Intranasal Administration of Perampanel Incorporated in a Non-Toxic Self-Microemulsifying Drug Delivery System Improves *in vivo* Anticonvulsant and Anxiolytic Activity

Meirinho S.[1*], Rodrigues M.[1,2], Santos A.O.[1], Falcão A.[3,4], Alves G.[1]

ORIGINAL ARTICLE

ABSTRACT

Introduction: Perampanel (PER) is a third-generation antiepileptic drug only available for oral administration. Regarding its potency and unique mechanism of action, PER can be effective beyond its approved therapeutic indications[1,2]. However, the oral route is not the best administration approach for some clinical conditions. Considering the growing interest of intranasal (IN) administration for brain delivery, PER could be envisioned as a good candidate for this route, especially if formulated in a lipidic nanosystem.

Methodology: We developed a non-toxic self-microemulsifying drug delivery vehicle (SMEDDS-FH5) for PER IN administration that shown ideal pharmaceutical characteristics[3]. Following IN administration of the SMEDDS to CD-1 mice (1 mg/kg), PER pharmacokinetics and brain biodistribution, together with its anticonvulsant and anxiolytic effects were herein studied. Additionally, olfactory and neuromotor toxicity after IN administration were evaluated. All protocols involving animals were approved by the local Animal Welfare and Ethical Review Body in agreement with European and Portuguese legislation.

Results: PER showed a rostral-caudal brain biodistribution pattern when administered intranasally. At the time that PER reached its maximal concentration after IN dosing (15 min), high PER concentrations were found in olfactory bulbs (olfactory bulbs/plasma ratios of 1.266 ± 0.183 and 0.181 ± 0.027 after IN and intravenous administrations, respectively), suggesting that a fraction of the drug reaches the brain directly through the olfactory pathway. In the maximal electroshock seizure test, IN PER protected 60% of mice against seizure development, a substantially higher value than the 20% protected after receiving oral PER. PER also demonstrated anxiolytic effects in open field and elevated plus maze tests. Neuromotor impairment was found in rotarod and open field tests 15 min and 2 hours after IN and oral administrations, respectively. Nevertheless, neuromotor performance was improved after 7 consecutive days of administration, with no signs of olfactory toxicity in the buried food-seeking test also after those repeated administrations.

Discussion: Altogether, the results suggest that the IN PER delivery through the developed SMEDDS-FH5 can be a safe and promising alternative to oral treatment, which support the design of clinical studies to evaluate the IN PER delivery to treat epilepsy and other neurological-related conditions as anxiety.

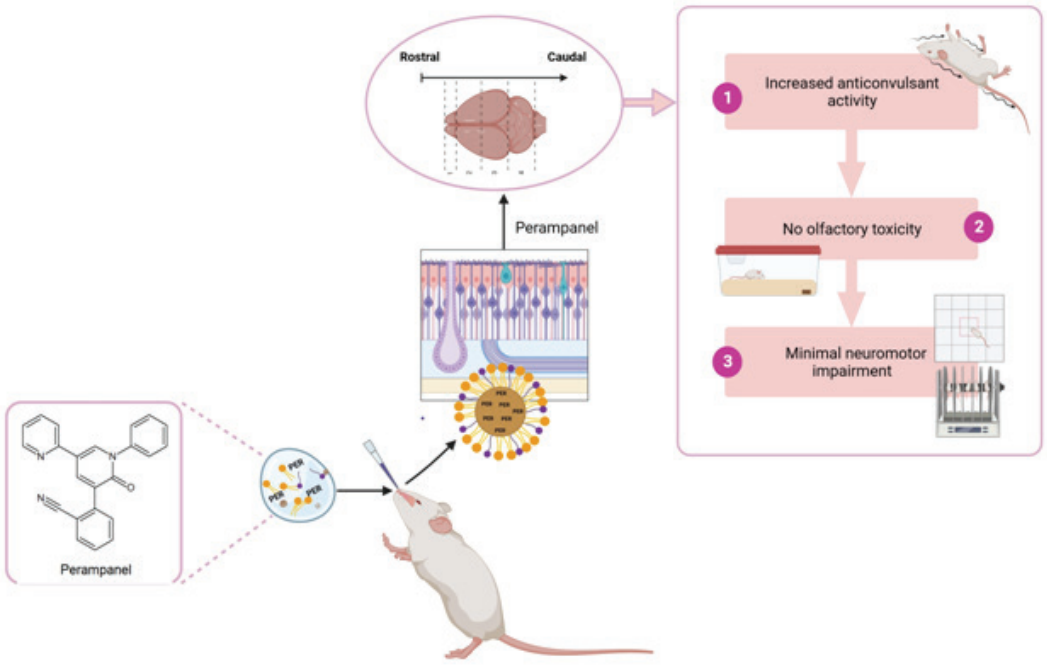
Keywords: Pperampanel, intranasal, self-microemulsifying drug delivery system.

[1] CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal.
[2] CPIRN-UDI-IPG-Center for Potential and Innovation of Natural Resources, Research Unit for Inland Development, Polytechnic Institute of Guarda, 6300-559 Guarda, Portugal.
[3] CIBIT – Coimbra Institute for Biomedical Imaging and Translational Research, University of Coimbra, Portugal.
[4] Laboratory of Pharmacology, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal.

*Corresponding author: sara.meirinho@ubi.pt.

 ISSN: 2182-3340

Graphical Abstract:



Acknowledgements: The authors acknowledge Fundação para a Ciência e a Tecnologia for the PhD fellowship SFRH/BD/136028/2018 and FEDER funds (CENTRO-01-0145-FEDER-000013 and CENTRO-01.0145-FEDER-000019).

Bibliographic References:

1. Chang FM, Fan PC, Weng WC, Chang CH, Lee WT. The efficacy of perampanel in young children with drug-resistant epilepsy. Seizure. 2020;75:82–6.

2. Patsalos PN. The clinical pharmacology profile of the new antiepileptic drug perampanel: A novel noncompetitive AMPA receptor antagonist Pharmacokinetics of Perampanel in Adults. Epilepsia. 2015;56(1):12–27.

3. Meirinho S, Rodrigues M, Ferreira CL, Oliveira RC, Fortuna A, Santos AO, *et al.* Intranasal delivery of lipid-based nanosystems as a promising approach for brain targeting of the new-generation antiepileptic drug perampanel. Int J Pharm. 2022; 622.

Targeting Lysyl Oxidase Like 2 to Prevent Breast Cancer Metastases

Ferreira S.[1,2], Ramos S.[1,2], Holota S.[3,4] Khyluk D.[5], Lesyk R.[3,6], Finyuk N.[7], dos Santos D.[1], Díaz-Lanza A.M.[2], Costa J.G.[1], Saraiva N.[1], Fernandes A.S.[1*]

ORIGINAL ARTICLE

ABSTRACT

Introduction: Lysyl oxidase (LOX) and LOX-like 1-4 (LOXL1-4) enzymes catalyze the cross-linking of elastin and collagen in the extracellular matrix, increasing its stiffness. Their activity is associated with cancer progression and the formation of metastases[1]. LOX inhibitors were recently suggested as potential anticancer drugs, especially in the context of breast cancer (BC). This study aims to investigate the therapeutic interest of targeting LOX enzymes in BC and to develop inhibitor drugs for this purpose.

Methodology: To explore the role of LOX enzymes on BC and its subtypes, a bioinformatic-based approach was followed using the The Cancer Genome Atlas database with the Gene Expression Profiling Interactive Analysis 2.0 and the Tumor Immune Estimation Resource 2.0[2]. A docking analysis of known LOXL2 binders was performed to unveil possible inhibitors' binding modes. Docking studies were also performed using a library of 4-thiazolidones, aiming at finding novel inhibitors. The inhibitory activity of selected compounds was evaluated using an Amplex Ultra Red-based technique[3]. Cell viability (thiazolyl blue tetrazolium bromide assay) was assessed in a panel of 10 cancer and normal-like cells. Cell migration was evaluated in MDA-MB-231 cells, using the wound healing assay for collective migration, transwell assay for chemotaxis/chemoinvasion, and single-cell tracking for random migration.

Results: Bioinformatic analysis showed higher levels of LOXL2 in BC compared with normal tissues. The overexpression of LOXL2 is associated with a negative impact on disease-free survival, particularly in basal BC. Docking studies allowed the identification of five potential LOXL-2 inhibitors. While four of those were inactive, CP5740, inhibited LOXL2 with an IC_{50} value in the micromolar range. This compound exhibited higher cytotoxicity for cancer cells, and particularly for MDA-MB-231 cells, than for normal-like cell lines. CP5740 did not significantly alter collective cell migration, but it reduced chemotaxis and chemoinvasion. A decrease in movement directionality was observed in the random migration analysis.

Discussion: Our data support the potential interest of inhibiting LOXL2 to prevent BC progression and describe a novel inhibitor that could be optimized towards a potential therapeutic use.

Keywords: lysyl oxidase-like 2 inhibitors, breast cancer, cancer progression.

[1] CBIOS, Universidade Lusófona's Research Center for Biosciences & Health Technologies, Lisbon, Portugal.
[2] Dpt. de Ciencias Biomédicas, Universidad de Alcala, España.
[3] Dpt. of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine.
[4] Dpt. of Organic Chemistry and Pharmacy, Lesya Ukrainka Volyn National University, Lutsk, Ukraine.
[5] Dpt. of Organic Chemistry, Medical University of Lublin, Poland.
[6] Dpt of Biotechnology and Cell Biology, University of Information Technology and Management, Rzeszow, Poland.
[7] Dpt. of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology of National Academy of Sciences of Ukraine, Lviv, Ukraine.

*Corresponding author: ana.fernandes@ulusofona.pt.

 ISSN: 2182-3340

Acknowledgements: FCT-Foundation for Science and Technology (UIDB/04567/2020 and UIDP/04567/2020); Universidade Lusófona/ILIND programs Fazer+ (LusoMod-LOX-ILIND/F+/EI/01/2020) and Seed Funding (CoSysCan-COFAC/ILIND/CBI-OS/1/2021).

Bibliographic References:

1. Ferreira S, Rijo P, Costa JG, Saraiva N, Santos B, Uriel C, *et al.* Evaluation of the Lysyl Oxidase-Like 2 (LOXL2) inhibitory activity of pimaranes and their glycosyl derivatives. Biomed Biopharm Res 2023; 20(1):1-12.

2. Ferreira S, Saraiva N, Rijo P, Fernandes, AS. LOXL2 Inhibitors and Breast Cancer Progression. Antioxidants 2021;10(2):312.

3. Ramos S, Ferreira S, Fernandes AS, Saraiva N. Lysyl Oxidases Expression and Breast Cancer Progression: A Bioinformatic Analysis. Front Pharmacol 2022; 13:883998.

Treatment Advances in Sepsis and Septic Shock: Modulating Pro-and Anti–Inflammatory Mechanisms

Marques A.[1*], Torre C.[1], Pinto R.[1,2], Sepodes B.[1], Rocha J.[1]

ORIGINAL ARTICLE

ABSTRACT

Introdution: Sepsis affects over 25 million people each year, resulting in millions of deaths worldwide. Although in-hospital mortality from sepsis has substantially decreased, traditional treatment strategies have been insufficient to curb long-term mortality[1]. Immunoadjuvant therapy has emerged as a promising way forward and research focus has largely shifted into targeting specific mechanisms of sepsis pathophysiology, but no sepsis-specific therapies have been approved thus far[2].

Methodology: In this scoping review, we summarize the major alterations in the host response during sepsis, provide a rationale for potential therapeutic interventions, as well as biomarkers to guide treatment[3]. We conducted a Pubmed search using terms such as "sepsis", "septic shock" and "immunotherapy" for English articles published in the last 10 years, and screened the reference lists of the selected papers for relevant articles. Given its parallels with traditional sepsis, we also explored the therapeutic approaches utilized to treat severe COVID-19, a sepsis-like illness characterized by an imbalanced immune system. We further searched four different clinical trial registries for completed and ongoing trials from the last 10 years that studied emergent therapeutic options in sepsis or septic shock. We excluded trials with terminated, suspended, unknown or withdrawn status.

Results: This search yielded 30 clinical trials, 11 of which are ongoing and largely focus on augmenting the immune response. Importantly, research in sepsis is well-known for highly promising studies in animals, which ultimately fail at a clinical level. This may be explained by the frequent omission of standard-of-care practices like antimicrobials, as well as the definition of outdated clinical endpoints in human trials.

Discussion: The host response during sepsis is extremely complex and non-linear, often resulting in emergent behavior that cannot be captured by single time points and isolated analyses of specific host response features. Both the early inflammatory and later immunosuppressive stages lead to a high risk of mortality. Consequently, clinical studies employing a combination of therapeutic interventions in each of these phases should add significant value in the improvement of clinical outcomes. Furthermore, as different immune phenotypes have been identified in sepsis, it's clear that critically ill patients included clinical trials should be stratified according to stage of disease and severity.

Keywords: sepsis, septic shock, immunomodulation.

[1] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisbon, Portugal.
[2] Joaquim Chaves Saúde, Joaquim Chaves Laboratório de Análises Clínicas, Miraflores, 1495-069 Algés, Portugal.

*Corresponding author: adriana.marques1@campus.ul.pt.

 ISSN: 2182-3340

Bibliographic References:

1. Hotchkiss RS, Moldawer LL, Opal SM, Reinhart K, Turnbull IR, Vincent J-L. Sepsis and septic shock. Nat Rev Dis Prim [Internet]. 2016 Jun 30;2(1):16045. Available from: https://www.nature.com/articles/nrdp201645.

2. Berg D, Gerlach H. Recent advances in understanding and managing sepsis [version 1; peer review: 3 approved]. F1000Research. 2018;7(0):1–8.

3. Marques A, Torre C, Pinto R, Sepodes B, Rocha J. Treatment Advances in Sepsis and Septic Shock: Modulating Pro-and Anti-Inflammatory Mechanisms. J Clin Med [Internet]. 2023 Apr 15;12(8):2892. Available.

Managing Hypertension: Sex Differences in Angiotensin-Converting Enzyme Inhibition by Natural Bioactive Peptides

Ribeiro-Oliveira R.[1,2*], Rodríguez-Rodríguez P.[3], Ruvira S.[3], Sousa J.B.[1], Ferreira I.M.P.[2], Arribas S.M.[3], Diniz C.[1]

ORIGINAL ARTICLE

ABSTRACT

Introduction: Increasing evidence appoint for a differential response of the cardiovascular (CV) system in men and women, highlighting the relevance of gender in CV therapeutic strategies including the management of hypertension as a major CV risk factor[1]. Bioactive peptides, such as those derived from brewing by-products, are natural promising anti-hypertensive agents, acting as inhibitors of angiotensin-converting enzyme (ACE)[2]. However, its efficacy in inhibiting endogenous ACE as well as its impact on gender responses remains to be verified. Accordingly, we aimed to evaluate the impact of bioactive peptides from a mixture (MIX) of brewing by-products on the vasocontractile response to the ACE substrate angiotensin (Ang) I on different arteries from male and female spontaneously hypertensive rats (SHR).

Methodology: Experiments were approved by ORBEA (number: 409/2022/ORBEA). Large iliac (distribution) and small mesenteric (resistance) arteries from male/female SHR (6.2±0.8 month-years old) were isolated and mounted at an organ bath or wire Mulvany myograph to measure the vascular isometric tension[3]. After ensuring the vascular function viability (with 120 mM KCl) and the endothelium integrity (with 10^{-9}-10^{-4} M acetylcholine in pre-contracted vessels), cumulative curves to Ang I (10^{-9}-10^{-6} M) were performed after a 30 min incubation with Krebs solution in the presence or absence of MIX (0.87 mg/mL) or captopril (1 μM, an ACE-inhibitory drug; positive control). Differences of means were compared using one-way ANOVA followed by Tukey *t*-test, or by Student *t*-test.

Results: The contractile response obtained after addition of Ang I varied according to the arterial type and between sex. Thus, captopril and MIX-mediated effects were normalized regarding the response to Ang I. MIX revealed a vast discrepancy between sex, acting as an ACE-inhibitor in SHR male arteries, statistically similarly as captopril, reducing near 95% and 47% the constriction in mesenteric and iliac arteries, respectively. By opposition, in females SHR arteries, MIX surprisingly increased the vasoconstriction response to Ang I in a paradoxical effect.

Discussion: MIX bioactive peptides display a sex-selective impact, being able to inhibit ACE in male but not in female SHR arteries, which may suggest the usefulness of these peptides to prevent and/or treat hypertension but only in man.

Keywords: Brewer's spent grain, brewer's spent yeast, renin-angiotensin system.

[1] LAQV/REQUIMTE, Laboratory of Pharmacology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal.
[2] LAQV/REQUIMTE, Laboratory of Bromatology and Hydrology, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal.
[3] Department of Physiology, Faculty of Medicine, Universidad Autónoma de Madrid, Madrid, Spain.

*Corresponding author: up201303483@edu.ff.up.pt.

 ISSN: 2182-3340

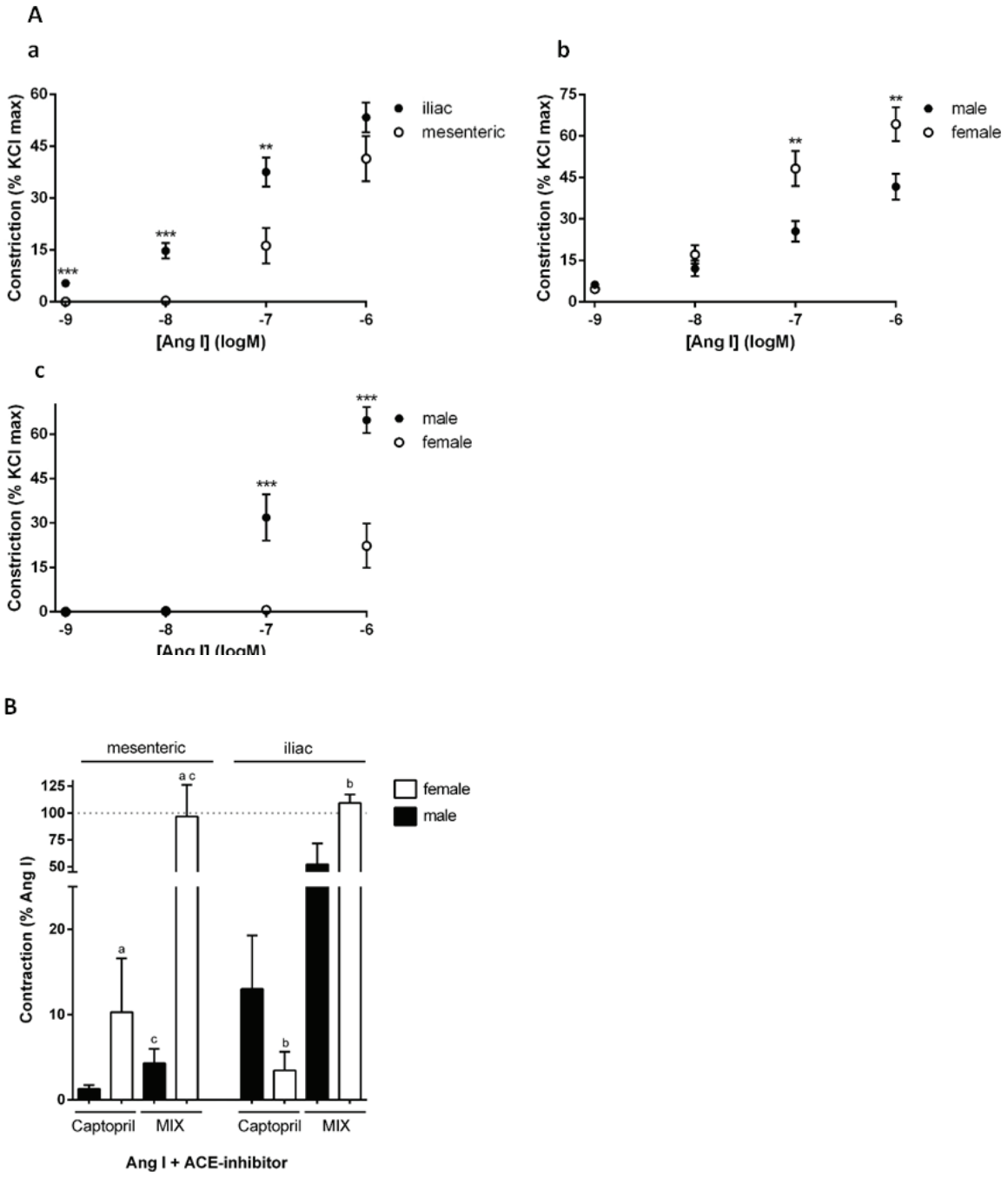


Figure 1. A: Concentration-response curves to angiotensin-converting enzyme substrate (angiotensin I – Ang I) on the vasoconstriction of iliac and mesenteric arterial segments from male and female SHR: (a) separation by arterial type; (b) separation by sex on iliac arteries; (c) separation by sex on mesenteric arteries. Constriction expressed as a percentage of maximal contraction to 120 mM KCl. Data presents means ± SEM from ≥6 independent experiments (animals) in each group. Significant differences between groups for each Ang I concentration: ** $p < 0.01$ and *** $p < 0.001$ (Student t-test). B: Impact of peptides on angiotensin-converting enzyme from iliac and mesenteric arterial segments from males and females SHR. Constriction elicited by angiotensin-converting enzyme substrate angiotensin I (10-7 M). Peptides tested: 0.86 mg/mL of MIX natural bioactive peptides and 1 μM of captopril drug. Constriction expressed as a percentage of angiotensin I alone. Data presents means ± SEM from ≥5 independent experiments (animals) in each group. Significant differences ($p < 0.05$) between different groups displayed as different letters (One-way ANOVA followed by post hoc Tukey *t*-test).

Acknowledgements: To the Portuguese Foundation for Science and Technology (FCT) for the financial support UIDB/50006/2020 and to Ministerio de Ciencia, Innovación y Universidades (Spain; grant number RTI2018-097504-B-I00), co-financed with FEDER funds. Rita Ribeiro-Oliveira thanks the FCT for the Ph.D. grant SFRH/BD/146243/2019, funded by the European Social Fund of the European Union and national funds FCT/MCTES through the Norte's Regional Operational Programme.

Bibliographic References:

1. Song J-J, Ma Z, Wang J, Chen L-X, Zhong J-C. Gender Differences in Hypertension. J Cardiovasc Transl Res. 2020 Feb 1;13(1):47–54.

2. Ribeiro-Oliveira R, Martins ZE, Faria MÂ, Sousa JB, Ferreira IMPLVO, Diniz C. Protein Hydrolysates from Brewing By-Products as Natural Alternatives to ACE-Inhibitory Drugs for Hypertension Management. Life. 2022 Oct 7;12(10):1554.

3. Rodríguez-Rodríguez P, Ragusky K, Phuthong S, Ruvira S, Ramiro-Cortijo D, Cañas S, *et al.* Vasoactive Properties of a Cocoa Shell Extract: Mechanism of Action and Effect on Endothelial Dysfunction in Aged Rats. Antioxidants. 2022 Feb 21;11(2):429.

Development of Palladium-Spermine Complex (Pd2Spm) for Triple-Negative Breast Cancer Cells – *in vitro* and *in vivo* Preclinical Development

Vojtek M.[1*], Batista de Carvalho A.L.M.[2], Ferreira I.M.[3], Mota-Filipe H.[4], Marques M.P.[2,5], Diniz C.[1*]

ORIGINAL ARTICLE

ABSTRACT

Introduction: Triple-negative breast cancer (TNBC) is a breast carcinoma subtype with aggressive behaviour and poor prognosis. Current treatment options with platinum-(Pt)-based chemotherapeutics yield unsatisfactory results due to severe toxicity/resistance that encouraged the development of novel metal-based compounds with improved efficacy/safety profiles, such as palladium-(Pd)-based agents. This work aimed at studying the potential of Pd-Spermine complex (Pd_2Spm) as an anticancer agent against TNBC.

Methods: *In vitro* assays in TNBC cells (MDA-MB-231- sensitive and resistant to cisplatin) and in MCF-12A non-neoplastic cells were used to evaluate the impact of Pd_2Spm on cell proliferation, migration and cellular death compared to cisplatin. In vivo xenograft and pharmacokinetic studies were also performed to assess the efficacy/safety of Pd_2Spm. Data were analysed by one- or two-way ANOVA and nonparametric Kruskal-Wallis test. Ethics Committee of U.Porto approved the study.

Results and Discussion: Pd_2Spm selectively induced cellular death in MDA-MB-231 cells sensitive and resistant to cisplatin, via a non-apoptotic pathway, and reduced cellular migration. When focusing on cell death, incubation of Pd_2Spm with either Necrostatin-1 (necroptosis inhibitor), Z-VAD (apoptosis inhibitor) or 3-Methyladenine (3-MA, autophagy inhibitor) showed that 3-MA can rescue Pd_2Spm-induced growth inhibition in MDA-MB-231 and MDA-MB-231/R cells. Furthermore, in MDA-MB-231 cells, Pd_2Spm triggered higher LC3-II levels and more profound Beclin-1 inhibition than cisplatin. Regarding apoptotic cell death, Pd_2Spm did not induce caspase-3 cleavage and co-incubation with both Pd_2Spm and Z-VAD yielded only marginal effects in preventing the phosphatidylserine externalization compared to cisplatin. Moreover, a similar pharmacokinetic profile and efficacy to inhibit tumour growth in mice-TNBC xenograft were obtained for Pd_2Spm compared to cisplatin. Notwithstanding, in vivo Pd_2Spm presented lower toxicity than cisplatin. This work reveals an encouraging potential of Pd_2Spm as an anticancer agent to treat TNBC (including in cisplatin-resistant TNBC).

Keywords: TNBC, breast cancer, palladium.

[1] LAQV/REQUIMTE, Laboratory of Pharmacology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal.
[2] Molecular Physical-Chemistry R&D Unit, Department of Chemistry, University of Coimbra, 3004-535 Coimbra, Portugal.
[3] LAQV/REQUIMTE, Laboratory of Bromatology and Hydrology, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal.
[4] iMed.ULisboa, Faculty of Pharmacy, University of Lisbon, 1649-003 Lisbon, Portugal.
[5] Department of Life Sciences, Faculty of Science and Technology, University of Coimbra, 3000-456 Coimbra, Portugal.

*Corresponding authors: matovoj@gmail.com (M.V.), cdiniz@ff.up.pt (C.D.).

 ISSN: 2182-3340

Funding: Funded by FCT: UIDB/50006/2020.

Bibliographic References:

1. Vojtek M, Gonçalves-Monteiro S, Šeminská P, Valová K, Bellón L, Dias-Pereira P, Marques F, Marques MPM, Batista de Carvalho ALM, Mota-Filipe H, et al. Pd2Spermine Complex Shows Cancer Selectivity and Efficacy to Inhibit Growth of Triple-Negative Breast Tumors in Mice. Biomedicines. 2022; 10(2):210.
2. Vojtek M, Gonçalves-Monteiro S, Pinto E, Kalivodová S, Almeida A, Marques MPM, Batista de Carvalho ALM, Martins CB, Mota-Filipe H, Ferreira IMPLVO, *et al*. Preclinical Pharmacokinetics and Biodistribution of Anticancer Dinuclear Palladium(II)-Spermine Complex (Pd2Spm) in Mice. Pharmaceuticals. 2021; 14(2):173.

Ultra High Performance Liquid Chromatography Technique to support Pharmacokinetic monitoring of Losartan, Irbesartan and Valsartan

Gouveia F.[1,2,3,4*], Bicker J.[1,2], Carona A.[1,2], Ettcheto M.[3,4,5], Cruz M.T.[1,6,7], Fortuna A.[1,2]

ORIGINAL ARTICLE

ABSTRACT

Introduction: Nowadays, Alzheimer's disease (AD) stands as the most prevalent form of dementia for which a definitive cure remains unmet. Our group has already demonstrated the potential of drugs targeting the renin angiotensin system (RAS) in delaying the progression of AD[1,2]. However, angiotensin receptor blockers (ARBs) have different efficacy profiles owing to their distinct ability to cross the blood brain barrier[3]. As a result, there is a pressing need for the development of new strategies aimed at enhancing the brain targeting of ARBs. This demands for pharmacokinetic studies that require the accurate quantification of ARBs in plasma, brain and other tissues. Therefore, it was herein developed and full validated an ultra high performance liquid chromatographic (UHPLC) technique with diode-array detection for three ARBs, losartan, irbesartan and valsartan in human plasma. A partial validation was also performed in mice plasma, brain and lung, in order to support future preclinical pharmacokinetic studies.

Material & Methods: Blank samples (100 μL for plasma and lung tissue; 200 μL for brain tissue) were spiked with internal standard, perampanel and each ARB solution to prepare the calibration standard. The analytes were extracted by solid phase extraction (SPE) carried on Oasis PRIME HLB cartridges. Chromatographic separation was accomplished within 20 min on the InfinityLAb Poroshell® 120 C18 column (4.6 mm×100 mm, 2.7 μm) at 35 ºC with a mobile phase composed of water 0.1% trifluoroacetic acid (TFA)/acetonitrile 0.1% TFA in a gradient elution (1.0 mL/min). The method was validated according to the international guidelines[4].

Results: The technique was linear in human plasma: 50 - 5000 ng/mL (irbesartan and losartan) and 100 - 5000 ng/mL (valsartan); in mice plasma and lung: 50 - 5000 ng/mL (irbesartan) and 100 - 5000 ng/mL (valsartan and losartan); and mice brain: 25 - 2500 ng/mL (irbesartan) and 50 - 2500 ng/mL (valsartan and losartan). Intra- and inter-day accuracy was within 0.02 - 18%, while precision was lower than 16%. Recoveries were consistent and varied between 73 to 104%.

Discussion: To our knowledge,the UHLPC-DAD method herein successfully developed and validated was the first to simultaneously quantify 3 ARBs in 4 different matrices - human plasma and mice plasma, brain and lung - making it extreme important for further pharmacokinetic studies.

Keywords: HPLC, angiotensin, pharmacokinetics.

[1] Laboratory of Pharmacology, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal.
[2] CIBIT/ICNAS – Coimbra Institute for Biomedical Imaging and Translational Research, University of Coimbra, Coimbra, Portugal.
[3] Department of Pharmacology, Toxicology and Therapeutic Chemistry, Faculty of Pharmacy and Food Science, University of Barcelona, Barcelona, Spain.
[4] Institute of Neurosciences, University of Barcelona, Barcelona, Spain.
Biomedical Research Networking Centre in Neurodegenerative Diseases (CIBERNED), Carlos III Health Institute, Madrid, Spain.
[5] Biomedical Research Networking Centre in Neurodegenerative Diseases (CIBERNED), Carlos III Health Institute, Madrid, Spain.
[6] CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal.
[7] CIBB-Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal.

*Corresponding author: filipafdhgouveia@gmail.com.

 ISSN: 2182-3340

Acknowledgments: This work was supported by the FCT, Fundação para a Ciência e a Tecnologia, Portugal (Ph.D. research Grant 2020.04442. BD).

Bibliographic References:

1. Gouveia F, Ettcheto M, Camins A, Silva A, Cruz MT, Fortuna A. Abstract book - International Multi Brain Congress [Internet]. 2022. Available from: https://www.ub.edu/UBneuroCongress2022/wp-content/uploads/2022/11/abstract-book_multibrain.pdf.

2. Gouveia F, Camins A, Ettcheto M, Bicker J, Falcão A, Cruz MT, et al. Targeting brain Renin-Angiotensin System for the prevention and treatment of Alzheimer's disease: Past, present and future. Ageing Res Rev. 2022;77(August 2021).

3. O'Caoimh R, Healy L, Gao Y, Svendrovski A, Kerins DM, Eustace J, et al. Effects of Centrally Acting Angiotensin Converting Enzyme Inhibitors on Functional Decline in Patients with Alzheimer ' s Disease. J Alzheimar's Dis. 2014;40:595–603.

4. European Medicines Agency. ICH Guideline M10 on Bioanalytical Method Validation. Sci Med Heal [Internet]. 2019;44(March):57. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-m10-bioanalytical-method-validation-step-5_en.pdf%0Ahttps://www.ema.europa.eu/en/ich-m10-bioanalytical-method-validation#current-version-section

Risco de Interações Medicamentosas em Idosos em Ambiente Familiar

Auxtero M.D.[1*], Brito J.[1], Costa I.M.[1]

ORIGINAL ARTICLE

RESUMO

Introdução: Em 2022, 23,8% da população Portuguesa tinha idade igual ou superior a 65 anos, e 6.9% ultrapassavam os 80 anos[1]. Estas faixas etárias estão associadas a comorbilidades e à polimedicação, com risco aumentando de interações medicamentosas (IM)[2]. Na Europa o problema é semelhante, com uma população expectável de 520 milhões em 2070. IM são responsáveis por hospitalizações geriátricas, com elevada taxa de morbilidade e mortalidade[3]. Pretendeu-se avaliar a terapêutica de idosos, em ambiente familiar, com mais de 65 anos, a tomar no mínimo dois medicamentos, em termos de risco de IM.

Metodologia: participaram no estudo transversal realizado em 2023 (aprovado pela C. ética da Egas Moniz), idosos (≥ 65 anos), e a tomar pelo menos dois medicamentos. Foram recolhidos dados demográficos como idade e sexo e analisadas as cartonagens da terapêutica. Avaliou-se o risco de IM usando o *Medscape Interaction Checker*. As IM foram classificadas em 3 graus: IM1 – Séria (usar alternativa); IM2- Monitorizar e IM3- Menor. Considerou-se uma partição da amostra com base nos quartis do número de fármacos tomados: Q1 = 3; Q2 = 4; Q3 = 6. Estes quatro grupos foram comparados entre si para o número de IM1, IM2 e IM3, mediante aplicação dos testes de Kruskal-Wallis e Mann-Whitney com correcção de Bonferroni.

Resultados: participaram no estudo 104 idosos (65,4% mulheres e 34,6% homens) com 78,4 anos de média de idades. Identificaram-se 13 idosos (12.5%) com risco de IM1 (total de 15 IM1), 53 idosos (51%) com IM2 (total de 155 IM2) e 27 idosos (26%) com IM3 (total de 44 IM3). As diferenças no número de IM1 não atingem significância estatística entre grupos (p=0,065), mas a sua existência é sugerida pelos dados. Sujeitos que tomam até 3 fármacos apresentam número de IM2 significativamente menor do que sujeitos que tomam mais do que 4 e até 6 fármacos (p< 0,001), e sujeitos que tomam mais do que 6 fármacos (p<0,001). Para além disso, neste último grupo, o número de interações é significativamente mais elevado do que em sujeitos que tomam 4 fármacos (p=0,006). Sujeitos que tomam até 3 fármacos apresentam significativamente menos IM3 do que sujeitos que tomam mais do que 4 e até 6 fármacos (p = 0,006), e sujeitos que tomam mais do que 6 fármacos (p<0,001). Das 15 IM1, as mais frequentes são a sinvastatina com amlodipina (n=4); sertralina com quetiapina (n=2) e perindopril com ácido acetilsalicílico (AAS). Os fármacos mais envolvidos em IM1 são a sinvastatina e amlodipina (n=4) e AAS, quetiapina e clopidogrel (n=3). Relativamente a IM2, o AAS é o mais frequentemente envolvido (n=37), seguido do bisoprolol (n=30) e do valsartan (n=18). A terapêutica foi maioritariamente prescrita pelo médico (98%) e 2% aconselhada pelo farmacêutico.

Discussão: Os resultados corroboram a conclusão de que é muito importante monitorizar a terapêutica dos idosos, especialmente quando o número de fármacos excede 4, para reduzir o risco de IM.

Palavras-chave: interações medicamentosas, idosos, polimedicação.

[1] Centro de Investigação Interdisciplinar Egas Moniz (CiiEM); Egas Moniz School of Health & Science, 2829-511 Caparica, Almada, Portugal.

*Autor correspondente: mauxtero@egasmoniz.edu.pt.

 ISSN: 2182-3340

Referências Bibliográficas:

1. dos Santos FFM. População residente: total e por grupo etário [Internet]. PORDATA. [cited 2023 Aug 9]. Available from: https://www.pordata.pt/portugal/populacao+residente+total+e+por+grupo+etario-10.

2. Kantor ED, Rehm CD, Haas JS, Chan AT, Giovannucci EL. Trends in prescription drug use among adults in the United States from 1999-2012. JAMA [Internet]. 2015 Nov 3;314(17):1818–31. Available from: http://dx.doi.org/10.1001/jama.2015.13766.

3. Petrini E, Caviglia GP, Pellicano R, Saracco GM, Morino M, Ribaldone DG. Risk of drug interactions and prescription appropriateness in elderly patients. Ir J Med Sci [Internet]. 2020 Aug;189(3):953–9. Available from: http://dx.doi.org/10.1007/s11845-019-02148-8.

Inflammation Inhibition with Carvone Wnantiomers: Two Sides of a Coin with Different Targets

Sousa C.[1,2,3], Neves B.M.[4], Leitão A.J.[1,2,3], Mendes A.F.[1,2,3*]

ORIGINAL ARTICLE

ABSTRACT

Introduction: Chronic low-grade inflammation has been reported as a relevant mechanism in age-related diseases[1]. Thus, signalling pathways involved in inflammation are relevant targets for the development of new therapeutic strategies to tackle those diseases. Our previous work showed that (S)-(+)- and (R)-(-)-carvone have anti-inflammatory properties, namely by inhibiting the expression of inflammatory mediators[2]. Therefore, the aim of this work was to elucidate the mechanism of action of carvone enantiomers responsible for their anti-inflammatory effects, namely their effect in the two major inflammatory pathways: the Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF- kB) and Mitogen-Activated Protein Kinases (MAPK).

Methods: The murine macrophage cell line, Raw 264.7, stimulated with bacterial lipopolysaccharide (LPS) was used as cell model of inflammation. The effects of carvone enantiomers on MAPK and NF-kB activation were evaluated by Western Blot and immunocytochemistry.

Results: The results obtained show that carvone enantiomers inhibited LPS-induced JNK1 phosphorylation, but not that of p38 and ERK1/2. Moreover, carvone enantiomers also did not affect LPS-induced NF-kB/p65 nuclear translocation. Nevertheless, carvone enantiomers decreased NF-kB/p65 acetylation on lysine (Lys) 310.Deacetylation of that Lys residue is dependent on the activity of Sirtuin 1 (SIRT1). Using an *in vitro* fluorimetric assay, (S)-(+)-carvone was found to directly activate SIRT1 whereas (R)-(-)-carvone did not affect its activity. Both compounds did not interfere with SIRT1 protein levels.

Discussion: Taking together, these results show that carvone enantiomers inhibit JNK1 and the transcriptional activity of NF-kB without interfering with its canonical activation pathway. However, (S)-(+)-carvone affects NF-kB transcriptional activity by directly activating SIRT1. This work highlights the relevance of enantiomerism and the diversity of molecular mechanisms that can be involved in the anti-inflammatory activity of monoterpenes.

Keywords: (S)-(+)-carvone, (R)-(-)-carvone, NF-kB.

[1] Centre for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal.
[2] Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal. Currently at NOVA Medical School|Faculdade de Ciências Médicas (NMS|FCM), NOVA University of Lisbon, Portugal.
[3] Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal.
[4] Department of Medical Sciences and Institute of Biomedicine – iBiMED, University of Aveiro, Aveiro, Portugal.

*Corresponding author: afmendes@ff.uc.pt.

 ISSN: 2182-3340

Acknowledgements: This work was supported by the ERDF, through the Centro 2020 and through the COMPETE 2020 and Portuguese national funds via FCT under projects POCI-01-0145-FEDER-028424 (CARTILFACTORY), UIDB/04539/2020, UIDP/04539/2020 and LA/P/0058/2020 and the PhD fellowship SFRH/79600/2011 to C. Sousa.

Bibliographic References:

1. Robinson WH, Lepus CM, Wang Q, Raghu H, Mao R, Lindstrom TM, et al. Low-grade inflammation as a key mediator of the pathogenesis of osteoarthritis. Nat Rev Rheumatol. 2016 Oct 19;12(10):580–92.

2. Sousa C, Leitão AJ, Neves BM, Judas F, Cavaleiro C, Mendes AF. Standardised comparison of limonene-derived monoterpenes identifies structural determinants of anti-inflammatory activity. Sci Rep. 2020;10(1):1–14.

Galectin-3 and α-Klotho as Indicators of Peritoneal Dialysis Outcomes

Sousa C.[1,2], Calça R.[1,2,3], Martins A.R.[3], Teixeira-Santos L.[1,2], Mateus C.[3], Jervis M.J.[3], Gomes D.P.[3], Azeredo-Lopes S.[1,4], Civantos E.[5], Mas-Fontao S.[5], Gaspar A.[3], Ramos S.[3], Rodrigues A.[6,7], Branco P.[1,2,3], Pereira S.A.[1,2*]

ORIGINAL ARTICLE

ABSTRACT

Introduction: Peritoneal dialysis (PD) is a renal replacement therapy that is based on the dialytic properties of the peritoneal membrane (PM) and is a self-care treatment performed at home, facilitating patient's autonomy[1]. However, loss of PM integrity and progression to fibrosis is still a major complication[2]. The PM microenvironment is variable among patients and reflects their systemic uremic profile, which might be a factor for PM integrity, patient survival and long-term PD outcomes, e. g. major cardiovascular events (MACE) and loss of residual renal function. Thus, the aim of this study was to investigate PM status, clinical data, and aging-related molecules, such as α-klotho and galectin-3, as predictors of PD long-term outcomes.

Methods: A 5-year prospective study was conducted at the PD Unit of Santa Cruz Hospital, Centro Hospitalar de Lisboa Ocidental, where 58 incident patients with biopsy at the study baseline were included. This study was approved by the Ethics Committee of the NOVA Medical School, Faculdade de Ciências Médicas, NOVA University of Lisbon (Approval number 50/2019). The following endpoints were evaluated:(a) PD failure and time until PD failure, (b) MACE and time until MACE. PM histomorphology and aging-related indicators were assessed before the start of PD and investigated as predictors of study endpoints.

Results: Fibrosis of the PM was associated with MACE occurrence and earlier MACE, but not with the patient or membrane survival. Serum α-Klotho bellow 742 pg/mL was related to the submesothelial thickness of the PM. This cutoff stratified the patients according to the risk of MACE and time until MACE. Uremic levels of galectin-3 were associated with PD failure and time until PD failure.

Discussion: To sum up, this work points out galectin-3 and α-klotho as molecular indicators to tailor patient management in this home-based renal replacement therapy and potential therapeutic targets. However, further studies are needed to better understand the subjacent mechanisms.

Keywords: chronic kidney disease, fibrosis, peritoneal membrane.

[1] NOVA Medical School|Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, Lisboa, Portugal.
[2] Centro Clínico Académico de Lisboa, Lisboa, Portugal.
[3] Hospital de Santa Cruz, Centro Hospitalar de Lisboa Ocidental, Lisboa, Portugal.
[4] Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.
[5] IIS-Fundación Jiménez Díaz, Universidad Autónoma de Madrid, Spanish Biomedical Research Centre in Diabetes and Associated Metabolic Disorders (CIBERDEM), Madrid, Spain.
[6] UMIB—Unidade Multidisciplinar de Investigação Biomédica, ITR-Laboratory for Integrative and Translational Research in Population Health, Porto, Portugal.
[7] ICBAS—Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Centro Hospitalar Universitário do Porto, Porto, Portugal.

*Corresponding author: catia.moreirasousa@nms.unl.pt.

 ISSN: 2182-3340

Acknowledgements: Sociedade Portuguesa de Nefrologia funded a project and A.R.M. (MD) Nephrology fellow for a residence at Jiménez Díaz Foundation University Hospital, Madrid under the scope of novel serum biomarkers of CKD.

Bibliographic References:

1. Bello AK, Okpechi IG, Osman MA, Cho Y, Cullis B, Htay H, et al. Epidemiology of peritoneal dialysis outcomes. Nat Rev Nephrol. 2022 Dec 16;18(12):779–93.

2. Terri M, Trionfetti F, Montaldo C, Cordani M, Tripodi M, Lopez-Cabrera M, et al. Mechanisms of Peritoneal Fibrosis: Focus on Immune Cells–Peritoneal Stroma Interactions. Front Immunol. 2021 Mar 29;12.

Comparación de Perfiles de Disolución de Amiodarona-HCl en Tabletas utilizando Medios de Relevancia Fisiológica

Reyes-Ramírez F.D.[1], Ruiz-Segura J.C.[1], Medina-López J.R.[1*]

ORIGINAL ARTICLE

RESUMEN

Introducción: La amiodarona-HCl es un fármaco de baja solubilidad y alta permeabilidad ampliamente utilizado para tratar enfermedades cardiacas[1]. Debido a las características de la molécula y a la alta variabilidad observada en humanos por efecto de primer paso es necesario evaluar el desempeño de liberación *in vitro* de las formulaciones genéricas disponibles[2] , respecto al medicamento de referencia, a fin de contar con medicamentos seguros y eficaces. El objetivo del presente trabajo consiste en comparar la velocidad y grado de disolución de amiodarona-HCl en tabletas comerciales utilizando el Aparato 2 USP (paletas) y medios de disolución de relevancia fisiológica.

Metodología: Se utilizaron dos formulaciones (referencia y genérico) de amiodarona-HCl (tabletas 200-mg). Los perfiles de disolución se obtuvieron con el Aparato 2 USP a 75 rpm y 900 ml de medio. Se utilizó HCl 0.1 N y soluciones reguladoras de pH 4.5y 6.8 como medio de disolución adicionadas con lauril sulfato de sodio al 1%. Se tomaron muestras cada 5 min durante 60 min. El grado de fármaco disuelto se calculó por UV (240 nm) y curvas de calibración en cada medio de disolución (1.87-30 μg/ml). Los perfiles se compararon con los valores de eficiencia de disolución, tiempo medo de disolución y porcentaje disuelto a los 60 min (t-Student). Se consideraron diferencias significativas valores de $p<0.05$. Algunos datos de disolución se ajustaron a diferentes modelos matemáticos y la ecuación que mejor describe el comportamiento in-vitro se escogió con el mayor valor de coeficiente de correlación ajustado y menor valor de criterio de información de Akaike.

Resultados: En la figura 1 se presentan los perfiles de disolución de amiodarona-HCl obtenidos bajo las condiciones previamente descritas. En HCl 0.1 N ninguna formulación superó el 71% disuelto al término de la prueba y al comparar los perfiles con los parámetros de disolución estos fueron similares. Los datos en ácido ajustaron a la función de Weibull y la comparación de β corrobora el resultado anterior. En los medios de pH 4.5 y 6.8 el grado disuelto fue <76% y los perfiles de disolución no se consideraron similares ($p<0.05$).

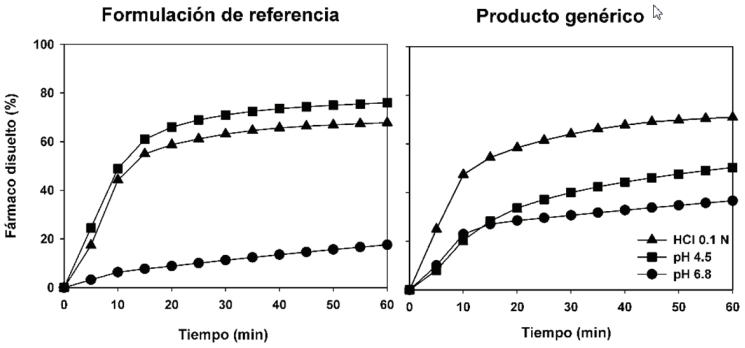


Figura 1. Perfiles de disolución de amiodarona-HCl en tabletas.

[1] Departamento Sistemas Biológicos, Universidad Autónoma Metropolitana-Xochimilco, Ciudad de México, México.

*Autor correspondiente: rmlopez@correo.xoc.uam.mx.

 ISSN: 2182-3340

Discusión: La diferencia en los perfiles de disolución entre ambas formulaciones sugiere variaciones en la absorción y, por lo tanto, en la manifestación del efecto terapéutico esperado del medicamento genérico. Es necesario llevar a cabo estudios en humanos para confirmar o no la intercambiabilidad de las formulaciones de amiodarona-HCl disponibles a la población.

Palabras clave: amiodarona-HCl, disolución, genéricos.

Referencias Bibliográficas:

1. Prieto Guerra Maidelis, Gómez Fernández Mario Jesús, Vila García Elena Esther. Amiodarona: fortalezas y debilidades. Rev cubana med [Internet]. 2022 Jun [citado 2023 Sep 15] ; 61(2): e2589. Disponible en: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0034-75232022000200012&lng=es. Epub 01-Jun-2022.

2. Rubén, Camarena G, Moreno E. ACCIONES FARMACOLÓGICAS INDICACIONES DOSIFICACIÓN AMIODARONA Antiarrítmico clase III [Internet]. Available from: https://anestesiar.org/WP/uploads/2013/12/prev_1387103800.pdf.

Utilidad del Método de Celda de Flujo Continuo para Estimar los Niveles Plasmáticos de Ketoprofeno

Reyes-Ramírez F.D.[1], Ruiz-Segura J.C.[1], Medina-López J.R.[1*]

ORIGINAL ARTICLE

RESUMEN

Introducción: El ketoprofeno es un fármaco analgésico-antiinflamatorio de baja solubilidad y alta permeabilidad (Clase II del SCB) que ha presentado problemas de absorción por lo que la información generada con estudios de disolución es una herramienta importante para evaluar el desempeño *in vivo* de medicamentos orales que contienen este fármaco[1]. Los estudios se llevan a cabo con los equipos estandarizados y los datos se tratan de forma matemática para generar valores hipotéticos de concentraciones plasmáticas. El procedimiento da una idea del nivel de absorción y de las condiciones *in vitro* en las que se predicen de mejor forma parámetros farmacocinéticos importantes[2]. El objetivo del presente trabajo es estimar niveles plasmáticos de ketoprofeno a partir de datos de disolución de tres productos genéricos y la referencia.

Metodología: La velocidad y grado de liberación de ketoprofeno (cápsulas 100-mg) se determinó con el método de celda de flujo continuo (Aparato 4 USP) en flujo laminar a 16 ml/min. Adicionalmente, y a manera de comparación con un equipo más conocido, se utilizó el Aparato 2 USP (paletas) a 50 rpm y 900 ml de medio. En ambos equipos se utilizó solución amortiguadora de fosfato pH 7.5 como medio de disolución. Se tomaron muestras cada 5 min durante 45 min y el fármaco se cuantificó por espectrofotometría (260 nm) con apoyo de curvas de calibración. Con los datos *in vitro* de los cuatro medicamentos e información farmacocinética publicada del fármaco se simularon los perfiles plasmáticos por el método de convolución. Finalmente se calcularon los valores de predicción del error para los datos de estimados de C_{max} y $ABC_{0\text{-inf}}$.

Resultados: En la figura 1 se presentan los perfiles de disolución de los medicamentos evaluados en los dos equipos de disolución. En ambos equipos los medicamentos genéricos A y C y el medicamento de referencia alcanzaron más del 80% de fármaco disuelto a los 45 min. En el Aparato 4 USP el medicamento B no superó el 30% al término de la prueba. Al estimarlos niveles plasmáticos y calcular los valores de predicción del error en el Aparato 2 USP el medicamento genérico C y en el Aparato 4 USP el medicamento de referencia presentaron valores <10% en los parámetros farmacocinéticos C_{max} y $ABC_{0\text{-inf}}$.

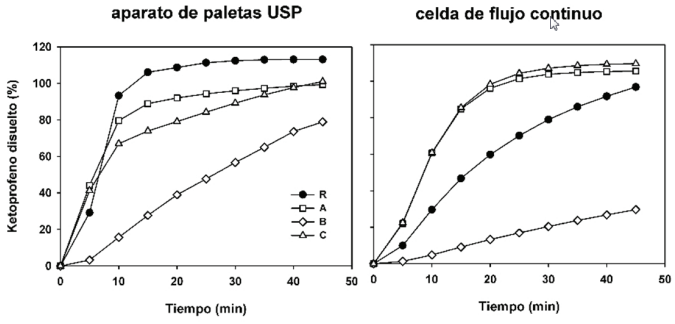


Figura 1. Perfiles de disolución de ketoprofeno en cápsulas.

[1] Departamento Sistemas Biológicos, Universidad Autónoma Metropolitana-Xochimilco, Ciudad de México, México.

*Autor correspondiente: rmlopez@correo.xoc.uam.mx.

 ISSN: 2182-3340

Discusión: La predicción de niveles plasmáticos, con resultados parecidos a los reportados en un estudio de biodisponibilidad, del medicamento de referencia usando el método de celda de flujo continuo permiten establecer las condiciones ideales para la comparación de productos farmacéuticos orales de ketoprofeno antes que éstos manifiesten fallas terapéuticas por problemas de absorción.

Palabras clave: ketoprofeno, celda de flujo continuo, convolución.

Referencias Bibliográficas:

1. Lobos M. Evaluación de la solubilidad y disolución de ketoprofeno en cápsulas de 50 mg para sustentar la eximición de estudios in vivo en la determinación de su equivalencia terapéutica [Internet]. Santiago, Chile: Universidad de Chile - Facultad de Ciencias Químicas y Farmacéuticas; 2013 [citado: 2023, septiembre]. Disponible en: https://repositorio.uchile.cl/handle/2250/114222.

2. Carrillo Esper Raúl, Zavaleta Bustos Miriam, Álvarez Alcántara Haidee, Carrillo Córdova Dulce María, Carrillo Córdova Carlos Alberto. La importancia de los parámetros farmacocinéticos y farmacodinámicos en la prescripción de antibióticos. Rev. Fac. Med. (Méx.) [revista en la Internet]. 2013 Jun [citado 2023 Sep 15] ; 56(3): 5-11. Disponible en: http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0026-17422013000700002&lng=es.

Predicción del Desempeño *in vivo* de Tabletas de Furosemida: Influencia del Medio y Equipo de Disolución

Medina-López J.R.[1*], Reyes-Ramírez F.D.[1], Ruiz-Segura J.C.[1]

ORIGINAL ARTICLE

RESUMEN

Introducción: La furosemida es un fármaco diurético de baja solubilidad y baja permeabilidad cuya intercambiabilidad debe ser demostrada con estudios de bioequivalencia. Los estudios de disolución bajo diferentes condiciones son un factor clave en la predicción de esta propiedad. Diversos autores han documentado problemas en el proceso de liberación *in vitro*[1] por lo que el objetivo de este trabajo es simular el desempeño *in vivo* de furosemida en tabletas con datos de disolución obtenidos en los Aparatos 2 y 4 USP y medios de relevancia fisiológica. El desempeño será expresado en términos de similitud de resultados con datos farmacocinéticos publicados[2].

Metodología: Los datos *in vitro* de dos medicamentos (referencia y genérico, tabletas 40-mg) se obtuvieron con el Aparato 2 USP (50 rpm y 900 ml de medio) y Aparato 4 USP (flujo laminar a 16 ml/min)3. Se utilizó HCl 0.1 N y soluciones amortiguadoras de pH 4.5 y 6.8. Se tomaron muestras hasta 60 min y el porciento disuelto se calculó por espectrofotometría (274 nm). Con los datos *in vitro* e información *in vivo* se procedió estimar por convolución los perfiles plasmáticos. Un valor de predicción del error <10% en C_{max} y $ABC_{0\text{-}inf}$ indica un desempeño muy parecido al observado en estudios en humanos.

Resultados: En la figura 1 se presentan los perfiles de disolución. Estos se compararon con los valores de eficiencia de disolución y tiempo medio de disolución (t-Student). Las formulaciones no presentaron similitud en sus perfiles en ningún medio ni con ningún equipo (p<0.05), incluso en HCl 0.1 N se disolvió <25%. En pH 4.5 y 6.8 los resultados mejoraron ya que la liberación del fármaco se explicó con la función de Weibull. Al simular los niveles plasmáticos solo se encontraron valores de predicción del error <10% para C_{max} y $ABC_{0\text{-}inf}$ con los datos del medicamento de referencia a pH 6.8 en el Aparato 4 USP.

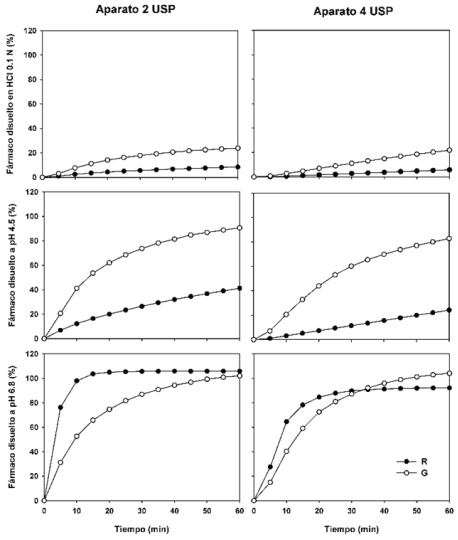


Figura 1. Perfiles de disolución de furosemida en tabletas de referencia (R) y genérico(G).

[1] Departamento Sistemas Biológicos, Universidad Autónoma Metropolitana-Xochimilco, Ciudad de México, México.

*Autor correspondiente: rmlopez@correo.xoc.uam.mx.

 ISSN: 2182-3340

Discusión: Los estudios de disolución que involucren condiciones lo más parecido a las observadas en humanos son una herramienta importante en la predicción del desempeño *in vivo* de fármacos con problemas de solubilidad. El Aparato 4 USP resultó la mejor opción para simular los niveles plasmáticos de furosemida.

Palabras clave: furosemida, perfiles, Aparato 4 USP.

Referencias Bibliográficas:

1. Granero GE, Longhi MR, Mora MJ, Junginger HE, Midha KK, Stavchansky S, Dressman JB, Barends DM. Biowaiver monographs for immediate release solid oral dosage forms: furosemide. J Pharm Sci. 2010;99(6):2544-2556.
2. Najib N, Idkaidek N, Beshtawi M, Bader M, Admour I, Alam M, Zaman Q, Dham R. Bioequivalence evaluation of two brands of furosemide 40 mg tablets (Salurin and Lasix) in healthy human volunteers. Biopharm Drug Dis. 2003;24(6):245-249.
3. Fotaki, N. Flow-through cell apparatus (USP Apparatus 4): operation and features. Dissolut Technol. 2011;18(4):46-49.

Simulación de los Perfiles Plasmáticos de Verapamilo-HCl utilizando Datos de los Aparatos 2 y 4 USP

Medina-López J.R.[1*], Contreras-Jiménez J.M.[1], Hurtado M.1, Helmy S.[2,3], Reyes-Ramírez F.D.[1], Ruiz-Segura.[1] J.C.

ORIGINAL ARTICLE

RESUMEN

Introducción: El verapamilo-HCl es un fármaco utilizado para tratar enfermedades cardiacas y presenta alta variabilidad biológica debido a su baja biodisponibilidad[1]. Los medicamentos de referencia son el producto de comparación al evaluar medicamentos genéricos por lo que es importante documentar el desempeño *in vitro* que presentan estos productos a fin de predecir el desempeño *in vivo*[2] y evitar problemas en la práctica clínica. El objetivo del presente estudio es simular los perfiles plasmáticos de verapamilo-HCl utilizando datos de disolución generados en los Aparatos 2 y 4 USP e información *in vivo* publicada en la literatura especializada[3].

Metodología: Se determinaron los perfiles de disolución de verapamilo-HCl del medicamento de referencia (tabletas 40-mg) con el Aparato 2 USP a 50 rpm y 900 ml de medio de disolución y el Aparato 4 USP con flujo laminar a 16 ml/min. Se utilizó HCl 0.1 N y soluciones amortiguadoras de pH 4.5 y 6.8. El fármaco se cuantificó por diferencia de la absorbancia (278 y 300 nm). Se tomaron muestras filtradas a los 5, 10, 15, 20 y 30 min. Con esta información y datos farmacocinéticos se procedió a simular los niveles plasmáticos de verapamilo-HCl por el método de convolución. Se calculó el valor de predicción del error para los parámetros estimados de Cmax y $ABC_{0\text{-inf}}$. Un valor <15% indica parámetros estimados parecidos a los observados en estudios *in vivo*.

Resultados: En ambos equipos de disolución y con HCl 0.1 N así como con el Aparato 4 USP y medio de pH 4.5 las tabletas liberaron más de 85% de fármaco a los 30 min. En la figura 1 se presenta los perfiles plasmáticos estimados con datos de disolución en HCl 0.1 N (A), pH 4.5 (B) y pH 6.8 (C). En ambos equipos y con HCl 0.1 N los valores de predicción del error fueron <15%. Con el Aparato 4 USP y medio de pH 4.5 también el error no superó el 15%.

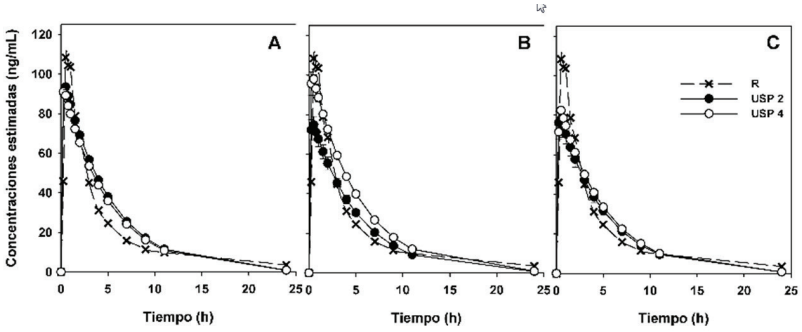


Figura 1. Perfiles hipotéticos de verapamilo-HCl. Media, n=6.

[1] Departamento Sistemas Biológicos, Universidad Autónoma Metropolitana-Xochimilco, Ciudad de México, México.
[2] Department of Clinical and Hospital Pharmacy, Faculty of Pharmacy, Taibah University, AL-Madinah AL-Munawarah, Kingdom of Saudi Arabia.
[3] Department of Pharmaceutics, Faculty of Pharmacy, Damanhour University, Damanhour, Egypt.

*Autor correspondiente: rmlopez@correo.xoc.uam.mx.

 ISSN: 2182-3340

Discusión: El Aparato 4 USP demostró generar datos de disolución que permiten simular de mejor forma el desempeño *in vivo* del fármaco. Es necesario llevar a cabo la correlación *in vitro/in vivo* con este medicamento para corroborar la capacidad del Aparato 4 USP en la predicción de los perfiles plasmáticos de verapamilo-HCl.

Palabras clave: verapamilo, convolución, genéricos.

Referencias Bibliográficas:

1. Vogelpoel H, Welink J, Amidon GL, Junginger HE, Midha KK, Möller H, Olling M, Shah VP, Barends DM. Biowaiver monogrpahs for immediate release solid oral dosage forms based on biopharmaceutics classification system (BCS) literatura data: verapamil hydrochloride, propranolol hydrochloride and atenolol. J Pharm Sci. 2004;93(8):1945-1956.

2. Qureshi SA. *In vitro–in vivo* correlation (IVIVC) and determining drug concentrations in blood from dissolution testing – a simple and practical approach. Open Drug Deliv J. 2010;4(2):38-47.

3. Haeri A, Javadian B, Saadati R, Dadashzadeh S. Metabolite parameters as an appropriate alternative approach for assessment of bioequivalence of two verapamil formulations. Iran J Pharm Res. 2014;13(2):383-391.

Eficacia del Cannabidiol Frente al Glioblastoma: Monoterapia y Terapia Combinada con Temozolomida o Carmustina

Lázaro L.G.[1*], Blanco J.A.[1], Sabroso C.M.[1], Sánchez A.I.F.[1], Rubio M.C.M.[2], Marcos C.H.[2], Suárez A.I.T.[1]

ORIGINAL ARTICLE

RESUMEN

Introducción: El glioblastoma es uno de los tumores con mayor incidencia y agresividad del sistema nervioso central (SNC), su tasa de supervivencia de 10-20 meses. Debido a su alto porcentaje de recurrencia y a la falta de terapias efectivas por la dificultad de muchos fármacos de acceder al SNC[1]. El tratamiento actual combina temozolomida (TMZ) o carmustina (BCNU), como fármacos de primera y segunda línea respectivamente, con radioterapia[2]. El cannabidiol (CBD), ha demostrado poseer efecto citotóxico en distintos tipos de cáncer[3], por lo que su uso en el tratamiento del glioblastoma primario en combinación con los quimioterápicos actuales podría suponer un aumento en la eficacia de estos, al tiempo que permitiría reducir su dosis y efectos secundarios. El objetivo de este trabajo es el estudio del efecto citotóxico *in vitro* e *in ovo* del CBD en monoterapia y del efecto citotóxico *in vitro* del CBD combinado con TMZ/BCNU sobre células de glioblastoma humano.

Metodología: El efecto citotóxico del CBD se evaluó sobre las células U-87-MG (ATCC® HTB-14™) tanto in vitro (ensayo MTT a 24 y 48h), como en el modelo in ovo (CAM model) donde se aplicó el tratamiento sobre tumores formados en la membrana corioalantoidea de embriones de pollo, observándose el efecto tras 48h. Posteriormente se evaluó el efecto citotóxico *in vitro* (ensayo MTT a 24 y 48h) de las combinaciones CBD+TMZ y CBD+BCNU.

Resultados y discusión: El CBD posee efecto citotóxico sobre las células U-87 MG de glioma humano con un valor de IC_{50} de 37.08 μM (34.00-40.44) y de 28.71 μM (26.25-31.41) a las 24 y 48 h de tratamiento, respectivamente (N=3). Respecto a los ensayos in ovo, a las 48h, se observó una reducción del tamaño tumoral de un 43% frente al control (N=5). La combinación CBD+TMZ presentó un efecto sinérgico a las 24 y a las 48 h. Por el contrario, la combinación CBD+BCNU resultó ser antagónica. En conclusión, El CBD podría considerarse una alternativa eficaz en la terapia frente a glioblastoma tanto en monoterapia como en combinación con TMZ (figura 1).

[1] Dpto. de Farmacia Galénica y Tecnología Alimentaria, Universidad Complutense de Madrid, España.
[2] Dpto. de Ciencias Farmacéuticas y de la Salud, Universidad CEU San Pablo de Madrid, España.

*Autor correspondiente: lgomez14@ucm.es.

 ISSN: 2182-3340

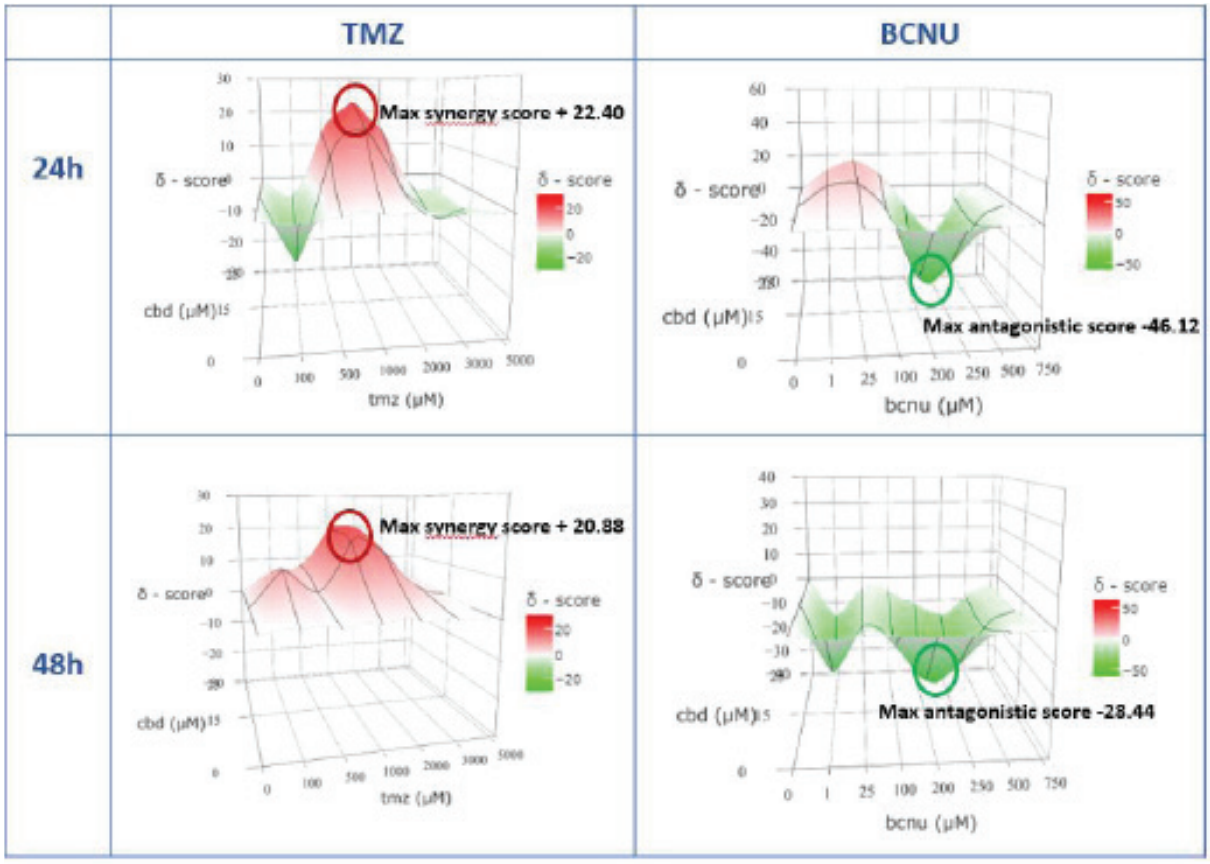


Figura 1. Screening de combinaciones en modelos 2D. Estudio del efecto citotóxico en modelos in vitro 2D de las combinaciones CBD+TMZ y CBD+BCNU sobre las células U-87-MG de glioblastoma humano a las 24 y a las 48h de tratamiento. Los mapas 3D de las combinaciones se obtuvieron mediante el software *Synergyfinder*.

Palabras clave: cannabidiol, temozolomida, carmustina.

Agradecimientos: Ministerio de Ciencia, Innovación y Universidades. Ref. PID2019-105531RB-I00/AEI/10.13039/501100011033. Beca Santander UCM CT82/20-CT83/20).

Referencias Bibliográficas:

1. Robilliard LD, Yu J, Anchan A, Finlay G, Angel CE, Graham ES. Comprehensive Assessment of Secreted Immuno-Modulatory Cytokines by Serum-Differentiated and Stem-like Glioblastoma Cells Reveals Distinct Differences between Glioblastoma Phenotypes. Int J Mol Sci. 2022;23(22).

2. Aparicio-Blanco J, Sanz-Arriazu L, Lorenzoni R, Blanco-Prieto MJ. Glioblastoma chemotherapeutic agents used in the clinical setting and in clinical trials: Nanomedicine approaches to improve their efficacy. Int J Pharm. 2020;581(February).

3. Heider CG, Itenberg SA, Rao J, Ma H, Wu X. Mechanisms of Cannabidiol (CBD) in Cancer Treatment: A Review. Biology (Basel). 2022;11(6).

Tumor Mutational Burden in Colorectal Cancer: Implications for Treatment

Marques A.[1*], Torre C.[1], Pinto R.[1,2], Sepodes B.[1], Rocha J.[1]

ORIGINAL ARTICLE

ABSTRACT

Introduction: Colorectal cancer (CRC) is the third most common and second most lethal cancer worldwide. Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of several advanced solid cancers, but only a minority of CRC patients seem to benefit from these innovative therapies. For this specific subgroup of patients—those with deficient mismatch repair or high microsatellite instability (dMMR/MSI-H)—the FDA and EMA have approved three checkpoint-blocking antibodies[1]. Deficiencies in DNA repair cause increased mutational load, leading to a higher diversity of neoantigens and subsequent infiltration of immune cells. However, MSI status does not adequately identify all CRC patients who might benefit from treatment with ICIs: for example, POLE/POLD[1] mutations also result in a hypermutated phenotype[2]. Tumor mutational burden (TMB), which reflects the number of mutations present in the genome of cancer cells, has emerged as a potential predictor of ICI benefit. In fact, following its approval for patients with dMMR/MSI-H tumors, pembrolizumab has been granted a second tissue-agnostic approval for patients with high TMB by the FDA. Although TMB holds promise for identifying new subpopulations of patients who may benefit from ICIs, current evidence is insufficient to recommend its routine application in clinical practice[3].

Methodology: In this review, we explore the potential of TMB as a predictor of ICI benefit, address the limitations associated with its widespread implementation, and evaluate its utility in metastatic CRC. To explore common patterns in TMB-based stratification in colorectal cancer, we compiled a dataset of CRC or pan-cancer studies utilizing numerical thresholds for high TMB.

Results: Our research confirms the lack of standardization reported when it comes to TMB as a predictive biomarker: the information regarding the details of TMB assessment and definition was often unclear or entirely absent. The clinical application of TMB in this context has been further hindered by the inconsistency of stratification thresholds across studies, cancer types, and ICI agents. In the 45 included studies, TMB-H cut-offs varied from 6.88 to 41 mut/Mb, and were most often set at 10, 17 or 20 mut/Mb.

Discussion: Harmonization of TMB methodologies and cutoff values is critical to ensure adequate translation between study results and real-life applicability.

Keywords: colorectal cancer, immune checkpoint inhibitors, tumor mutational burden.

[1] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisbon, Portugal.
[2] Joaquim Chaves Saúde, Joaquim Chaves Laboratório de Análises Clínicas, Miraflores, 1495-069 Algés, Portugal.

*Corresponding author: adriana.marques1@campus.ul.pt.

 ISSN: 2182-3340

Bibliographic References:

1. Ooki A, Shinozaki E, Yamaguchi K. Immunotherapy in Colorectal Cancer: Current and Future Strategies. J Anus, Rectum Colon. 2021;5(1):11–24.

2. Ogunwobi OO, Mahmood F, Akingboye A. Biomarkers in colorectal cancer: Current research and future prospects. Int J Mol Sci. 2020;21(15):1–20.

3. Jardim DL, Goodman A, de Melo Gagliato D, Kurzrock R. The Challenges of Tumor Mutational Burden as an Immunotherapy Biomarker. Cancer Cell. 2021;39(2):154–73.

Unveiling Thioxanthones: an *in vitro* Investigation of P-gp Modulation at the Kidney Level

Veiga-Matos J.[1,2*], Sousa E.[3,4], Morales A.I.[2], Prieto M.[2], Remião F.[1], Silva R.[1]

ORIGINAL ARTICLE

ABSTRACT

Background: P-glycoprotein (P-gp) is an efflux transporter located at the apical membrane of important barrier tissues, like the proximal tubular epithelium, playing a crucial role in the detoxification of endobiotics and xenobiotics. (Thio)xanthonic derivatives have been shown to enhance P-gp expression and/or activity at the intestinal level, promoting an increase in the amount of transported substrate, thus limiting the intracellular accumulation of harmful P-gp substrates and, consequently, reducing their toxicity[1-3]. However, no studies exist on the ability of such compounds for P-gp modulation in kidney cells, which could have therapeutic applications in mitigating drug-induced nephrotoxicity. The aim of this study was to assess, in vitro, the ability of 5 thioxanthones (TX1-5) to activate/induce P-gp at the kidney level, using the HK-2 cells as *in vitro* model.

Methodology: Rhodamine 123 (RHO123) accumulation assay was performed to evaluate TX1-5 (5, 10 and 20 μM) effects on P-gp activity. Two different experimental approaches were used: 1) exposure to TX1-5 for 90 minutes with simultaneous incubation with the P-gp fluorescent substrate RHO123 (5 μM), a protocol used to detect immediate effects on P-gp activity (direct P-gp activation); and 2) exposure to TX1-5 for 24 hours, followed by their removal and exposure to RHO123 (5 μM, for 90 minutes), a protocol used to detect effects on P-gp activity resulting from an increased P-gp expression (P-gp induction).

Results: After 90 minutes of exposure to TX1-5, a significant increase in P-gp activity was observed for all TX, except TX4, reaching a maximum of 141% in the presence of TX2 (5 μM). Moreover, although less expressive, the exposure to TX1-5 for 24 hours also induced a significant increase of P-gp activity, except for TX4, achieving the maximum of 121% for TX3 (5 μM).

Conclusions: The present study confirmed that thioxanthonic derivatives can increase P-gp transport activity at different time points in HK-2 cells, suggesting that these compounds act as P-gp activators and/or inducers, potentially facilitating the removal of toxic P-gp substrates from kidney's proximal tubules. This highlights their therapeutic potential for reducing the nephrotoxic effects of P-gp substrates.

Keywords: nephrotoxicity, HK-2 cells, Rhodamine 123.

[1] UCIBIO, Laboratório de Toxicologia, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal.
[2] Unidad de Toxicología, Universidad de Salamanca; Group of Translational Research on Renal and Cardiovascular Diseases (TRECARD); Instituto de Investigación Biomédica de Salamanca (IBSAL); Salamanca, España.
[3] Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal.
[4] CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, Matosinhos, Portugal.

*Corresponding author: jessicamatos1996@outlook.com.

 ISSN: 2182-3340

Acknowledgements: Funding: This research was funded by FCT (Fundação para a Ciência e Tecnologia), UIDB/04423/2020, UIDP/04423/2020, grant number 2021.06177.BD, through national funds.

Bibliographic References:

1. Silva R, Vilas-Boas V, Carmo H, Dinis-Oliveira RJ, Carvalho F, De Lourdes Bastos M, *et al*. Modulation of P-glycoprotein efflux pump: Induction and activation as a therapeutic strategy. Vol. 149, Pharmacology and Therapeutics. Elsevier Inc.; 2015. p. 1–123. Available from: http://dx.doi.org/10.1016/j.pharmthera.2014.11.013.

2. Martins E, Silva V, Lemos A, Palmeira A, Puthongking P, Sousa E, et al. Newly synthesized oxygenated xanthones as potential P-glycoprotein activators: In vitro, *ex vivo*, and *in silico* studies. Molecules. 2019;24(4). Available from: https://doi.org/10.3390/molecules24040707.

3. Silva V, Gil-Martins E, Rocha-Pereira C, Lemos A, Palmeira A, Puthongking P, et al. Oxygenated xanthones as P-glycoprotein modulators at the intestinal barrier: *in vitro* and docking studies. Med Chem Res. 2020;29(6):1041–57. Available from: http://dx.doi.org/10.1007/s00044-020-02544-1.