

P293 Toxicity profiling of cardiac transcription factor-targeted compounds in various cardiac and stem cell types reveals cell type- and compound-dependent toxicity

ST. Karhu¹ ; M. Valimäki¹ ; M. Jumppanen² ; L. Pohjola¹ ; R. Leigh¹ ; G. Foldes³ ; G. Boije Af Gennas² ; J. Yli-Kauhaluoma² ; H. Ruskoaho¹ ; V. Talman¹ 1

University of Helsinki, Faculty of Pharmacy, Drug Research Program and Division of Pharmacology and Pharmacotherapy, Helsinki, Finland; 2 University of Helsinki, Faculty of Pharmacy, Drug Research Program and Division of Pharmaceutical Chemistry and Technology, Helsinki, Finland; 3 Imperial College London, National Heart and Lung Institute, London, United Kingdom

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Introduction: Cardiac transcription factors, such as GATA4, NKX2-5, and TBX5, are key regulators of cardiac development. They also play an important role in the development of pathological conditions such as cardiomyopathy and left ventricular hypertrophy and are therefore considered attractive drug targets. We have recently described a new family of GATA4-targeted compounds that have shown potential against myocardial remodeling. To reduce the risk of toxicity-dependent failure at later stages of drug development, effective toxicity screening using precise cell models can be useful.

Purpose: The purpose of this study was to investigate the in vitro toxicity and structure-toxicity relationships of the novel GATA4-targeted compounds, and to compare different cardiac and stem cell models in toxicity testing.

Methods: Cell viability was studied in eight cell types: the H9c2 myoblast cell line, primary neonatal rat ventricular cardiomyocytes and fibroblasts, mouse embryonic stem cells (mESCs), mouse embryonic fibroblasts, mESC-derivatives from day 5 embryoid bodies, human induced pluripotent stem cells (hiPSCs) and hiPSC-derived cardiomyocytes (hiPSC-CMs). The cells were exposed to test compounds for 24 hours and the lactate dehydrogenase (LDH) and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays were carried out to investigate necrosis and mitochondrial redox metabolism, respectively. Based on these results two compounds were chosen for high-content analysis of cell viability and proliferation using hiPSCs and hiPSC-CMs. For this, cell viability was assessed with staining for mitochondrial membrane potential, whereas proliferation was investigated with bromodeoxyuridine and Ki67 stainings.

Results: None of the compounds induced necrotic cell death even at the highest concentration studied (30 μ M). The MTT assay however revealed significant structure-dependent and cell type-specific toxicity profiles for the structurally related compounds. None of the compounds were toxic to H9c2 cells, cardiomyocytes or fibroblasts, while compounds possessing a 6-member ring in the southern part of the molecule were concentration-dependently toxic to stem and progenitor cells. As an example, the lead compound 3i-0666 induced a significant reduction in cell viability in hiPSCs (93–94% at ≥ 3 μ M, $P < 0.001$) but had no effect on cell viability in hiPSC-CMs (Fig. 1). It also induced a 2.7-fold ($P = 0.100$) increase in cells positive for a fluorescent caspase reporter, indicating that stem cell death caused by some of the GATA4-targeted compounds is at least partially caspase-dependent.

Conclusions: The cell types used for toxicity screening have a major impact on the results and should thus be chosen carefully. Stem cells provide the most sensitive model for toxicity screening. Identification of structural features responsible for stem cell toxicity in GATA4-targeted compounds allows further drug development towards non-toxic derivatives.