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

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INTRODUCTION

- A diverse set of chemical compounds, including some pharmaceuticals and insecticides, have the potential to perturbate the functionality of calcium channels.
- Among the different types of calcium channels, the L-type calcium channel (LTCC) is responsible for the excitation-contraction coupling of skeletal, smooth, and cardiac muscle. Chemicals that unintentionally block this channel may impair cardiovascular functions.
- Pharmaceuticals that act as calcium channel blockers (CCBs) are also present in the aquatic environment at very low concentrations[1]. However, very little is known about the potential effects of CCBs on non-target species.
- Relevant mechanistic multi-scale models may represent useful tools to support hazard and risk assessment of these compounds for both humans and wildlife.
- The aim of our research was to develop an Adverse Outcome Pathway (AOP) that describes the causal links between LTCC blockade and adverse cardiovascular effects.

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 a) heart failure via the decrease of cardiac contractility, and b) disruption of cardiac electrophysiology.
- We proposed 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.
- The two AOPs have been developed using mammalian data. However, the high degree of evolutionary conservation of LTCC suggests that the domain of taxonomic applicability could be extended to fish species, enabling their application to ecotoxicology research questions.

METHODOLOGICAL APPROACH

Problem formulation

Literature review & Data extraction

- Review papers
- CCBs exposure studies
- Genetic manipulation studies

In vivo, ex vivo, in vitro

Database generation

Key event identification & evaluation

- Quantification of effect direction & responsiveness
- Identification of the most sensitive endpoints
- Assessment of the influence of disease state on each key event

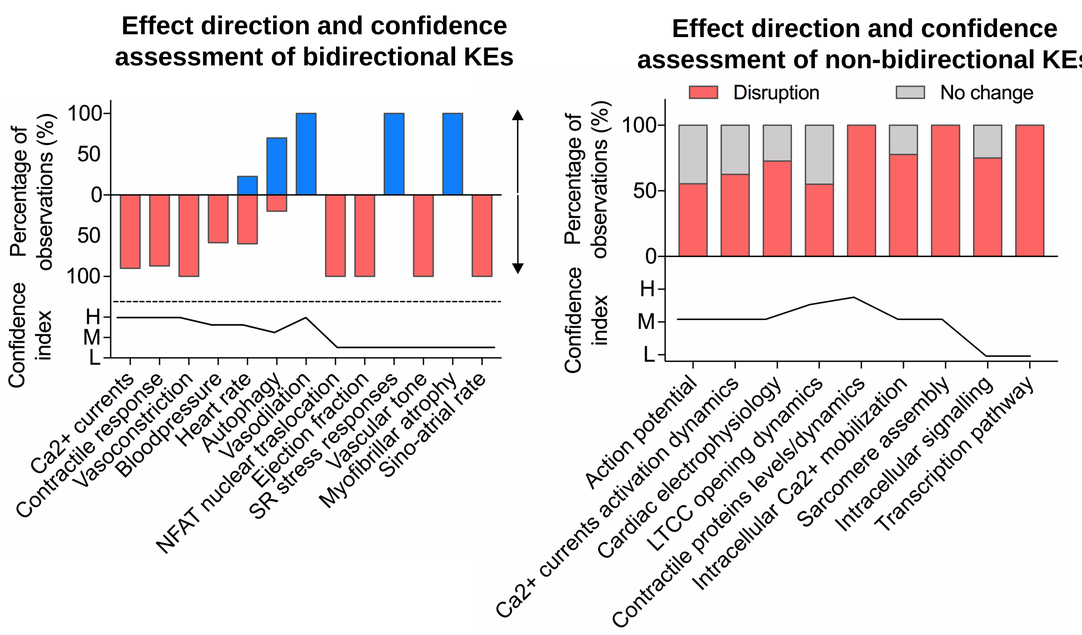
AOP development and assessment

Assessment of Key Event essentiality, biological plausibility of inter-key event relationships, & empirical support

RESULTS

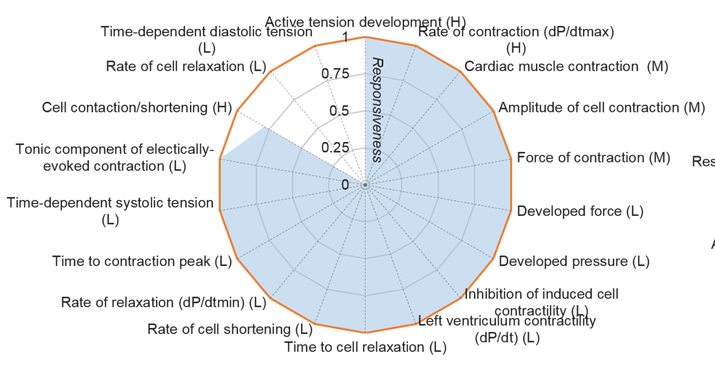
We extracted data from over 150 primary publications that investigated a) the effects of CCBs on different components of the cardiovascular system, and b) the effects of genetic manipulations of the Molecular Initiating Event (MIE). We generated a database containing over 1,100 *in vitro, ex vivo* and *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.



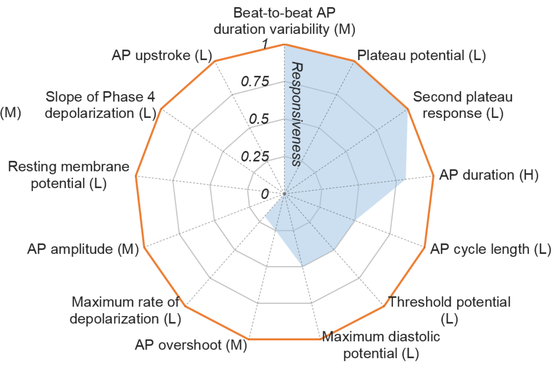
In some cases, KEs can be quantified using a variety of specific endpoints. The analysis of the responsiveness of each endpoint subsequent to LTCC blockade provided clear indications of the sensitivity of each measurement, and of the frequency at which statistically significant effects were observed. 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.

KE: Contractile response, Disruption



No. of datapoints = 93

KE: Action potential, Disruption

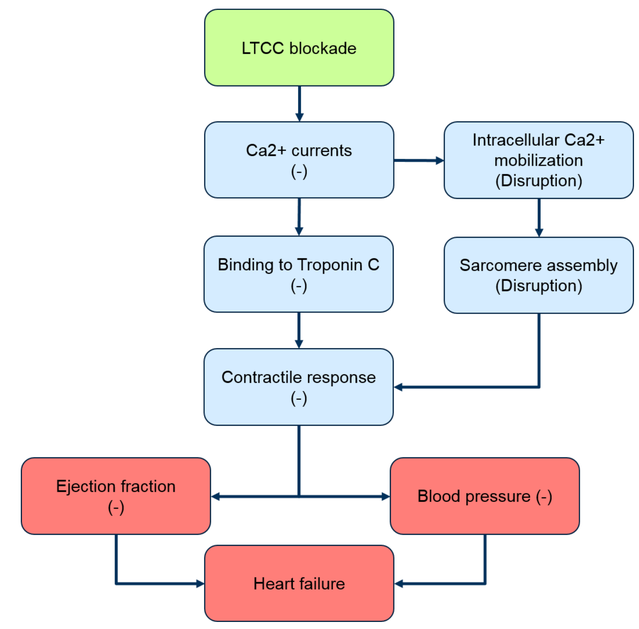


No. of datapoints = 65

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 inter-KE relationship is strong in most of the cases, and moderate in others. A full description of the evidence assessment is available 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.

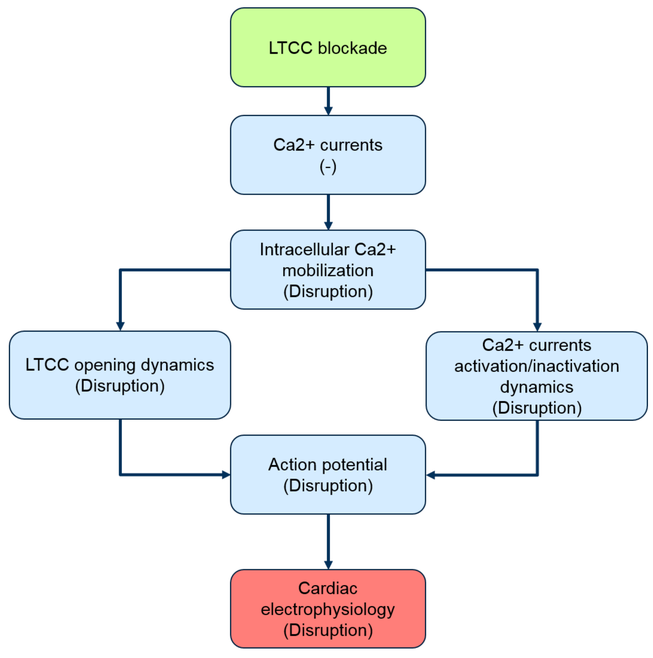
AOP 1

L-Type calcium channel blockade leading to heart failure



AOP 2

L-Type calcium channel blockade leading to the disruption of cardiac electrophysiology



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[1] Saari et al. (2017) Chemosphere 189, 466-478. [2] Passini et al. (2017) Frontiers in Physiology 8:668 [3] Land et al. (2017) Journal of Molecular & Cellular Cardiology 106: 68–83

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