REVIEW

by

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Regarding the dissertation "Identification, analysis and evaluation of pharmacokinetic and pharmacodynamic drug interactions" with author Assoc. Kaloyan Dobrinov Georgiev, D.Sc., for the degree of Doctor of Science in the field of scientific education 7.0 Health and sports, professional field 7.3. Pharmacology, specialty "Pharmacology (including pharmacokinetics and chemotherapy)"

The dissertation submitted for defence consists of 7 chapters, conclusions, references for contributions and publications related to the dissertation, as well as an annex, with a total volume of 320 standard typing pages. The dissertation work is extremely richly illustrated with 78 tables, 77 figures and 32 equations for better understanding. The chapters of the presented author's own results are composed of a brief introduction to the topic, goals, materials and methods, results and discussion, conclusion and literature. A total of 441 titles are sources throughout the bibliography, which are correctly cited in the text. The considerable number of literature sources used clearly indicates the author's high level of awareness regarding the issues under study.

Short biographical data

Kaloyan Dobrinov Georgiev was born in 1978 in Varna. In 2003 he graduated from the Faculty of Pharmacy, Sofia with a degree in Pharmacology. Since 2005, after winning an assistant competition, he has been appointed to the Department of Pharmacology at MU-Varna. In 2010, Kaloyan Georgiev was admitted for a PhD student at the Department of Pharmaceutical Design and Biochemical Pharmacology at the Bulgarian Academy of Sciences (BAS) in Sofia. He has successfully defended his dissertation on Design, Synthesis and Pharmacological Characterization of Peptide Mimetics of Endomorphin-2, Morphiceptin and RGD with Analgesic and Antitumor Activity. He acquired a specialty in Pharmacology in 2010 and Clinical Pharmacy in 2018. From 2015 until now, he holds the academic position of Associate Professor and is the

Head of the Department of Pharmaceutical Technologies, Faculty of Pharmacy, Medical University - Varna.

The actuality of the problem

The dissertation presented is a thorough research in the field of drug interactions. They are extremely relevant nowadays as more and more drugs are being introduced into clinical practice and this contributes to complicating of the used therapies. There is also an increase in patients' consumption of so-called natural products that are described as safe but in many cases may adversely affect the therapy used. Identifying, analyzing, and evaluating such interactions are extremely valuable in order to screen out the beneficial interactions. That is why I think that Assoc. Prof. Kaloyan Georgiev considers a really actual problem in the field of pharmacology and clinical pharmacy in theory and practice. The dissertation is written in accessible language, with good knowledge of specialized literature and practice, and on this basis correct conclusions and actual recommendations are formulated that can be applied in real practice.

The **literature** review covers the main terms and concepts used in the description of drug interactions and examines examples of pharmacokinetic and pharmacodynamic drug interactions. The dissertation's in-depth look at drug-related problems is striking. This chapter is presented on 49 pages with 124 literature sources used.

The purpose of the study is to determine the place of the computer technology in drug interaction studies by applying pharmacometric methods using in vitro, in vivo and in populo data. It is clearly defined, logically substantiated and set out according to the thesis. A total of 11 tasks have been set, which follow the content structure of the dissertation and help to accomplish the goal. This shows that Assoc. Prof. Georgiev is thoroughly familiar with the problem, which he has researched and has the necessary experience and knowledge to accurately analyze the results achieved.

Chapter III is entitled, "Selection, Isolation and Analysis of Plant Extracts and Fractions. Design, synthesis and analysis of oligopeptides". Plant fractions, for which there is no evidence of their involvement in drug interactions, were isolated and analyzed. In addition, in this Chapter, oligopeptide substances have been synthesized and analyzed, which have shown promising results from in vitro and in vivo testing from previous dissertation studies (dissertation for the

acquisition of a Ph.D.). The dissertant demonstrates enviable skills in the processes of drug design, peptide synthesis and analysis, isolation of plant fractions and analysis of biologically active substances.

Chapter IV, "Pharmacokinetic Drug Interaction Studies", is the most comprehensive and detailed in the dissertation (101 pages in total and 97 references). The dissertation in this chapter demonstrates good handling of in vitro screening techniques, with brand new isolated plant fractions and newly synthesized oligopeptides being screened using Vivid kits for possible inhibition of both major cytochrome isoforms - *CYP3A4* and *CYP2C9*, their IC_{50} values, the mechanism of inhibition and their inhibitory constants (Ki) are determined. Then, using common algorithms from executive agencies (such as FDA, EMA, etc.) - basic and mechanistic (static and dynamic) models, the whole pathway for characterizing a substance, with proven inhibition of a drug-metabolizing enzyme, was traced determining its potential to induce drug interactions in clinical situations. A physiology-based pharmacokinetic model (PBPK) has been applied to prepare a PK profile through the *SimCYP* and *ADMEWORKS DDI* platforms of the methylxanthine fraction and oligopeptides. Mastering the complexity of working with these two software, especially the first one (SimCYP), used by every pharmaceutical company in the top 10 in the world, demonstrates excellent handling of sophisticated computer platforms - the skill the dissertant gained during a training course conducted by Certara® (SimCYP).

In this Chapter, the author outlines some peculiarities in the implementation of the mentioned algorithms; in some cases the base models used may not imply a risk of drug interactions, while the applied mechanistic models will detect one. In addition, the increased risk of using static-mechanistic models can be neglected when applying dynamic models. The choice of the appropriate analysis software is also essential, as there may be discrepancies in the results obtained with the individual software on the market.

In addition, it is worth mentioning the challenges the dissertant has undertaken: on the one hand building PBPK models of plant fractions based on a major component, and on the other hand, oligopeptides for which there is insufficient physical-chemical and PK data collected. This demonstrates the excellent theoretical background and confidence of the author and his ability to combine the knowledge he has acquired in the educational degrees heretofore obtained - Master of Pharmacy, Specialist in Pharmacology and Doctor of Chemistry.

In **Chapter V**, "Pharmacodynamic Drug Interactions", plant-derived plant fractions (Bancha, Pu-erh and L.barbarum) were tested on tumor cells in vitro by breast cancer (major) and the combination effect with doxorubicin was determined. Experimental animals (rats) were also used to demonstrate the organoprotective activity of these fractions on cardio- and nephrotoxicity induced by doxorubicin. Of particular interest is the combination of these two activities – enhancement of the therapeutic effect of one hand and organoprotective on the other is demonstrated and completely adequately selected methods by the dissertant. This outlines the need to monitor not only the beneficial synergistic effects observed with respect to the pharmacological target, but also the need to evaluate the effects on the so-called, off-targets, when using a combination.

In **Chapter VI**, Study and Analysis of Pharmacokinetic and Pharmacodynamic Drug Interactions, Clinical Practice, the author selected a population of patients with heart failure (HF). The value of labor lies in identifying and analyzing the main potential drug-drug interactions (pDDIs) in this population using one of the most commonly used clinical drug analysis software – Lexicomp® drug interactions. The risk factors that lead to an increase in their frequency have been identified. The author draws serious attention to the need to introduce clinical pharmacists into clinical practice, especially in high-risk patients that will provide pharmaceutical care and increase patient involvement and adherence to prescribed treatment. In addition, the author also uses the SimCYP simulator to analyze the pharmacokinetic interactions associated with CYP2C9, CYP3A4, and P-gp, responsible for the most common detected interactions between standard therapy used in HF patients and low therapeutic index drugs, as this demonstrates the broad applicability of this platform - both in the preclinical as well as in the clinical stages of single drug monitoring.

Conclusions and contributions

The contributions and conclusions of the thesis are extremely valuable and are formulated based on many experimental and clinical studies, combined with the addition of computerized simulation methods, and can be summarized as follows:

- Pharmacokinetic interactions of isolated methylxanthine fractions from Bancha and Pu-erh tea leaves, isolated fractions (polysaccharide, pectin-free and total extract) from

L.barbarum (Goji berry), as well as newly synthesized endomorphine-2 analogues have been characterized for the first time with respect to CYP3A4 and CYP2C9 isoenzymes.

- PBPK models of plant fractions with proven major components as well as newly synthesized oligopeptides based on in silico data were prepared and simulations for possible drug interactions with CYP3A4 substrates were performed.
- The individual algorithms and models for tracking proven enzyme inhibition in the development of new drugs have been evaluated, as well as the individual simulation programs used to evaluate drug interactions.
- For the first time, the antiproliferative effects of isolated methylxanthine fractions from Bancha, Pu-erh and L.barbarum fractions on breast cancer cell lines were determined. The combination potential of doxorubicin co-treatment and the organoprotective effect in rats of doxorubicin-induced organotoxicity were determined. The results indicate that these fractions could be combined with doxorubicin in the treatment of mammary tumors, as they increase its antitumor activity on the one hand and reduce its dose-limiting side effects (cardio- and nephrotoxicity) on the other.
- Patients with heart failure are elderly, with many concomitant diseases, taking many drugs (polypharmacy), which makes them more likely to develop drug interactions. The physicians of these patients should always be on the lookout for potential drug interactions. To reduce the risk of potential drug interactions, appropriate software should be used to analyze the prescribed therapy and to include a clinical pharmacist in the multidisciplinary team.

Scientometric indicators

The dissertant presents 19 publications, eight of which have been used in previous competitions and eleven that have not been used. In 16 of them, Assoc. Prof. Georgiev is the first author, in 2 - second author and in 1 - third author, which clearly shows the main contribution of the author in the presented works. Two of the publications are in Impact Factor journals - 4.011 (Scientific reports) and 1.69 (International journal of clinical pharmacy). This complies with the requirements for the "Doctor of Science" degree.

Part of the dissertation work was funded by a project from the Science Foundation 2016 at the Medical University of Varna - "Study of drug interactions at the level of biotransformation".

Critical notes and recommendations

In dissertation it is not specified the scientific forums in which the dissertation data were reported.

Conclusion

The dissertation work of Assoc. Prof. Georgiev "Identification, analysis and evaluation of pharmacokinetic and pharmacodynamic drug interactions" is up-to-date and meets the scientometric criteria, as well as the rules for academic development of the Medical University - Varna for awarding the scientific degree "Doctor of Sciences".

For this reason, I recommend the Honorable members of the Scientific Jury to vote positively for the degree "Doctor of Science" in Pharmacology to Assoc. Prof. Kaloyan Dobrinov Georgiev, Ph.D.

17.02. 2020

Reviewer:

/Prof. Dr. Marieta Georgieva, MD, PhD/