal carcinomas (pT1a) and 9 widely invasive carcinomas (pT>1) were analysed by immunohistochemistry for E-cadherin, Ki67, Bcl-2, p53, pSrc and ALDH1A. All pT1a lesions showed typical signet ring cells (SRCs), absence of p53 and Ki-67 expression. In contrast, pT>1 carcinomas were composed by a mixture of SRCs and pleomorphic cells, characterized by high Ki-67 proliferation index (89%) and p53 overexpression (56%) in the pleomorphic component. E-cadherin immunoexpression was heterogeneous, from absent/decreased to cytoplasmic. Expression of ALDH1 and pSsrc decreased from early (100% and 58%, respectively) to advanced carcinomas (44% and 33%, respectively). Bcl-2 was expressed only in one case. We verified that early HDGCs present with an “indolent” phenotype (SRCs; Ki67–; p53–), while advanced carcinomas display an “aggressive” phenotype (pleomorphic cells; Ki67+;p53+). This is the first evidence of phenotypic heterogeneity in HDGC lesions and may help define predictive biomarkers of progression from indolent to widely invasive carcinomas.
ENVIRONMENTAL AZOLE FUNGICIDE, PROCHLORAZ, CAN INDUCE CROSS-RESISTANCE TO MEDICAL TRIAZOLES IN CANDIDA GLABRATA

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Abstract

Acquisition of azole resistance by clinically relevant yeasts in nature may result in a significant, yet undetermined, impact in human health. The main goal of this study was to assess the development of cross-resistance between agricultural and clinical azoles by Candida spp. An in vitro induction assay was performed, for a period of 90 days, with Prochloraz (PCZ) – an agricultural antifungal. Afterwards, the induced molecular resistance mechanisms were unveiled.

MIC value of PCZ increased significantly in all Candida spp. isolates. However, only C. glabrata developed cross-resistance to fluconazole and posaconazole. The increased MIC values were stable. C. glabrata azole resistance acquisition triggered by PCZ exposure involved the upregulation of the ATP binding Cassette multidrug transporter genes and the transcription factor, PDR1. Single mutation previously implicated in azole resistance was found in PDR1 while ERG11 showed several synonymous single nucleotide polymorphisms.

These results might explain why C. glabrata is so commonly less susceptible to clinical azoles, suggesting that its exposure to agricultural azole antifungals may be associated to the emergence of cross-resistance. Such studies forward potential explanations for the worldwide increasing clinical prevalence of C. glabrata and the associated worse prognosis of an infection by this species.
EXPRESSION OF OSTEOPONTIN ISOFORMS IS RELATED WITH THYROID CANCER GROWTH AND INVASION

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Abstract

Osteopontin (OPN) is a matricellular protein highly expressed in cancer cells, which is able to modulate tumorigenesis and metastasis in several malignancies, including follicular cell-derived thyroid cancers (TC). OPN is one of the gene products aberrantly expressed in TC, but the contribution of each OPN isoform (OPNi), named as OPNa, OPNb and OPNc, is currently unknown. This study aims to analyze the expression profile of OPNi in TC tissue samples, correlate its expression with molecular and clinicopathologic features, and evaluate the role of OPNi in TC cell lines. The expression profiles of OPNi in TC cell lines and in thyroid tissues were evaluated by qRT-PCR and immunohistochemistry. In order to address the putative roles of OPNi in TC, we overexpressed OPNi in c643 and 8505c TC cell lines. Functional assays were also performed. We found that the OPNa and the OPNb are expressed in higher levels in classic papillary thyroid carcinoma (cPTC) than in non-tumoral thyroid, adenomas and follicular thyroid carcinoma tissues. Conversely, OPNc levels are similar among samples from the aforementioned pathologies. In cPTC, high OPNa and OPNb expression levels were significantly associated with higher tumor size, presence of vascular invasion and BRAFV600E mutation. In TC cell lines, we observed differential expression of the OPNi, of which c643 expressed the lower levels of OPNi. Higher proliferation, migration and motility were associated with c643 and 8505c overexpressing OPNa. In both cell lines overexpressing OPNa, we observed an increase of matrix metalloproteinase 2 in the extracellular medium. Further, in vivo CAM assay we found that cells overexpressing OPNa are significantly more invasive when compared to the control cells. Taken together, our data indicate that both OPNa and OPNb are overexpressed in cPTC and OPNa is significantly associated to promotion of cell growth, migration, motility and invasion in TC cells.
**POLYPHENOL CONSUMPTION MODULATES ANGIOGENIC PATHWAYS IN DIABETES MELLITUS TYPE 2**

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Metabolismo: Clínica e Experimentação  
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**Abstract**

Diabetes mellitus (DM) is responsible for metabolic deregulation leading to inflammation and oxidative stress, causing angiogenic derangements. An angiogenic paradox is present, with distinct patterns in different organs, namely an increase of kidney neovascularization and the opposite on the left ventricle (LV), but the mechanism has not been described. Beer polyphenols, as xanthohumol (XN) and 8-prenylnaringenin (8-PN), modulators of angiogenesis, could ameliorate DM related complications. This study aimed to verify if polyphenols consumption affects angiogenic paradox and if this is related with metabolic changes in diabetic mice. For this purpose, C57Bl/6 mice were divided in 5 groups: standard diet, high fat diet (HFD), HFD and ethanol, HFD and XN, and HFD and 8-PN during 20 weeks. Kidney and LV were collected to evaluate microvessel density (MVD) and the expression of angiogenic receptors and related pathways. Statistically significance was assessed by ANOVA followed by Bonferroni test. We confirm the presence of the angiogenic paradox in diabetic animals, with an increase of MVD in kidney of HFD-fed animals, and a reduction of the neovascularization in the LV. Polyphenol ingestion led to a significant reduction in neovessel formation and VEGFR-2 expression and VEGF-A levels in kidney and an increase of those parameters in LV. These results are accompanied by reduced VEGF-B levels and VEGFR-1 expression in both tissues and plasma, which may be related to a decrease in endothelial-to-tissue lipid metabolism, reducing ectopic lipid accumulation, and by a reduction of PFKFB3, a glycolytic activator in endothelial cells. Beer polyphenols had benefits on DM angiogenic paradox with distinct effects on different tissues and prevents the development of metabolic dysregulation associated with diabetes. It would be of great interest to
clarify the interplay of angiogenesis and metabolism for the development of novel therapeutic strategies.

SEXUAL EDUCATION OF YOUNG PEOPLE AND PREVENTION OF UNWANTED PREGNANCIES

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Abstract
The clandestine abortion is an important health problem in Angola, estimating that is responsible for 10 to 15% of maternal mortality.

Two fundamental factors related to this problem: 1) the high index of unwanted pregnancy due to poor sexual education of adolescents and young people and 2) the highly restrictive legislation on abortion. The consequences are obvious: Before the determination of interrupting the pregnancy, patients opt for any resource to achieve it.

In addition to be proclaimed by UNESCO as a right of all young people, studies demonstrated that sexual education well conducted promotes a more responsible sexual behavior, guided not by individual feelings and the mere sexual biological instinct, but by the good morals and standards of society.

As part of a coherent strategy forwarded to introduce sexual education in schools of Angola, I conceived this manual as didactic material of a basic course designed in a first step for secondary school students.

The manual, written in a simple language and slick, is structured with an introduction, 6 chapters and an epilogue. The chapters deal in a didactic sequence the following topics:
Chapter I: Why and what for this manual?
Chapter II: anatomy and physiology of human reproduction.
Chapter III: human reproduction.
Chapter IV: the essential aspects of the biological development relating to sexuality.
Chapter V: Meaning and development stages of sexuality.
Chapter VI: Safety sexual and reproductive health.

This Manual, recommended by the Ministry of Education for all educational institutions, especially of secondary education, will come to light, in the coming weeks with the seal of Editora Mayamba and it is a part of a research project for the doctorate.

**DIETARY POLYPHENOLS AFFECT UPTAKE OF 14C-FRUCTOSE BY INTESTINAL EPITHELIAL CACO-2 CELLS**

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**Abstract**
The incidence of metabolic syndrome has been dramatically increasing during the last decades. Intake of high-fructose products is closely related with the development of metabolic syndrome and interestingly a substantial increase in the consumption of this sugar has been observed during the last 30 years. Several polyphenols are known to interfere with glucose intestinal absorption, but little is known concerning the effect of these phytochemicals on fructose intestinal absorption. So, we decided to investigate if polyphenols can interfere with fructose intestinal absorption, by testing both the acute (26 min) and the chronic (24h) effect of 27_distinct_polyphenols, belonging to distinct classes, on the uptake of 14C-fructose (100 nM) by Caco-2 cells.
Several polyphenols induced a significant decrease on the uptake of 14C-fructose. But Quercetin (100 µM), chrysin (100 µM) and apigenin (100 µM) caused the most marked effect (a 25% decrease in uptake). The inhibitory effect of these polyphenols was not related to a cytotoxic effect, as these compounds did not affect cell viability (determined with the MTT and the LDH assays). When combined, the effect of the polyphenols having the most marked chronic inhibitory effect was similar to the effect of the single polyphenols. GLUT5 is the main carrier involved in the apical uptake of fructose by enterocytes. By using a specific inhibitor of GLUT5 (L-Sorbose-Bn-OZO 10 µM) we could conclude that quercetin 100 µM, apigenin 100 µM and chrysin 100 µM do not appear to interfere with this transporter. On the other hand, by using phloretin 1 mM, an inhibitor of GLUT2, we could conclude that these polyphenols appear to interfere with this transporter. In conclusion, several polyphenols were found to be effective inhibitors of 14C-fructose uptake by Caco-2 cells. This suggests that these compounds might decrease the intestinal absorption of fructose, with beneficial effects on metabolic syndrome. Results obtained with the GLUT5 and GLUT2 inhibitors lead us to conclude that polyphenols appear to interfere with GLUT2-mediated 14C-fructose uptake.

INCOMPLETE REVERSE REMODELING IN MICE AFTER SURGICAL REMOVAL OF CHRONIC PRESSURE-OVERLOAD

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Abstract
Incomplete myocardial reverse remodelling (MRR) is a major determinant for heart failure patient worse outcome. We aim to establish and characterize an animal model that mimics the structural and functional changes in MRR after chronic pressure-
overload (PO) relief. Pressure-overload was established in 7-weeks-old C57BL/6-mice by ascending aortic constriction. An echocardiographic and hemodynamic evaluation was performed to assess cardiac function after 7-weeks. Subsequently, a surgical debanding (DEB) procedure was performed in half of the banding (BA) and control (SHAM) animals (BA_DEB and SH_DEB). Cardiac function was re-evaluated 2-weeks later. Interstitial fibrosis and cardiomyocyte hypertrophy were quantified. Comparing to SHAM, PO induced concentric left ventricle hypertrophy (LVH) as confirmed by increased values of LV mass (135.60±6.84 vs 176.80±9.66 mm/cm², p=0.0034), LV maximum pressure (85.17±3.94 vs 126.30±9.42 mm/cm², p=0.0019) and LV cardiomyocytes sectional area (467.50±4.81 vs 510.40±5.01 mmHg, p).

**IMPAIRMENT OF SENSORY FIBERS BY ONABOTULINUMTOXIN/A (ONABOT A) IMPROVES NEUROGENIC DETRUSOR OVERACTIVITY (NDO) FOLLOWING SPINAL CORD INJURY (SCI)**

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**Abstract**

SCI often leads to NDO due to synaptic reorganization of circuits involving sensory nerves. NDO is characterized by high frequency of voiding contractions and increased intravesical pressure being a priority when managing SCI. A current therapy for NDO consists in OnabotA bladder injections, which impair both sensory and parasympathetic nerves coursing the bladder. However, the marked decrease of parasympathetic outflow frequently causes urinary retention in patients maintaining voluntary voiding. We hypothesized that the selective impairment of sensory nerves
by OnabotA would improve NDO without the risk of urinary retention. Rats underwent T9 complete spinal cord transection and an intrathecal (IT) catheter was implanted at L5/L6 spinal segments. Sham animals were used as controls. Four weeks later, rats received IT OnabotA (2U in 50 μL of saline) (n=5) or saline (n=5). Cystometries were performed 48h later. L5/L6 spinal segments and dorsal root ganglia (DRG) were collected and processed for immunohistochemistry against cleaved (c) SNAP-25 (OnabotA end-product), CGRP (sensory marker) and ATF3 (neuronal damage marker). CGRP and ATF3 expression at the L5/L6 DRG were quantified by western-blots. Rats developed NDO 4 weeks after SCI. IT saline did not induce any alteration in bladder function. IT OnabotA significantly decreased the frequency of voiding contractions and basal intravesical pressure. Urinary retention was not observed in OnabotA-treated rats. Immunolabelling for cSNAP-25 was only found in OnabotA treated rats and it was mainly observed in dorsal horns, suggesting a restricted effect on sensory nerves. CGRP labelling was more intense in SCI saline-treated rats comparing to sham. IT OnabotA significantly reduced the expression of CGRP at the spinal cord dorsal horns and DRG and increased ATF3 DRG expression. IT OnabotA improved NDO through a selective activity on sensory afferents an effect that may involve the impairment of neurotransmitter release in the dorsal horn and neuropeptide DRG synthesis.

EXPLORING THE SYNERGISTIC INTERACTION BETWEEN LIPOSOMAL AMPHOTERICIN B AND COLISTIN

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Abstract
Patients at risk of invasive fungal infections often receive simultaneously or sequentially antifungals and antibacterial agents, without full knowledge of the consequences of
drug interactions. The literature has demonstrated that colistin (COL) exhibits a synergistic effect with some antifungals. Considering the clinical relevance of liposomal amphotericin B (L-AMB), this work aims to evaluate the association between L-AMB and COL against Candida and Aspergillus spp. and elucidate the mechanism involved in this interaction. Four clinical isolates of Candida spp. and two of Aspergillus fumigatus with different susceptibility profiles to L-AMB were used. In order to evaluate the combined effect of L-AMB and COL, the MIC to L-AMB was determined in the presence of 1-, 2-, 4- and 10-fold plasma concentration of COL (3 mg/L). MIC to COL alone was also determined. Cellular physiological status induced by the association of L-AMB/COL was assessed by flow cytometry in a time-course assay. Additionally, computational molecular dynamic studies were performed to clarify the molecular interaction that occurs between L-AMB/COL. Single colistin was completely inactive against all the fungal isolates studied but when associated with L-AMB a maximum synergistic effect was reached at 4-fold serum concentrations of COL (12 mg/L), with a L-AMB MIC reduction of 2-3 dilutions for Candida and 4-5 dilutions for Aspergillus. L-AMB/COL association induced: an increase of cell membrane permeability; an increase of cellular metabolic activity soon after 1h of exposure; an increase of ROS production up to 24h. The molecular dynamic studies suggested that L-AMB and COL molecules act together on fungal cells, forming a strong complex. We demonstrated that COL interacts synergistically with L-AMB, inducing an increase of cell membrane permeability and metabolic activity, which can be related with an increase of endogenous ROS production as a stress response. L-AMB fungicidal activity is improved by the complex formed with COL.

UROCORTIN-2 IMPROVES RIGHT VENTRICULAR FUNCTION AND ATTENUATES EXPERIMENTAL PULMONARY ARTERIAL HYPERTENSION

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Abstract

Urocortin (UCN)-2 has shown promising therapeutic effects in humans and animal models with heart failure (HF). This study analyzed the effects of UCN-2 treatment in an animal model of right ventricle (RV) HF, secondary to pulmonary arterial hypertension (PAH). Male Wistar rats received monocrotaline (MCT, 60mg/Kg) or vehicle. Additionally, in order to differentiate indirect from direct myocardial effects of UCN-2 treatment, we used a model of RV hypertrophy without PAH (pulmonary artery banding-PAB). Thus, another set of rats were subjected to PAB or sham operation. The study resulted in 7 groups: CTRL (n=9), CTRL+UCN-2 (n=9), MCT (n=7), MCT+UCN-2 (n=10), SHAM (n=8), PAB (n=9), PAB+UCN-2 (n=9). After 2 weeks, animals received UCN-2 (5µg/Kg/day) or vehicle. Functional studies and samples collection were performed 4 weeks after MCT injection/PAB operation. Hemodynamic studies revealed that MCT group developed PAH, as shown by increased RV end-systolic pressure, end-diastolic pressure, RV dilation, decreased cardiac output, and ejection fraction. UCN-2 treatment resulted in attenuation of these changes. Moreover, the survival rate for UCN-2 treated rats was higher than for MCT rats and UCN-2 treatment was able to increase exercise tolerance in animals with PAH. PAH rats presented RV hypertrophy, as shown by the morphometrical analysis (RV weight/tibia length ratio) and by histology (cardiomyocyte cross-sectional area), and UCN-2 treatment attenuated RV remodeling. Molecular studies showed that MCT group presented increased UCN-2 expression and decreased CRHR2 expression in the RV, which was reversed by UCN-2 treatment. The increased expression of pathology markers in MCT animals (BNP, ET-1 and HIF-1α), as well as markers of apoptosis (caspase-3 and caspase-8) were attenuated by UCN-2. Moreover, UCN-2 treatment also reverted RV morpho histological changes in animals submitted to PAB. UCN-2 treatment attenuates PAH and RV dysfunction and increases survival in MCT-induced PAH, and has direct anti-remodelling effects on the pressure-overloaded RV. UCN-2 has a relevant role in the pathophysiology of PAH, and might be a new treatment option in this condition.