Development of an Adverse Outcome Pathway for cardiotoxicity mediated by the blockade of L-type calcium channels

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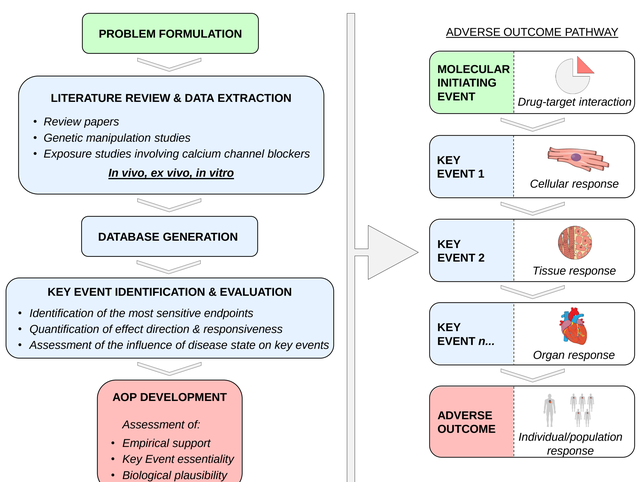
PROJECT BACKGROUND & AIM

- In 2012, the OECD launched an ambitious programme aimed at mapping the cascades of causally-related events that link the perturbation of molecular targets (e.g. drug-target interactions) to the manifestation of adverse health effects at higher levels of biological organisation. A conceptual multi-scale model, called Adverse Outcome Pathway (AOP), has been proposed as the pragmatic tool that would enable this large-scale mapping exercise[1].
- The vision underlying the AOP development programme is that weight-of-evidence-based mechanistic knowledge, centralised using a Wiki format, may improve the prediction of adverse effects caused by exposure to chemicals. The AOP knowledgebase may also facilitate the implementation of the 3Rs vision in current and future safety testing strategies.
- So far, AOP development efforts have been driven by the safety assessment of industrial chemicals and personal care products. The work described here is one of the first attempts to explore the applicability of the AOP concept to drug safety assessment.
- Cardiotoxicity was identified as an area of potential interest for AOP development by a network of experts convened by the UK NC3Rs and the European Union Reference Laboratory for alternatives to animal testing (EURL-ECVAM) in 2015. The blockade of L-type calcium channels (LTCCs) was proposed as one of the priority molecular initiating events (MIE) that may benefit from the AOP vision.
- LTCCs are responsible for the excitation-contraction coupling of skeletal, smooth, and cardiac muscle. Pharmaceuticals that unintentionally block these channels in cardiac cells may impair heart function and health.
- The aim of our research was to develop an AOP that describes the causal links between LTCC blockade and adverse cardiovascular effects.

METHODOLOGICAL APPROACH

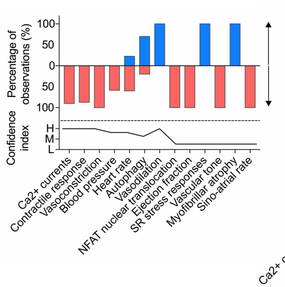


RESULTS

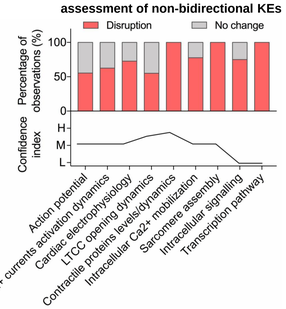
We extracted data from more than 150 primary publications that investigated a) the effects of calcium channel blockers (CCBs) on different components of the cardiovascular system, and b) the effects of genetic manipulations of the drug target. We generated a database containing over 1,100 *in vitro, ex vivo* and *in vivo* data points. The database was used to identify potential Key Events (KEs).

For each KE, we performed a quantitative analysis to determine effect direction and degree of responsiveness, as displayed in the figures below. We also assigned a database-specific confidence index to each KE, according to the degree of reproducibility of the effect (H: High; M: Medium; L: Low). This approach was used in combination with the assessment of biological plausibility to identify the final KEs characterized by the highest level of confidence.

Effect direction and confidence assessment of bidirectional KEs



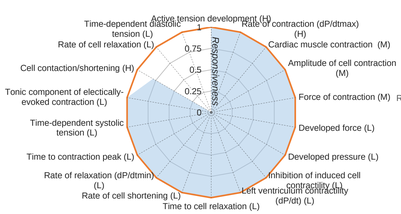
Effect direction and confidence assessment of non-bidirectional KEs



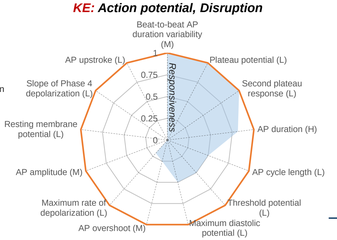
In some cases, KEs can be quantified using a variety of specific endpoints. The analysis of the responsiveness of each endpoint subsequent to LTCC blockade provided clear indications of the sensitivity of each measurement, and of the frequency at which statistically significant effects were observed. This knowledge has both biological and methodological significance. In the latter case, it can effectively inform the development of suitable testing strategies aimed at maximizing the probability to detect changes in a given KE. An example of responsiveness analysis is provided below for two different KEs.

ENDPOINT-SPECIFIC RESPONSIVENESS ANALYSIS

***KE:* Contractile response, Disruption**



***KE:* Action potential, Disruption**



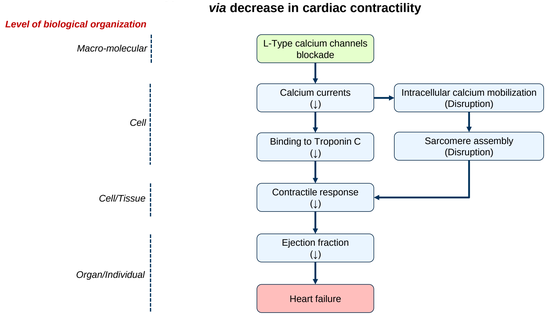
No. of data points = 93 **No. of data points = 65**

The development strategy used in our study led to the identification of two different AOPs that are relevant to cardiovascular physiology. The two AOPs share the same MIE and two different KEs, and can be considered either individually or as a functional network. The evidence underlying each qualitative inter-KE relationship is strong in most of the cases, and moderate in others. A full description of the evidence assessment is described in the related AOPWiki projects (AOP 261 & 262).

Several *in silico* methods are currently available to determine the quantitative relationship between various KEs in the two proposed AOPs in humans[2,3]. Future development efforts will be aimed at combining those different methods to develop a fully quantitative AOP network.

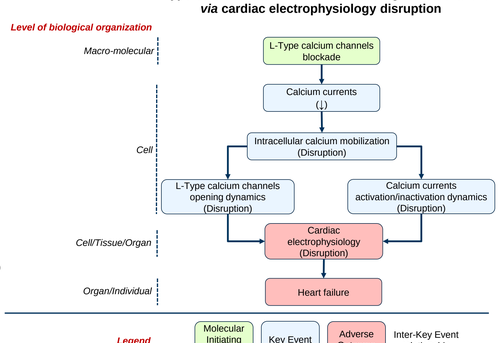
AOP 1

L-Type calcium channel blockade leading to heart failure *via* decrease in cardiac contractility



AOP 2

L-Type calcium channel blockade leading to heart failure *via* cardiac electrophysiology disruption



TAKE-HOME MESSAGES

- We developed two different AOPs that describe the adverse cardiovascular effects triggered by the blockade of L-type calcium channels (LTCC). Specifically, LTCC blockade leading to heart failure *via* a) decrease in cardiac contractility, and b) disruption of cardiac electrophysiology.
- We developed a novel data visualisation approach to support Weight of Evidence analysis, and guide the identification of the most appropriate testing strategies for the quantification of each Key Event, in line with the 3Rs vision.
- Several *in silico* methods are currently available to determine the quantitative relationship between various Key Events in the two proposed AOPs. Future development efforts will be aimed at combining those different methods to develop a quantitative AOP network.

ACKNOWLEDGEMENTS

This work was funded by a UK NC3Rs Strategic Award granted to Dr Luigi Margiotta-Casaluci (NC/R001243). The full details of the development work presented here will be described in the AOPWiki projects 261 & 262. An additional AOP (LTCCs blockade leading to the disruption of vascular tone maintenance) has been developed during the project, but it is not shown in this poster. A dedicated AOPWiki Project for this additional AOP will be available in 2019.

We would like to thank Professor Chris Denning (University of Nottingham, UK) and the participants to the NC3Rs Cardiovascular Showcase Event 2018 for the valuable feedback.

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GET IN TOUCH

We are very interested to explore the potential applicability of the AOPs presented here to realistic drug safety assessment scenarios. This project was only the first step of a more ambitious programme that will ideally lead to the development of a quantitative AOP network able to support decision-making.

If you have any feedback, or would like to discuss potential collaborations for future development phases, please contact Dr Luigi Margiotta-Casaluci (Luigi.Margiotta-Casaluci@brunel.ac.uk).

REFERENCES

[1] *Villeneuve et al. (2014) Adverse Outcome Pathway (AOP) Development I: Strategies and Principles. Toxicological Sciences, 142: 312–320.* ***[2]*** *Passini et al. (2017) Human in silico drug trials demonstrate higher accuracy than animal models in predicting clinical pro-arrhythmic cardiotoxicity. Frontiers in Physiology 8:668* ***[3]*** *Land et al. (2017) A model of cardiac contraction based on novel measurements of tension development in human cardiomyocytes. Journal of Molecular & Cellular Cardiology 106: 68–83.*