IBJ Plus IberoAmerican Journals
IBJ IberoAmerican Journals
ISSN: 2531-0151

IBJ Plus

Volume 1, Special Issue 1, March 2018

Abstracts of 5th Symposium on Biomedical Research.

Advances and Perspectives
In Pharmacology, Drug Toxicity and Pharmacogenetics
Madrid, March 15th 2018

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The Symposium

Programme & Poster

5th Symposium on Biomedical Research
Advances and Perspectives
In Pharmacology, Drug Toxicity and Pharmacogenetics

PROGRAMME

15th March, 2018

8:00-8:30. *Registration / Recogida de documentación*

AULA MAGNA, FACULTAD DE MEDICINA

8:30-8:45. *Opening* ***Rafael Garesse (Chancellor of the UAM)***

8:45-10:45. ***Plenary Session 1: Chairperson María Monsalve (IIBM)***

8:45-9:15 ***Maribel Lucena***. Departamento de Farmacología, UMA, Málaga.
"Drug-Induced Liver injury (DILI) prediction, detection and adjudication"
9:15-9:45 ***José A. García-Agundez***. Instituto Universitario de Biomarcadores de Patologías Moleculares, UNEX, Cáceres.
"Development of guidelines for the implementation of pharmacogenetic biomarkers – CPIC-"
9:45-10:15 ***Teresa Bellón.*** Instituto de Investigación Sanitaria de Hospital Universitario La Paz, IdiPAZ, Madrid.
"Identification of genetic and immunologic risk factors for severe cutaneous adverse reactions to medications. The experience of PIELenRed"
10:15-10:45 ***Arturo Soto Matos-Pita*** . Director of Clinical Development, Pharmamar, Madrid.
"Rational drug development in oncology"

10:45-11:30. *Coffee Break & Poster viewing*

11:30-13:15. ***Plenary Session 2: Chairperson Ana Guadaño (IIBM)***

11:30-12:00 ***Sara Eyal.*** The Hebrew University, Jerusalem
"Precision and stratified medicine: the promise and the barriers"
12:00-12:30 ***Mati Berkovitch.*** Assaf Harofeh Medical Center's, Jerusalem
"Medical Cannabidiol (Cannabis) in Pediatrics, Pregnancy and Lactation: Pharmacological Aspects"
12:30-13:00 ***David De Lorenzo.*** NIMGenetics, Madrid
"Role of genome-environment interactions in precision medicine"
13:00-13:15 ***Selected Short talk 1 Vanesa Sánchez.*** ISCIII, Madrid
"Antitumoral effects of Hispanolone derivatives in glioblastoma cells"

13:15-15:30. *Lunch / Almuerzo*

15:30-16:45. ***Plenary Session 3: Chairperson Paloma Sánchez Aparicio (IIBM)***

15:30-16:00 ***Alberto Borobia.*** Facultad de Medicina (UAM), Madrid
"Implementation of Pharmacogenetic Testing in La Paz Hospital: Strategy and Experience Over 3 Years"
16:00-16:15 ***Selected Short talk 2 Roberto Corchado.*** USAL, Salamanca
"Identification of genetic and molecular determinants associated with cardiotoxicity by anthracyclines and taxanes according to age"
16:15-16:30 ***Selected Short talk 3 Irene García.*** IdiPAZ, Madrid
"Drug induced cholestasis: assessment of drug causality with algorithm versus Lymphocyte Transformation Test"
16:30-16:45 ***Selected Short talk 4 Assel Sarsenbayeva.*** UU, Uppsala
"Effects of olanzapine and aripiprazole on lipolysis in healthy human subcutaneous adipocytes during short incubations"

16:45-17:45. *Poster viewing & Coffee*

16th March, 2018

SALÓN DE ACTOS, IIBM

8:30-08:45. *Welcome:* ***Antonio Cuadrado (Head of Biochemistry Department, UAM)***

8:45-11:15. ***Plenary Session 4: Chairperson Pilar López (UAM)***

8:45-9:15 ***John Jones***. CNC, Coimbra.
"What is metabolism?"
9:15-9:45 ***Ángela M. Valverde.*** IIBM, CSIC-UAM & CIBERDEM-ISCIII, Madrid.
"Insights on molecular mechanisms of paracetamol-mediated hepatotoxicity"
9:45-10:15 ***Manuela G. López.*** Facultad de Medicina, UAM, Madrid.
"Melatonin and melatonin derivatives as a therapeutic strategy for neurodegeneration"
10:15-10:45 ***Ana Catarina Fonseca***. CNC, Coimbra.
"Regulation of proteostasis and mitochondrial function in human epicardial fat"
10:45-11:15 ***Francisco Abad-Santos***. Instituto de Investigación Sanitaria Hospital Universitario de la Princesa, UAM, Madrid.
"Identification and validation of biomarkers that predict efficacy and toxicity: Example of psoriasis"

11:15-12:00. *Coffee Break*

12:00-13:30. ***Complementary Training Course: Chairperson Concha Peiró (UAM)***

12:00-12:30 ***Flora de Pablo.*** CIB-CSIC & CIBERDEM-ISCIII, Madrid.
"The Way Forward: Women in Science"
12:30-13:00 ***Simon Barlett.*** CNIC, Madrid.
"Scientific Writing Skills"
13:00-13:30 ***Rafael Dal-Ré***. Instituto de Investigación sanitaria-Hospital Universitario Fundación Jiménez Díaz, UAM, Madrid.
"Are clinical trial results falsified?"

13:30-13:45. *Closing Remarks:* ***Lisardo Boscá (Director of IIBM)***

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15th-16th March, 2018

Deadline for registration & abstract submission: 28th of February, 2018

5th Symposium on Biomedical Research

Advances and Perspectives

In Pharmacology, Drug Toxicity and Pharmacogenetics

***Organized by:* IIBM & UAM School of Medicine**

15/3 Aula Magna, School of Medicine UAM
Arzobispo Morcillo 4, 28029-Madrid

16/3 Salón de Actos, IIBM (CSIC-UAM)
Arturo Duperier 4, 28029-Madrid

Invited Speakers

José A. García-Agúndez, UNEX
David de Lorenzo, NIMGenetics
Francisco Abad, UAM
Alberto Borobia, UAM
Simon Bartlett, CNIC
Flora de Pablo, CIB
Ana Catarina Fonseca, CNC
Arturo Soto, Pharma Mar

Information and registration (Free of charge):

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Abstracts

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Fludarabine inhibits KV1.3 currents in human B lymphocytes.

Vera-Zambrano A[1,2,*], de la Cruz A[2], Peraza D A[2], Zapata J M[2,3], Valenzuela C[2], Perez-Chacon G[2,3], Gonzalez T[1,2,3]

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Introduction: Fludarabine (F-ara-A) is a purine analogue commonly used in the treatment of indolent B cell malignancies that interferes with different aspects of DNA and RNA synthesis. KV1.3 potassium channels are membrane proteins involved in the control of K+ homeostasis and in the maintenance of the resting potential of the cell, thus controlling signalling events, proliferation and apoptosis in lymphocytes. The aim of this study was to determine if F-ara-A modulates KV currents in human B lymphocytes.

Material and methods: We assessed the expression, the activity and the effect of F-ara-A on the KV1.3 channel in BL2 and Dana B cell lines. Currents were registered by whole-cell patch-clamp. Statistical significance was determined by t-Student test or by nonparametric Mann-Whitney test.

Results: We show that KV1.3 is expressed in both BL2 and Dana B cell lines, although total KV1.3 levels were higher in BL2 compared to those in Dana cells. However, KV currents in the plasma membrane were similar in both cell lines. These KV currents were abrogated by the specific KV1.3 channel inhibitor PAP-1, indicating that most KV currents in these B cell lines are controlled by KV1.3. F-ara-A (3.5 µM), a concentration similar to that achieved in the plasma of fludarabine phosphate-treated patients (3 µM), inhibited KV1.3 currents by 61±6.3% and 52.3±6.3% in BL2 and Dana B cells, respectively. The inhibitory effect of F-ara-A was concentration dependent and showed an IC50 value of 0.36±0.04 µM and a nH value of 1.07±0.15 in BL2 cells and 0.34±0.13 µM (IC50) and 0.77±0.11 (nH) in Dana cells. This inhibitory effect of F-ara-A on the activity of plasma membrane KV1.3 was observed in these cells irrespective of their cytotoxic effect. F-ara-A had no effect on heterologously expressed KV1.3 channels, suggesting an indirect mechanism of inhibition.

Conclusions: Fludarabine (F-ara-A), a chemotherapeutic drug extensively used in clinics, strongly inhibits KV1.3 currents in B lymphoma and lymphoblastoid cells. Although this inhibitory activity is not sufficient to induce cell death, it might still contribute to the cytotoxic effect of the drug.

Keywords: Fludarabine, F-ara-A, KV1.3, chronic lymphocytic leukemia, B lymphocyte
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Vera-Zambrano, de la Cruz A, Peraza D A, Zapata J M, Valenzuela C, Perez-Chacon G, Gonzalez T. Fludarabine inhibits KV1.3 currents in human B lymphocytes. IBJ Plus 2018 (S1):e0001 doi: 10.24217/2531-0151.18v1s1.00001.
Funding: This study was supported by MINECO (SAF2013-45800-R, SAF2016-75021-R, RD12/0042/0019, CB/11/00222) and ISCIII (PI12/01135 and PI16/00895).
Competing Interests: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Deficiency in the transcription factor NRF2 worsens inflammatory parameters in a mouse model with combined tauopathy and amyloidopathy.

Ana I Rojo[1*], Marta Pajares[1], Angel J García-Yagüe[1], Izaskun Buendia[2], Fred Van Leuven[3], Masayuki Yamamoto[4], Manuela G Lopez[2], Antonio Cuadrado[1,5*]

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Chronic neuroinflammation is a hallmark of the onset and progression of brain proteinopathies such as Alzheimer disease (AD) and it is suspected to participate in the neurodegenerative process. Transcription factor NRF2, a master regulator of redox homeostasis, controls acute inflammation but its relevance in low-grade chronic inflammation of AD is inconclusive due to lack of good mouse models. We have addressed this question in a transgenic mouse that combines amyloidopathy and tauopathy with either wild type (AT-NRF2-WT) or NRF2-deficiency (AT-NRF2-KO). AT-NRF2-WT mice died prematurely, at around 14 months of age, due to motor deficits and a terminal spinal deformity but AT-NRF2-KO mice died roughly 2 months earlier.

NRF2-deficiency correlated with exacerbated astrogliosis and microgliosis, as determined by an increase in GFAP, IBA1 and CD11b levels. The immunomodulatory molecule dimethyl fumarate (DMF), a drug already used for the treatment of multiple sclerosis whose main target is accepted to be NRF2, was tested in this preclinical model.

Daily oral gavage of DMF during six weeks reduced glial and inflammatory markers and improved cognition and motor complications in the AT-NRF2-WT mice compared with the vehicle-treated animals. This study demonstrates the relevance of the inflammatory response in experimental AD, tightly regulated by NRF2 activity, and provides a new strategy to fight AD.

Keywords: Tauopathy, neuroinflammation, Alzheimer disease, sclerosis.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Ana I Rojo, Marta Pajares, Angel J García-Yagüe, Izaskun Buendia, Fred Van Leuven, Masayuki Yamamoto, Manuela G Lopez, Antonio Cuadrado. IBJ Plus 2018 (S1):e0002 doi: 10.24217/2531-0151.18v1s1.00002.
Funding: The authors declare no funding involved.
Competing Interests: The authors declare no conflicts of interest.

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Alterations in the stimulus-secretion coupling related to aging in the murine model of accelerated senescence SAMP8.

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Introduction: The nervous system is especially vulnerable to aging. Its vulnerability is manifested by the existence of neurodegenerative pathologies like Alzheimer's disease (AD), Parkinson's disease (PD) or Amyotrophic Lateral Sclerosis (ALS). During these diseases, alterations of neurotransmitter systems has been reported. Although, numerous changes can also be observed in many individuals during non-pathological aging. Our working hypothesis suggests that, with the progression of age, alterations in the stimulus-secretion coupling can occur, compromising the release of neurotransmitters and causing cognitive deficits.

Materials and Methods: In this work, mice of the senescence-prone strain 8 (SAMP8) have been used which show age-related behavioural deterioration such as deficits in learning and memory, emotional disorders and altered circadian rhythm, being therefore used as a model of spontaneously occurring Alzheimer's disease. In parallel, their resistant senescence (SAMR1) brothers have been used as a control. We used the chromaffin cell as a model of neurosecretion in SAMP8 and SAMR1 mice at 2, 6 and 12 months of age. By means of the patch-clamp technique, we have studied the ionic currents involved in the secretory process of catecholamines and in the transmission of the nerve impulse (nicotinic, Na+, Ca2+ and K+ currents). We have also assessed cell excitability by measuring membrane potential and spontaneous and triggered action potentials. Moreover, we have studied the release of the neurotransmitters by the amperometric technique using K+ as stimuli. Finally, we have used the Y-maze to evaluate the cognitive behaviour of the mice.

Results: We have observed that there is an increase in all ionic currents with the age in both, SAMP8 and SAMR1 mice, but this increase occurs before and more remarkable in SAMP8 mice. Regarding membrane potential and spontaneous and triggered action potentials we have observed that there is a hyperpolarization of membrane potential in both types of mice during the aging, moreover, the depolarization that ACh produces is lower throughout the age and again, this phenomenon occurs before and is more remarkable in SAMP8 mice. Furthermore, the number of action potentials generated at rest and those produced by depolarizing pulses decreases during the aging, being higher the amplitude and posthyperpolarization area. Although, the number of action potentials evoked by ACh increases with aging due the less depolarization being more remarkable in SAMP8 mice.

Regarding the release of the neurotransmitters we have seen that when we stimulate with K+ there is an increase in the catecholamine secretion with the aging, happening before in SAMP8 mice. Moreover, this increase in the catecholamine release is accompanied by changes of secretory spikes.

Finally, with Y-maze, we have seen that these alterations in the release of neurotransmitters are associated with a cognitive deficit, since the SAMP8 mice explore all the arms equally, whereas the R1 always explore more the new arm.

Conclusion: We have found that during the aging the mechanisms of exocytosis of neurotransmitters is altered. These alterations could be correlated with the changes that occurs in some neurodegenerative diseases and also causing cognitive deficits.

Keywords: exocytosis, chromaffin cell, aging, Alzheimer.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Andrés M. Baraibar, Carmen Nanclares, Inés Colmena, Isabel Gameiro-Ros, Iris Álvarez-Merz, Alicia Muñoz-Montero, Jesús M. Hernández-Guijo, Luis Gandía. Alterations in the stimulus-secretion coupling related to aging in the murine model of accelerated senescence SAMP8. IBJ Plus 2018 (S1):e0003 doi: 10.24217/2531-0151.18v1s1.00003.
Funding: SAF2013-44108-P and SAF2016-78892-R.
Competing Interests: The authors declare no conflicts of interest.

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Nicotinic receptor subtypes involved in the tobacco-mediated resistance to cisplatin in human non-small cell lung carcinoma.

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Introduction: Cigarette smoking not only correlates with the onset and progression of a wide variety of smoking-related tumors, but the continued tobacco use after cancer diagnosis is known to decrease the effectiveness of adjuvant chemotherapy. These tumors express several nicotinic acetylcholine receptor (nAChR) subtypes whose activation by certain components of tobacco appears to underlie both deleterious effects. One of the tumors most associated with smoking is non-small cell lung carcinoma (NSCLC), a tumor that accounts for 75-85% of all cases of lung cancer. Nicotine, the addictive component in tobacco, plays a key role in tobacco-mediated chemoresistance through the activation of a still unknown nAChR subtype, which triggers signaling pathways that lead to the inhibition of apoptosis. The aim of this study is to elucidate the nAChR subtype and the apoptotic signaling pathways underlying nicotine-mediated chemoresistance in NSCLC, as well as to assess whether another component of tobacco, such as the nitrosamine NNK, may also be involved in the above effect.

Material and Methods: All assays were carried out on A549 cells, a human lung adenocarcinoma cell line. Cell viability and apoptosis assays were measured using the MTT and Annexin V-FITC kits, respectively. Gene silencing of the nAChR subunits was performed with small interference RNA (siRNA). Molecules of the pro- and anti-apoptotic signaling pathways triggered by cisplatin, in the absence or presence of nicotine or NNK, were analyzed by Western Blot.

Results: Nicotine and NNK inhibited cisplatin-induced apoptosis in A549 cells in a dose-dependent manner. The silencing of the α7 or α5 subunits of the nAChR significantly reduced the above effect of the tobacco components, while silencing of the dupα7-nAChR subunit increased it. Compared with cells exposed to cisplatin, those also treated with nicotine and NNK showed an upregulation of anti-apoptotic signaling (pAkt and Akt) and downregulation in the pro-apoptotic protein Bax. The above effects of nicotine and NNK on the apoptotic signaling pathway were suppressed in cells with the α7 or α5 subunits silenced and they were increased after the silencing of dupα7 subunits.

Conclusion: These results reveal that some components of tobacco induce resistance to cisplatin by an anti-apoptotic mechanism and that nAChR subtypes containing α7 or α5 subunits play a key role in the above effect. In addition, our data also suggest that high dupα7 gene expression levels in NSCLC could reduce tobacco-mediated chemoresistance through interference with nAChRs containing α7 subunits.

Keywords: nicotinic receptors, tobacco, chemotherapy, resistance, lung adenocarcinoma.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Anna Bordas Sánchez, María Extremera Mazuela, José Luis Cedillo Mireles, Carolina Martín-Sánchez, Carmen Montiel López. Nicotinic receptor subtypes involved in the tobacco-mediated resistance to cisplatin in human non-small cell lung carcinoma. IBJ Plus 2018 (S1):e0004 doi: 10.24217/2531-0151.18v1s1.00004.
Funding: SAF2014-56623-R grant to C.M.; FPI-UAM grant to A.B., Universidad Autónoma de Madrid, Spain.
Competing Interests: The authors declare no conflicts of interest.

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Effects of olanzapine and aripiprazole on lipolysis in healthy human subcutaneous adipocytes during short incubations.

Assel Sarsenbayeva[1*], Cátia Marques[1], Gretha Boersma[1], Maria João Pereira[1], Jan Eriksson[1]

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Introduction: Second-generation Antipsychotics (SGAs) have become the treatment of choice over the typical antipsychotics as they provide excellent efficacy and fewer extrapyramidal symptoms. However, the compliance of the patients to SGAs is negatively affected by their ability to induce or aggravate metabolic syndrome, namely, weight gain, insulin resistance, and Type 2 Diabetes. The exact underlying mechanism of metabolic effects of SGAs is not fully elucidated and it is assumed to be at least partially due to their effect on central nervous system. However, whether SGAs have a direct effect on insulin action in the tissues is still to be elucidated. The effect of SGAs on body metabolism varies and we have chosen two drugs, Olanzapine and Aripiprazole, which are associated with high and low risk of metabolic side-effects, respectively.

Our research is focused on studying the effect of both SGAs on insulin resistance in human adipose tissue. Aside from lipid storage function, adipose tissue has been recognised, as an endocrine organ, producing hormones, such as adiponectin and leptin, indispensable for energy homeostasis. The set of experiments performed as a part of this study includes measuring the effect of Olanzapine and Aripiprazole on the lipolysis in human isolated adipocytes.

Methods: Biopsies of subcutaneous adipose tissue (SAT) were collected from 6 patients (3 men, 3 women; age: 20-76 years; BMI: 20.9-34.5 kg/m2). Subjects were free of antidepressants or antipsychotics treatment. At the moment, only the effect of Olanzapine has been tested and measured, the experiments with Aripiprazole are in progress.

A 6% adipocyte suspension was incubated with olanzapine (0.004, 0.04, 0.1, 0.2, 2 and 20 µM) or aripiprazole (0.02, 0.2, 0.5, 1, 10 and 100 µM). This was followed by 10 minutes incubation with 4 concentrations of insulin (0.1 µU; 1.0 µU; 10 µU; 100 µU) and then incubated with 0.5 µM ß-adrenergic receptor agonist, Isopretenerol, for 1h 50 min. ß-adrenergic stimulation activates hormone-sensitive lipase (HSL) enzyme via cAMP-dependent pathway. HSL, in turn, hydrolyses tritriacylglycerol (TAG), diacylglycerol (DAG) or monoacylglycerol (MAG) molecules producing free fatty acids and glycerol. The supernatant was then collected and used for glycerol measurement.

Results: Short incubations of adipocytes with therapeutic concentrations of Olanzapine show no effect in lipolysis. The highest concentration of the drug hints at a reduced rate of lipolysis in adipocytes by more than 50% for each insulin concentration ($p<0.0001$) and in control conditions ($p<0.01$).

Conclusions: Therefore, it seems that short-term incubation of adipocytes with 20 µM Olanzapine reduces the rate of lipolysis, while the therapeutic concentrations do not seem to alter lipolysis in adipocytes.

Keywords: Adipocytes, olanzapine, aripiprazole.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Assel Sarsenbayeva, Cátia Marques, Gretha Boersma, Maria João Pereira, Jan Eriksson. Effects of olanzapine and aripiprazole on lipolysis in healthy human subcutaneous adipocytes during short incubations. IBJ Plus 2018 (S1):e0005 doi: 10.24217/2531-0151.18v1s1.00005.
Funding: This work is being supported by Marie Skłodowska Curie Actions (H2020-MSCA-ITN-2016).
Competing Interests: The authors declare no conflicts of interest.

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Ageing alters the kinetics of exocytosis of both wildtype and the 3xTg-AD mouse model of Alzheimer´s disease.

Carmen Nanclares[1], Inés Colmena[1], Andrés M. Baraibar[1], Alicia Muñoz-Montero[1], Iris Álvarez-Merz[1], Isabel Gameiro-Ros[1], Antonio G. García[1] and Luis Gandía[1*].

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Introduction: Alzheimer´s disease (AD) is the most common form of dementia. The alteration of several neurotransmitter systems has been reported. These alterations in the neurotransmission processes could be correlated with changes in the synthesis, storage or release of neurotransmitters.

Materials and Methods: In this study we have used a triple transgenic murine model of AD (3xTg-AD). This animal model contains mutations in the gene encoding the amyloid precursor protein (βAPPSwe), presenilin-1 (PS1M146V) and tauP301L, which mimics the development of the disease on AD patients. We propose to study here the last steps of exocytosis in chromaffin cells of 3xTg-AD mice of different ages and their controls (WT) using the amperometric technique. Different ionic currents involved in the physiological release of catecholamines will be studied using the patch-clamp technique.

Results: We have found significant changes in the exocytosis of catecholamines occurring in 3xTg-AD when compared with wildtype (WT) mice. These changes show an increase of the number of amperometric spikes during the development of the disease, as well as an increase in the quantal catecholamine content. Kinetic analysis of secretory spikes shows that as mice age amperometric spikes are faster in triggering and shorter in duration. However, the release of catecholamines is slower and longer in duration when comparing 3xTg-AD with WT mice.

Using the patch-clamp technique we found no changes in nicotinic currents, a decrease in sodium currents and an increase in potassium currents. Some alterations were also found in calcium currents.

Taken together, these findings suggest that throughout the development of 3xTg-AD mice and as Alzheimer's disease is established there are changes in chromaffin cell excitability, which causes an increase in neurotransmission.

Conclusion: Although ageing caused some coincident alterations in the kinetics of exocytosis, there were also clear differences between WT and 3xTg-AD mice. These alterations could have an impact on the response that the organism offers in a stressful situation.

Keywords: Alzheimer´s disease, exocytosis, catecholamine
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Carmen Nanclares, Inés Colmena, Andrés M. Baraibar, Alicia Muñoz-Montero, Iris Álvarez-Merz, Isabel Gameiro-Ros, Antonio G. García and Luis Gandía. Ageing alters the kinetics of exocytosis of both wildtype and the 3xTg-AD mouse model of Alzheimer´s disease. IBJ Plus 2018 (S1):e0006 doi: 10.24217/2531-0151.18v1s1.00006.
Funding: This work was supported by SAF2016-78892-R
Competing Interests: The authors declare no conflicts of interest.

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Histone modifications associated with nonconventional systemic drug response in moderate-to-severe psoriasis.

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Introduction: Epigenetic factors play an important role in psoriasis onset and development. There are different drugs available to treat moderate-to-severe psoriasis patients resistant to conventional systemic drugs. Although these drugs are safe and effective for moderate-to-severe psoriasis treatment, some patients do not show an adequate response to them. Therefore, it is necessary to find biomarkers that could predict patients´ response to these drugs. Objective: To analyse the association between nonconventional systemic drugs (ustekinumab, secukinumab, adalimumab, ixekizumab and apremilast) response and histone modifications in moderate-to-severe psoriasis patients.

Materials and methods: Peripheral blood mononuclear cells (PBMCs) were isolated from psoriasis patients treated with nonconventional systemic drugs before and after the administration of these therapies. PBMCs obtained from healthy subjects have been used as controls. Afterwards, histones were extracted from PBMCs. Four different histone modifications (H3 and H4 acetylation, H3K4 and H3K27 methylation) were determined by Enzyme-Linked ImmunoSorbent Assay (ELISA). Data were analyzed by IBM SPSS v.23.

Results and conclusions: Psoriasis patients presented reduced levels of acetylated H3 and H4 and increased H3K4 methylated levels compared to healthy controls. Global changes in any of the histone modifications analyzed were not observed in psoriasis patients between before and after treatment administration. Nevertheless, significant changes in methylated H3K27 were found between responders and non-responders to nonconventional systemic drugs. These results should be validated in large-scale studies before implementation in clinical practice.

Keywords: pharmacoepigenetics, psoriasis, biologic drugs, histone modifications, epigenetics.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: M.C. Ovejero-Benito, A. Reolid, P. Sánchez-Jiménez, M. Saiz-Rodríguez, E. Muñoz-Aceituno, M. Llamas-Velasco, S. Martín-Vilchez, T. Cabaleiro, M. Román, D. Ochoa, E. Daudén, F. Abad-Santos. Histone modifications associated with nonconventional systemic drug response in moderate-to-severe psoriasis. IBJ Plus 2018 (S1):e0007 doi: 10.24217/2531-0151.18v1s1.00007.
Funding: This study was supported by Instituto de Salud Carlos III PI 13/01598, PI 14/01751, PI 17/01972, Fundación Teófilo Hernando, the Ministry of Science and Innovation and the European Regional Development's funds (FEDER).
Competing Interests: F Abad-Santos and D Ochoa have been a consultant or investigator in clinical trials sponsored by the following pharmaceutical companies: Abbott, Alter, Chemo, Farmalíder, Ferrer, Galenicum, GlaxoSmithKline, Gilead, Janssen-Cilag, Kern, Normon, Novartis, Servier, Teva and Zambon. E Daudén and M. Llamas Velasco have potential conflicts of interest (advisory board member, consultant, grants, research support, participation in clinical trials, honoraria for speaking, and research support) with the following pharmaceutical companies: AbbVie (Abbott), Amgen, Janssen-Cilag, Leo Pharma, Novartis, Pfizer, MSD, Lilly and Celgene.

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Effects of olanzapine and aripiprazole on glucose uptake in healthy human subcutaneous adipocytes during short incubations.

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Introduction: Second-generation Antipsychotics (SGAs) are preferable pharmacological treatment for patients with Schizophrenia, mainly due to their efficacy and reduced risk of extrapyramidal effects when compared with first generation antipsychotics. However, there are metabolic side effects associated with the administration of SGAs, namely weight gain, dyslipidaemia and impaired glucose metabolism. Literature reports that even in the absence of antipsychotic treatment patients with Schizophrenia have a propensity to develop metabolic changes that can lead to cardiovascular diseases, insulin resistance, obesity and Type 2 Diabetes. Olanzapine and aripiprazole belong to SGAs and have been reported as drugs with the highest and the lowest risk of inducing metabolic changes, respectively. However, the pharmacological mechanisms underlying their metabolic side effects remain unclear and they will be the main focus of our study.

Adipose tissue is not only specialized in storing lipids but it is also an endocrine organ that produces and secretes numerous biological active compounds that regulate metabolic homeostasis.

Our aim is to evaluate the effect of olanzapine and aripiprazole in the glucose uptake on human isolated adipocytes.

Methods: Biopsies of subcutaneous adipose tissue were collected from 16 healthy volunteers (4 men, 12 women; age: 20-76 years; BMI: 20.9-34.5 kg/m2). Subjects taking antidepressants or antipsychotics were not included. The effect of short-term incubation (30 min) with different concentrations of olanzapine (0.004, 0.04, 0.1, 0.2, 2 and 20 µM) or aripiprazole (0.02, 0.2, 0.5, 1, 10 and 100 µM) on basal and insulin-stimulated (25 and 1000 µU/ml) D-[U-14C]-glucose uptake of isolated adipocytes was measured and compared with control.

Results: Short incubation of adipocytes with olanzapine or aripiprazole showed no effect on basal or insulin-stimulated glucose uptake, with the exception of supra-physiological concentrations of aripiprazole (10 and 100 µM) where we see a systematic decrease of basal and insulin-stimulated glucose uptake; 10 µM by ≈20-25% ($p<0.05$) and 100 µM by ≈60-70% ($p<0.01$).

Conclusions: Short-term treatment of isolated adipocytes with therapeutic doses of olanzapine or aripiprazole did not affect glucose uptake, suggesting no acute alteration in insulin activity. These data suggest that the plasma glucose increase seen in patients taking Olanzapine cannot be justified by acute alteration in insulin-signalling in adipocytes. Incubation with aripiprazole at 10 and 100 µM decreased the adipocyte glucose uptake but this might be justified by cell death, which will be explored in future experiments.

Keywords: Adipocytes, olanzapine, aripiprazole.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Cátia Marques, Assel Sarsenbayeva, Gretha Boersma, Angela Valverde, Eugénia Carvalho, Maria João Pereira, Jan Eriksson. Effects of olanzapine and aripiprazole on glucose uptake in healthy human subcutaneous adipocytes during short incubations. IBJ Plus 2018 (S1):e0008 doi: 10.24217/2531-0151.18v1s1.00008.
Funding: This work is being supported by Marie Skłodowska Curie Actions (H2020-MSCA-ITN-2016).
Competing Interests: The authors declare no conflicts of interest.

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Dasatinib reversibly disrupts endothelial vascular integrity by increasing non-muscle myosin II contractility in a ROCK-dependent manner.

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Purpose: Dasatinib is a short-acting dual ABL/SRC family tyrosine kinase inhibitor (TKI), which is frequently used to treat chronic myeloid leukemia. Although very effective, dasatinib often displays severe adverse effects, including pleural effusions and increased risk of bleeding primarily in the gastrointestinal tract. The actual causes of these side effects are currently undetermined. We hypothesize that endothelial cells (ECs) that line the inner walls of blood vessels and control the traffic to the underlying tissues, might be involved.

Experimental design: The effects of TKIs on ECs were studied by various assays, such as real-time cell impedance measurements, live-cell microscopy, wound healing, western blot and an in vivo model.

Results: Dasatinib uniquely causes a profound, dose-dependent disorganization of the EC monolayers. Dasatinib promoted the disassembly of cell-cell contacts, altered cell-matrix contacts and further altered the wound healing. A key observation is that this effect is fully reversible after drug washout. In line with these in vitro observations, intraperitoneal administration of dasatinib to mice caused significant vascular leakage in the intestine. The underlying molecular mechanism of dasatinib-induced reorganization of the actin involves ROCK activation, which increases the amount of the phosphorylation of myosin light chain and consequently activates the non-muscle myosin II.

Conclusions: Our data are consistent with a scenario in which dasatinib triggers a transient increase in vascular leakage that probably contributes to adverse effects such as bleeding diathesis and pleural effusions.

Keywords: Dasatinib, adverse effects, endothelial integrity, cytoskeleton, myosin light chain.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Anna Kreutzman, Beatriz Colom-Fernández, Ana Marcos Jiménez, Mette Ilander, Carlos Cuesta-Mateos, Yaiza Pérez-García, Cristina Delgado Arévalo, Oscar Brück, Henna Hakanen, Jani Saarela, Alvaro Ortega-Carrión, Ana de Rosendo, Alba Juanes-García, Juan Luis Steegmann, Satu Mustjoki, Miguel Vicente-Manzanares, Cecilia Muñoz-Calleja. Dasatinib reversibly disrupts endothelial vascular integrity by increasing non-muscle myosin II contractility in a ROCK-dependent manner. IBJ Plus 2018 (S1):e0009 doi: 10.24217/2531-0151.18v1s1.00009.
Funding: AK is a Sigrid Jusélius postdoctoral fellow. Grants PI12/00494P and PI15/02085 from the Fondo de Investigaciones Sanitarias to CMC supported this work. CMC was co-financed by FEDER funds. MVM is an investigator from the Ramon y Cajal Program (RYC-2010-06094) and is supported by grants SAF2014-54705-R from MINECO, Marie Curie CIG-293719 from the EU, CIVP16A1831 from Ramon Areces Foundation and the BBVA Foundation. SM is supported by the Finnish Cancer Institute, Academy of Finland, Sigrid Jusélius Foundation and Finnish Cancer Associations.
Competing Interests: JLS has received honoraria from Ariad, BMS, Novartis and Pfizer. SM has received honoraria and research grants from BMS, Novartis and Pfizer. CMC have received research grants from BMS. CMC was co-financed by FEDER funds.

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Changes in the Gene Expression Profile of Multiple Myeloma cell line in response to Immunomodulatory (IMIDs) Drugs: MYB Gen Target.

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Introduction: The immunomodulatory drug (IMiD) thalidomide and its structural analogs lenalidomide and pomalidomide are highly used to treat multiple myeloma (MM), however the molecular mechanism of IMiDs' action is not well-established. There before, we performed gene expression profiling analyses using microarray technologies to determine changes in genes involved in cellular biological pathways of MM induced by IMiDs in monotherapy.

Material and Methods: We used MM1S cell line with concentrations of lenalidomide (1µM) and pomalidomide (100nM) in six-well plates for 1 and 5 days at 37 °C. After treatment, MM1S cells were evaluated by flow cytometry using Annexin-V and propidium iodide after selective labelling of plasma cells with CD38 and CD138 antibodies. The samples were analyzed using Affimetrix Gene-chip Expression Arrays. The absolute expression values for each probe were calculated using the MAS 5.0 software. The comparative analyzes were carried out using the Dchip program, the SAM algorithm, and the Ingenuity Pathway Analysis software. Expression changes were deemed significant if they were ±2-fold. Ingenuity Pathway Analysis was used to identify the most relevant biological mechanisms, pathways, and functional categories in the data set of genes selected by statistical analysis.

Results: After 1-day, in vitro treatment with lenalidomide (1µM), with 5-7% apoptosis, significantly deregulated 10 genes whereas treatment with pomalidomide (100nM), with 8-10% apoptosis, deregulated 19 genes. Among these genes, 6 genes were exclusively deregulated by lenalidomide, 15 were deregulated by pomalidomide, and 4 genes were regulated by both lenalidomide and pomalidomide. After 5-days, in vitro treatment with lenalidomide (1µM), with 20-22% apoptosis, significantly deregulated 39 genes whereas treatment with pomalidomide (100nM), with 25-27% apoptosis, deregulated 359 genes. Among these genes, 8 genes were exclusively deregulated by lenalidomide, 328 were deregulated by pomalidomide, and 31 genes were regulated by both lenalidomide and pomalidomide. These dysregulated genes are involved in the most relevant biological pathways for MM.

Conclusion: We observed changes in the gene expression profile of MM cells after treatment with lenalidomide and pomalidomide, resulting pomalidomide with greater anti-MM effect. The under-expression of the MYB oncogene — regulator of transcription with an essential role in the control of the proliferation and differentiation of hematopoietic progenitor cells and their tumorigenesis— was commonly deregulated in both IMiDs lenalidomide and pomalidomide in monotherapy. Future studies may consider MYB as a new pharmacogenetic therapeutic target in MM.

Keywords: Multiple Myeloma, immunomodulatory drugs, Gene Expression.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Fernández Lázaro Diego, Fernández Lázaro César Ignacio, San-Segundo Laura, Ocio San Miguel Enrique. Changes in the Gene Expression Profile of Multiple Myeloma cell line in response to Immunomodulatory (IMIDs) Drugs: MYB Gen Target. IBJ Plus 2018 (S1):e0010 doi: 10.24217/2531-0151.18v1s1.00010.
Funding: The present work was funded by a grant from the Spanish Ministry of Science and Technology (FIS PI 11/01465); the Association Española Contra el Cancer (AECC) grant (GCB120981SAN); and Cooperative Research Thematic Networks (RTICC) grant numbers RD06/0020/0006, RD12/0036/0058 and RD12/0036/0003. EDR has also been awarded with an AECC grant.
Competing Interests: The authors declare no conflicts of interest.

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Anticholinergics otilonium and pinaverium trigger mitochondrial-mediated apoptosis in rat cortical neurons.

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Introduction: In the frame of a Repositioning Drug Programme with cholinergic medicines in current medical use, searching for neuroprotection, we found that anticholinergics otilonium (OTI) and pinaverium (PIN) had the opposite properties that is, neurotoxic effects. Here we have explored the mechanism underlying such neurotoxicity.

Material and methods: All experiments were carried out according with European and Spanish Directive for laboratory animal experimentation. Primary cultures of rat brain embryo cortical neurons were used. Cell viability was estimated with MTT and apoptosis with flow cytometry. Cytosolic Ca2+ was monitored with Fluo-4 AM.

Results: Fig.1 Shows microphotographs of cortical neurons and neurons incubated with otilonium and pinaverium at different times; note the cell damage elicited by both compounds.

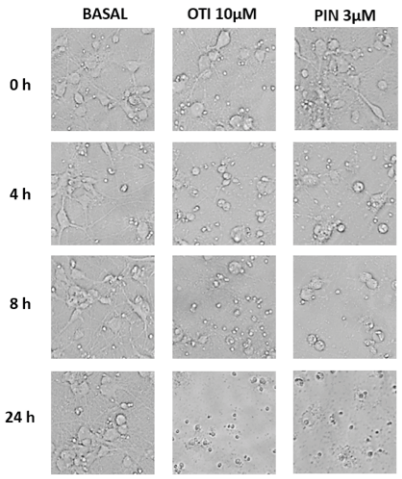

Fig. 1 Microphotographs at 20X of cultured rat embryonic cortical cells of 8 DIV, in the first column cells under basal conditions, in the second and third with cells exposed to OTI 10µM and PIN 3µM at different times 0,4,8 and 24 hours.

Cell damage was not mimicked by other cholinergic drugs and was unaffected by catalase, glutathione, N-acetyl-cysteine or melatonin. Cell death was elicited by apoptosis and necrosis. The apoptotic cell death elicited by OTI and PIN was prevent by cyclosporine A (CSA) a blocker of the mitochondrial transition pore and by Ac-LEHD-CHO an inhibitor of caspase-3 and caspase-9.

Conclusion: Data are compatible with the idea that OTI and PIN caused neuronal cell death by activating the intrinsic apoptotic pathway linked to mitochondria. These compounds may be used as new chemical tools to elicit neuronal apoptosis.

Keywords: otilonium, pinaverium, cortical neurons, neurotoxicity, apoptosis.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: F. García-Alvarado, A.G. García. Anticholinergics otilonium and pinaverium trigger mitochondrial-mediated apoptosis in rat cortical neurons. IBJ Plus 2018 (S1):e0011 doi: 10.24217/2531-0151.18v1s1.00011.
Funding: The author declared that no grants were involved in supporting this work.
Competing Interests: The authors declare no conflicts of interest.

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Studies on the anti-tumoral activity of 3-substituted indoles and azaindoles in B cell malignancies

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Indole-3-carbinol (3-hydroxymethylindole; I3C) is a natural product found in broadly consumed plants of the Brassica genus, such as broccoli, cabbage, and cauliflower, which exhibits anti-tumor effects through poorly defined mechanisms. I3C can be orally administered and clinical trials have demonstrated that I3C is safe in humans. We have described that I3C efficiently induces apoptosis in cell lines derived from EBV-positive Burkitt's lymphomas (EBV+BL) (Perez-Chacon et al, 2014 Pharmacol. Res. 89:46) and in leukemic cells from chronic lymphocytic leukemia (CLL) patients. Interestingly, I3C was able to induce cell death of CLL cells and to synergize with fludarabine even in cells from patients with refractory CLL (Perez-Chacon et al, 2016. Clin. Cancer Res 22:134).

We have studied whether other 3-substituted indoles also have a deleterious effect on the viability of the EBV+ BL cell lines Raji and BL60.2, and of CLL cells. Our results show that 3-substituted indoles with either carboxylic, methylcarboxylic, aminocarboxylic, cyanomethyl, carboxaldehide, dimethylaminomethyl, methoxymethyl or carboxamide groups failed to have any effect on the viability of the EBV+BL and CLL cells at concentrations up to 200 µM. Interestingly, not even 3-hydroxyethyl-indole and indole-2-methanol showed any cytotoxic effect, thus underlying the key role of the hydroxylmethyl group at 3 position for the anti-tumoral activity of I3C. It is noteworthy that indole-3-ol was cytotoxic in some of the tested cell lines. However, while I3C cytotoxicity was prevented by the caspases inhibitor zVAD-fmk, thus indicating that I3C induces apoptosis, zVAD-fmk failed to prevent indole-3-ol-induced cell death, thus suggesting that I3C and indol-3-ol kill by different mechanisms.

In addition, we have also demonstrated that 7-azaindole and 3-hydroxymethyl-7-azaindole lack of any deleterious effect on the viability of B cell malignancies under study. This result indicates that the replacement of C for N at 7-position of the indole ring abolishes its anti-tumor activity.

Finally, we have also observed that C6-methylated-I3C was more potent than I3C in inducing cell death in both EBV+ BL cell lines and in CLL cells, thus suggesting that substitution(s) at the benzene-fused ring (positions 4, 5, and/or 6) might enhance the anti-tumor activity of I3C and open the possibility to develop new I3C derivatives with improved anti-tumor activity.

Keywords: indoles, azaindoles, anti-tumoral.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Gema Pérez-Chacón, Andrea Unión, Kaline Arnauts, Andrea de Andrés, Cristóbal de los Rios, Juan M. Zapata. Studies on the anti-tumoral activity of 3-substituted indoles and azaindoles in B cell malignancies. IBJ Plus 2018 (S1):e0012 doi: 10.24217/2531-0151.18v1s1.00012.
Funding: The author declared that no grants were involved in supporting this work.
Competing Interests: The authors declare no conflicts of interest.

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Mitochondria function and morphology alterations precede neurosecretion impairment in chromaffin cells of the SOD1^{G93A} mouse model of amyotrophic lateral sclerosis.

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Introduction: Amyotrophic lateral sclerosis (ALS) is characterized with a selective loss of motor neurons that cause paralysis and respiratory failure. Hyperexcitability and Ca2+-dependent glutamate excitotoxicity has been hypothesized to be involved in ALS pathogenesis. Exploring the chromaffin cell (CC) of SOD1^{G93A} mouse model of familiar ALS, we found that the fusion pore kinetics of exocytosis is slowed but with higher catecholamine quantal size when the disease is already established (Calvo-Gallardo et al., Am J Physiol Cell Physiol 2015;308:C1-C19). To go further in the study of these neurosecretion alterations, we investigate the exocytosis before the disease onset (30 days postnatal), and we focus in the study of mitochondrial ultrastructure and function as a crucial organelle involved in the process.

Material and Methods: Mitochondrial ultrastructure was explored by transmission electron microscopy (TEM) and analyzed with ImageJ software. Luminometer was used to measure ATP levels in CC cultures with the commercial CellTiter-Glo® kit. Reactive oxygen species (ROS) production was monitored 30 minutes with the fluorescent dye H2DCFDA. Fusion pore kinetic was studied by amperometry, eliciting exocytosis by 1 minute acetylcholine stimulus.

Results: TEM showed that mitochondria from SOD1^{G93A} CCs have the following alterations with respect to wildtype CCs: i) more number and small sized; ii) increased mitochondrial intermembrane space; iii) lower number and swollen cristae. These ultrastructural changes suggesting mitochondrial fission and ultrastructure damage were accompanied by lower ATP production and a higher rate of ROS generation. However, we fail in observe such significant differences in the fusion pore kinetics.

Conclusion: The described mitochondrial alterations shown an interesting non-motor neuron degeneration in this ALS model at presymptomatic stages. However, the kinetic of the exocytotic fusion pore have not been affected, contrary to the slowed secretion observed once the paralysis is already established. These results evidence that this mitochondrial alterations precede the functional changes linked to neurotransmitter release. In spite of having lower clinical relevance than in later stages, it could generate some clues about the initiation and progression of the disease. Our data consolidate the mitochondria as a potential target and the sympathetic-adrenal system affectation as an interesting new approach for ALS diagnosis.

Keywords: ALS, SOD1^{G93A}, chromaffin cell, mitochondrial ultrastructure, fusion pore, exocytosis.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Iago Méndez-lópez, Carmen Martínez-Ramírez, Antonio G. García, Fernando Padín Nogueira. Mitochondria function and morphology alterations precede neurosecretion impairment in chromaffin cells of the SOD1^{G93A} mouse model of amyotrophic lateral sclerosis. IBJ Plus 2018 (S1):e0013 doi: 10.24217/2531-0151.18v1s1.00013.
Funding: SAF 2013-44108-P grant to AGG; FPI BES-2014-069005 grant to I.M-L., MINECO, Spain.
Competing Interests: The authors declare no conflicts of interest.

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Prediction Models for Voriconazole Pharmacokinetics Based on Pharmacogenetics: An Exploratory Study Nested to a Bioequivalence Trial of Two Voriconazole Formulations of 200 mg.

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Introduction: Individualization of the therapeutic strategy for the oral antifungal voriconazole (VCZ) is highly important for treatment optimization. This is due to its narrow therapeutic range and its wide interpatient variability in serum concentrations, which are directly related to both VCZ efficacy and the occurrence of adverse drug reactions (ADRs). Part of this variability is explained by genetic variation in genes coding enzymes involved in the metabolic pathway of VCZ. To date, the European Clinical Pharmacogenetics Consortium and the US Food and Drug Administration include CYP2C19 as the only major PGx biomarker in their dosing guidelines and protocols; however, the effect of other genes might be important for VCZ dosing prediction. We developed an exploratory pharmacogenetics (PGx) study nested to a bioequivalence trial of two VCZ formulations of 200 mg to identify new biomarkers related to VCZ pharmacokinetics (PK) that could be included in clinical practice.

Materials and Methods: Forty-six healthy volunteers were included. Cmax, Tmax and AUC0-∞ were obtained from VCZ PK analysis and Cmax and AUC0-∞ were adjusted to dose/weight administered. Molecular analysis was performed for the selected SNPs among the CYP2C19, ABCB1, CYP3A4, CYP3A5, CYP2C9, POR, NR1I2, FMO3 genes using the custom SNP-array genotyping platform PharmArray®. After molecular and PK analysis, we first designed a Clinical Practice VCZ AUC0-∞ prediction model based on CYP2C19 to be used as a reference model for comparison. We then performed a statistical analysis in three steps to design new prediction models by the incorporation of additional biomarkers.

Results: Due to the strong influence of CYP2C19 on VCZ AUC0∞ heterogeneity we propose that its major effect might be masking the influence of other metabolic enzymes and transporters in VCZ metabolism and that this influence could be unmasked by a stratification of the study population into CYP2C19 phenotypic subgroups. We here propose three different predictive models that improve the clinical practice model predictive rates by the incorporation of complementary biomarkers to CYP2C19.

Conclusion: Due to the small sample size, further research is needed for cross-validation of the proposed models. However, the CYP2C9 gene was proposed as a consistent biomarker for VCZ AUC prediction in addition to the well-established CYP2C19 gene.

Keywords: Pharmacogenetics, Pharmacokinetics, Voriconazole, AUC-prediction models, CYP2C19, CYP2C9.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Irene Dapía, Irene García, Jose Carlos Martinez, Pedro Arias, Pedro Guerra, Lucía Díaz, Alberto García, Jair Tenorio, Elena Ramírez, Gema Gordo, Jesús Frías, Pablo Lapunzina, Antonio J Carcas, Alberto M Borobia. Prediction Models for Voriconazole Pharmacokinetics Based on Pharmacogenetics: An Exploratory Study Nested to a Bioequivalence Trial of Two Voriconazole Formulations of 200 mg. IBJ Plus 2018 (S1):e0014 doi: 10.24217/2531-0151.18v1s1.00014.
Funding: The author declared that no grants were involved in supporting this work.
Competing Interests: Alberto Borobia and Jesús Frías are the executive deputy editors of Ibjournals. Antonio J. Carcas is the editor in-chief of Ibjournals.

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Agranulocytosis after metamizole use: assessment of drug causality with algorithm versus Lymphocyte Transformation Test.

García García I[1*], Díaz García L[1], Rodríguez Mariblanca A[1] , Hernández Zabala R[1] , Queiruga Parada J[1], Ramírez García E[1]

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INTRODUCTION: Metamizole is a non-narcotic analgesic/antipyretic widely used in some countries but prohibited in others due to suspected risk of agranulocytosis. The mechanism of this side effect has not been completely clarified, but it is generally accepted that it is immune-mediated. The lymphocyte transformation test (LTT) has been proposed as a diagnostic method to determine if a drug is the causal agent of an immune-mediated drug reaction. We present a case of drug-induced agranulocytosis in which Spanish Pharmacovigilance System probability algorithm of drug causality was applied and in vitro LTT was performed.

CLINICAL CASE: A 30-year-old woman was admitted to our hospital with fever and severe weakness. Eighteen days before she had received knee surgery and was prescribed metamizole (MTZ) along with dexketoprofeno (DK) alterned every four hours. She was also on esomeprazole (ESO) and bemiparine treatment since the surgical procedure. Two days after the onset of treatment she presented fever up to 37.8ºC which was followed by increasing weakness in the next days. On day 6 after surgery, she was diagnosed tonsillitis and started therapy with amoxicillin-clavulanic. Body temperature values kept rising and weakness remained after amoxicillin-clavulanic therapy had been completed. Not showing any clinical improvement, she decided to come to our Hospital. Laboratory tests revealed severe neutropenia of 0.03×10e3/µL. Blood smear showed no alterations of the red cells, scarce neutrophils and no evidence of dysplasia. Haematological, infectious diseases and other alternative causes were excluded leading to drug suspicion. Possible causal drugs were discontinued and antibiotic prophylaxis in addition to filgastrim were started. The algorithm of Spanish pharmacovigilance system was conducted to MTZ, DK, ESO and bemiparine.

RESULTS: Applying the algorithm, MTZ, DK and ESO obtained 4 points, which implies “possible causality”. Bemiparine pointed a “conditional causality” (2 points), and was consequently excluded. After drug withdrawal of (MTZ, DK, ESO) and filgastrim therapy, she showed clinical and analytical improvement and was discharged from Hospital. We performed LTT to the remaining suspected drugs. The test had a positive result for MTZ, and was negative for DK and ESO. A recommendation was made to the patient and stated on clinical records not to rechallenge to metamizole or other pyrazolones again.

CONCLUSIONS: This is to our concern the first case of agranulocytosis by metamizole in which LTT has been performed. The positive result elucidated metamizole as causal agent and supports the hypothesis of immunological mechanism for this adverse reaction.

Keywords: Agranulocytosis, metamizole, Lymphocyte Transformation Test, Spanish Pharmacovigilance System probability algorithm, drug causality.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: García García I, Díaz García L, Rodríguez Mariblanca A, Hernández Zabala R, Queiruga Parada J, Ramírez García E. Agranulocytosis after metamizole use: assessment of drug causality with algorithm versus Lymphocyte Transformation Test. IBJ Plus 2018 (S1):e0015 doi: 10.24217/2531-0151.18v1s1.00015.
Funding: No funding was received for this work.
Competing Interests: The authors declare no conflicts of interest.

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Drug induced cholestasis: assessment of drug causality with algorithm versus Lymphocyte Transformation Test.

García García I[1*], Rodríguez Mariblanca A[1], Díaz García L[1], Martínez De Soto L[1], Hernández Zabala R[1], Ramírez García E[1]

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INTRODUCTION: Cholestatic drug-induced liver injury (DILI) can be a diagnostic challenge due to the varying clinical and laboratory features, in addition to the extensive differential diagnosis. It can be divided into two categories regarding the underlying pathogenic mechanism: direct hepatic injury caused by the drug itself or idiosyncratic. The majority of cases are related to idiosyncratic reactions that develop independently of drug dose, route, or duration of administration. Its pathogenesis is not yet clear, but among other hypothesis it includes immunological mechanisms. We present a case of drug induced cholestasis in a patient with rechallenge to suspected drugs in which RUCAM (Roussel Uclaf Causality Assessment Method) algorithm was applied and in vitro LTT (Lymphocyte Transformation Test) was performed.

CLINICAL CASE: A 88-year-old woman was admitted to our hospital presenting right hypochondrium pain. She was diagnosed right kidney ischemia which was conservatively treated. Relevant history included cephalosporin allergy, hypertension, hyperlipidaemia, type 2 diabetes diet controlled, cholelithiasis, arthrosis and sixth nerve ischemic neuropathy fully recovered. Her medication included hydrochlorothiazide, atenolol and salicylic acid. Six years before she suffered atlas and wrist fracture and was prescribed metamizole (MTZ), acetaminophen (ACP) and pantroprazole (PAN). Eighteen days later she was diagnosed dissociated cholestasis, which was related to MTZ, ACP and PAN treatment. A recommendation was made not to rechallenge to this drugs. The patient claimed not had been re-exposed.

During the current hospitalization she received atenolol, salicylic acyd, MTZ, ACP, omeprazole (OMZ), metoclopramide (MTC), acenocumarol, amoxicillin clavulanic, enalapril, enoxaparin, furosemide and ranitidine. Thirteen days after the onset of treatment alkaline phosphatase, alanil transferase and aspartate aminotransferase began to rise.

RESULTS: Alternative causes for dissociated cholestasis were excluded. Taking into account rechallenge, MTZ, ACP and OMZ were classified as probable (7 points) by RUCAM algorithm. MTC obtained 0 points and was excluded. The rest of medication was excluded considering dates of administration and rise of liver enzymes. After drug withdrawal of MTZ, ACP and OMZ liver enzymes levels began to lower. LTT was performed to MTZ, ACP and OMZ with a positive result for MTZ, and with lower values to OMZ. A recommendation was made to the patient and stated on clinical records not to rechallenge to pyrazolones or proton pump inhibitors.

CONCLUSIONS: LTT is of great help to elucidate the culprit agent when several drugs are suspected, allowing us to make more precise recommendations.

Keywords: Drug induced cholestasis, Lymphocyte Transformation Test, metamizole, omeprazole, Roussel Uclaf Causality Assessment Method, RUCAM.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: García García I, Rodríguez Mariblanca A, Díaz García L, Martínez De Soto L, Hernández Zabala R, Ramírez García E. Drug induced cholestasis: assessment of drug causality with algorithm versus Lymphocyte Transformation Test. IBJ Plus 2018 (S1):e0016 doi: 10.24217/2531-0151.18v1s1.00016.
Funding: No funding was received for this work.
Competing Interests: The authors declare no conflicts of interest.

IBJ Plus
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Drug induced hypersensitivity by simvastatin: peripheral polyneuropathy, eosinophilia, fever and skin eruption.

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INTRODUCTION: Statins adverse effects are infrequent, and although myopathy is well known by clinicians, few attention is paid to its other adverse reactions, which makes it difficult to establish drug causality. Through our Prospective Pharmacovigilance Program from Laboratory Signals at Hospital, we detect abnormal laboratory values which indicate severe reactions that are frequently related to the use of drugs. We are reporting a case of induced hypersensitivity caused by simvastatin (SMV).

CLINICAL CASE: Laboratory Signals are reviewed each week; among others, peripheral eosinophilia >700x10e3/µL is detected and prospectively evaluated. We identified eosinophilia in a 58 year old male patient. His medical history was remarkable for hypertension, hyperlipidaemia under SMV 10 mg once a day treatment and type 2 diabetes mellitus diet controlled. In 2015 he received surgery and radiotherapy for a non-functional pituitary adenoma. Symptoms started in 2017 with two episodes of urticaria followed by dissociated cholestasis, fever up to 39ºC, weakness, loss of weight and numbness in his right hand with subsequent pain, cramps, paresthesia and hypoesthesia of his feet. Infectious or inflammatory diseases, malignant diseases and other alternative diagnosis were excluded. Electromyography showed predominantly axonal sensory-motor peripheral neuropathy. When a definitive diagnosis had not yet been established he started treatment with corticosteroids, showing 24 hours later, improvement in constitutional symptoms and normalization of body temperature. Taking all this into account SMV was established as suspected cause. We made contact with Internal Medicine Service and SMV therapy was discontinued maintaining corticosteroids treatment.

RESULTS: On review three weeks after the withdrawal of SMV, he presented resolution of weakness and fever, along with weight gain. Two months later he showed improvement of peripheral neuropathy symptoms, muscle cramps had reduced and he had no pain, although numbness and sensation of pins and needles in his feet persisted.

Eosinophilia, urticaria, fever and neuropathy are described as side effects of SMV in the drug label and while a literature review revealed several cases reported, no patient has been described as having all of them at the same time. The clinical and laboratory features suggest a Drug reaction with eosinophilia and systemic symptoms (DRESS), with an atypical presentation regarding that nervous system damage is an unusual manifestation of DRESS. A lymphocyte transformation test will be performed to confirm SMV as causal agent.

CONCLUSIONS: Drug adverse reactions comprise a wide range of entities and we should always take account of medication once alternative diagnosis are excluded.

Keywords: Drug induced hypersensitivity, Drug reaction with eosinophilia and systemic symptoms , simvastatin, lymphocyte transformation test.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: García García I, Herrero Gil C, Rodríguez Mariblanca A, Monserrat Villatoro J, Rodríguez Dávila MA, Ramírez García E. Drug induced hypersensitivity by simvastatin: peripheral polyneuropathy, eosinophilia, fever and skin eruption. IBJ Plus 2018 (S1):e0017 doi: 10.24217/2531-0151.18v1s1.00017.
Funding: No funding was received for this work.
Competing Interests: The authors declare no conflicts of interest.

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A Cell-Permeable Peptide Corresponding to the Calmodulin-Binding Domain of Grb7 Inhibits Proliferation and Migration of A431 Cells.

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Introduction: Grb7 (growth factor receptor bound 7) is a mammalian adaptor protein that transduces signals from tyrosine kinase receptors. This protein is overexpressed, together with ErbB2, in different human carcinomas. Grb7 is implicated in cell migration, contributing to the invasiveness and metastatic capacity of tumour cells. Also, Grb7 controls cell proliferation and tumour-associated angiogenesis. Our group demonstrated that calmodulin (CaM) binds to the proximal region of the pleckstrin homology (PH) domain of human Grb7 in a Ca2+-dependent manner, comprising the sequence 243RKLWKRFFCFLRRS256 [1, 2]. A deletion mutant of Grb7, lacking its CaM-binding domain (CaM-BD), impairs the adhesion and migratory capacity of transfected cells [3]. Moreover, the expression of this deletion mutant in rat C6 glioblastoma cells strongly inhibited its proliferation, the growth of brain tumours derived from stereotaxically implanted tumour cells, and tumour-associated angiogenesis [4]. Therefore, the CaM-BD of Grb7 could be a potential target to inhibit tumour cells invasiveness and metastasis development [5].

Materials and Methods: Human carcinoma epidermoid A431 cells were used in all the experiments. Custommade synthesis of peptides (>99% purity) with the amino acid sequence RKLWKRFFCFLRRS, and a derivative with a myristoyl group at its N-terminus (Myr-RKLWKRFFCFLRRS) to facilitate cell internalization, were manufactured by Wuxi Nordisk Biotech Ltd. (China). Cell migration was measured by artificial wound healingassays and time-lapse microscopy, and cell proliferation was measured by Crystal Violet staining. The assays were done in the absence and presence of 10 nM EGF, and 20-50 µg/ml of the different peptides, or 10-50 µM W-7 and W-12 (CaM antagonists).

Results: The addition of EGF first induced a 12-18 h lag phase in which the migration of A431 cells was arrested, and a significant retraction of the border of the artificial wound was observed. Thereafter, the migration rate of the cells increased several folds as compared to the rate of migration of cells in the absence of EGF. The RKLWKRFFCFLRRS peptide inhibited the migration of A431 cells both in the absence and presence of EGF.

This inhibitory effect was more evident using the Myr-RKLWKRFFCFLRRS peptide. The high affinity CaM antagonist W-7, and in lesser extent its low affinity analogue W-12, incremented the lag phase in which the migration of the cells was arrested upon addition of EGF and decreased its subsequent migration rate. As previously demonstrated [6], EGF induced a significant inhibition of cell proliferation in A431 cells, and both the RKLWKRFFCFLRRS and Myr-RKLWKRFFCFLRRS peptides further inhibited the proliferative response.

Conclusion: The mechanism of action of the peptides under study could be due to the capacity to sequester CaM and/or to the prevention of Grb7 dimerization, possibilities that are under study. These peptides, upon selected delivery to tumour cells, could have potential therapeutic effects against cancer and metastasis development.

Keywords: Calmodulin, cell migration, cell permeable peptides, cell proliferation, Grb7.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Juan Alcalde, María González-Muñoz, Iván Madroñal, Antonio Villalobo. A Cell-Permeable Peptide Corresponding to the Calmodulin-Binding Domain of Grb7 Inhibits Proliferation and Migration of A431 Cells. IBJ Plus 2018 (S1):e0018 doi: 10.24217/2531-0151.18v1s1.00018.
Funding: No funding was received for this work.
Competing Interests: The authors declare no conflicts of interest.

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Pharmacogenetic testing in a suspected drug-induced parkinsonism related with antipsychotic treatment: a case report.

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Introduction: The establishment of a pharmacogenetics unit with the intention of facilitating the integration of pharmacogenetic testing into clinical practice is important to personalise treatments and clinical recommendations and to carry out pre-emptive genotyping in risk populations. In our Clinical Pharmacogenetics Unit at La Paz University Hospital we classified pharmacogenetics tests into three groups. One of these groups are drugs with well-known evidence but without a well-defined protocol. In these cases, we evaluate a specific therapeutic problem and determine whether the pharmacogenetics test is recommended. We presented the case of a patient with suspected drug-induced parkinsonism (DIP) related with antipsychotic treatment.

Material and Methods: DIP is the most common movement disorder induced by antipsychotics and reduces quality of life favouring non-compliance of treatments in patients with psychotic disorders with risk of morbidity and mortality. We evaluated a patient being treated with several drugs (sertraline, paroxetine, risperidone, quetiapine, zuclopenthixol, haloperidol and aripiprazole) who had developed parkinsonism one year earlier. Information about the pharmacogenetic testing variants and their impact on drug response was gathered mostly from the variant and clinical annotations in PharmGKB. We analysed single nucleotide polymorphism (SNP) associated with the metabolism and response of these drugs. We selected CYP2D6 and CYP2C19 as the most important biomarkers involved (all drugs have metabolism by CYP2D6, except sertraline which has metabolism mainly by CYP2C19). Pharmacogenetic test was performed and 21 SNP were analysed with OpenArray technology (PharmArray®).

Results: Our patient presented the genotype CYP2C19*1/*17 (Rapid Metabolizer) and CYP2D6*4/*41 (Intermediate Metabolizer). So, sertraline metabolism is increased and the metabolism of the other antipsychotic drugs are reduced and the higher plasma concentrations may increase the probability of side effects, among them DIP.

Conclusions: These results could explain the higher risk of DIP observed in our patient due to the pharmacokinetic interaction at the level of CYP450. We are aware of the limitations of the CYP2D6 technique analysis but in the case of our patient it is important to consider these results in the evaluation of clinical symptoms due to the severity of the side effects of this type in therapeutic efficacy and the optimal treatment of antipsychotic disorders. In addition, it is important to point out the implementation of clinical pharmacogenetics programs in the clinical practice to carry out individualization of clinical recommendations and pre-emptive genotyping in risk populations.

Keywords: Drug-induced parkinsonism, antipsychotics, side effects, pharmacogenetics test, single nucleotide polymorphism, CYP2D6, CYP2C19, clinical pharmacogenetics programs.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Díaz García L, Dapia I, Garcia Garcia I, Arias P, Borobia Alberto M, Carcas Antonio J. Pharmacogenetic testing in a suspected drug-induced parkinsonism related with antipsychotic treatment: a case report. IBJ Plus 2018 (S1):e0019 doi: 10.24217/2531-0151.18v1s1.00019.
Funding: No funding was received for this work.
Competing Interests: Alberto Borobia is the executive deputy editors of Ibjournals. Antonio J. Carcas is the editor in-chief of Ibjournals.

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KV1.3 channel inhibition by indolic compounds in chronic lymphocytic leukemia cells.

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Introduction: Chronic lymphocytic leukemia (CLL) is the most common leukemia in western countries and it is based on B cell clonal expansion. This disease has no cure and the appearance of pharmacological resistance is very common. We have previously identified indole-3-carbinol (I3C) and its main metabolite, 3,3'-diindolylmethane (DIM), as active compounds with pharmacological activity against CLL. KV1.3 potassium channels are involved in B and T cells function controlling plasmatic membrane potential, Ca2+ entry and cellular proliferation. Therefore, these channels could be a new therapeutic target against CLL. Here, we have analysed if these indolic compounds can act, in part, by modulating the KV1.3 function.

Material and Methods: we assessed the activity and effect of the indolic compounds on KV1.3 channels in CLL cells that are either sensitive or resistant to I3C cytotoxicity. Currents were registered by whole-cell patch-clamp. Statistical significance was determined by t-Student test or by nonparametric Mann-Whitney test.

Results: The KV1.3 current magnitude on CLL cells correlated with their sensitivity to the cytotoxic effect of I3C; I3C resistant CLL (IR-CLL) cells exhibiting ≈2.5-fold higher KV1.3 current amplitude than sensitive (IS-CLL) cells. Both I3C and DIM inhibited KV1.3 current in CLL cells, DIM being ≈4-fold more potent than the parent compound. However, a non-cytotoxic compound, indole-3-carboxylic acid (I3CA), did not inhibit the KV1.3 current.

Conclusions: IR-CLL cells exhibit greater KV1.3 current magnitude than IS-CLL cells, which can be due to the existence of different canalosomes in these cells. The KV1.3 inhibitory effect of the indolic compounds tested, correlates with their cytotoxic effect on CLL cells. Our results suggest that KV1.3 channel could be involved in CLL cells pharmacological resistance mechanisms and its inhibition could be part of the cytotoxic effect of I3C and DIM, which open new venues to the treatment of this disease.

Keywords: Chronic lymphocytic leukemia (CLL), KV1.3, indole-3-carbinol (I3C), 3,3'-diindolylmethane (DIM).
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Baena-Nuevo M, Vera-Zambrano A, Martinez-Laperche C, Muñoz-Calleja C, Buño I, Zapata JM, Perez-Chacon G, Gonzalez T. KV1.3 channel inhibition by indolic compounds in chronic lymphocytic leukemia cells. IBJ Plus 2018 (S1):e0020 doi: 10.24217/2531-0151.18v1s1.00020.
Funding: This study was supported by MINECO (SAF2013-45800-R, SAF2016-75021-R, RD12/0042/0019, CB/11/00222) and ISCIII (PI12/01135 and PI16/00895).
Competing Interests: The authors declare no conflicts of interest.

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Sirolimus monitoring in airway compromise vascular tumor: a case report.

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Introduction: Neonates with vascular anomalies causing airway compromise require early initiation of therapy. Treatment options are limited. In the last years, sirolimus has shown to be an effective treatment. Correct dosing in these patients, who have a reduced hepatic metabolism of sirolimus, is difficult. Standard dosing can cause supratherapeutic levels in patients under 7 months of age.

Methods: Female preterm, born at 34 weeks in a gemelar delivery, with a policystic multicompartimental lymphatic malformation with cervical airway compromise, required intubation. Sirolimus dosing in neonates is not clear, but therapy with 0.8mg/m2 every 12 hours was started the day after the born. After five days the through concentration was supratherapeutic (>90ng/ml), being the goal trough 5-15ng/ml. The dose was decreased to 0.1mg/kg once daily, but supratherapeutic levels persisted for three more days. Sirolimus was discontinued to avoid toxicity. Moreover, the pharmacogenetics study was carried out in order to pick up some mutation which could explain the supratherapeutic levels.

Results: Sirolimus levels started to go down the fourth day after the suspension. Ten days after the last dose, the through had decreased until 9.21ng/ml. Estimated sirolimus half-life, calculated with the last concentration points after discontinuation, was 55 hours, which is similar to the half-life observed previously. After that, sirolimus 0,05mg/kg/day was restarted, remaining in the within the therapeutic range. The genotype determination of CYP3A4 (rs55785340, rs4646438, rs2740574, rs35599367, rs28371759, rs138105638, rs67666821), CYP3A5 (rs776746, rs55965422, rs10264272, rs41303343, rs41279854), CYP3A7 (rs45465393, rs11568824, rs45494802, rs45575938, rs45467892, rs11568825, rs11568826, rs45446698, rs55798860, rs28451617, rs2257401, rs779179631) and CYP2C8 (rs11572080, rs10509681, rs1058930, rs11572103) was performed. It did not show any mutation. Sirolimus is a substrate of CYP3A4, whose expression is very low in neonates, instead they express CYP3A7. Maturation of hepatic enzyme composition occurs over the first 6 months of life, with rapid changes in the first 3-6 weeks. Some studies have modelled the pharmacokinetic of sirolimus in infants and children, demonstrating significant variability attributed to body size and organ maturation processes, including bioavailability and hepatic metabolism.

Conclusion: The cause of the supratherapeutic sirolimus levels was probably the immaturity, as the genetic test showed a normal metabolizer genotype. Important uncertainty exists about the appropriate dosage and schedule in the first postnatal days/weeks. More studies are needed to clarify these questions and others like the efficacy and safety.

Keywords: Vascular anomalies, neonates, sirolimus, supratherapeutic levels, pharmacogenetics.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Rodríguez Mariblanca A, Díaz García L, García García I, Borobia A, Carcas Sansuán AJ. Sirolimus monitoring in airway compromise vascular tumor: a case report. IBJ Plus 2018 (S1):e0021 doi: 10.24217/2531-0151.18v1s1.00021.
Funding: No funding was received for this work.
Competing Interests: Alberto Borobia is the executive deputy editors of Ibjournals. Antonio J. Carcas is the editor in-chief of Ibjournals.

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Cyclosporine-induced lymphoproliferative disorder in toxic epidermal necrolysis (net): a case report.

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Introduction: Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are a delayed-type hypersensitivity reaction to drugs. They are medical emergencies and an early recognition and appropriate management is decisive. The mortality rates have decreased, but may be as high as 20-30%. It is very important the immediate withdrawal of causative drugs and prompt referral to a burn unit for specific supportive treatment. Several immunomodulatory agents are used in the treatment of TEN, but evidence of their efficacy is limited, only retrospective observational open-label single-centre studies are available. Glucocorticoids and intravenous immunoglobulin were not found to be superior to supportive care. Cyclosporine is associated with a mortality reduction in a systematic review with a meta-analysis of TEN.

Description of the case: Female 58-year-old patient was moved to La Paz Burn Care Unit from Caceres, suspecting a NET. Complementary studies didn't show an infectious causative of TEN, a palmeatte hair lotion was considered the culprit drug. Cyclosporine therapy 175mg every 12 hours was started the same day she was admitted to our centre. It was keep out for 9 days. Supratherapeutic cyclosporine levels were obtained after nine days (869,8ng/ml), being withdrawn. It was restarted 50mg every 12 hours 3 days later, remaining in goal trough until re-epithelialization. The genotype determination of CYP3A4 and CYP3A5 was performed in order to detect a possible mutation related with the metabolism of cyclosporine. It did not show any mutation. After nine months since the patient was discharged, she was diagnosed with lymphoma. In the tests done during the hospitalization, we found a positive PCR for Epstein-Barr virus (EBV) and several adenopathies in the body computed tomography.

Results: Cyclosporine seems to be a promising therapy in TEN, but it has been well documented in the literature an increased incidence of neoplastic disorders, specifically lymphoproliferative disorders. We have not found any other case of cyclosporine induced-lymphoma in TEN because of the low TEN incidence, but cyclosporine has been widely used as immunosuppressive agent for transplant recipients. Most lymphomas associated with cyclosporine have occurred within 1 year of initiating the drug, being lymph nodes one of the predominant sites. It is well known an association between EBV and polyclonal lymphoproliferation.

Conclusion: The CT and EBV results suggest the lymphoma is related to cyclosporine therapy. The increased incidence of lymphoproliferative disorders is related to high blood levels not to a specific cyclosporine dose. Acyclovir use and/or decrease the dose of cyclosporine may decrease the proliferation of lymphomas.

Keywords: Toxic Epidermal Necrolysis, cyclosporine, lymphoma, Epstein-Barr virus, supratherapeutic levels.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Rodríguez Mariblanca A, García García I, Martínez de Soto L, Díaz García L, Ramírez García E. Cyclosporine-induced lymphoproliferative disorder in toxic epidermal necrolysis (net): a case report. IBJ Plus 2018 (S1):e0022 doi: 10.24217/2531-0151.18v1s1.00022.
Funding: No funding was received for this work.
Competing Interests: The authors declare no conflicts of interest.

IBJ Plus
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CYP2C19 defines clopidogrel response in patients undergoing percutaneous neurointervention procedure.

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Introduction: Clopidogrel is a widely prescribed thienopyridine prodrug which inhibits platelet aggregation. It is prescribed to prevent atherothrombotic and thromboembolic events in patients who are given a stent implant in carotid, vertebral or cranial arteries. CYP2C19 is the most studied enzyme involved in clopidogrel metabolism. The most common CYP2C19 no function polymorphisms (*2 and *3) have been associated with hyporesponse to clopidogrel, showing lower levels of the active metabolite. On the contrary, the presence of the increased function allele (*17) has demonstrated enhanced platelet inhibition and clopidogrel hyperresponse.

Methods: This observational retrospective study assessed antiplatelet response and clinical events after clopidogrel treatment in patients who underwent percutaneous neurointervention, related to CYP2C19 metabolizer status (normal (NM), intermediate/poor (IM-PM) and ultra-rapid (UM); inferred from *2, *3 and *17 allele determination by real-time PCR).

Results: One hundred twenty-three patients were analysed, of which 83% had cardiovascular risk factors. The most common type of intervention was angioplasty with stent. According to the aggregation value, 58.7% of the patients were responders to clopidogrel; moreover, 4.1% required dose reduction and 12.2% change of treatment. CYP2C19 IM-PM had higher aggregation value (201.1 vs 137.6 NM, 149.4 UM, $p<0.05$) and lower response rate (37.5% vs. 69.8% NM, 61.1% UM), along with higher treatment change rate (25% vs. 5.7% NM, 10.5% UM). Moreover, 20% of the patients suffered from a subsequent clinical event. The highest ischemic events incidence occurred in NM (11.3% vs. 6.3% IM, 10.5% UM; $p=0.358$) and haemorrhagic events in UM (13.2% vs. 0% IM and 3.8% NM; $p=0.041$). No differences found regarding ischemic events' onset time, while haemorrhagic events' frequency in UM was higher with shorter onset time ($p=0.047$). Additionally, 53% of the patients were receiving concomitant treatment with proton-pump inhibitors (PPIs), which showed significantly higher aggregation value when compared to those not receiving PPI concomitant treatment (178.1 vs. 134.4; $p=0.009$).

Conclusion: CYP2C19 no function and increased function alleles defined clopidogrel response. CYP2C19 genotyping and platelet reactivity quantification help to determine whether a patient could be at risk of ischemic or haemorrhagic event. CYP2C19 UM patients have increased bleeding risk after percutaneous neurointervention. Therapeutic recommendations should include an alternative therapeutic option in IM-PM or UM patients.

Keywords: CYP2C19; phenotype, antiplatelet; clopidogrel; neurointervention, haemorrhage, ischemia.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Miriam Saiz-Rodríguez, Daniel Romero-Palacián, Carlos Villalobos-Vilda, José Luis Caniego, Carmen Belmonte, Dora Koller, Eduardo Bárcena, María Talegón, Francisco Abad-Santos. CYP2C19 defines clopidogrel response in patients undergoing percutaneous neurointervention procedure. IBJ Plus 2018 (S1):e0023 doi: 10.24217/2531-0151.18v1s1.00023.
Funding: M. Saiz-Rodriguez was co-financed by Consejería de Educación, Juventud y Deporte from Comunidad de Madrid and Fondo Social Europeo. D. Koller is co-financed by the H2020 Marie Sklodowska-Curie Innovative Training Network 721236 grant.
Competing Interests: F. Abad-Santos has been consultant or investigator in clinical trials sponsored by the following pharmaceutical companies: Abbott, Alter, Chemo, Cinfa, FAES, Farmalíder, Ferrer, GlaxoSmithKline, Galenicum, Gilead, Janssen-Cilag, Kern, Normon, Novartis, Servier, Silverpharma, Teva, and Zambon. The remaining authors declare no conflicts of interest.

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Modulation of SIRT1 by IL-1β/NFκB signaling during Acetaminophen-induced hepatotoxicity.

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Introduction: The liver is the main organ in charge of drug catabolism and also the major site susceptible to drug injury. Sirtuin 1 (SIRT1), a NAD+-dependent histone deacetylase, is a key player in liver physiology and a therapeutic target against hepatic inflammation. In this study, we evaluated the role of SIRT1 in the pro-inflammatory context and oxidative stress during acetaminophen (APAP)-mediated hepatotoxicity.

Material and Methods: SIRT1 expression was analyzed in APAP-induced liver failure in humans and mice. Hepatotoxicity was assessed in wild-type and transgenic mice overexpressing SIRT1 (SIRT1 Tg) poisoned with APAP (300 mg/kg). Raw 264.7 and peritoneal macrophages were treated with APAP and conditioned medium (CM) was added to mouse hepatocytes. siRNA was used to reduce inflammatory mediators in hepatocytes.

Results: SIRT1 protein levels decreased in human and mouse livers following APAP overdose. SIRT1-Tg mice maintained higher levels of SIRT1 upon APAP injection than wild-type mice and were protected against hepatotoxicity by modulation of antioxidant systems and restrained inflammatory responses, with decreased oxidative stress, pro-inflammatory cytokine mRNA levels, nuclear factor kappa B (NFκB) signaling, and cell death. Mouse hepatocytes stimulated with conditioned medium of APAP-treated macrophages (APAP-CM) showed decreased SIRT1 levels; an effect mimicked by interleukin 1β (IL1β), an activator of NFκB. This negative modulation was abolished by neutralizing IL1β in APAP-CM or silencing p65-NFκB in hepatocytes. APAP-CM of macrophages from SIRT1-Tg mice failed to downregulate SIRT1 protein levels in hepatocytes. In vivo administration of the NFκB inhibitor BAY 11-7082 preserved SIRT1 levels and protected from APAP-mediated hepatotoxicity.

Conclusion: SIRT1 protein levels are downregulated by IL1β/NFκB signaling in APAP hepatotoxicity, resulting in inflammation and oxidative stress. Thus, maintenance of SIRT1 during APAP overdose by inhibiting NFκB might be clinically relevant. Our work evidenced the unique role of SIRT1 in APAP hepatoprotection by targeting oxidative stress and inflammation.

Keywords: paracetamol; SIRT1; oxidative stress; inflammation; hepatotoxicity; interleukin 1β.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Patricia Rada, Virginia Pardo, Maysa A. Mobasher, Irma García-Martínez, Laura Ruiz, Águeda González-Rodríguez, Cristina Sanchez-Ramos, Jordi Muntané, Susana Alemany, María Monsalve, M. Pilar Valdecantos, Ángela M. Valverde. Modulation of SIRT1 by IL-1β/NFκB signaling during Acetaminophen-induced hepatotoxicity. IBJ Plus 2018 (S1):e0024 doi: 10.24217/2531-0151.18v1s1.00024.
Funding: This work was funded by SAF2015-65267-R (MINECO/FEDER), CIBERdem (ISCIII, Spain), INFLAMES (ISCIII PIE14/00045, co-funded by ERDF, "Investing in your future") to A.M.V; IJCI-2014-19381 (MINECO/FEDER) to P.R and A.M.V; SAF2014-52009-R (MINECO/FEDER) to S.A; SAF2015-63904-R (MINECO/FEDER) to M.M.; CP14/00181 (ISCIII/FEDER) to A. G-R; PI13/00021 (ISCIII/FEDER) to J.M. CIBERehd (ISCIII, Spain) to A. G-R and J.M; Grant 37/371 from Al Jouf University (to M.A.M). We also acknowledge H2020 Marie Sklodowska-Curie ITN-TREATMENT (Grant Agreement number 721236) (European Commission).
Competing Interests: The authors declare no conflicts of interest.

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Neuroprotective effects of new compounds directed to PP2A, a promising therapeutic target for Alzheimer's disease.

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Introduction: Alzheimer's disease (AD) is a progressive neurological disease that causes a progressive memory loss. Main histopathological hallmarks of AD are senile plaques, and neurofibrillary tangles, generated by aggregation of the microtubule associated protein tau. In addition to these pathological characteristics, there are other alterations, such as oxidative stress or loss of cholinergic transmission, among many others. One of the most promising approaches in AD treatment is to inhibit neurofibrillary degeneration produced by an abnormal tau hyperphosphorylation. In this sense, serine/threonine phosphoprotein phosphatase 2A (PP2A) is the major phosphatase in brain that accounts for over 70% of tau dephosphorylation. It has been shown that PP2A activity is significantly decreased in postmortem AD brains, partly due to the increase of endogenous inhibitors that bind the PP2A catalytic subunit C.

Hypothesis: Okadaic acid (OA) is a natural toxin capable of inhibiting PP2A, leading to tau hyperphosphorylation. Our working hypothesis is based on antagonizing the inhibitory effects of OA on PP2A by designing analogues of OA, capable to bind to PP2A but without exerting inhibition, and thus preventing the attachment of endogenous inhibitors.

Material y Methods: The compounds synthesized, analogues to C19-C27 OA fragment, have been pharmacologically studied, evaluating them in several in vitro models of AD, such as: tau hyperphosphorylation induced by OA, oxidative stress caused by the toxic cocktail rotenone and oligomycin A or the glutamate induced excitotoxicity, all of them by the MTT method. Furthermore, we have measured the cellular phosphatase activity by the pNPP method. In order to carry out these objectives, we have used SH-SY5Y neuroblastoma cells and rat embryonic cortical neurons. Finally, we confirm our theory by docking studies.

Results and conclusions: Our molecules are not toxic in SH-SY5Y cells or in cortical neurons, and they are capable of reducing the neurotoxicity induced by OA. Some of them also showed good profile in the cell stress model induced by R/O A in SH-SY5Y cells, and in the glutamate-induced excitotoxicity in cortical neurons. The new compounds maintained the serine/threonine phosphatase activity, depressed by the action of two PP2A inhibitors: OA and citostatin. Molecular docking studies indicated that compound ITH12680 is capable of binding to PP2A similarly to OA, but it does not interact with the catalytic site, thus confirming our starting hypothesis. Taking into account these results, we conclude that our compounds have potential indication for the treatment of neurodegenerative diseases based on the maintenance of PP2A activity, which prevents tau hyperphosphorylation.

Keywords: tau hyperphosphorylation, PP2A, okadaic acid.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Raquel L. Arribas, Rocío Lajarín Cuesta, Cristóbal de los Ríos Salgado. Neuroprotective effects of new compounds directed to PP2A, a promising therapeutic target for Alzheimer's disease. IBJ Plus 2018 (S1):e0025 doi: 10.24217/2531-0151.18v1s1.00025.
Funding: We thank Instituto Fundación Teófilo Hernando for its continuous support. R.L.A.and R.L.-C. thanks Universidad Autónoma de Madrid for predoctoral fellowships. This study was supported by Proyectos de Investigación en Salud from Instituto de Salud Carlos III (Madrid, Spain, PI13/00789; PI16/01041) to C.d.l.R.
Competing Interests: The authors declare no conflicts of interest.

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Identification of genetic and molecular determinants associated with cardiotoxicity by anthracyclines and taxanes according to age.

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Introduction: Cardiotoxicity due to anthracyclines (CDA) is a very common problem in cancer patients, with great repercussion on their quality of life, which limits chemotherapy treatment and has consequences in the final prognosis of the oncologic disease itself. The susceptibility and degree of cardiotoxicity by anthracyclines is very heterogeneous among patients, and who will suffer this complication is unknown. CDA is a complex trait, thus follows a model of quantitative genetics, whose polygenic component is mostly unidentified. In addition, as a complex trait, CDA heterogeneity is explained by the variability among subphenotypes that would participate in its pathogenesis. Thus, anthracyclines exert their toxicity through DNA damage, so that among these sub-phenotypes would be the molecular pathways involved in the response to it, and a series of signaling pathways that promote and others that protect from that heart damage anthracyclines have a pro-genotoxicity effect. Differences in these pathways with the genetic variants linked with them could contribute to different susceptibility to CDA among individuals.

Material and Methods: we identified the genetic and molecular determinants of cardiotoxicity in a simplified model of controlled genetic and phenotypic heterogeneity, generated by a backcross of two mouse strains of divergent phenotypic behavior, FVB and C57BL/6. We evaluated cardiac damage at histopathological level and also quantified different subphenotypes such as signaling pathways associated with cardiac damage and protection, genotoxicity pathways, TGFβ levels, telomere length, and expression of miRNAs in the myocardium.

Results: We quantified anthracycline cardiotoxicity in a heterogeneous cohort of mice with breast cancer generated by a backcross. Cardiotoxicity was higher in old mice, was higher in the combined treatment with taxanes, was higher in the subendocardial zone and was influenced by the genetic background. We have identified multiple QTL associated with CDA in these different conditions studied. Differences in the grade of anthracycline cardiotoxicity at the histopathological level were accompanied by differences in the molecular levels in the myocardium of different molecular components of the pathways of response to damage at DNA, signaling pathways, miRNAs and telomere length. QTLs associated with these subphenotypes help to define CDA variability. Lastly, we have also defined CDA by multivariate models.

Conclusion: The identification of genetic and molecular factors responsible for the increased risk of CDA will contribute to a better understanding of their pathophysiology, which could lead to new approaches to predict, prevent and treat this serious complication of chemotherapy.

Keywords: Anthracyclines, Cardiotoxicity, Backcross.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Gómez Vecino A, Corchado-Cobos R, Blanco-Gómez A, Prieto C, Patino-Alonso C, Galindo-Villardón P, Fraile-Martín S, Rodrigues-Teixeira T, García-Macías C, Galvis-Jiménez M, García-Sánchez A, Isidoro-García M, García-Cenador MB, García-Criado FJ, García-Sancha N, Holgado-Madruga M, Sánchez Fernández PL, Pérez Losada J. Identification of genetic and molecular determinants associated with cardiotoxicity by anthracyclines and taxanes according to age. IBJ Plus 2018 (S1):e0026 doi: 10.24217/2531-0151.18v1s1.00026.
Funding: JPL was partially supported by FEDER and the MICINN (SAF2014-56989-R, SAF2017-88854R), the Instituto de Salud Carlos III (PIE14/00066), “Proyectos Integrados IBSAL 2015” (IBY15/00003), the Sandra Ibarra Foundation “de Solidaridad Frente al Cáncer” Foundation and “We can be heroes” Foundation.
Competing Interests: The authors declare no conflicts of interest.

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Using omics approaches to develop a biomarker signature for anti-psychotic drug toxicity.

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Introduction: Schizophrenia (SCZ) is a chronic brain disorder with symptoms of hallucinations, delusions, disturbed behavior and emotional and cognitive impairment. These symptoms are managed by administration of antipsychotic drugs (APDs), either as a single APD or a combination of different APDs depending on the individual. It is always recommended to take a maintenance dose of APD even after the reduction of SCZ symptoms which makes it a lifelong treatment. APD treatment is currently known to be the only effective treatment against SCZ. However, they are observed to trigger metabolic dysfunctions such as insulin resistance, obesity, coronary heart diseases and dyslipedemia in SCZ patients at the later stage during APD treatment. The main aim of the project is to identify a biomarker signature that could be detected in blood to diagnose the metabolic dysfunction due to APD administration at an early stage. The talk will provide an insight into omic approaches for biomarker discovery and a worked example of these approaches using relevant existing data sets from public repositories.

Materials and Methods: The public repository Array Express (AE) (https://www.ebi.ac.uk/arrayexpress/) was used to shortlist the datasets from transcriptomic studies of primary human hepatocytes (PHH) that has been treated with any antipsychotic drug. R studio was used to process the data and shortlist genes that had a minimum fold change of 2. Bioinfominer online tool (https://bioinfominer.com/) was used for Gene ontology enrichment analysis.

Results and conclusion: We found a microarray dataset of PHH that has been treated with Chlorpromazine (APD) from AE (AE id: E-MTAB-1747). The deregulated gene list from PHH that were treated with CPZ was significantly grouped under inflammatory and immune response from the gene ontology term enrichment analysis. Since the gene products of the inflammatory/immune response are mostly secreted, they are likely to be detected in blood which makes them potential biomarker candidates. However, these candidates were identified from PHH isolated from a single donor, so more work would be required to confirm them as valid biomarkers. Despite that caveat, the gene list can be considered as a useful reference list for future experiments.

Keywords: Biomarkers; Metabolic dysfunction; Schizophrenia; Transcriptomics.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Sangeetha Bupalan, Afshin Samali, Adrienne M. Gorman. Using omics approaches to develop a biomarker signature for anti-psychotic drug toxicity. IBJ Plus 2018 (S1):e0027 doi: 10.24217/2531-0151.18v1s1.00027.
Funding: H2020-MSCA-ETN-2016-72123.
Competing Interests: The authors declare no conflicts of interest.

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Impaired signaling of Transcription factors NF-κB and NRF2 in CX3CR1-deficient microglia leads to altered neuroinflammation and phagocytosis: implications in tauopathies.

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Introduction: TAU protein aggregation is the main characteristic of a group of age-related neurodegenerative diseases called tauopathies. One of the key hallmarks associated with neurodegeneration is the presence of low-grade chronic inflammation, indicating a crosstalk between damaged neuron and glial cells. Previously we have shown that TAUP301L overexpressing neurons released CX3CL1 that activates anti-inflammatory NRF2 signalling in microglial cells in vitro and in vivo. However, the potential role of CX3CR1 in the context of TAU pathology and its implication neuroinflammation are poorly described.

Material and Methods: Pro-inflammatory markers in immortalized microglia cells (IMG) treated with CX3CL1 were analysed. We also studied mRNA expression levels of NF-κB, anti-inflammatory Nrf2 signalling and TAM receptors (TYRO3, AXL and MER) in CX3CR1-deficient primary microglia cells. Finally, the effect of sulforaphane (SFN), a NRF2 inducer, was examined on neuroinflammation in Cx3cr1+/+ and Cx3cr1-/- mice stereotaxically injected in the right hippocampus with an adeno-associated vector expressing human TAUP301L and treated daily with SFN (50mg/kg, i.p) during three weeks.

Results: In this study we show that CX3CL1 treatment induced NF-κB-p65 and pro-inflammatory cytokines expression. On the other hand, CX3CR1-deficient primary microglia cells present impaired NF-κB mRNA expression levels and decreased anti-inflammatory NRF2 signalling, suggesting a dual role of CX3CL1/CX3CR1 axis in neuroinflammation. Lack of CX3CR1 microglia exhibit decreased mRNA expression levels of TAM receptors (TYRO3, AXL and MER) that functionally results in a deficiency in phagocytosis. SFN treatment reverses astrogliosis in Cx3cr1+/+ and Cx3cr1-/-, whereas at microglial level we did not see any improvement in the Cx3cr1-/- mice.

Conclusions: These findings suggest that CX3CR1-NRF2 axis activation is essential in the modulation of microglial activation associated with tauopathy, and that the associated polymorphisms of CX3CR1 must be taken into account in the design of pharmacological strategies for the treatment of taupathies.

Keywords: tauopathies, sulforaphane, NRF2, NF-κB, microglia, inflammation.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Sara Castro-Sánchez, Ángel J. García-Yagüe, Marcos Galán-Ganga, Tresa López-Royo, Sebastian Kügler, Isabel Lastres-Becker. Impaired signaling of Transcription factors NF-κB and NRF2 in CX3CR1-deficient microglia leads to altered neuroinflammation and phagocytosis: implications in tauopathies. IBJ Plus 2018 (S1):e0028 doi: 10.24217/2531-0151.18v1s1.00028.
Funding: No funding was received for this work.
Competing Interests: The authors declare no conflicts of interest.

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Antitumoral effects of Hispanolone derivatives in glioblastoma cells.

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Introduction: Glioblastoma multiforme (GBM) constitutes the most frequent and aggressive primary brain tumor in adults. Even vast efforts have been made to develop effective treatments, including the combination of surgery, chemotherapy, and radiotherapy; the prognosis for the patients is extremely poor, with mean survival of about 14 months. Therefore, there is still an urgent need for novel and effective therapies for treating these tumors. On this issue, natural product-based molecules represent interesting therapeutic alternatives. Hispanolone derivatives have been shown to induce apoptosis in several human cancer cells. Nevertheless, the activity of these compounds against glioblastoma cells remains unclear. In the present study, we aimed to investigate the effects of a hispanolone derivative, α-hispanolol, on proliferation as well as on the migration and invasion of human glioblastoma cells.

Material y Methods: Cytotoxicity of α-hispanolol was determined against glioblastoma cancer cells, using the MTT assay. Flow cytometry was performed to analyze the changes in cell cycle and apoptotic effect, if any. Cells were also studied for their wound healing and invasive potential as well as for Western blotting of apoptotic genes.

Results: Our results show that α-hispanolol induced cell morphological changes and decreased the cell viability of U87 and U373 cells in a dose- and time-dependent manner. This inhibitory effect was found to be linked to arrest of cell cycle at the G0/G1 phase, along with induction of apoptosis and accumulation in the sub-G1 phase. Moreover, α-hispanolol also induced caspases activities and increased the pro-apoptotic protein (Bax and Bid), and inhibited the anti-apoptotic proteins (Bcl-2 and Bcl-xl) in GBM cells. In addition, α-hispanolol displayed an inhibitory effect on the migration and invasion of glioma cells by inhibiting the expression and activity of MMPs.

Conclusion: Our findings suggest that α-hispanolol may have therapeutic potential for the treatment of glioblastomas.

Keywords: α-hispanolol, glioblastoma, antitumoral, apoptosis.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Vanesa Sanchez-Martin, Beatriz de las Heras, Sonsoles Hortelano. Antitumoral effects of Hispanolone derivatives in glioblastoma cells. IBJ Plus 2018 (S1):e0029 doi: 10.24217/2531-0151.18v1s1.00029.
Funding: This study was supported by grant PI11/00036, PI14/00055, and PI17/00012 from Instituto de Salud Carlos III to S. Hortelano.
Competing Interests: B. de las Heras and S. Hortelano are inventors on a Spanish patent application on labdane diterpenoids as anti-tumoral agents. The other authors declared no conflict of interest.

Treatment with Brusatol inhibits oncogenic transcription factors NRF2 and TAZ, reducing glioma stem cells survival.

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Glioblastomas are nervous system solid tumours with poor prognosis and hard treatment, since they present glioma stem cells (GSCs), a subpopulation of cells responsible for resistance to chemotherapy and radiotherapy, which lead to tumour recurrence. NRF2 and TAZ transcription factors are involved in tumour development, but their role in regulation of cancer stem cells (CSCs) and their possible crosstalk have not been explored. Our studies demonstrate a correlation between NRF2 and TAZ expression and the prognosis of patients with glioma. Knock-down of NRF2 in human glioblastoma explants and glioblastoma cell lines, decreased messenger RNA and protein levels of TAZ and its transcriptional signature. In addition, we identified functional NRF2- binding sites (Antioxidant Response Element, ARE) in the promoter region of the TAZ encoding gene (WWTR1). Besides, NRF2 knock-down reduces cell growth both in vitro and in vivo, being rescued with TAZ overexpression.

Consequently, we conclude that at least part of the tumorigenic capacity of NRF2 is TAZ-dependent. Following this evidence, we have used NRF2 inhibitor Brusatol. This treatment reduced TAZ levels and GSCs growth in the same way as NRF2 knock-down. Because of this, we propose NRF2 and TAZ pharmacological inhibition as a novel glioblastoma therapy.

Keywords: Brusatol, TAZ, NRF2, Glioblastomas.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Diego Lastra, Marta Pajares, Natalia Robledinos-Antón, Ricardo Gargini, Antonio Cuadrado, Maribel Escoll. ITreatment with Brusatol inhibits oncogenic transcription factors NRF2 and TAZ, reducing glioma stem cells survival. IBJ Plus 2018 (S1):e0030 doi: 10.24217/2531-0151.18v1s1.00030.
Funding: No funding was received for this work.
Competing Interests: The authors declare no conflicts of interest.

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Measurement of polymorphic P-glycoprotein activity in cell cultures: a review.

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Introduction: The P-glycoprotein (P-gp) is an efflux pump widely expressed in the organism that exports xenobiotic compounds out of the tissue where it is expressed. It plays a central role in the Blood-Brain Barrier permeability, being responsible of Central Nervous System side-effects or ineffectiveness of many drugs. Several single-nucleotide polymorphisms (SNPs) in ABCB1 (the gene encoding for P-gp) have been identified. The most relevant ones, C3435T, C2677T/A and C1236T have been associated with variable pharmacokinetic parameters in healthy volunteers that received single oral doses of antidepressants and antipsychotics. There is no consensus regarding the in vivo effect they have. Here, we compile relevant information in order to simplify the understanding of materials, methods and cell lines classically used to assess polymorphic P-gp activity in in vitro cell culture models.

Methods: A comprehensive research of the studies performed in this regard has been accomplished. More than 389 articles have been reviewed, corresponding to the topics “ABCB1 polymorphisms”, “assessment of P-gp function” and “P-gp expression in cell cultures” published in PubMed Search Engine.

Results: Twenty-four articles have been summarised and classified. Site-directed mutagenesis has been acknowledged as a convenient approach to obtain cell lines expressing mutant P-gp. Ten different techniques have been identified as key in the assessment of P-gp function: Transfection; Western Blot; Flow Cytometry; Transcriptional Analysis; TEER measurements; Calcein-AM, Rhodamine-123 or Radioactivity based accumulation and transport assays; Transwell® inserts; MTT viability assays.

Conclusion: Site-directed mutagenesis performed in plasmids that contain wild-type ABCB1, followed by transfection of the plasmid into cells (HeLa, Caco-2 cells) may lead to cell lines expressing P-gp with the SNPs of interest (C3435T, C2677T/A and C1236T). Assessment of P-gp function may be accomplished by Calcein-AM or Rhodamine-123 accumulation assays. The actual effect of these SNPs in P-gp on antidepressants or antipsychotics efflux through membranes could be assessed by Transwell® insert transport assays.

Keywords: ABCB1, P-glycoprotein, P-gp, cell cultures, Transwell, Transfection, site-directed mutagenesis, antidepressants, antipsychotics.
Published April 10, 2018.
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Editor: Alberto M. Borobia Pérez.
Cite as: Pablo Zubiaur, Miriam Saiz-Rodríguez, Dora Koller, María C Ovejero-Benito, Francisco Abad-Santos. Measurement of polymorphic P-glycoprotein activity in cell cultures: a review. IBJ Plus 2018 (S1):e0031 doi: 10.24217/2531-0151.18v1s1.00031.
Funding: P. Zubiaur is co-financed by Consejería de Educación, Juventud y Deporte from Comunidad de Madrid and Fondo Social Europeo. D. Koller is co-financed by the H2020 Marie Sklodowska-Curie Innovative Training Network 721236 grant.
Competing Interests: F. Abad-Santos has been consultant or investigator in clinical trials sponsored by the following pharmaceutical companies: Abbott, Alter, Chemo, Cinfa, FAES, Farmalíder, Ferrer, GlaxoSmithKline, Galenicum, Gilead, Janssen-Cilag, Kern, Normon, Novartis, Servier, Silverpharma, Teva, and Zambon. The remaining authors declare no conflicts of interest.