Review of Clinical Pharmacology and Pharmacokinetics

INTERNATIONAL EDITION

VOLUME 34, 2020 ❁ No 2

Issue Devoted to Papers Presented at the

18th Medicinal Chemistry Conference

Drug Discovery and Design

Organized by the
Departments of Chemistry, Medicine and
Pharmacy of the University of Patras
Patras, Hellas

October 30-31, 2017
Patras, Hellas

ISSN 1011-6583

Review of Clinical Pharmacology and Pharmacokinetics

INTERNATIONAL EDITION

Articles published in this Journal are ***Indexed*** or ***Abstracted*** in:

• ***Chemical Abstracts*** • ***Elsevier' Bibliographic Databases: Scopus, EMBASE, EMBiology, Elsevier BIOBASE*** • ***SCImago Journal and Country Rank Factor***

Further informations regarding the Journal can be seen in the web address: http://pharmakonpress.gr

Review of Clinical Pharmacology and Pharmacokinetics
INTERNATIONAL EDITION

☸

Published three times a year by PHARMAKON-Press

Publisher Responsible According to the Law
Dr Helen S. Plessa
9A Kanari str., GR-15239, Nea Penteli, Athens, Hellas
Tel.- Fax 00302109756332
Email: splessas@otenet.gr & stplessas@hotmail.com

☸

☸

Original Papers, Review Articles, as well as short preliminary communications will be considered for publication and should be sent to the Editors-in Chief

☸

Online ISSN 1011-6583

Articles published in this Journal are ***Indexed*** or ***Abstracted*** in:

• ***Chemical Abstracts*** • ***Elsevier's Bibliographic Databases: Scopus, EMBASE, EMBiology, Elsevier BIOBASE*** • ***SCImago Journal and Country Rank Factor***

VOLUME 34 ❁ 2020 No 2

CONTENTS

GENERAL INFORMATION

REVIEW OF CLINICAL PHARMACOLOGY AND PHARMACOKINETICS INTERNATIONAL EDITION

The Journal aims to promote optimum drug therapy by providing original papers and review articles covering important aspects of clinical and applied Pharmacology and Therapeutics. The focus of the Journal comprises drug evaluation reviews, which provide a detailed focus on different properties, i.e. dosage, toxicology, drugs interactions and a place in therapy of both newer and established drugs. Other Review Articles offer state-of-the-art literature surveys covering broader topics. Practical Therapeutics Articles and Leading Articles provide recommendations for specific situations of connections or emerging areas, respectively.

The Journal publishes, in special issues, papers presented at:
- the *Conferences with International Participation Medicinal Chemistry: Drug Discovery and Design organized by the Departments of Chemistry and Pharmacy of the University of Patras, Hellas*
- the *Panhellenic Congresses of Pharmacology organized by the Hellenic Society of Pharmacology*

The *scientific standard* of the papers, which are accepted for publication, is controlled by the Editorial Board or by other Experts in the various fields of Pharmacology, Pharmacokinetics and Thepapeutics.

INSTRUCTIONS TO AUTHORS

English is the preferred language for all papers. However, papers in French, German or other European languages can also be submitted, provided they are accompanied by an English summary.

FORMAT: Summary, Introduction, Materials and Methods, Results, Discussion
Acknowledgements and References

Manuscripts: These should mention, on the first page, the *Title*, *Author(s)* and the *Name of the Institution* at which the work was done. The complete *address* of the author, including Postal area code number, should be given under the rubric *Send reprint requests to*. Papers should follow the general form: *Introduction, Materials and Methods, Results, Discussion* and *References*. Drugs must be referred to by their generic or chemical name, but may be identified by trade name in parenthesis or o footnote. All papers should be submitted in duplicate.

Summary: A summary in English (maximum length 200 words) must accompany all manuscripts.

Key words: A list of key words should be submitted, after summary

References: These should be numbered in the paper and listed under *References* in order of their appearance in the text. The author(s) surname followed by the initials should be given first, then the complete title of the article, the name of the Journal or Magazine (abbreviated according to the Index Medicus), the volume number, page numbers and year of publication in parenthesis.

Letter from the Guest Editor

The Medicinal Chemistry Graduate Program “Drug Discovery and Design” of the University of Patras has completed its 20th year of operation (1998-2018). The program, a joint collaboration of the Departments of Chemistry, Medicine and Pharmacy, has been successful with outstanding research and academic activities in the field of Medicinal Chemistry. The Program has attracted the interest of world leading scientists for participation and research collaboration. Each year a distinguished scientist is honored for his/her contribution to Biomedical Research and Science.

This year in our 18th Med Chem Conference Guests of Honor are two distinguished Scientists Professor Christos Zerefos, Member of the Academy of Athens and Professor George Chrousos Emeritus and former Chairman of the Department of Pediatrics at the Athens University Medical School, Greece. Their Participation was a great Honor for our Conference and for the University of Patras.We are happy that our Medicinal Chemistry Program continues successfully the tradition of our annual Conferences. This is the 18th Medicinal Chemistry Meeting since 2000 and I wish that we will go on with this successful tradition for many many years.

The success of the Medicinal Chemistry program was the result of collective efforts and was based on the cooperation, dedication, hard work, vision and high objectives. Most of all, on the potential of our graduate students. The credibility of the research team relied on the excellent research work, published in leading peer-reviewed scientific journals. Behind all, was the love for the University, for the Students, for Research and the Vision. Biosciences in Greece are a priceless treasure. Innovation and Excellence in Greek Universities and Institutions are the way for the Development and Prosperity of the Country.

On behalf of the Post Graduate Program I wish to express my gratitude to all who have contributed to the great success of this program, in particular esteemed invited scientists, colleague teachers, our graduate students, collaborators in research. I would like also to thank Elizabeth Diamantopoulou, Biology Student and Elias Theodoropoulos, Economics Student, Maria Tsikrika, Department of Material Sciences and Maria Tsiligianni a Philosophy Student for their great input in editing this Issue.

The Guest Editor, on behalf of the Postgraduate Program Committee, wishes to express his deep appreciation to all contributors in this book. We also thank the Editorial Board of Review of Clinical Pharmacokinetics. In particular Journal Editors Prof. S. T. Plessas and Dr C. T. Plessas for invitation and for providing the suitable and high-standard forum through which important finding of this research will come available to the scientific community.

The Guest Editor
John Matsoukas
Professor of Chemistry

Issue Devoted to Papers Presented at the

18th Medicinal Chemistry Conference

Archaeological Museum of Patras, Patras

October 30-31, 2017

Drug Discovery, Design & Development

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 34: 57 (2020)

18th Conference, No 1

Montreal Protocol: 30 years of success

Christos S. Zerefos, Academician

Honorary Member and former President, International Ozone Commission

This year, 2018 marks the 30th anniversary of the Montreal Protocol, which controls the substances depleting the protective ozone layer on earth. The Protocol and its many amendments has been efficient from its very beginning, reducing significantly the rates of ozone decrease and the subsequent increases in the harmful to life UV-B solar radiation. The system has been seriously complicated in the past decade by the accelerating effect of global warming which is linked to the cooling and destabilization further of the ozone layer. The ozone odyssey appears to continue, in spite the fact of positive departures observed in the past few years.

References

1. Eleftheratos, K., C. Zerefos, D. Balis, M.E. Koukouli, J. Kapsomenakis, D. Loyola, P. Valks, M. Coldewey-Egbers, C. Lerot, S. Frith, A. Søvde-Haslerud, I. Isaksen, S. Hassinen, "The use of QBO, ENSO and NAO perturbations in the evaluation of GOME-2/MetopA total ozone measurements", **Atmos. Meas. Techn.,** 12, 987-1011, 2019.
2. Fountoulakis, I., C.S. Zerefos, A.F. Bais, J. Kapsomenakis, M.E. Koukouli, N. Ohkawara, V. Fioletov, H. De Backer, K. Lakkala, T. Karppinen, A.R. Webb, "25 years of spectral UV-B measurements over Canada, Europe and Japan: trends and effects from 1 changes in ozone, aerosols, clouds and surface reflectivity", **Comptes Rendus Geoscience,** 350, 393-402, 2018.
3. Zerefos, C., J. Kapsomenakis, K. Eleftheratos, K. Tourpali, I. Petropavlovskikh, D. Hubert, S. Godin-Beekmann, W. Steinbrecht, S. Frith, V. Sofieva, B. Hassler, "Representativeness of single lidar stations for zonally averaged ozone profiles, their trends and attribution to proxies", **Atmos. Chem. Phys.**, 18(9), 6427-6440, 2018.
4. Zerefos, C. S., K. Eleftheratos, J. Kapsomenakis, S. Solomos, A. Inness, D. Balis, A. Redondas, H. Eskes, V. Amiridis, C. Repapis, M. Allaart, R. Engelmann, A. Dahlback, V. De Bock, H. Diémoz, P. Eriksen, J. Gröbner, A. Heikkilä, J. Jarosławski, W. Josefsson, T. Karppinen, U. Köhler, C. Meleti, C. Repapis, J. Rimmer, V. Savinykh, V. Shirotov, A. M. Siani, A. R. D. Smedley, M. Stanek, and R. Stübi, "Detecting volcanic sulfur dioxide plumes in the Northern Hemisphere using the Brewer spectrophotometer, other networks, and satellite observations", **Atmos. Chem. Phys**., 17, 551–574, 2017.
5. Papayannis, A., A. Argyrouli, A. Bougiatioti, E. Remoundaki, S. Vratolis, A. Nenes, S. Solomos, M. Komppula, E. Giannakaki, J. Kalogiros, R. Banks, K. Eleftheriadis, E. Mantas, E. Diapouli, C.G. Tzanis, S. Kazadzis, I. Binietoglou, L. Labzovskii, J. Vande Hey, C.S. Zerefos, "An overview from hygroscopic aerosols to cloud droplets: The HygrA-CD campaign in the Athens basin", **Sci. Total Environ.**, 574, 21-223, 2017.
6. Diémoz, H., K. Eleftheratos, S. Kazadzis, V. Amiridis, and C. S. Zerefos, Retrieval of aerosol optical depth in the visible range with a Brewer spectrophotometer in Athens, **Atmos. Meas. Techn.**, 9, 1871-1888, doi:10.5194/amt-9-1871-2016, 2016.

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 34: 58-59 (2020)

18th Conference, No 2

Stress, Genetics and Epigenetics in Human Evolution and Development

George R. Chrousos

National and Kapodistrian University of Athens, Athens, Greece

Nowadays, we frequently associate the fields of Evolution, aka Genetics and Phylogeny, and Development, aka Epigenetics and Ontogeny, and use the abbreviated term Evo-Devo to refer to both fields. The human organism and the societies it forms are complex systems that, given the enormous impact of human cognitive and emotional empathy on survival of both the species and the individual, should be considered together. As such systems, humans and their societies are in a relatively «stable disequilibrium» or homeostasis, that is maintained by extrinsic energy. Complex systems respond adaptively to exogenous or endogenous threats, the stressors, and the state of disturbed homeostasis, or stress, represents a condition that has the power to shape the ability of a species or individual to survive and reproduce. Hence, both evolution and development are influenced by stress. Major evolutionary and developmental stressors include starvation, dehydration or hemorrhage, injurious agents, presence of adversaries, tissue injury and societal disruption. We have adapted our physiology and behavior, both as a species and as individuals, to respond to these stressors as successfully as possible. Now, we have the benefit of the stupendous progress in biology and genetics to understand the mechanisms through which our species has evolved by adapting to and surviving through major evolutionary and developmental stressors. These selective pressures explain, to a great extent, the appearance of the modern chronic diseases of humanity, such as obesity, the metabolic syndrome, hypertension, allergies, autoimmune disorders, anxiety, depression, the pain and fatigue syndromes, and sociopathic behaviors. The term Epigenesis was first employed by Aristotle to suggest the process of de novo changes in organismal responses to environmental conditions, as opposed to the inner preformation theory of Plato, who had proposed that all developmental processes were predetermined and unfolded over time. The modern definition of Epigenetics was proposed by C. H. Waddington in 1942, as “the causal interactions between genes and their products to bring the phenotype into being”. Even though epigenetics represent acquired properties that are obtained by the organism over its lifetime, i.e., during ontogeny, some may cross generations or even lead to genetically inheritable changes. The epigenetic process is effected by

covalent bonds on the DNA without changes in the base sequence of the molecule, post-translational modification of proteins, DNA-binding proteins or protein complexes, miRNAs, piRNAS and other noncoding RNAs, as well as by formation of "super-enhancers", which appear to play major organizational roles in tissue differentiation. Methylation vs. demethylation, as well as acetylation vs. deacetylation, of DNA and chromatin proteins represent key molecular changes in epigenesis. Epigenetic functions include embryonic cell differentiation, genomic imprinting, X-chromosome inactivation, retrotransposon repression, somatic cell differentiation, immune function, puberty, sexual orientation, right/left handedness, labor and delivery, maternal and perinatal stress, brain plasticity, memory formation and stress-related behaviors. Behavioral disorders, such as depression and schizophrenia, have a strong epigenetic component. We should note that epigenetic control mechanisms evolve, there is a Lamarckian dimension in evolution, and imprints and methylation marks are erased and reestablished de novo stochastically twice, at the gamete and blastocyst stage, in each generation.

References

1. Gassen NC, Chrousos GP, Binder EB, Zannas AS. Life stress, glucocorticoid signaling, and the aging epigenome: Implications for aging-related diseases. Neurosci Biobehav Rev. 2017 Mar;74(Pt B):356-365. doi: 10.1016/j.neubiorev.2016.06.003. Epub 2016 Jun 23. Review. PMID:27343999

2. Gassen NC, Fries GR, Zannas AS, Hartmann J, Zschocke J, Hafner K, Carrillo-Roa T, Steinbacher J, Preißinger SN, Hoeijmakers L, Knop M, Weber F, Kloiber S, Lucae S, Chrousos GP, Carell T, Ising M, Binder EB, Schmidt MV, Rüegg J, Rein T.Chaperoning epigenetics: FKBP51 decreases the activity of DNMT1 and mediates epigenetic effects of the antidepressant paroxetine. Sci Signal. 2015 Nov 24;8(404):ra119. doi: 10.1126/scisignal.aac7695.

3. Zannas AS, Chrousos GP.Epigenetic programming by stress and glucocorticoids along the human lifespan. Mol Psychiatry. 2017 May; 22(5):640-646. DOI 10.1038/mp.2017.35. Epub 2017 Mar 14.PMID: 28289275

4. Zannas AS, Stein MB, Chrousos GP Editorial: Molecular Mechanisms in Stress and Trauma Related Disorders. Front Psychiatry. 2020 Mar 3;11:103. doi: 10.3389 /fpsyt. 2020.00103. eCollection 2020. Entire issue

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 34: 60-61 (2020)

18th Conference, No 3

New Strategies in the immunotherapy of Multiple Sclerosis

John Matsoukas[1], Vasso Apostolopoulos[2]

[1]NewDrug., Patras Science Park, Platani, Patras 26504, Greece
[2]Centre for Chronic Disease, College of Health and Biomedicine, Victoria University, Melbourne VIC 3030, Australia

The treatment of multiple sclerosis (MS) has changed over the last 20 years. The majority of immunotherapeutic drugs target relapsing remitting MS (RRMS) and it still remains a medical challenge in MS to develop a treatment for progressive forms. The most common injectable disease-modifying therapies in RRMS include β-interferons 1a or 1b and glatiramer acetate. However, one of the major challenges of injectable disease-modifying therapies has been poor treatment adherence with approximately 50% of patients discontinuing the therapy within the first year.

Current disease-modifying therapies in the armament against Multiple Sclerosis are interferons, glatiramer acetate, dimethyl fumarate, teriflunomide, fingolimod, mitoxantrone, humanized monoclonal antibodies natalizumab, ofatumumab, ocrelizumab, alemtuzumab, daclizumab. Emerging immune modulating approaches for the treatment of MS include stem cells, DNA vaccines, nanoparticles, altered peptide ligands. Herein, we go back to the basics to understand the immunopathophysiology of MS by sheding light to Myelin epitopes implicated in triggering disease in order to gain insights in the development of new improved drug treatments.

New strategies developed in our laboratories which focus on therapies based on Myelin Epitope Peptides or mutants conjugated to mannan. These strategies are promising therapies as they target T-cells which trigger disease (1-9).

References

1. Effect of Linear and Cyclic Peptide Analogues of Myelin Basic Protein Epitope MBP 72-85 on Human T-cell Activation, S. Thymianou, K. Chatzantoni, M. Karakantza, T. Tselios, P. Papathanassopoulos, J. Matsoukas, A. Mouzaki, Drug Discovery and Design: Medical Aspects J. Matsoukas and T. Mavromoustakos (Eds.), IOS Press, 241, 2002

2. Structural requirements for binding of myelin basic protein (MBP) peptides to MHC II: effects on immune regulation, ED Mantzourani, TM Mavromoustakos, JA Platts, JM Matsoukas, Current medicinal chemistry 12 (13), 1521-1535, 2005

3. A double mutation of MBP83–99 peptide induces IL-4 responses and antagonizes IFN-γ responses, M Katsara, E Yuriev, PA Ramsland, G Deraos, T Tselios, J Matsoukas, V. Apostolopoulos, Journal of neuroimmunology 200 (1-2), 77-89, 2008

4. Mannosylation of mutated MBP83–99 peptides diverts immune responses from Th1 to Th2, M Katsara, E Yuriev, PA Ramsland, G Deraos, T Tselios, J Matsoukas, V. Apostolopoulos, Molecular immunology 45 (13), 3661-3670, 2008

5.Design and Synthesis of a Cyclic Double Mutant Peptide (cyclo(87−99) [A91,A96]MBP87−99) Induces Altered Responses in Mice after Conjugation to Mannan, M Katsara, G Deraos, T Tselios, MT Matsoukas, I Friligou, J Matsoukas, V. Apostolopoulos, Journal of medicinal chemistry 52 (1), 214-218, 2008

6. Cyclization of PLP139-151 peptide reduces its encephalitogenic potential in experimental autoimmune encephalomyelitis, A Lourbopoulos, MT Matsoukas, M Katsara, G Deraos, A Giannakopoulou, J Matsoukas, V. Apostolopoulos, Bioorganic & medicinal chemistry 26 (9), 2221-2228, 2018

7. Cyclic MOG35–55 ameliorates clinical and neuropathological features of experimental autoimmune encephalomyelitis, A Lourbopoulos, G Deraos, MT Matsoukas, J Matsoukas, V. Apostolopoulos, O Touloumi, Bioorganic & medicinal chemistry 25 (15), 4163-4174, 2017

8. Round and round we go: cyclic peptides in disease, M Katsara, T Tselios, S Deraos, G Deraos, MT Matsoukas, E Lazoura, J Matsoukas, V. Apostolopoulos, Current medicinal chemistry 13 (19), 2221-2232, 2006

9. Design and Synthesis of a cyclic Double Mutant Peptide (cyclo(87-99)[A91,A96]MBP87-99) Induce Altered Responses in Mice after Conjugation to Mannan: Implication in the Immunotherapy of Multiple, Sclerosis, M Katsara, G Deraos, T Tselios, M-T Matsoukas, I Friligou, J Matsoukas, V Apostolopoulos, J. Med. Chem, 52, 214-218, 2009

10. Mannan-conjugated Myelin Peptides Prime Non-Pathogenic Th1 and Th17 Cells and Ameliorate Experimental Autoimmune Encephalomyelitis, V Tseveleki , T Tselios , I Kanistras, O Koutsoni, M Karamita , S Vamvakas, V Apostolopoulos , E Dotsika , J Matsoukas , H Lassmann , L Probert , Journal of experimental Neurology 10, 019, 2014

11. In vivo evaluation and in vitro metabolism of leuprolide in mice—mass spectrometry-based biomarker measurement for efficacy and toxicity, Z Sofianos, T Katsila, N Kostomitsopoulos, V Balafas, J Matsoukas, T Tselios, C Tamvakopoulos, Journal of mass Spectroscopy 43, 1381-1392, 2008

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 34: 62-63 (2020)

18th Conference, No 4

Macromolecular X-ray Powder Diffraction & Protein-based drug screening: Achievements & Perspectives

Valmas Alexandros, Karavassili Fotini, Margiolaki Irene

Department of Biology, Section of Genetics, Cell Biology and Development, University of Patras, GR-26500, Patras, Greece.

Knowledge of 3D structures of pharmaceutical compounds, from small organics to proteins, plays a major role in both designing drugs and understanding biochemistry of life. Various molecules often form microcrystalline precipitates either accidentally, as byproducts of crystallization experiments or on purpose by batch precipitation, as it is the case in microcrystalline pharmaceutical compounds[1]. The average size of individuals crystallites (~0.1-10 μm) in such precipitates is usually far too small for single-crystal diffraction studies, but it is in many cases ideal for X-ray Powder Diffraction (XRPD)[2]. The continuous evolution during the last fifteen years of macromolecular powder techniques has upgraded XRPD from being an ambitious suggestion for adventurous crystallographers to a respectable as well as powerful practice[3].

To date, a series of experiments and data analyses have been carried out which establish the validity of the method[4-6]. Powder diffraction applied to proteins is an effective technique which can be implemented for various purposes such as observing phase transitions, characterizing bulk pharmaceuticals[7], determining structures via the molecular replacement method and detecting ligands in protein–ligand complexes[8-9]. In this presentation we demonstrate the value of in-house and synchrotron XRPD as an analytical tool in industrial protein-based drug screening, and its potential to help troubleshooting the production process providing information for further refining the manufacturing of pharmaceuticals. Selected examples will be presented regarding studies of pharmaceutical compounds and their complexes with organic ligands including proteins (human insulin, urate oxidase) as well as peptides (octreotide)[10] and small organic molecules (sartans).

References

1. Acta Cryst. D 66, 539-548 (2010), I. Collings, Y. Watier, M. Giffard, S. Dagogo, R. Kahn, F. Bonnete, J. P. Wright, A. N. Fitch, I. Margiolaki.
2. Acta Cryst. A64, 169-180 (2008). I. Margiolaki & J. P. Wright.
3. Protein & Peptide Letters, 23 (3):232-41 (2016), F. Karavassili & I. Margiolaki.

4. Acta Cryst. D68, 1632-1641 (2012), F. Karavassili, A. E. Giannopoulou, E. Kotsiliti, L. Knight, M. Norrman, G. Schluckebier, L. Drube, A. N. Fitch, J. P. Wright & I. Margiolaki.
5. Acta Cryst. D69, 978-990 (2013), I. Margiolaki, A. E. Giannopoulou, J. P. Wright, L. Knight, M. Norrman, G. Schluckebier, A. N. Fitch & R. B. Von Dreele.
6. Acta Cryst. F72, 877-884 (2016), S. Fili, A. Valmas, M. Christopoulou, M. Spiliopoulou, N. Nikolopoulos, J. Lichiere, S. Logotheti, F. Karavassili, E. Rosmaraki, A. Fitch, J. Wright, D. Beckers, T. Degen, G. Nenert, R. Hilgenfeld, N. Papageorgiou, B. Canard, B. Coutard & I. Margiolaki.
7. Biomolecules 2017, 7 (3), 63 (OPEN ACCESS), F. Karavassili, A. Valmas, S. Fili, C. Georgiou & I. Margiolaki.
8. J. Am. Chem. Soc. 129, 11865 (2007) & ESRF Press release. I. Margiolaki, J. P. Wright, M. Wilmanns, A. N. Fitch & N. Pinotsis.
9. “Macromolecular Powder Diffraction”, Book Chapter for the International Tables of Crystallography- Volume H: Powder Diffraction, chapter 7.1, 718-736 (2019), I. Margiolaki.
10. Acta Cryst. B75, 611-620 (2019): Structural Science, Crystal Engineering and Materials, S. Fili, A. Valmas, M. Spiliopoulou, P. Kontou, A. Fitch, D. Beckers, T. Degen, Kl. Barlos, K. Barlos, F. Karavassili and I. Margiolaki.

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 34: 64-65 (2020)

18th Conference, No 5

Synthesis of natural products and designed organic molecules in anticancer drug development

Veroniki P. Vidali

Natural Products Synthesis and Bioorganic Chemistry Laboratory, Institute of Nanotechnology and Nanoscience, NCSR "Demokritos", Ag. Paraskevi, Athens, Greece.

Email: v.vidali@inn.demokritos.gr

Natural products are primary and secondary metabolites derived from plants, animals and microorganisms. In medicinal chemistry, the term is usually restricted in "secondary metabolites". These compounds exhibit one or more biological activities and they have driven the development of anticancer, antibiotic and other drugs. However, in most cases, the amounts isolated from nature are extremely limited. Thus, their chemical synthesis is essential. The great variety and complexity met in natural products, have played a crucial role in organic chemistry development. In this class of compounds, strained carbocyclic systems, including tertiary and/or quaternary carbon centers, are especially challenging synthetic targets. In such molecules, the sequence in which carbon centers are introduced and rings are constructed may define the stereochemical outcome.

Modern computational methods have also contributed significantly to drug development, accelerating drug-lead discovery through high-throughput screening. Specifically, organic molecules are selected and/or designed in silico, in order to show a targeted biological activity. In all cases, flexible and effective synthesis of proper bioactive compounds, of natural or not origin, is essential for further drug development.

This presentation includes a description of the key points of synthetic strategies, towards anticancer natural products, as well as the knowledge obtained from their development in our lab. Such compounds are adociasulfate-2 and other strained carbocyclic systems. Moreover, examples of synthetic strategies towards designed heterocyclic compounds for the development of selective oncoprotein inhibitors, will be described.

References

1. M. Cragg , D. G. I. Kingston, D. J. Newman, Anticancer agents from natural products, G 2nd Edition, CRC Press, 2012.
2. Novel Stereocontrolled Approach to *syn*- and *anti*- Oxepene-Cyclogeranyl *trans*-Fused Polycyclic Systems. Asymmetric Total Synthesis of (–)-Aplysistatin, (+)-Palisadin A, (+)-Palisadin B, (+)-12-

Hydroxy-Palisadin B and the AB Ring System of Adociasulfate-2 and Toxicol A. E. A. Couladouros, *V. P*, *Vidali, Chem. Eur. J.* 2004, *10*, 3822–3835.

3. Therapeutic effects of an anti-Myc drug on mouse pancreatic cancer D. Stellas, M. Szabolcs, S. Koul, Z. Li, A. Polyzos, C. Anagnostopoulos, Z. Cournia, C. Tamvakopoulos, A. Klinakis, A. Efstratiadis, J. Natl. Cancer Inst. 2014, 106.

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 34: 66-67 (2020)

18th Conference, No 6

Creation of tolerogenic dendritic cells for individualized therapy in multiple sclerosis patients

Maria Rodi, Anne-Lise de Lastic and Athanasia Mouzaki

Laboratory of Immunohematology, Division of Hematology, Department of Internal Medicine, Medical School, University of Patras, Patras, Greece

Dendritic cells (DCs) are cells of the immune system that bridge innate and adaptive immunity. The term “dendritic” is derived from the greek word “dendro” that means “tree” (due to their morphology), and was attributed to them in 1973 by Ralph Steinman and Zanvil Cohn.

DCs function as educators for T lymphocytes, capturing, processing and presenting antigens to T cells. DCs are mainly encountered in one of three profiles: a) immature DCs (imDCs), b) mature/immunogenic DCs (matDCs) and c) DCs that induce immunological tolerance (tolDCs). The manipulation of the dendritic cell profile can lead to the induction of cells with desired attributes according to the required applications. Such a DC population can be applied to individualized cellular therapy in disorders where the host’s pathological immune responses are involved.Studies, including our own [1], have shown that in the case of cancer cells that express unique antigens on their surface, mature DCs can be used to induce antigen-specific effector T cell-mediated immune responses against the disease. In contrast, in the case of autoimmune conditions such as multiple sclerosis (MS), where the mechanisms of immune tolerance have been disrupted, the use of tolDCs can be beneficial at restoring the immunological balance by inducing the proliferation of regulatory T cells and suppressing the harmful effects of autoreactive effector T cells.In our laboratory we have developed novel in vitro systems of human DC generation, differentiation and antigen presentation. Our aim is the study of antigen presentation of peptidic epitopes of myelin, that are the main antigenic targets in MS, and the use of tolerogenic DCs to alter the course of the disease and protect from its reemergence [2-5].

References:

1. de Lastic A-L, Rodi M, Mouzaki A. 2016. Effect of dendritic cell state and antigen-presentation conditions on resulting T-cell phenotypes and Th cytokine profiles. Immunobiology, 221(8):862-8
2. Regulatory cell populations in relapsing-remitting multiple sclerosis (rrms) patients: effect of disease activity and treatment regimens, Maria Rodi, Nikolaos Dimisianos, Anne-Lise de Lastic, Panagiota Sakellaraki, George Deraos, John Matsoukas, Panagiotis Papathanasopoulos, Athanasia Mouzaki, International journal of molecular sciences, 17 (9), 1398, 2016

3. Properties of myelin altered peptide ligand cyclo (87-99)(Ala91, Ala96) MBP87-99 render it a promising drug lead for immunotherapy of multiple sclerosis, George Deraos, Maria Rodi, Hubert Kalbacher, Kokona Chatzantoni, Fotios Karagiannis, Loukas Synodinos, Panayiotis Plotas, Apostolos Papalois, Nikolaos Dimisianos, Panagiotis Papathanasopoulos, Dimitrios Gatos, Theodore Tselios, Vasso Apostolopoulos, Athanasia Mouzaki, John Matsoukas, 101, 13-23, 2015
4. Parissi T, Chatzantoni K, Deraos S, Matsoukas I & Mouzaki A. 2004. Synthetic peptides mapping to epitopes of the extracellular domain of the IL-2 receptor (IL-2R) beta chain to inhibit T-cell activation. Transplant. Proc., 36(6):1723-1727, 2004
5. Mouzaki A, Koutsokera M, Dervilli Z, Rodi M, Kalavrizioti D, Dimisianos N, Matsoukas I & Papathanasopoulos P. 2010. Remitting-relapsing multiple sclerosis patient refractory to conventional treatments and bone marrow transplantation who responded to natalizumab. Int. J. Gen. Med., 3:313-32, 2010

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 34: 68-69 (2020)

18th Conference, No 7

Using tandem mass spectrometry for metabolite identification in untargeted liquid chromatography-mass spectrometry metabolomics

Charikleia Kaltsi[1,2], Matthaios-Emmanouil Papadimitropoulos[1,3], Theodore Tselios[2], Maria I. Klapa[1]

[1]Institute of Chemical Engineering Sciences, Foundation for Research & Technology-Hellas (FORTH/ICE-HT), Patras, Greece,
[2]Department of Chemistry, University of Patras, Greece,
[3]Department of Biology, University of Patras, Greece

Untargeted metabolomics refers to the high throughput analysis of the metabolic network state of a biological system through the simultaneous measurement of the concentrations of all (measurable) free metabolites that act as reactants or products in the metabolic reactions. The metabolite concentration profile constitutes the metabolic profile of the biological system at the particular physiological conditions. A major issue in mass spectrometry (MS) based metabolomics in general and liquid chromatography-MS metabolomics more particular is the large number of unidentified metabolite peaks, which hinders the extrapolation of the relevant profiles into biological knowledge. There is a need for analytical methodologies, which in combination with metabolic network reconstruction and analysis protocols and relevant databases, could contribute to unknown metabolite identification. In LC-MS analyses, the most common method to ionize the analytes is Electron Spray Ionization (ESI), which usually does not result to the fragmentation of the metabolite and leads to the measurement of its molecular ion, the latter not providing substantial structural information which could help in its identification. Therefore, Tandem Mass Spectrometry (MS/MS) is used for further fragmentation of the molecular ions in order to acquire information for the metabolite identification. In this study, we aimed at designing and standardizing a methodology for metabolite identification that combines LC-MS/MS metabolomics with metabolic network analysis tools and information from metabolic and metabolomic databases and the physiological context of the investigated biological sample. We applied this methodology in the identification of two unknown peaks in the MS-reconstructed liquid chromatogram of a tomato leaf metabolic profile, achieving to propose their potential identity with a substantially high probability.

References

1. Patti, G. J., Yanes, O. & Siuzdak, G. 2012. Innovation: Metabolomics: the apogee of the omics trilogy. Nat Rev Mol Cell Biol, 13, 263-9.
2. Kanani, H., Chrysanthopoulos, P. K. & Klapa, M. I. 2008. Standardizing GC-MS metabolomics. *Journal of chromatography B, Analytical technologies in the biomedical and life sciences,* 871**,** 191-201.
3. Maga-Nteve C., Klapa MI. 2016. Streamlining GC-MS metabolomic analysis using the M-IOLITE software suite. *IFAC-PapersOnLine* 49:286-288.
4. Kebarle, P. & Verkerk, U. H. 2009. Electrospray: From Ions in Solution to Ions in the Gas Phase, What We Know Now. *Mass Spectrometry Reviews,* 28**,** 898-917.
5. Dooley, K. C. 2003. Tandem mass spectrometry in the clinical chemistry laboratory. *Clin Biochem,* 36**,** 471-81.
6. Kaltsi Ch. 2017. Master's Thesis. Interdepartmental Graduate Program "Medicinal Chemistry", Department of Chemistry, University of Patras, Patras, Greece (in Greek; abstract in English).

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 34: 70-71 (2020)

18th Conference, No 8

The role of the transcriptional silencer Ets-2 in the pathogenesis of Multiple Sclerosis

Ioannis Panagoulias[1], Ioanna Aggeletopoulou[1], Evangelia Kourepini[2], Fotios Karagiannis[1], Panagiota Davoulou[1], Panagiotis Papathanasopoulos[3], Vily Panoutsakopoulou[2] and Athanasia Mouzaki[1]

[1]Laboratory of Immunohematology, Division of Hematology, Department of Internal Medicine, Medical School, University of Patras, Patras, Greece,
[2]Cellular Immunology Laboratory, Center for Basic Research, Biomedical Research Foundation of the Academy of Athens, Athens, Greece,
[3]Neurology Clinic, Medical School, University of Patras, Patras, Greece (present address: Olympion Therapeutirion, Patras, Greece)

T helper (Th) cells are key players in controlling specific immune responses to foreign antigens. Malfunctions of their antigen-recognition, activation and/or function, lead to pathological conditions that include autoimmune diseases. An important unresolved issue of the pathogenesis of autoimmune diseases is at which stage of Th cell differentiation errors occur, at the molecular level, that result in the development of pathological Th cell clones.

Multiple sclerosis (MS) is a chronic, demyelinating disease of the central nervous system. In MS, pathogenic effector Th cells, mainly Th1 and Th17, recognize myelin antigens and contribute to the damage to the central nervous system that results in the neurological problems that people with MS experience.

We recently showed that in naive Th cells, the transcription factor Ets-2 binds to the ARRE-2 element of the promoter of IL-2, the first cytokine produced when naive Th cells are activated, and keeps the gene silent. In activated or differentiated Th cells, Ets-2 changes its function, possibly to facilitate a quick response when Th cells re-encounter antigen. This mechanism seems to be defective in Th cells of MS patients, and we aim to show that this is a major cause for disturbed downstream events in Th cell differentiation leading to the development of pathological cells.

Our preliminary results showed low-level synthesis of Ets-2 mRNA and protein levels in Th cells of MS patients with remitting-relapsing multiple sclerosis compared to healthy controls. In parallel, we observed high levels of constitutive cytokine gene expression, in particular IL-2 and IL-17A in naive Th cells and IFN-γ and TNF-α in memory Th cells, and downregulation of IL-4 in naive Th cells of MS patients. Our preliminary results also showed decreased

Ets-2 binding capacity to the ARRE-2 element of the IL-2 promoter of naive Th cells from MS patients that coincides with elevated levels of IL-2 gene expression in these cells.

In addition, our preliminary results from mice showed significantly higher ets-2 expression in undifferentiated Th cells compared to in vitro differentiated Th cell populations, as well as elevated ets-2 expression in Th cells from mice resistant to experimental autoimmune encephalomyelitis (EAE).

We are currently investigating (1) the molecular mechanisms that control Ets-2 expression in naive, effector and regulatory Th cells in MS patients vs healthy controls, and (2) whether low-level synthesis and dysfunction of Ets-2 in Th cells of MS patients are responsible for the increase in pathological Th cell clones.

Our working hypothesis is that Ets-2 is implicated in MS pathogenesis. Ets-2.

References

1. Panagoulias I, Georgakopoulos T, Aggeletopoulou I, Agelopoulos M, Thanos D, Mouzaki A. Transcription Factor Ets-2 Acts as a Preinduction Repressor of Interleukin-2 (IL-2) Transcription in Naive T Helper Lymphocytes. J Biol Chem. 2016 Dec 23;291(52):26707-26721.
2. Argyropoulos C, Nikiforidis GC, Theodoropoulou M, Adamopoulos P, Boubali S, Georgakopoulos TN, Paliogianni F, Papavassiliou AG, Mouzaki A. Mining microarray data to identify transcription factors expressed in naïve resting but not activated T lymphocytes. Genes Immun. 2004 Jan;5(1):16-25.
3. Mouzaki A, Theodoropoulou M, Gianakopoulos I, Vlaha V, Kyrtsonis MC, Maniatis A. Expression patterns of Th1 and Th2 cytokine genes in childhood idiopathic thrombocytopenic purpura (ITP) at presentation and their modulation by intravenous immunoglobulin G (IVIg) treatment: their role in prognosis. Blood. 2002 Sep 1;100(5):1774-9.
4. Mouzaki A, Rungger D. Properties of transcription factors regulating interleukin-2 gene transcription through the NFAT binding site in untreated or drug-treated naive and memory T-helper cells. Blood. 1994 Oct 15;84(8):2612-21.
5. Panagoulias I. Role of Ets-2 and FoxP3 in Multiple Sclerosis Pathogenesis. EBioMedicine. 2015 Oct; 2(10): 1266–1267.

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 34: 72-73 (2020)

18th Conference, No 9

Neurophysiological effects of multiple sclerosis: More than changes in neuronal excitability

Costas Papatheodoropoulos

Department of Medicine, Lab of Physiology, University of Patras, Patras, Greece

Multiple sclerosis is a progressive inflammatory and ultimately neurodegenerative disease of the central nervous system (brain and spinal cord). Though clinical signs of multiple sclerosis are heterogeneous depending on the brain regions that are affected, is characterized by axonal demyelination and damage of neuronal cell axons. Demyelination hampers the ability of nerve cells to conduct their electrical signals, a condition described as reduced neuronal excitability that produces impairments in communication with other nerve cells and causes neurological deficits. A detailed knowledge of the pathologies that accompany multiple sclerosis will allow effective symptom management and development of new treatments for the prevention of progression of the disease. Importantly, a large proportion of patients (~50%) are affected by cognitive dysfunction and cognitive decline can occur even in the absence of neurological impairments. For instance, the ability to form new declarative memories can become compromised. Lasting changes in synaptic transmission between neurons (i.e. synaptic plasticity) is thought to be fundamental for the formation of new memories. It has been recently shown that the disease symptoms in a model of multiple sclerosis (experimental autoimmune encephalomyelitis) are accompanied by a loss of the ability to form hippocampus-dependent memories and an impaired ability to maintain long-term strengthening of synaptic connections. Additional evidence points to the possibility that long-term synaptic potentiation may be a basic adaptive mechanisms that increases neuronal excitability by increasing the efficacy of synaptic communication. This evidence opens new roads for multiple sclerosis research, including the development of new treatments that could be targeted toward preventing synaptic plasticity impairments or enhancing recovery in synaptic transmission and excitability by promoting synaptic plasticity in the brain.

References

1. Di Filippo M, Chiasserini D, Gardoni F, Viviani B, Tozzi A, Giampa C, Costa C, Tantucci M, et al. (2013), Effects of central and peripheral inflammation on hippocampal synaptic plasticity. Neurobiology of disease 52:229-236.

2. Kim DY, Hao J, Liu R, Turner G, Shi FD, Rho JM (2012), Inflammation-mediated memory dysfunction and effects of a ketogenic diet in a murine model of multiple sclerosis. PloS one 7:e35476.
3. Kojovic M, Bologna M, Kassavetis P, Murase N, Palomar FJ, Berardelli A, Rothwell JC, Edwards MJ, et al. (2012), Functional reorganization of sensorimotor cortex in early Parkinson disease. Neurology 78:1441-1448.
4. Mosayebi G, Soleyman MR, Khalili M, Mosleh M, Palizvan MR (2016), Changes in Synaptic Transmission and Long-term Potentiation Induction as a Possible Mechanism for Learning Disability in an Animal Model of Multiple Sclerosis. International neurourology journal 20:26-32.
5. Nistico R, Mango D, Mandolesi G, Piccinin S, Berretta N, Pignatelli M, Feligioni M, Musella A, et al. (2013), Inflammation subverts hippocampal synaptic plasticity in experimental multiple sclerosis. PloS one 8:e54666.
6. Novkovic T, Shchyglo O, Gold R, Manahan-Vaughan D (2015), Hippocampal function is compromised in an animal model of multiple sclerosis. Neuroscience 309:100-112.
7. Prochnow N, Gold R, Haghikia A (2013), An electrophysiologic approach to quantify impaired synaptic transmission and plasticity in experimental autoimmune encephalomyelitis. Journal of neuroimmunology 264:48-53.

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 34: 74 (2020)

18th Conference, No 10

Ethical Dilemmas in Clinical Trials for Psychiatric Drugs

Paraskevopoulou Stavroula

Postdoctoral Researcher, National and Kapodistrian University of Athens, Greece

Medicines' role is important to mental illnesses' therapy and rehabilitation and their contribution is unquestionable to promotion of mental health and quality of life. However production of new, more effective and safer drugs requires clinical trials on human subjects, which creates ethical dilemmas. The main dilemmas are related to the risks that these entail and the issue of participants' autonomy. Although these issues have been already adequately addressed by codes of conduct and international declarations, in the case of psychiatric drugs there are some additional difficulties inherent in experimental planning. Main difficulties are risks associated with relapse and worsening of disease in severe cases such as dementia or schizophrenia, especially in placebo trials, but also effect of mental illnesses on the ability of these patients to make decision and give informed consent for participating in research. Legitimate application of scientific knowledge and codes of conduct could contribute in overcoming of these dilemmas that will ensure patients' autonomy and at the same time will lead to production of new therapies that will contribute to wellbeing of society.

References

1. Friedman L., Furberg C., DeMets D., *Fundamentals of clinical trials*, 4th edition, Springer, 2010.
2. Beauchamp T. & Childress J., *Principles of biomedical ethics*, 4th edition, New York: Oxford University Press, 1994.
3. World Medical Association, *Declaration of Helsinki: Ethical Principles for Medical Research involving human subjects*, Bulletin of the World Health Organization, 79(4), 373-374, 2001.
4. National Bioethics Advisory Commission, *Research involving persons with mental disorders that may affect decisionmaking capacity*, 1998. (from www.bioethics.gov)

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 34: 75-76 (2020)

18th Conference, No 11

Investigating the Genetic Architecture of Hypertension through Integrated Analysis of GWAS and the Human Protein Interaction Network

Evridiki-Pandora Tsare[1,2], Aris Gioutlakis[1,2], Maria I. Klapa[2] and Nicholas K. Moschonas[1,2]

[1] Laboratory of General Biology, School of Medicine, University of Patras, Greece,
[2] Metabolic Engineering & Systems Biology Laboratory, FORTH/ICE-HT, Patras, Greece

OBJECTIVE: A major objective of medical genetics concerns the elucidation of the genetic architecture of diseases. Especially for multifactorial diseases, this effort could be greatly assisted by combining Genome-Wide Association Studies (GWAS) for the determination of gene loci linked with the disease with information derived from the analysis of the human Protein-Protein Interaction (PPI) network. Protein interactions are fundamental for the regulation of cellular processes, and the PPI network provides a comprehensive representation of the intracellular molecular function interconnectivity. High blood pressure (hypertension) is a major cardiovascular disease and stroke risk factor. To-date many gene loci associated to blood pressure regulation have been suggested through polycentric GWAS. The objective of this study is the exploration of all currently available GWAS data in combination with the relevant protein interaction network towards unveiling blood pressure regulatory mechanisms. To this end, we aimed at: a) the systematic collection of GWAS combined with expression Quantitative Trait Loci (eQTL) hypertension associated data, b) data integration via the human PPI network, and c) hypertension related PPI network topology analysis and determination of gene loci with higher probability being predisposing to hypertension.

SOURCES AND METHODS: High Blood Pressure (BP) GWAS data were retrieved from six recent large polycentric studies [1-6], referring collectively to more than a million samples. BP-associated gene loci combined with available eQTL data were mapped to the integrated human protein-protein interaction network of the PICKLE meta-database version 2.1 [www.pickle.gr; 7-8], comprising 120882 direct PPIs for 14134 UniProt IDs supported by 35752 publications. Network analysis and data visualization was performed using the Cytoscape software [9]. The DAVID platform [10,11] was used for functional annotation analysis and gene clustering.

RESULTS: About 300 hypertension-associated gene loci have been determined by GWAS; most (297) of them encode proteins, among which 261 belong to the PICKLE-reconstructed direct PPI network in human. Interestingly, the search for interactions showed that 67 hypertension-associated proteins form a functional subnetwork with its members directly

interacting with each other. Moreover, some of them are known anti-hypertensive drug targets. Functional annotation analysis of the subnetwork revealed four cell signaling pathways associated to blood pressure regulation connected through a core of 10 proteins.Noted that one of the 10 central proteins is RXRA (Retinoid X Receptor Alpha) involved, among other biological processes, in cardiac muscle cell proliferation and cholesterol metabolism.

CONCLUSIONS: The integrated analysis of BP GWAS data within the context of the integrated human protein interaction network may advance our understanding of the molecular pathophysiology of the disease and indicate candidate predisposing gene variants and disease modules playing a central role in the abnormal regulation of blood pressure.

References

1. Ehret GB, Ferreira T, Chasman DI, et al. The genetics of blood pressure regulation and its target organs from association studies in 342,415 individuals. Nat Genet, 48(10):1171-1184 (2016).
2. Ehret G., Munroe P., Rice K. et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature 478, 103–109 (2011).
3. Warren HR, Evangelou E, Cabrera CP, et al. Genome-wide association analysis identifies novel blood pressure loci and offers biological insights into cardiovascular risk. Nat Genet., 49(3):403-415 (2017).
4. Surendran P, Drenos F, Young R, et al. Trans-ancestry meta-analyses identify rare and common variants associated with blood pressure and hypertension. Nat Genet., 48(10):1151-1161 (2016).
5. Liu C, Kraja AT, Smith JA, et al. Meta-analysis identifies common and rare variants influencing blood pressure and overlapping with metabolic trait loci. Nat Genet., 48(10):1162-1170 (2016).
6. Levy D, Ehret GB, Rice K, et al. Genome-wide association study of blood pressure and hypertension. Nat Genet., 41(6):677-687 (2009).
7. Klapa MI, Tsafou K, Theodoridis E, Tsakalidis A, Moschonas NK. Reconstruction of the experimentally supported human protein interactome: what can we learn?. BMC Syst Biol., 7:96 (2013).
8. Gioutlakis A, Klapa MI, Moschonas NK. PICKLE 2.0: A human protein-protein interaction meta-database employing data integration via genetic information ontology. PLoS ONE 12(10): e0186039 (2017).
9. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res., 13(11):2498-2504 (2003).
10. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc., 4(1):44-57 (2009).
11. Dennis, G., Sherman, B.T., Hosack, D.A. et al. DAVID: Database for Annotation, Visualization, and Integrated Discovery. Genome Biol 4, R60 (2003).

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 34: 77-78 (2020)

18th Conference, No 12

Design and Synthesis of Targeted Inhibitors of PI3Kα as Candidate anti- Cancer Drugs

Anna Kapella[1,2], Paraskevi Gkeka[3], Dimitris Stellas[3], Veroniki P. Vidali[1], Alexandra Papafotika[4], Savvas Christoforidis[4], Zoe Cournia[3], Elias A. Couladouros[1,2]

[1] Natural Products Synthesis and Bioorganic Chemistry Laboratory, Institute of Nanoscience and Nanotechnology, NCSR Demokritos 15310 Ag. Paraskevi, Attiki, POB 60228, Athens, Greece
[2] Chemistry Laboratories, Agricultural University of Athens, 75 Iera Odos Str., 11855 Athens, Greece
[3] Biomedical Research Foundation of the Academy of Athens, Soranou Efesiou, 11527 Athens, Greece
[4] Institute of Molecular Biology and Biotechnology-Biomedical Research (IMBB-BR) & Laboratory of Biological Chemistry, University of Ioannina, Medical School, 45110 Ioannina, Greece

Correspondence e-mail: zcournia@bioacademy.gr

PI3K family enzymes are mutated in a range of human cancers, comprising therefore an important target for drug development. However, a key challenge in targeting PI3K pathway is the high similarity of PI3K isoforms in their active site, which often leads to the development of non-selective PI3K drug candidates that may yield undesirable side effects. Allosteric modulators typically bind to less conserved sites compared to the active site of an enzyme, and thus, they may confer greater specificity in kinase regulation [1]. Our research focuses on the H1047R mutation of the PI3Kα catalytic subunit p110α, which is one of the two hotspot mutations observed in breast and colon cancers [2,3]. Morpholino chromone PIK-108 was recently found to occupy a second binding pocket near the H1047R mutation site [4]. Our aim was to verify whether this new pocket is allosteric and further discover new allosteric pockets and inhibitors of PI3Kα. To investigate the potential allosteric action of PIK-108, the molecule was synthesized [5] and tested in a cell-free assay.

Using advanced computational techniques we developed mutant specific lead compounds with allosteric inhibitory action. Over 2M small molecules were evaluated in silico for binding to identified allosteric pockets of the mutated PI3Kα; Subsequently 17 compounds were selected, purchased and tested in suitable protein cell-free

assays. Thus, two lead- compounds were identified: the benzimidazole derivative PIK-010 and the naphthyl sulfonylamine PIK-104. PIK-010 and PIK-104 were also synthesized and tested with a cell-free assay to assess their allosteric behavior. In order to increase the selectivity of our lead compounds, we performed lead optimization of PIK-010 and PIK-104. Synthesis of 170 designed optimized analogues followed. Out of the compounds tested so far, the cell-free assay verified an optimized analog of PIK-010, PIK-021, which exhibited 100-fold more potent inhibition of the mutated protein compared to the WT. Finally, we performed a preliminary evaluation of the anti- tumor efficacy of compounds PIK-010 and PIK-021 using mouse xenograft models.

References

1. P. Gkeka , A. Papafotika, S. Christoforidis, Z. Cournia, “Exploring a Non-ATP Pocket for Potential Allosteric 2. Modulation of PI3Kα”, Journal of Physical Chemistry B, vol 119, pp.1002-1016, 2015.
2. S. B. Gabelli, C.- H. Huang, D. Mandelker, O. Schmidt- Kittler, B. Vogelstein, L. M. Amzel, “Structural effects of oncogenic PI3Kα mutations”, Current Topics in Microbiology and Immunology, vol.347, pp.43-53, 2010.
3.]P.K. Vogt, S. Kang, M.-A. Elsliger, M.Gymnopoulos, “Cancer- specific mutations in Phosphatidylinositol 3- kinase”, Trends in Biochemical Sciences, vol. 32, pp. 342-349,2007.
4. W.- C. Hon, A. Berndt, R. Williams, “Regulation of lipid binding underlies the activation mechanism of class IA PI3-kinases”, Oncogene vol 31, pp. 3655-3666, 2012.
5. Z. A. Knight et al., “A Pharmacological Map of the PI3K Family Defines a Role for p110a in Insulin Signaling”, Cell, vol.125, pp. 733-747, 2006.
6. Z. Cournia, S Christoforidis, A Kapella, E Couladouros, A Efstratiadis, “Method of preparation and use of phosphoinositide 3-kinase inhibitors in treating cancer”, 2018, OBI Patent Application Page 18 number 20180100392 and 2019, International Patent Application 69028P WO/BBbja

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 34: 79-80 (2020)

18th Conference, No 13

A theragnostic device with photo regulated drug dosing and cancer microenvironment sensing character

Christos Chatzigiannis, Andreas Tzakos

Section of Organic Chemistry & Biochemistry, Medicinal Chemistry Program,
Department of Chemistry, University of Ioannina, Ioannina, Greece

Gemcitabine is a well-known and efficient cytotoxic drug and more specifically a nucleoside analogue which expresses its anticancer activity mainly, through incorporation to the DNA[1-6]. Although, major limitations confine the therapeutic ability of gemcitabine like the lack of selectivity which in result kills normal cells, the poor bioavailability caused by the enzymatic deamination from the cytidine deaminase enzyme (CDA) leading to inactive uridine metabolites. Another problem is the induced resistance of cancer cells caused from chronic administration of the drug and as an outcome, deficiency of nucleoside transporters is observed so, due to gemcitabine's hydrophilic nature is unable to enter the cells. Another form of resistance expressed is the inactivation of the deoxycytidine kinase (DCK) inhibiting the rate limiting step of the first phosphorylation in the path leading to apoptosis of the cell[4,7-11]. To surmount these drawbacks, with rational design, in an effort to overcome the limitations of gemcitabine and take advantage of its therapeutic properties we assembled a theranostic device with photo-regulated and cancer sensing compartments. This device consists of four different moieties to whom it owes the synergistic and complex action. The first part in order of appearance, is specific biomarker which is selectively cleaved by GSH whose levels are much greater in cancer cells than normal cells and is being used as a way to discover cancer cells[12,13]. Upon the cleavage triggered by GSH the fluorescent dye is revealed and it is linked with ''click'' chemistry with a linker which is well known to be photolabile[14-16]. Upon the fluorescent ''mark'' of the fluorescent dye the tumor microenvironment is recognized and upon irradiation at a tissue non-destructive wavelength, release the toxic warhead which "photocaged" and linked through a traceless carbonate linker with the photolabile group.

To proof of our concept, we have used an array of techniques and assays such as UV, Fluorescence and 1D-2D NMR spectroscopy. We have mapped precisely the degradation of our device until the time of the drug administration. So, we present a novel theranostic prodrug (GCPG) that is described in three axes the diagnosis, the photocontrolled drug dosing and the selective therapy.

References

1. Moysan, E.; Bastiat, G.; Benoit, J.-P., Gemcitabine versus Modified Gemcitabine: A Review of Several Promising Chemical Modifications. *Molecular Pharmaceutics* **2013**, 10, 430-444.
2. Pasut, G.; Canal, F.; Dalla Via, L.; Arpicco, S.; Veronese, F. M.; Schiavon, O., Antitumoral activity of PEG–gemcitabine prodrugs targeted by folic acid. *Journal of Controlled Release* **2008**, 127, 239-248.
3. Pratt, S. E.; Durland–Busbice, S.; Shepard, R. L.; Donoho, G. P.; Starling, J. J.; Wickremsinhe, E. R.; Perkins, E. J.; Dantzig, A. H., Efficacy of Low-Dose Oral Metronomic Dosing of the Prodrug of Gemcitabine, LY2334737, in Human Tumor Xenografts. *Molecular Cancer Therapeutics* **2013**, 12, 481-490.
4. Rudin, D.; Li, L.; Niu, N.; Kalari, K. R.; Gilbert, J. A.; Ames, M. M.; Wang, L., Gemcitabine Cytotoxicity: Interaction of Efflux and Deamination. *Journal of drug metabolism & toxicology* **2011**, 2, 1-10.
5. Wickremsinhe, E.; Bao, J.; Smith, R.; Burton, R.; Dow, S.; Perkins, E., Preclinical Absorption, Distribution, Metabolism, and Excretion of an Oral Amide Prodrug of Gemcitabine Designed to Deliver Prolonged Systemic Exposure. *Pharmaceutics* **2013**, 5, 261-276.
6. Wong, A.; Soo, R. A.; Yong, W.-P.; Innocenti, F., Clinical pharmacology and pharmacogenetics of gemcitabine. *Drug Metabolism Reviews* **2009**, 41, 77-88.
7. Achiwa, H.; Oguri, T.; Sato, S.; Maeda, H.; Niimi, T.; Ueda, R., Determinants of sensitivity and resistance to gemcitabine: The roles of human equilibrative nucleoside transporter 1 and deoxycytidine kinase in non-small cell lung cancer. *Cancer Science* **2004**, 95, 753-757.
8. Bergman, A. M.; Pinedo, H. M.; Talianidis, I.; Veerman, G.; Loves, W. J. P.; van der Wilt, C. L.; Peters, G. J., Increased sensitivity to gemcitabine of P-glycoprotein and multidrug resistance-associated protein-overexpressing human cancer cell lines. *British Journal of Cancer* **2003**, 88, 1963-1970.
9. Bildstein, L.; Dubernet, C.; Marsaud, V.; Chacun, H.; Nicolas, V.; Gueutin, C.; Sarasin, A.; Bénech, H.; Lepêtre-Mouelhi, S.; Desmaële, D.; Couvreur, P., Transmembrane diffusion of gemcitabine by a nanoparticulate squalenoyl prodrug: An original drug delivery pathway. *Journal of Controlled Release* **2010**, 147, 163-170.
10. Réjiba, S.; Bigand, C.; Parmentier, C.; Hajri, A., Gemcitabine-Based Chemogene Therapy for Pancreatic Cancer Using Ad-dCK::UMK GDEPT and TS/RR siRNA Strategies. *Neoplasia (New York, N.Y.)* **2009**, 11, 637-650.
11. Saiki, Y.; Yoshino, Y.; Fujimura, H.; Manabe, T.; Kudo, Y.; Shimada, M.; Mano, N.; Nakano, T.; Lee, Y.; Shimizu, S.; Oba, S.; Fujiwara, S.; Shimizu, H.; Chen, N.; Nezhad, Z. K.; Jin, G.; Fukushige, S.; Sunamura, M.; Ishida, M.; Motoi, F.; Egawa, S.; Unno, M.; Horii, A., DCK is frequently inactivated in acquired gemcitabine-resistant human cancer cells. *Biochemical and Biophysical Research Communications* **2012**, 421, 98-104.
12. Redy-Keisar, O.; Kisin-Finfer, E.; Ferber, S.; Satchi-Fainaro, R.; Shabat, D., Synthesis and use of QCy7-derived modular probes for the detection and imaging of biologically relevant analytes. *Nature Protocols* **2013**, 9, 27.
13. Zhang, X.; Hang, Y.; Qu, W.; Yan, Y.; Zhao, P.; Hua, J., Diketopyrrolopyrrole-based ratiometric fluorescent probe for the sensitive and selective detection of cysteine over homocysteine and glutathione in living cells. *RSC Advances* **2016**, 6, 20014-20020.
14. Holmes, C. P., In; Google Patents: 1998.
15. Holmes, C. P., In; Google Patents: 1999.
16. Wieboldt, R.; Ramesh, D.; Jabri, E.; Karplus, P. A.; Carpenter, B. K.; Hess, G. P., Synthesis and Characterization of Photolabile o-Nitrobenzyl Derivatives of Urea. *The Journal of Organic Chemistry* **2002**, 67, 8827-8831.

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 34: 81-82 (2020)

18th Conference, No 14

Nanotechnology in DNA analysis

Despina Kalogianni

Department of Chemistry, University of Patras, Greece

Nanotechnology refers to nanomaterials from 1 to 100 nm in size. Today, nanotechnology is considered to be the leading technology and the main tool of modern technologies, as well as the most active research field worldwide (1). It has provided scientists with new tools in all areas of research, including medicine, electronics, chemistry and the food industry (2). In order to overcome some of the limitations of conventional analytical methods, nanotechnology-based systems have been explored in a variety of applications. Nanomaterials are available in a variety of shapes (nanospheres, nanotubes, nanoparticles, etc.) and materials (silicon, gold, semiconductors, iron, oxides, and polymers).

Subsequently, paper-based DNA biosensors in dry-reagents format have been developed using various nanoparticles such as gold nanoparticles, carbon nanoparticles, quantum dots and polystyrene microspheres as reporters in a variety of applications.

These biosensors are distinguished for their low cost, rapid analysis (within 10 min) and their exceptional simplicity along with the increased detectability and repeatability,
without the need for expensive equipment or highly qualified personnel. Finally, an outstanding technique have been also reported. This method is based on hybridization tests that are performed on the surface of spectrally distinct fluorescent polystyrene microspheres, dramatically increasing the number of different analytes (up to 100) that can be simultaneously detected in a single reaction (3-8).

References

1. A state-of-the-art review of the application of nanotechnology in the oil and gas industry with a focus on drilling engineering. Jagar A. Ali, Abdullah M. Kalhury, Ayub N. Sabir, et al. Journal of Petroleum Science and Engineering (2020) 191: 107118
2. Nanotechnologies in food science: applications, recent trends, and future perspectives. Shivraj Hariram Nile, Venkidasamy Baskar, Dhivya Selvaraj et al. Nano-Micro Lett. (2020) 12: 45
3. Nanoparticle-based DNA biosensor for visual detection of genetically modified organisms. DP Kalogianni, T Koraki, TK Christopoulos, PC Ioannou. Biosensors and Bioelectronics (2006) 21 (7): 1069-1076
4. Dry-reagent disposable dipstick test for visual screening of seven leukemia-related chromosomal translocations. DP Kalogianni, V Bravou, TK Christopoulos, PC Ioannou, NC Zoumbos. Nucleic acids research (2007) 35 (4): e23

5. Lateral flow devices for nucleic acid analysis exploiting quantum dots as reporters. EA Sapountzi, SS Tragoulias, DP Kalogianni, et al. Analytica Chimica Acta (2015) 864: 48-54
6. Carbon nano-strings as reporters in lateral flow devices for DNA sensing by hybridization. DP Kalogianni, LM Boutsika, PG Kouremenou, TK Christopoulos, et al. Analytical and bioanalytical chemistry (2011) 400 (4): 1145-1152
7. Dipstick-type biosensor for visual detection of DNA with oligonucleotide-decorated colored polystyrene microspheres as reporters. DP Kalogianni, IK Litos, TK Christopoulos, PC Ioannou. Biosensors and Bioelectronics (2009) 24 (6): 1811-1815
8. Olive oil DNA fingerprinting by multiplex SNP genotyping on fluorescent microspheres. DP Kalogianni, C Bazakos, LM Boutsika, MB Targem, TK Christopoulos, et al. Journal of agricultural and food chemistry (2015) 63 (12): 3121-3128

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 34: 83-85 (2020)

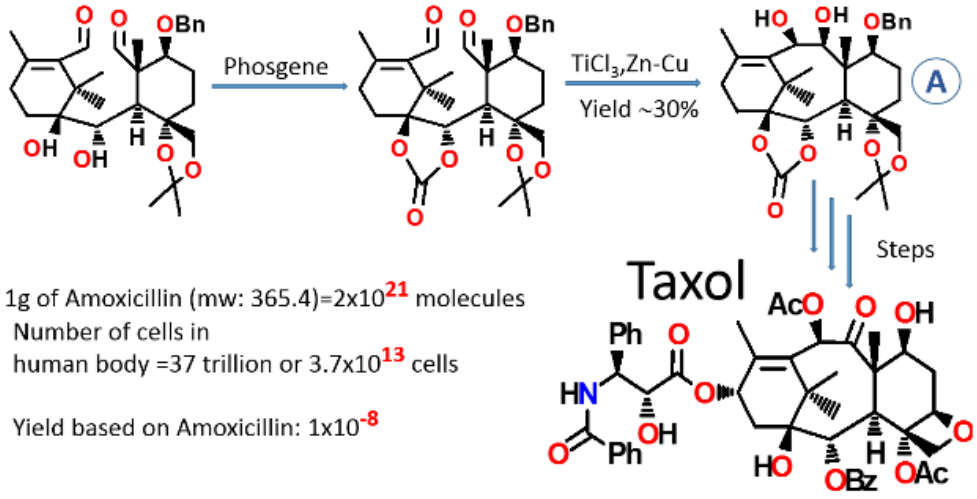
18th Conference, No 15

The Architectural Beauty of Bioactive Natural Products

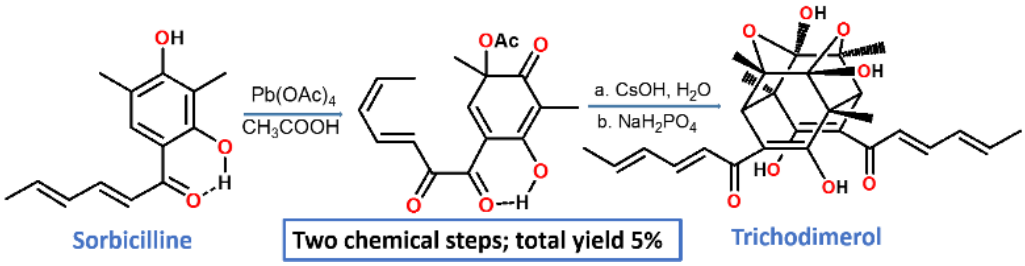
Elias A. Couladouros

Chemistry Laboratory, Agricultural University of Athens, Athens, Greece, 11855

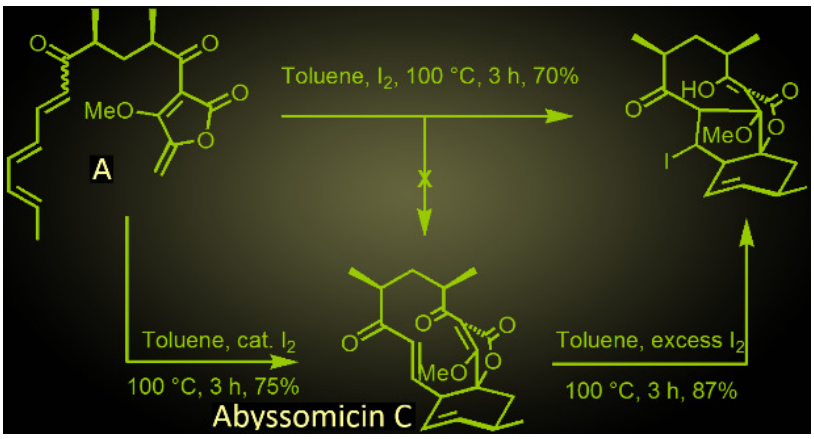
Synthetic Organic Chemistry is one of the sciences involved in the exploitation of natural products as therapeutic agents. Discovering new strategies and chemical reactions, chemists reveal beyond any doubt the complex structure of natural products and create synthetic analogues as possible weapons against diseases. Constructing these molecules step by step in the lab, chemists have the privilege to admire the architectural beauty of natural products and feel like artists.

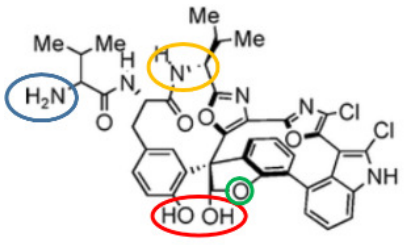


The construction of the anticancer drug Taxol was first achieved in the lab[1] using intermediate A which was constructed by forcing (under special experimental conditions) one electron of each of the two aldehydes to form a bond. The yield was "only" 30%, yet if we presume that in order to cure an infection we have to attack all the cells of a man with penicillin, even if we need one dose only, the yield for the treatment based on amoxicillin would be less than 0.00000001%!

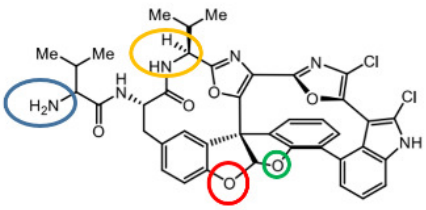


The complicated framework of trichodimerol (TNF-α inhibitor) was achieved biomimetically within two steps, starting from a simple natural product.[2]

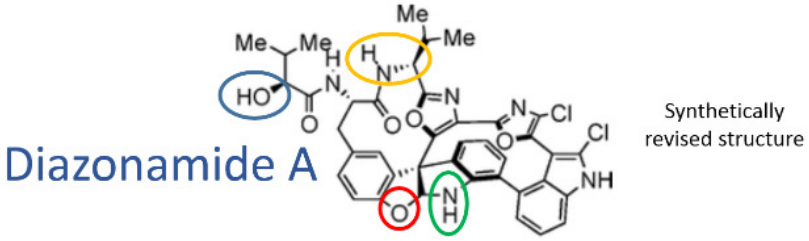




Originally proposed structure



X-ray revised structure



The tricyclic system of Abyssomicin C (inhibits biosynthesis of PABA) was made in one step from mono-cyclic intermediate A[3], using only catalytic amount of simple molecular iodine.

Finally, an imprecise example, indicating the importance of synthetic chemistry for the structure elucidation of the admirable architecture of biomolecules is the case of anti-mitotic Diazonamide[4].

References

1. a. Nicolaou, K.C.; Yang, Z.; Liu, J.J.; Ueno, H.; Nantermet, P. G.; Guy, R. K.; Claiborne, C. F.; Renaud, J.; Couladouros, E.A.; Paulvannan, K.; Sorensen, E.J.; *Nature* , 367,630-634, 1994.
b. Nicolaou, K. C.; Claiborne, C.F.; Nantermet, P. G.; Couladouros, E.A.; Sorensen, E. J.; *J. Am. Chem. Soc.,* 116, 1591-1592, 1994. **c.**Nicolaou, K.C.; Couladouros, E. A.; Nantermet, P.G.; Renaud, J.; Guy, R. K.; Wrasidlo, W.; *Angew. Chem., Int. Ed. Engl.*, 33, Int. Ed. Engl.33, 1994.
2. a. Nicolaou, K.C.; Vassilikogiannakis, G.; Simonsen, K.B.; Baran, P.S.; Vidali, V.P.; Pitsinos, E.N.; Couladouros, E.A. *J. Am. Chem. Soc.*, 122, 3071-3079, 2000.
b. Nicolaou, K.C.; Simonsen, K.B.; Vassilikogiannakis, G.; Baran, P.S.; Vidali, V.P.; Pitsinos, E.N.; Couladouros, E.A. *Angew. Chem. Int. Ed.*, 38, 3555-3559, 1999.
3. Couladouros, E.A.; Bouzas, E.A.; Magos, A.D.; Tetrahydron, 2006, 62, 5272-5279.
4. a. K.C. Nicolaou, David Y.-K. Chen, Xianhai Huang, Taotao Ling, Marco Bella, Scott A. Snyder; *J. Am. Chem. Soc.*, 2004, 126 (40), pp 12888–12896.
b. Nicolaou K.C., Bheema Rao P, Hao J, Reddy MV, Rassias G, Huang X, Chen DY, Snyder SA; *Angew Chem Int Ed Engl*. 2003 Apr 17;42(15):1753-8.