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Clinical biology

Multi-drug and extreme-drug resistance in clinical isolates of *Pseudomonas aeruginosa*

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Background: *Pseudomonas aeruginosa* has been globally implicated in healthcare-associated infection. The susceptibility pattern of clinical isolates of *Pseudomonas aeruginosa* to anti-pseudomonal antibiotics is reported.

Methods: Clinical samples namely blood, urine, tracheal aspirate, cerebrospinal fluid, wound swabs, high vaginal swabs, and eye and ear exudates were collected from patients and processed and identified using standard microbiological protocols. The antibiotic susceptibility testing was undertaken using the Kirby Bauer Disc diffusion method and the results were reported following Clinical and Laboratory Standards Institute guidelines.

Results: Of the 104 *Pseudomonas aeruginosa* isolates identified, a higher incidence was observed in males (52.88%) than in females (47.11%) patients. The highest prevalence was recorded from wound swabs [46 (44.23%)] followed by ear exudates [23 (22.12%)], urine [22 (21.15%)], while eye exudates and samples from the cerebrospinal fluid yielded the least [1 (0.96% each)]. From the antibiogram, imipenem had the highest antibiotic activity (91.3%) followed by polymyxin B (84.6%). The isolates exhibited the highest resistance to ceftazidime (73.1%) and piperacillin-tazobactam (61.5%). The antibiotic resistance pattern of *P. aeruginosa* isolates revealed 7.69% susceptible, 26% resistant, 61%

multidrug resistance (MDR), 5% extremely drug-resistant (XDR) and an absence (0%) of pandrug resistant phenotypes.

Conclusions: The study revealed an alarmingly high prevalence of MDR phenotypes and some XDR phenotypes of *P. aeruginosa* in UPTH. It will help to identify existing gaps in antimicrobial resistance surveillance and assist in improving public health policies regarding antibiotic stewardship, initiatives and interventions.

The in vitro effects of topical corticosteroids, intranasal saline, and antibiotics on biofilms of *Staphylococcus spp.* isolated from patients with chronic rhinosinusitis

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Background: Chronic rhinosinusitis (CRS) is a persistent inflammation of the nasal and paranasal sinuses mucosa, often accompanied by dysbiosis and bacterial infection. The disease has a multifactorial aetiology, and microbial biofilms have been implicated in the pathogenesis of chronic rhinosinusitis with nasal polyposis (CRSwNP). The presence of biofilms interferes with conventional therapy of sinusitis and is often a cause of antibiotic resistance due to selective

pressure from frequent antibiotic consumption in CRS patients.

Objectives: The aim of our study was to evaluate in vitro effects of two topical corticosteroids, isotonic and hypertonic nasal saline and two antibiotics commonly used in CRS treatment on biofilm formation by staphylococci isolated from the sinus tissue of CRSwNP patients.

Methods: The sinus mucosal specimens were harvested from the ethmoid cavity during endoscopic sinus surgery. The presence of biofilms was confirmed with histopathological examination of tissues, and identification of the strains was performed by MALDI-TOF MS System. Antibiotic-resistance profiles were assessed following the European Committee on Antimicrobial Susceptibility Testing recommendations. The biofilm-forming capacity of isolated bacteria was detected by the quantitative microtitre-plate method, and the effect of corticosteroids (mometasone and fluticasone), nasal salines (isotonic and hypertonic sodium chloride) and amoxicillin-clavulanic acid and levofloxacin on biofilm production was investigated in several concentrations by the recommended therapeutic dose when used in CRS.

Results: Five different strains of staphylococci were isolated from mucosal specimens of 40 patients, with a predominance of *Staphylococcus epidermidis* (42,5%) and *S. aureus* (35%). The most significant reduction of biofilm formation after treatment with corticosteroids and nasal salines was observed in strains of *S. aureus*. Fluticasone and isotonic saline were slightly effective in the suppression of biofilm formation in all tested species compared to the mometasone and hypertonic saline.

Subinhibitory concentrations of amoxicillin-clavulanic acid (1/2-1/8xMIC) and levofloxacin (1/2-1/4xMIC) significantly reduced staphylococcal biofilm formation ($p < 0.01$ and $p < 0.05$, respectively), with better efficacy of amoxicillin-clavulanic acid.

Suprainhibitory concentrations of both tested antibiotics (4-64 µg/ml) significantly eradicated mature biofilms of staphylococci ($p < 0.01$), with a more noticeable effect of levofloxacin, compared to the effect of amoxicillin-clavulanic acid ($p < 0.05$).

The inhibitory effect of all tested compounds is highly correlated with the amount of formed biofilm.

Conclusions: The topical steroids and nasal salines are shown to be potent antibiofilm agents in patients with CRSwNP, with better efficacy than fluticasone and isotonic saline. Amoxicillin-clavulanic acid was more effective in the prevention of biofilm formation, and levofloxacin in the eradication of mature biofilm. The effect of compounds tested in the study was dependent on bacterial species and the amount of formed biofilm.

Mediterranean-style diet intervention improves clinical, inflammatory, and mineral profiles in pre-diabetes patients

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Background: According to the World Health Organization (WHO), in 2030, diabetes will be one of the leading causes of death. In the early phases of the disease, a pre-diabetic stage develops with impaired fasting glucose associated with chronic low-grade inflammation. Lifestyle modifications while the patient is still in the pre-diabetes stage are effective in preventing or delaying the onset of diabetes. Herein, the main objective of this study is to evaluate the effects of Mediterranean diet-associated patterns and aerobic exercise on immunometabolism-related genes in overweight individuals with prediabetes.

Methods: All participants followed a low-energy diet (5.5 MJ/1315 kcal/daily) and aerobic exercise following WHO recommendation (at least 150 minutes of moderate-intensity aerobic physical activity each week) for 12 weeks. Participants were recruited from Marchena (Seville, Spain). Those eligible for inclusion were individuals with abdominal obesity (waist circumference ≥ 94 cm in men and ≥ 88 cm in women) and pre-diabetes (HOMA-IR $\geq 2,78$). Anthropometric and biochemical measures were carried out at baseline, 3, 6, and 12 weeks. At these points, peripheral blood mononuclear cells (PBMCs) were isolated and were used to analyse the effects of the nutritional and physical intervention on immunometabolism-related genes by using RT-qPCR. Finally, cytokines were evaluated by ELISA. Minerals were detected using colourimetric methods.

Results: In total, 20 individuals (15 women, and five men) attended the baseline visit and completed the follow-up visit. All the participants had a significant decrease in BMI, lean mass, fat mass, glucose, TC, TG, HDL-C and LDL-C. ($p < 0.05$). Likewise, cytokines (IL-10, IL-6, IL-1 β , TNF- α) were modulated substantially and mineral concentrations (Na, K, Cu, Mg, Cl, P, Ca, Zn, Fe) showed differences between the pre- and post-intervention analysis.

Conclusions: In overweight patients with pre-diabetes, Mediterranean diet-associated patterns and aerobic exercise modified mineral concentrations and improves clinical and inflammatory profiles.

The chronomodulatory potential of the therapeutic association of melatonin with irbesartan, on an experimental model of cardiovascular disease

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Background: The circadian pattern is greatly imprinted in human behaviour, in the physiological expression of every cell and the endocrine general setting. The majority of biochemical parameters change dramatically through a 24-hours cycle, following a strict code dictated by clock genes ubiquitarily expressed at the molecular level, that determine oscillatory protein synthesis.

From a pathological point of view, the cardiovascular system is the most exposed to circadian disruption consequences, among which hypertensive attacks depict a unique circadian rhythm with two important peaks, one in the early evening around 7 pm and the other one early in the morning, around 6 am.

Objectives: As conventional cardiovascular therapy does not target these particular time frames and neither the circadian disruption that usually accompanies this syndrome, the present study aimed to investigate, in a hamster experimental model, the chronomodulated antiatherogenic and antioxidant effects of irbesartan and melatonin. The therapeutical association between an angiotensin II receptor antagonist, irbesartan, with a chronobiotic hormone, melatonin, which is also one of the most important free radicals scavengers, is a completely new approach for managing the pathogenesis of cardiovascular diseases, aiming to moderate the hypertensive attacks, and nevertheless to adjust the chronobiological misalignments present in these syndromes.

Methods: The used experimental model is a classic one for inducing cardiovascular disorders in hamsters. It is accomplished exclusively by diet, mimicking human diseases, both in terms of metabolic and pathophysiological terms. A cholesterol, butter and salt-enriched diet, administered in hamsters, induces hypercholesterolemia and hypertension to give changes both in the values of the biochemical parameters and blood pressure, as well as the accumulation of lipids in the arterial wall and the aortic arch. Thus, the experimental animals throughout the study received a hypercholesterolemic, hypertensive, pro-atherogenic diet for three months.

The treatment protocol required the administration of irbesartan (10 mg/kg b.w./day) combined with or without melatonin (10 mg/kg b.w./day), orally administered by

gavage in different temporal frames, concerning the nocturnal species pattern.

Blood pressure, behavioural sleep-wake patterns and biochemical parameters involved in the etiopathogenesis of most cardiovascular diseases were determined: glucidic and lipidic profiles and oxidative stress levels.

Results: Irbesartan or melatonin monotherapy resulted in a significant reduction of blood pressure, and a mild restoration of the circadian rhythms. Furthermore, the combination therapy significantly decreased plasma levels of major pro-atherogenic markers, depicting a potentiating effect of the two drugs combination. This positive modulatory effect is also registered regarding the oxidative stress level and the chronobiotic restorative potential of this association. An interesting finding was the difference between the results obtained after administering this therapeutic combination of melatonin and irbesartan, in the two temporal frames considered relevant, which strongly impacted the endogenous clock system mechanism, improving blood pressure circadian rhythms.

Conclusions: The present study brings relevant and noteworthy conclusions regarding the potentiated effects of a new therapeutic combination, melatonin and irbesartan, and the tremendous significance of the time of administration for cardiovascular system targeting drugs, raising awareness of the importance of chronopharmacology and chronotherapy.

Neutralising antibodies and anti-COVID-19 vaccines

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Background: Problems and uncertainties generated by COVID-19 regarding different types of vaccines and the number of doses to be administered, led the Official Pharmacist Society of the Canary Islands (COFSCTFE) to carry out an effectiveness study according to the vaccine, number of doses and the presence or absence of diagnosed COVID 19 in healthcare personal.

Objectives: To detect and quantify neutralizing antibodies present in the serum of vaccinated volunteers. To estimate the protection generated considering vaccination.

Methods: 377 people participated in the study (342 were vaccinated with two doses and 35 were vaccinated with three doses). All of them were working in centres directed by a pharmacist.

The AFIAS COVID-19 nAb test was used, a competitive immunodetection method based on the work of Chee Wah Tan et al. in Nature Biotechnology.

Results: Of all the people vaccinated with two doses, 9,54% did not generate neutralizing antibodies. Janssen's vaccine, which proposed a single dose, obtained a 34,1% negative rate.

Conclusions: 1.- There is no difference by sex or age in the generation of neutralizing antibodies, regardless of the vaccine administered.

2.- The "RNA" vaccines are the ones that showed better results in terms of the generation of neutralizing antibodies.

3.- People diagnosed with COVID-19 had high values of neutralizing antibodies, regardless of the people with the two-dose vaccine.

4.- People with low percentages of neutralizing antibodies after the first two doses, with the third dose, the presence of nAb rises significantly.

5.- The administration of a single dose of Jansen's vaccine showed a less optimal result in the study.

6.- The results of the administration of Aztra-Seneca's vaccine and a second dose of an "RNA" vaccine resulted in higher protection.

Uncovering anti-infectious properties and radical scavengers of *Stenochlaena palustris* from Riau province, Indonesia

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Background: *Stenochlaena palustris*, also known as Kelakai, is ubiquitous in the province of Riau, Indonesia. This plant is primarily found in swamps and oil palm plantations, and it is consumed as a vegetable by some Riau Province villagers. In addition, it is believed to be effective as a traditional medicine for treating anaemia, stimulating breast milk production, reducing fever, and relieving skin pain on Borneo Island, Indonesia.

Objectives: The purpose of this research is to understand the antibacterial and radical scavenger properties of various species extracts.

Methods: Maceration of air-dried aerial parts of the species in methanol was followed by liquid-liquid extraction against n-hexane, dichloromethane, and ethyl acetate to obtain n-

hexane (HE), dichloromethane (DCME), ethyl acetate (EAE), and water extracts (WE). Pathogenic bacteria and human parasites were used to determine anti-infectious properties. In contrast, the antioxidant properties were evaluated against DPPH and nitric oxide radicals.

Results: By employing the Kirby-Bauer disc diffusion method, it was determined that the antibacterial activity of the extracts displayed an intermediate susceptibility against pathogenic bacteria. At a concentration of 500 µg/disc, both DCME and EAE demonstrated antibacterial activity against gram-positive bacteria, including gram-negative bacteria (*Listeria monocytogenes* and *Vibrio alginolyticus*). In addition, evaluations for in vitro anti-plasmodium properties were performed on two different strains of *Plasmodium falciparum* (3D7 and W2), and the results demonstrated that EA and DCME have promising activity at IC₅₀ concentrations of 11.06 and 21.68 µg/mL, respectively. In addition, the antioxidant activities of both extracts were demonstrated against DPPH and NO radicals with an IC₅₀ value ranging from 25.73 to 34.54 µg/mL.

Conclusions: These observations allow for a better understanding of the anti-infectious and radical scavenging properties of the *S. palustris* extracts, which can then serve as a basis for additional research into the isolation and bioactivity of secondary metabolites.

Fetal transfer of tadalafil is limited by breast cancer-resistant protein in the mouse

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Background: Tadalafil, a phosphodiesterase 5 (PDE5) inhibitor, is expected to be a therapeutic agent for fetal growth restriction and hypertensive disorders of pregnancy, and it is under clinical study. A perfusion study using the human placenta suggested that tadalafil is less transferred to the fetus than sildenafil. The fetal transfer across the placenta is regulated by the permeation of the syncytiotrophoblast layer, and the placenta barrier and several efflux transporters such as MDR1 and BCRP are involved in the permeation. Sildenafil is a substrate of MDR1 and BCRP, and Tadalafil is at least known as a substrate of MDR1.

Objectives: In this study, we aimed to clarify the fetal transfer of tadalafil in comparison with sildenafil in the mouse and the involvement of multidrug resistance protein 1 (MDR1) and breast cancer resistance protein (BCRP).

Methods: Tadalafil or sildenafil was administered to wild-type, Mdr1a/b double knockout or Bcrp knockout pregnant mice by continuous infusion from gestational day (GD) 14.5

to 17.5, and the transfer of drugs was evaluated by fetal to maternal plasma concentration ratio of the unbound form (unbound F/M ratio) at GD 17.5. Transcellular transport of tadalafil and sildenafil was evaluated in MDCKII-BCRP cells to clarify whether tadalafil is a substrate of BCRP or not.

Results: Unbound F/M ratio of tadalafil and sildenafil in the wild-type mice was 0.80 and 1.6, respectively, implying that fetal transfer of tadalafil across the placenta is relatively limited when compared to that of sildenafil. The unbound F/M ratio of tadalafil was increased to 1.1 and 1.7 in Mdr1a/b knockout mice and Bcrp knockout mice, respectively, while the ratio of sildenafil in Mdr1a/b knockout mice or Bcrp knockout mice was equal to or rather less than that in wild-type mice, respectively. A transcellular transport study revealed that basal-to-apical transport of tadalafil and sildenafil was significantly higher than those of the opposite direction in MDCKII-BCRP cells.

Conclusions: Our results suggest that tadalafil is a novel substrate of BCRP and fetal transfer of tadalafil is less than that of sildenafil in pregnant mice partly due to the involvement of BCRP in the fetus-to-mother transport of tadalafil across the placenta.

Design, identification, optimisation and validation of selective Casein Kinase II (CK2) modulator

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Introduction: The CK2 receptor has been acknowledged as a key contributor to carcinogenesis and has been identified as a druggable target in oncology because its inhibition slows down cellular metabolism. GO289 has been identified as a promising molecule for the mitigation of phosphorylation of circadian proteins and proliferation of malignant cells through CK2 inhibition. SAR studies indicate that the bromoguaiacol moiety of the GO289 molecule is a key moiety required for activity, and the phenyl group at the para position is modifiable.

Objectives: This study aimed to use dual approaches, specifically Virtual Screening and de novo design to identify novel CK2 modulators based on the GO289 scaffold.

Methods: For the virtual screening approach, the lead molecule GO289, and a second CK2 inhibitor were sequentially read into LigandScout, and a pharmacophoric structure was generated in each case. These were then superimposed and a consensus pharmacophore was modeled. This was used to query the ZincPharmer database, and Rule of three compliant hits were identified. The hit structures were docked into a CDK2 protomol generated in Sybyl-X, and ranked in order of affinity.

In the de novo design study, 2-dimensional topology maps describing the critical interactions between GO289 and the CDK2 receptor were generated using Discovery Studio. This data guided the generation of high-efficiency seed fragments based on the GO289 scaffold capable of sustaining molecular growth within the CDK2 ligand binding pocket. The novel structures were filtered for Lipinski Rule compliance, grouped in order of pharmacophoric similarity and ranked in order of Binding Affinity.

Virtual Screening is a more innovative approach when compared to the de novo design. The use of a consensus pharmacophore, and the fact that the hit structures were docked into the CDK2 protomol, which is the energetically unsatisfied space at the core of the CDK2 receptor supported the collection of a very structurally diverse molecular cohort. The disadvantage to protomol use is that it guarantees bioactivity less than does the use of the ligand binding pocket as described in the protein databank, which is known to be bioactive.

This limitation was addressed In the de novo approach, in which molecular growth was allowed exclusively in the area of the CK2 receptor known to be bioactive as described in the protein databank.

Results: The molecules obtained through virtual screening were structurally diverse, and exhibited an affinity for the protomol comparable to that of GO289. The molecules obtained through de novo design, being derived from a single molecular scaffold were structurally homologous, and had high ligand binding affinity for the CDK2 receptor with pKd ranging between 8.54 and 9.71 compared to that calculated for the lead-molecule (6.19).

Conclusions: The molecules with the highest affinity and the best physicochemical characteristics will be further validated in silico through molecular dynamics simulation studies.