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Translational trio of myocardial biomarkers for prediction, monitoring and controlling toxicologic response: Mechanistic (high content analysis), leakage (high-sensitivity cardiac troponin I) and function biomarkers (B-type natriuretic peptide)

Peter James O'Brien

Abstract

Myocardial toxic injury is characterized by a drug or chemical interference with a cellular process or structure that results in leakage of subcellular constituents with some loss of cardiomyocyte and cardiac function. Combined biomarkers of cytotoxicity, leakage and dysfunction that translate across cell-based to animal models to humans are needed to predict, detect, quantitate, monitor, understand, and assess clinical relevance of cardiotoxicity. High content analysis (HCA) of human, pluripotent, stem-cell-derived cardiomyocytes that have been exposed to toxic substances has been demonstrated to be the most-predictive model of human cardiotoxicity. *In vivo*, high-sensitivity cardiac troponin (cTn)assays are the most specific and sensitive biomarkers of myocardial injury. And B-type natriuretic peptides are the most sensitive biomarker of cardiac dysfunction. Recent developments in the evolution of these biomarkers is overviewed, including the recent qualification of cTn by the FDA for preclinical studies, the loss of cross-species reactivity of cTnT but gain for cTnI, the confounding and non-drug-related causes of cTn increase in animal studies, and the limitations of other cardiac injury biomarker assays that cross-react with skeletal muscle. Next steps in biomarker development are identified.

Addresses

Department of Clinical Pathology, School of Veterinary Medicine, University College Dublin, Ireland

Corresponding author: O'Brien, Peter James (Peter.James.Obrien@UCD.ie)

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Translational biomarker, Cardiotoxicity, Troponin, High-sensitivity, High content, Natriuretic peptide.

1. Introduction

Translational biomarkers of toxicologic response must be effective for both prediction and monitoring. They must identify and quantitate the specific and active pathology that they target and discriminate it from all other effects. They must translate bi-directionally, not just from animals to humans, or in reverse, but to live-cell models. Such full-range translation may allow engineering of next-generation drugs to eliminate or minimize cardiotoxicity potential and may allow better treatment of cardiotoxic side-effects that cannot be separated from drug efficacy, such as with cancer therapy. Such biomarkers have most-highly evolved for the myocardium, although they still have a long way to go. There is a trio of translational biomarkers for cardiotoxicity which are more specific and sensitive for injury and dysfunction, than have been developed for any other target-organ or cell-specific toxicity. The trio consist of mechanistic, leakage, and function biomarkers, namely a specific cytotoxicity, cardiac troponin I (cTnI) and natriuretic peptide (NTproBNP). Far from perfect, the trio biomarkers provide a current and useable model for management of drug-induced cardiotoxicity.

Firstly, regarding the *in vitro*, cell-based, cytotoxicity models, these historically have been largely ineffective in translating to *in vivo*. When they reveal cytotoxicity at a relevant concentration, they generally predict human toxicity [1]. However, they are insensitive, missing most toxic drugs [1]. This has biased the drug safety community against use of *in vitro* tests for predicting target-organ toxicity [2]. However, in the last decade, effectiveness of predictive cytotoxicity has grown exponentially with development of new, commercially-available technology within the pharmaceutical sector, namely high content analysis (HCA), but importantly, also human, pluripotent, stem-cell-derived cardiomyocytes [1–12]. Use of HCA and human cardiomyocytes by Drug Discovery and Drug Safety scientists has been rapidly growing and is likely making a significant dent into safety attrition in the marketplace. HCA cytotoxicity assessment should now be considered a necessary screen for cardiotoxicity potential. *In vitro* HCA assays may be applied *in vivo*, directly to peripheral blood mononuclear cells to assess a wide variety of specific, subcellular cytotoxicities, affecting mitochondria, lysosomes, cell membranes, DNA, or oxidative stress [2,3]. For example, mitochondrial toxicity has been demonstrated in peripheral blood lymphocytes using HCA

during chemotherapy of canine lymphoma [2,13–15]. It may be correlated with the concurrent cardiotoxicity due to similar, subcellular, toxic side-effects.

Secondly, troponin has long been the undisputed, gold-standard, leakage-biomarker of myocardial injury [16,17]. Understanding and refinement of the cardiac troponin I (cTnI) assay over the same time-frame as HCA development has caused marked growth in the effectiveness and application of what had already been a highly-effective biomarker both in humans and in animals [16–21]. Unfortunately, however, such development has resulted in decreased effectiveness of cardiac troponin T (cTnT) as a translational biomarker, due to substantial loss of cross-species reactivity [21–23], especially in the standard animal species for preclinical safety testing, namely the rat and dog. As high-sensitivity (hs) and tissue-specificity of cTnT (but not I) assay progressed for human application, species-specificity increased, at the expense of cross-species translation.

The third of the trio, natriuretic peptide, is a long-recognized specific biomarker of cardiac dysfunction, especially the B-type and especially as measured as the more stable, inactivated, prepropeptide, NTproBNP [24,25]. These natriuretic peptides are reliable biomarkers for monitoring acute cardiotoxicity and predicting late-onset cardiotoxicity [26]. They increase rapidly with ventricular wall distension. Technical difficulties have been limiting the assay translation, largely due to lack of cross-species reactivity and of the peptides' greater instability in rat and dog. Whereas both cTn and BNP assays are available on standard immunoanalysers found in most hospitals, only the cTnI will have cross-species activity. Typically, species-specific ELISA is needed for BNP in animals. In veterinary medicine, there is only one BNP assay for only one species, the dog, and it is controlled by one vendor – in contrast there are cTnI assays that work in all mammals. Recently, technical difficulties have been sufficiently overcome to allow practical, translational movement. And, recent studies found BNP changes may occur earlier than for cTnI, such as with carbon monoxide and minoxidil toxicity in rats [27,28].

2. High content analysis of human, pluripotent stem-cell-derived cardiomyocytes

This year, 2017, is the centenary of the first published study on cytotoxicity [reviewed in [3]]. After a century of study and development of the cytotoxicity assay, with more than a quarter million publications on its use, the pharmaceutical industry can now detect and partially elucidate most toxicological responses *in vitro* and predict their occurrence *in vivo* and the biomarkers for their monitoring [3]. The effective end-product of this research and development for direct myocardial toxicity is HCA of human, pluripotential, stem-cell-derived, functional cardiomyocytes. First developed and validated for idiosyncratic and other hepatotoxicities [1–3], HCA has now been extended and validated for the best *in vitro* models of the human myocardial cell [4–12]. It involves monitoring simultaneously the activities and images of multiple, live-cell activities and the structural features of large numbers of single cells. It uses automated, fluorescence microscopy in microtiter plate format and image analysis. Non-toxic, fluorescent intracellular dyes are used for monitoring several of a wide range of subcellular activities. These include: ionized calcium; mitochondrial number, DNA, size and function; lysosomal number and size; cell membrane permeability; apoptosis; oxidative stress, and cell proliferation. Even troponin has been used as an effective *in vitro* cytotoxicity biomarker.

Not surprisingly, the hepatocyte is highly effective in HCA tests for identifying cardiotoxicities that are based on inhibition of basic processes in homeostasis found in most cell types [1]. However, it does not have many of the types or ranges of activities as the cardiomyocyte. There is a combination of physiological activities of the myocardial cell that exist in no other cell type or single organelle that can be out-predictive of any other models and for which there are HCA biomarkers for toxicologic response. These include the massive cyclical calcium movements controlling the contraction–relaxation cycle, the unsurpassed rate of ATP turnover and associated oxidative stress, and the cyclical electrolyte movements the coordinate cell activity [4,23].

3. High-sensitivity cardiac troponin assays

After a generation of extensive study and application, cardiac troponin (cTn) has long become the universal, de facto, blood biomarker-of-choice for assessment of cardiac injury in human and veterinary medicine, cardiac research, and in the evaluation of the cardiac safety of chemicals and drugs [16–20]. Its effectiveness as a biomarker largely reflects its high, tissue specificity and concentration. Furthermore, its high conservation of structure and function across mammalian species makes it effective as a translational biomarker. As cardiac injury is a frequent and important occurrence with cardiovascular diseases, certain drug toxicities, and secondary to a wide range of other diseases, cTn has arguably become the most successful of all translational biomarkers [20,29]. It is useful to review the recent developments in knowledge and understanding, and technology, that are relevant to its application as a cardiotoxicity biomarker, especially with regard to limitations of its use in translation across species [30].

The characteristics of an effective, translational biomarker are generally well-defined, and include