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Interpreting In Vitro Pharmacological Profiling Data Requires Contextualisation of Risk in Relation to the Safety Margin for Different Off-target Interactions: Translational Pharmacology Case Studies

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A key element of drug discovery safety is in vitro pharmacological profiling of new chemical entities against a panel of molecular targets to understand off-target activity, and therefore likelihood of unwanted effects. When decisions to prioritise and progress compounds are made, contextualised understanding of how different off-target pharmacologies translate to specific safety risks is often missing.[PM1] A safety margin of 100-fold between off-target in vitro potency and either primary target potency or predicted efficacious exposures is generally applied. However, for some targets this may be misleading as translation from in vitro to in vivo can vary depending on the target. Here we describe an approach that provides semi-quantitative understanding of in vitro to in vivo translation for three targets with safety liabilities: alpha$_{1A}$ adrenoceptor antagonism for hypotension, ALK4/5 inhibition for heart-valve pathology and adenosine transporter inhibition for dysponea. We assessed the relationship between in vitro IC_{50}/K_i measured in a radioligand binding assay (alpha1A, AOT) or enzyme activity assay (ALK4/5) to the in vivo exposure required for these unwanted effects, using collated literature information on 4-5 pharmacologically active compounds per target. The outcome of this approach concluded coverage of in vitro potency for alpha$_{1A}$ adrenoceptor, ALK4/5 and AOT was 10-30x Ki, 75-180x IC50 and 0.3-5x Ki respectively, to elicit the safety effect. This illustrates translation of in vitro target interactions to in vivo effect varies across targets, likely due to the influence of biology and cellular pathways at play in vivo, and this must be considered when interpreting in vitro data.

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Inhibition of Bile Salt Export Pump (BSEP) in Relation to Systemic Exposure: A Risk Factor for Drug Induced Liver Injury (DILI)

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Background: Drug-induced Liver Injury (DILI) is one of the most frequent causes of drug failure. Inhibition of the bile salt export pump (BSEP) has been strongly implicated in the incidence of DILI.

Objectives: To determine a BSEP safety Margin (IC_{50}/free Cmax in plasma) for use in risk assessment during drug discovery and development.

Methods: BSEP IC_{50}s were determined in an everted vesicle assay. Marketed drug annotations were taken from the Liver Toxicity Knowledge Base (LTKB) and post-marketing adverse event database (FAERS) taken from Pharmapendium.

Results: 95% of 'No-DILI-concern' compounds (from LTKB) had an exposure margin of >500, whereas 75% of 'Most-DILI-concern' compounds had a margin of <500. Next we compared this same set of compounds with the percent clinical incidence of 'hepatic and hepatobiliary diseases' taken from FAERS. The 'No-DILI-concern' group had a mean of 1.9% incidence compared with 11% in the 'Most-DILI-concern' group. 100% of compounds with an incidence of <1.9% had an exposure margin >500 and 100% of compounds with an incidence of >11% had an exposure margin of <500. BSEP exposure margins did not correlate with the type of DILI (cholestatic or hepatocellular).

Conclusions: In the absence of local liver concentrations, free plasma Cmax is a good surrogate for calculating exposure margins. A safety margin of 500-fold is recommended. A low BSEP exposure margin will greatly increase the risk for DILI. BSEP inhibition and exposure margins should be determined as early as possible to allow mitigation prior to clinical candidate selection.

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Adverse Drug Reactions of Marketed Drugs: How Can We Use Them to Our Advantage?

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Background: All marketed drugs have adverse drug reactions (ADRs). Most unwanted effects are associated with particular targets, occurring in the same way as the desired therapeutic effects. Identification of target-ADR associations can help drug discovery programs to avoid them prior to development candidate selection. However, identifying such links is not trivial and is usually deduced from different sources, such as animal and human pharmacology, human genetic mutations and animal genetic manipulations.

Objectives: Can drug exposure information, coupled with clinical trial and post-marketing adverse event reports from FAERS, be used to support the evidence for individual target/ADR linkages and be used to determine safety margins for the support of off-target effect mitigation?

Methods: A marketed drug database has been set up with annotations for >2800 small molecule drugs. More than 1400 compounds have been tested through secondary pharmacology panels. Free Cmax values at the highest recommended dose were available for about 400 compounds. FAERS reports have been extracted from Pharmapendium.

Results: Examples of safety margins determined using FAERS, exposure and secondary pharmacology data will be presented (e.g. KDR and EGFR).

Conclusions: Drug exposure information is essential to provide translation between individual target based assays and ADRs. Clinical trial and post-marketing adverse event reports (FAERS) support the evidence for individual target/ADR linkages. Safety margins can be determined for use in off-target effect mitigation and risk assessment.

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Adult Human Primary Cardiomyocytes: An Integrative Translational Model for Preclinical Drug Testing

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Adult human cardiac tissues provide a much-needed integrative preclinical model to reliably assess the toxicity risks of new drugs. To

this aim, we have established methodologies that consistently allow the procurement and experimental interrogation of human heart tissue preparations. These ex-vivo cardiac models enable drug discovery projects to generate predictive human-based data at the preclinical stage. In order to further develop the throughput and scalability of the human ex-vivo heart model, we have developed novel methodologies for the isolation of adult human primary cardiomyocytes. Each isolation yields Ca^{2+}-tolerant cells that retain rod-shaped morphology, exhibit cross striations and contract/relax in response to field electrical stimulation. The cells also display the ability to adapt to changes in pacing frequency. To validate the use of these cells in predicting drug effects, we have assessed the effects of reference drugs on the excitation-contraction coupling. Specifically, we have measured the effects of reference drugs with known degrees of risk in human, similarly to the validation strategy adopted by the Comprehensive In Vitro Pro-arrhythmia Assay initiative. This initial validation of human primary myocytes for the assessment of drug risk potential will provide an important preclinical tool for risk assessment. In addition to the study of normal adult myocytes described in the present abstract, the opportunity now exists for the use of adult cardiomyocytes from highly prevalent disease conditions (diabetes, cardiac hypertrophy, heart failure, etc.), and therefore, for the ability to assess how cardiac toxicity risk may be affected by common comorbidities.

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Antioxidant Tiron Protects Against Doxorubicin-Induced Cardiotoxicity

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Doxorubicin is one of the most effective anti-cancer agents. However, its use is associated with adverse cardiac effects, including cardiomyopathy and progressive heart failure. Doxorubicin has been demonstrated to induce cardiac toxicity through the formation of free radicals (Davies and Doroshow, 1986 J Biol Chem 261:3060-3067). The antioxidant Tiron has been shown to protect against reactive oxygen species (ROS) related injury in skin cells (Oyewole et al, 2014, FASEB 28:485-494). Given our recently reported beneficial effects of Tiron against doxorubicin induced myocardial injury (Chiuzbaian et al, 2015 BPS pA_2 Online) we further investigated the effects of Tiron (0.25mM-0.5mM) on doxorubicin-induced (1μM) cardiac dysfunction in naïve and stressed conditions. Drug-induced effects were assessed using isolated cardiomyocytes and cell viability and ROS levels measured following drug-treatment. Human cancerous cell lines (HepG2 and HL60) were used to evaluate the effects of combined treatment of doxorubicin and Tiron on the cytotoxicity of doxorubicin in a cancer cell line. Doxorubicin caused a significant impairment of cardiac function and decreased the myocyte viability in both naïve and stressed conditions. Interestingly, co-treatment of doxorubicin with Tiron attenuated these detrimental effects of doxorubicin. Co-incubation of TIron with doxorubicin did not reduce the cytotoxicity of doxorubicin against HepG2 and HL60 cells. These data suggests that the antioxidant Tiron protects the heart against doxorubicin-induced cardiac injury. Data presented in this study highlights the potential of Tiron as protective adjunctive agent to ameliorate doxorubicin-induced cardiotoxicity without affecting its anti-cancer properties.

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Sunitinib-Induced Cardiotoxicity is Age Dependant and Involves Mitogen Activated Kinase Kinase 7 (MKK7)

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Introduction: Sunitinib is linked to adverse cardiovascular events. Mitogen activated kinase kinase 7 (MKK7) is involved in cardiac injury development. We investigated MKK7 involvement in age related, Sunitinib induced cardiotoxiciy by measuring haemodynamic parameters, cardiac-injury specific microRNAs and MKK7 expression in aged rats (12 week, 1 year and 2 year).

Methods: Langendorff perfused hearts were treated with Sunitinib (1μM) for 120 minutes and (a) triphenyl-tetrazolium chloride stained infarct assessment, (b) qRT-PCR analysis for myocardial injury associated miRNAs (miR-27a, miR-133a and miR-133b) or MKK7 mRNA or (c) Western blot analysis for p-MKK7 levels.

Results: Sunitinib treatment of all age groups significantly increased infarct size. Left ventricular developed pressure and heart rate reduced significantly following Sunitinib administration in 12 week and 1 year old rats. Sunitinib treatment (i) decreased the expression of miR-27a in both 12 week and 1 year old rats, (ii) increased the miR-133a expression in 12 week and decreased miR-133a expression in 1 year old rats, while (iii) miR-133b expression was increased in 12 week old rats. The MKK7 mRNA expression and p-MKK7 level was significantly decreased in 12 week old, MKK7 mRNA was significantly increased in 2 year old, while p-MKK7 was increased in 1 year old rats treated with Sunitinib.

Conclusion: This study highlights the complexity of drug-induced cardiotoxicity. This study indicates the in an acute model of drug administration aged rat hearts are less susceptible to Sunitinib-induced cardiac injury. Sunitinib-induced cardiotoxicity alters microRNAs linked to myocardial-injury and involves MKK7 at transcriptional and post-translational level.

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Assessment of Electrophysiology, Contractility and Viability Effects of Three UCB Compounds on Human Induced Pluripotent Stem Cell-Derived (hiPSC) Cardiomyocytes Using the Real Time Cell Analyzer (RTCA) CardioECR Platform

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The Comprehensive in vitro Proarrhythmia Assay (CiPA) is a novel cardiac safety paradigm intended to refine the current S7B and E14 guidelines. One of the CiPA components consists of assessing electrophysiological effects of candidate drugs in hiPSC-derived cardiomyocytes. Here, we tested the effects of 3 UCB compounds, which failed in (pre-)clinical phase due to cardiotoxicity, on contractility (i.e. impedance), field potential (FP) and cell viability on hiPSC-derived cardiomyocytes (Pluricyte® Cardiomyocytes) using the xCELLigence® RTCA CardioECR platform. Both acute and long-term (until 24h) effects of 4 concentrations of each compound were recorded in triplicates. For the 3 compounds, an extended dataset was available, including in vitro (cardiac channelogram), ex vivo (rabbit or dog Purkinje fibers (PF)), in vivo (ECG in telemetered