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Warsaw, 16. 12. 2018

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REVIEW REPORT OF THESIS SUBMITTED FOR DEGREE OF DOCTOR OF PHILOSOPHY

1. General Information

The title of the Thesis: Prediction of pro-arrhythmic activity in humans with the use of physiologically based pharmacokinetics and pharmacodynamics modelling

The Author: Nikunjkumar K. Patel

Supervisor: Dr hab. Sebastian Polak

Co-supervisor: Dr Barbara Wiśniowska

Doctoral dissertation submitted to: Faculty of Pharmacy, Jagiellonian University Medical College

Doctoral dissertation form: The doctoral dissertation was submitted in the form of a coherent collection of published articles according to Article 13 (2) to ACT of March 14, 2003 on academic degrees and academic title, and on degrees and title in the field of art.

Number of original publications: 4

Cumulative 5 years impact factor of publications applied for thesis preparation: 16.041

Submission date: September 2018

2. The Context, Aims and Scope of the Thesis

The drug safety assessment, in addition to the efficacy and quality, is one of the three fundamental elements of the pre-marketing evaluation of investigational medicinal products. The process of research and development of new molecular entities is inseparable from efficient and sensitive safety evaluation, it begins with preclinical *in vitro* and *in vivo* modeling and is carried out through all phases of human testing till detailed surveillance of postmarked use. One of the leading causes of investigative drug attrition during development is cardiotoxicity. As it was pointed by *Gintant et al.* „preclinical cardiotoxicity screening efforts should efficiently detect unsafe compounds sufficiently early on in the drug discovery process to influence the candidate drug selection, thus reducing later-stage attrition, risks to participants in clinical studies, development time and costs. However, over-simplified and highly sensitive (but not specific) approaches for the early detection of cardiac safety liabilities can result in unwarranted attrition of novel drug candidates owing to false-positive findings. The risk of such unwarranted attrition may also be increased by a lack of concordance between the effects of compounds in animals (or animal-derived tissues) and those in humans" (Nat. Rev. Drug Disc. 2016).

The strong focus in the current approach to cardiotoxicity assessment of drugs is put on drug-induced Torsades de Pointes (TdP) - life-threatening ventricular arrhythmia, which is connected with prolongation of the QT interval on a surface electrocardiogram (ECG). For the purpose of assessment of the proarrhythmic potential of new drugs, in July 2013, US Food and Drug Administration (FDA) introduced the initiative of Comprehensive in Vitro Proarrhythmia Assay (CiPA). The assay presents a fusion of various methodologies combining an assessment of *in vitro* drug effects on multiple cardiac

channels together with *in silico* reconstruction of cardiac action potential and confirmation using human stem-cell derived cardiomyocytes.

Mr Patel's PhD thesis focuses on novel approaches to application of existing translational tools together with the development of methodology, which allow to predict the risk of cardiotoxicity of active pharmaceutical ingredient (API) in terms of QT interval prolongation in the ECG. The work is directly linked to the assumptions of CIPA initiative. The concept of the thesis shows the application of Quantitative Systems Toxicology and Safety (QSTS) modeling strategies from early stages of drug discovery and development together with information obtained during life-cycle of medicinal products for assessment of cardiac safety.

The Author proposed the three main objectives of the work.

1. Development of QSTS model mimicking circadian variability in heart rate taking into account age and gender effects. The verification of the model was carried out with a widely accepted model drug used for positive control of Thorough QT (TQT) studies – moxifloxacin.
2. Preparation of mechanistic modelling based framework for screening molecules in early development stage as well as to optimize the clinical trials by initial simulations of virtual populations e.g. TQT trials. Application of developed modelling framework for postmarketing monitoring and understanding of personalized toxicity of drugs.
3. Application of comprehensive mechanistic modeling using Cardiac Safety Simulator (CSS) for evaluation of cardiac risk profile for active pharmaceutical ingredient and its electro-physiologically active metabolites.

3. Structure, Content, and Layout of the Thesis

The doctoral dissertation of Mr Patel was prepared on the base of four scientific articles published between 2013 and 2018 in high quality pharmaceutical journals. Three publications were published in *AAPS Journal* - the official journal of the American

Association of Pharmaceutical Scientists (5-year IF 4.911) and one was published in *Theoretical Biology and Medical Modeling* (5-year IF 1.308).

The thesis consists of six chapters, which present the clear description of the state of the art, concise methodology characterization, results description and discussion. The introduction and discussion are supported with references to 144 publications. The structure of the thesis is deliberate and transparent.

The concept of the dissertation is presented as a coherent collection of previously published articles, which were chosen to fulfill the main goals of the work.

In **publication I** (AAPS J. 2018 Jul 11;20 (5): 83) the virtual TQTsimulations is proposed as a tool applied prior clinical test, which could help, optimize, refine or avoid clinical study. As an example for virtual TQT simulations two related drugs Tolterodine (TOL) and fesoterodine (FESO) were applied. In case of TOL it was feasible to simulate the QT changes in the range of therapeutic and supratherapeutic doses within 95% confidence interval limits of observed data. The model was also able to predict differences between poor and extensive metabolisers. The example of FESO confirmed the model's ability to differentiate between QT prolongation induced by drug and its active metabolite 5-hydroxymethyl Tolterodine (5-HMT). The work demonstrated the usefulness of virtual TQT trials simulated with the QSTS approach.

Publication II (AAPS J. 2018 Mar 14;20 (3): 47) was focused on the application of PBPK- QST model of predictive risk assessment for novel compounds. The progressive refining of the model according to increasing clinical knowledge and enrichment in predict-learn-confirm cycles. Using moxifloxacin (MOXI) as a model drug with abundant clinical cardiac safety data. The PBPK simulations of drug concentrations in the systemic circulation and heart tissue together with *in vitro* measurements of inhibition of ion channel were used to predict QT prolongation in healthy patients. The predicted results closely reproduced the clinical observations. The arrhythmia was not observed even in case when exposure on MOXI increased 10 times. The combination of the same exposure levels with the presence of physiological risk factors, e.g., hypokalemia and tachycardia, caused the observation of arrhythmic events in simulations, which was consistent with reported

moxifloxacin-related TdP events. The results of the work showed that the application of a progressive PBPK-QST cardiac risk assessment methodology in early drug development stage could direct drug development decisions and in post-approval risk management it could be used for minimizing risk in clinical scenarios.

Publication III (AAPS J. 2017 Nov 27;20 (1): 6) deals with the personalization of the results of the developed methodology. For this purpose interesting concept of „virtual twin" for real patient was proposed, the QST model for citalopram (CT) was established, which allowed to predict cardiotoxic effects of clinical scenarios previously reported in the literature. The results of predictions were then compared with the clinical data of real patients. The QST model took under consideration CT and its electophysiologically active metabolites – desmethylcitalopram and didesmethylcitalopram. The results of the work showed the importance of a multifactorial approach to modeling of cardiotoxic effects of drug on various levels of exposures (therapeutic and supratherapeutic). This mechanistic modeling approach presented in the work could bridge the gap between preclinical cardiac safety assessment and clinical toxicology.

Last publication in the cycle and the oldest one – **publication IV** (Theor Biol Med Model. 2013 Feb 9;10:7) presents the development of the circadian model of heart rate as a covariate of age and gender. The model was established by application of publicly available data set describing the circadian changes of the heart rate of 18 healthy subjects. Its external validation was carried out with the reference to clinical research database containing heart rate measurements derived from 67 healthy subjects. The developed model was integrated with ToxComp platform. Its performance was assessed using MOXI as a model drug.

In the publications I-III Mr Patel is the first author, in the publication IV he is on the second place in autorship list.

4. The Main Findings of the Thesis and Contribution to the Discipline

The evaluation of the balance between toxicological risks and therapeutic benefits in the early stage of development of novel molecules is challenging and usually it is connected

with inherent uncertainty. The researcher always suffers from the lack of the information, from the other hand the ethical and financial reasons never allow to collect the satisfactory data set. Moreover, when translating information acquired at the preclinical stage into clinical situation, a number of factors at the human physiology and population levels have to be taken into account. It is especially difficult in the case of cardiotoxicity evaluation. From this point of view, the development and improvement of *in silico* methods, which allow to fill the gap between preclinical studies and post-approval monitoring of drug safety is crucial. The current thesis develops these issues on various levels, to the most important achievements of work should be included.

- Improvement of existing methodology with the introduction of additional factors to improve its performance – the work proposes development of the circadian model of heart rate.
- The proposal of the iterative approach to QSTS modelling with the advancement of the model through „learn-confirm-predict-apply" paradigm.
- The inclusion into the model the evaluation of cardiotoxicity of active metabolites.
- The presentation of methodology of personalization of the results by „virtual twin" generation.

The presented data are of great importance and value. For a special distinction deserves methodology of data collection presented in the work. It must be pointed out that it is practically impossible to find the data sets, which would be sufficient and could be directly applied for the model development. Therefore, the information is collected from various sources and included into the model. The work presents a modern approach to the chosen complex problem of cardiotoxicology, which include integration of preclinical and clinical data, detailed physiology descriptions of the population. Presented approach – is in my opinion – the future of preclinical studies and subsequent post-marketing safety risk assessment.

5. Questions and Critical Remarks

Even though the thesis was prepared correctly, there are some questions and minor errors.

Questions:

- In various stages of the studies, four various substances were chosen. What was the base of such a choice? Why it was not referred to substances presented in List of 28 compounds, which were chosen to be tested in Comprehensive In Vitro Proarrhythmia Assay paradigm?
- The work is focused on the cardiotoxic effects of drugs and their active metabolites. Why the cardiotoxic impurities of active substances were not taken under consideration or even mentioned in the discussion?
- On page 25, there is an information that the IC_{50} for I_{Kr} inhibiton by MOXI ranged from 0.93μm to 398μm. Why the value 29μm for I_{Kr} inhibition was chosen as most biorelevant?
- The methodology of development of „virtual tween" for CT (p. 27) is an excellent example of effective application of data originated from various sources. How the data obtained from different publications was validated or verified?
- What was the age range estimations in particular models?
- What were the limitations of proposed methodology?
- On page 43 the Author states that „the slope of simulated ΔΔQtcF-concentration relationship (10-15ms/ng/mL) were very similar to reported from clinical data analysis". How the similarity of the slopes was assessed?

Critical remarks:

- The work contains a number of abbreviations, which are only partially listed in the Abbreviation list (p. 9).
- Page 20: misspelling -5MHT instead of 5HMT.

The shortcomings do not substantially affect the quality of the work and do not disqualify it in any way.

6. Conclusions

Since the first discussion of CiPA in 2013, significant progress has been made to define, standardize, and validate more comprehensive, mechanistic-based testing system. The dissertation of Mr Nikunjkumar K. Patel contains original and valuable scientific results and is in line with current trends in the discipline. In my opinion the requirements concerning PhD thesis defined by Polish law have been fulfilled and I recommend to admit Mr Patel to further steps of the PhD procedure.

I request the High Council of Faculty of Pharmacy of Jagiellonian University Medical College to recognize the doctoral thesis with *summa cum laude* award.

Zastępca Dyrektora
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