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Assaying cardiac biomarkers for toxicity testing using biosensing and cardiomyocytes derived from human embryonic stem cells

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ABSTRACT

Human embryonic stem cell (hESC) derived cardiomyocytes are in the present study being used for testing drug-induced cardiotoxicity in a biosensor set-up. The design of an *in vitro* testing alternative provides a novel opportunity to surpass previous methods based on rodent cells or cell lines due to its significantly higher toxicological relevance. In this report we demonstrate how hESC-derived cardiomyocytes release detectable levels of two clinically decisive cardiac biomarkers, cardiac troponin T and fatty acid binding protein 3, when the cardiac cells are exposed to the well-known cardioactive drug compound, doxorubicin. The release is monitored by the immuno-biosensor technique surface plasmon resonance, particularly appropriate due to its capacity for parallel and high-throughput analysis in complex media.

1. Introduction

Human pluripotent stem cells have unique ability to multiply indefinitely for differentiation into virtually any specialized cell type (Améen et al., 2008). These features can be harnessed to generate large amounts of target cells difficult or impossible to source from human tissues (Stummann and Bremer, 2008). One example is the cardiomyocyte for which there is a huge need in toxicity testing, but where the access of primary cells from the human heart is very limited. However, human embryonic stem cells (hESCs), differentiated into cardiomyocyte-like cells, can potentially replace the animal derived primary cells and cell lines currently used in cardiotoxicity testing (Steel et al., 2009). The main advantages with the hESC-derived cardiomyocytes are (1) their human origin, (2) reproducible and limitless supply, and (3) functional similarity to their adult counterparts. It is expected that the use of cells of human origin may improve the predictivity of human cardiotoxicity tests by avoiding inter-species differences (Stummann et al., 2009). However, a detailed understanding of the physiology of the hESC-derived cardiomyocytes is important to fully exploit their potential for use in cardiotoxicity testing of drugs.

hESC-derived cardiomyocytes have previously been used to assess drug-induced perturbations of the cells' electrophysiological properties (He et al., 2003; Satin et al., 2004; Reppel et al., 2005; Caspi et al., 2009). These data lend support to further develop and validate assays using of hESC-derived cardiomyocytes for safety assessments in pharmacology.

Besides effects on the electrophysiology of the heart, drugs can also be cardiotoxic without any direct interactions with ion channels (Altena et al., 2009). This can be explained by drug-induced formation of reactive oxygen species, apoptosis, or altered molecular signaling in the cells. However, studies of these mechanisms in human cardiomyocytes have been hampered due to limited access of relevant cells; for this the hESC-derived cardiomyocytes could provide a valuable help.

Also, testing strategies based on established biomarkers of cardiac damage (e.g. troponins) may considerably facilitate large-scale cardiotoxicity screening (Dolci et al., 2008). However, issues related to the predictivity, standardization, and validation must be properly addressed.

Troponin T (cTnT) and fatty acid binding protein 3 (FABP3 or cFABP) are two critical biomarkers for assaying adverse cardiotoxic responses of drugs and are cardiac. Normally, these are

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clinical indicators of myocardial infarction but they are also considered sensitive translational safety biomarkers for drug-induced toxicity. (Nakata et al., 2003; Key et al., 1999a,b; Okamoto et al., 2000; O'Brien, 2008). FABP3, being the less studied of these two, is released at high levels early during myocardial infarction and may exhibit a more sensitive response also in toxicity testing (Nakata et al., 2003; O'Donoghue et al., 2006).

Sensitive and accurate bioanalytical tools for these biomarkers are necessary to develop for high precision *in vitro* assays. Especially useful alternatives for quantification of proteins in complex biological media could be surface-based immuno-biosensors (Marks et al., 2007). Examples of attractive techniques are the acoustic wave, surface plasmon resonance (SPR), and total internal refection fluorescence methods (Liedberg et al., 1983; Kunz et al., 1996; Spangler et al., 2001; Dmitriev et al., 2003).

In particular SPR is appropriate due to its elaborate surface design, and possibility for parallel sample detection and computation of affinity interaction data (Karlsson et al., 1991). The microenvironment of the SPR dextran chip negates unspecific effects, a most valuable feature when dealing with small signals and unpredictable interferences. Importantly, the SPR allows multi-target analysis of several relevant biomarkers provided that antibodies for critical epitopes have been raised and isolated. In addition, analysis time per sample is short and regeneration straightforward. Also, cardiac TnT responses for cardiotoxic drugs using the murine cardiac cell line HL-1 have recently been demonstrated (Andersson et al., in press).

So far, SPR, or any other surface-based biosensor, has not been used with hESC-derived cells for developing *in vitro* toxicity assays. In this article we propose the use of SPR biosensing for monitoring cardiotoxicity effects on hESC-derived cardiomyocytes, thereby taking advantage of the high specificity achieved on the sensor chip coupled ligands and the ability of the sensor chips to carry out parallel monitoring of biomarkers on a miniaturized format. We show that hESC-derived cardiomyocytes can serve as a key component in an immuno-based biosensor assay for *in vitro* drug-induced toxicity testing due to their human-like characteristics. To demonstrate the principle, the above two described cardiac biomarkers cTnT and cFABP were applied together with a well-known cardiotoxic drug, doxorubicin. However, the principle is applicable to any immunoassay provided that there is a suitable and specific antibody type available.

2. Materials and methods

2.1. Cell culture and human cardiac tissue samples

The hESC line SA002 (Cellartis AB, Gothenburg, Sweden) was propagated and differentiated into cardiomyocytes essentially as described previously (Synnergren et al., 2008). Spontaneously contracting cardiomyocyte clusters were identified by visual inspection and harvested by mechanical dissection. The clusters were dissociated using trypsin and the cells were seeded into 96-well plates. The cells were allowed to recover for at least 3 days before use for subsequent analysis.

Human biopsies from auricle of right atrium were obtained after written, informed consent from patients undergoing heart surgery and after ethical approval by the Research Ethics Board at the University of Gothenburg and the Sahlgrenska Academy, Gothenburg, Sweden.

2.2. Quantitative gene expression analysis

Total RNA was extracted from hESC-derived cardiomyocytes and human heart tissue using RNeasy Kit (Qiagen). Preparation of cDNA and real-time qPCR was performed as described (Sandstedt et al., 2010) using the following human TaqMan Gene Expression Assays: NKX2.5 Hs00231763_m1, TNNT2 Hs00165960_m1, MYH6 Hs01101425_m1, MYH7 Hs00165276_m1, KCNH2 Hs00165120_m1, HCN4 Hs00175760_m1. CREBBP (Hs00231733_m1) was used as an endogenous reference gene (Synnergren et al., 2007). All samples were analyzed in duplicates and the relative comparative CT method was used to analyze the real-time qPCR data (User Bulletin #2, Applied Biosystems). A common reference sample was also included in each analysis which allows for direct inter-experiment comparisons.

2.3. Immunocytochemistry

The hESC-derived cardiomyocytes were harvested from the 96-well plates using trypsin and seeded onto 24-well cell culture plates and fixed in 4% paraformaldehyde. Following permeabilization and blocking using 0.5% Triton X solution (Sarstedt) and 2% BSA in PBS (Invitrogen) respectively, the cells were incubated with primary antibody solution with a monoclonal antibody to cardiac troponin I (Abcam 52862) overnight at 4 °C. Incubation with an Alexa 594 conjugated anti-rabbit secondary antibody was performed for 60 min at room temperature. Nuclei were counter stained with DAPI (Sigma–Aldrich). The stained cells were inspected using a Nikon Eclipse TE-2000 U fluorescence microscope (Nikon, Tokyo, Japan), and compared to matched controls prepared without the primary antibody.

2.4. Toxicity testing procedure

A 70 mM stock solution of doxorubicin (Sigma) in H_2O was made, and further dilutions to indicate concentrations were made in cell culture medium. At the start of the experiment, the overnight medium was replaced by the drug-containing medium. Medium containing solvent only was included as control. After 24 h, the medium was removed from the cells, centrifuged at 13,000 × *g* for 10 min, and immediately analyzed on a Biacore 2000 or Elecsys 2010. Medium from untreated cells was used as background.

2.5. Surface plasmon resonance assay

The antibodies for cardiac troponin T [7G7], [1F11] and fatty acid binding protein 3 [9F3] (all from Abcam) were covalently immobilized on a CM5 sensor chip (GE Healthcare AB, Uppsala, Sweden) using the amine coupling procedure. Measurements on the chip were carried out in a commercial surface plasmon resonance instrument (Biacore 2000™, GE Healthcare AB). As running buffer HBS-EP (0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% surfactant P20) was used at a continuous flow of 5 μl/min. The carboxylated dextran matrix of the sensor chip was activated by 0.1 M NHS/0.4 M EDC (GE Healthcare) in a 1:1 ratio and exposed to the chip for 7 min. The antibodies were subsequently coupled to the surface during a 10 min injection (150 μg/ml in 10 mM sodium acetate buffer, pH 4.5). Subsequently unbound groups were deactivated by a 7 min injection of 1 M ethanolamine pH 8.5 (GE Healthcare). The surface was then washed repeatedly with 10 mM glycine–HCl, pH 2.5 (GE Healthcare) for 5× 3 min. The immobilization levels of antibody ligands varied between 9000 and 13,000 resonance units (RU).

For measurement of samples from the cell experiments, a continuous flow of HBS-EP at 5 μl/min was maintained and the samples were injected for 3 min. Cell culture medium was analyzed undiluted. The sensor chip surface was regenerated by injection of 10 mM glycine–HCl pH 2.5 for 1 min.

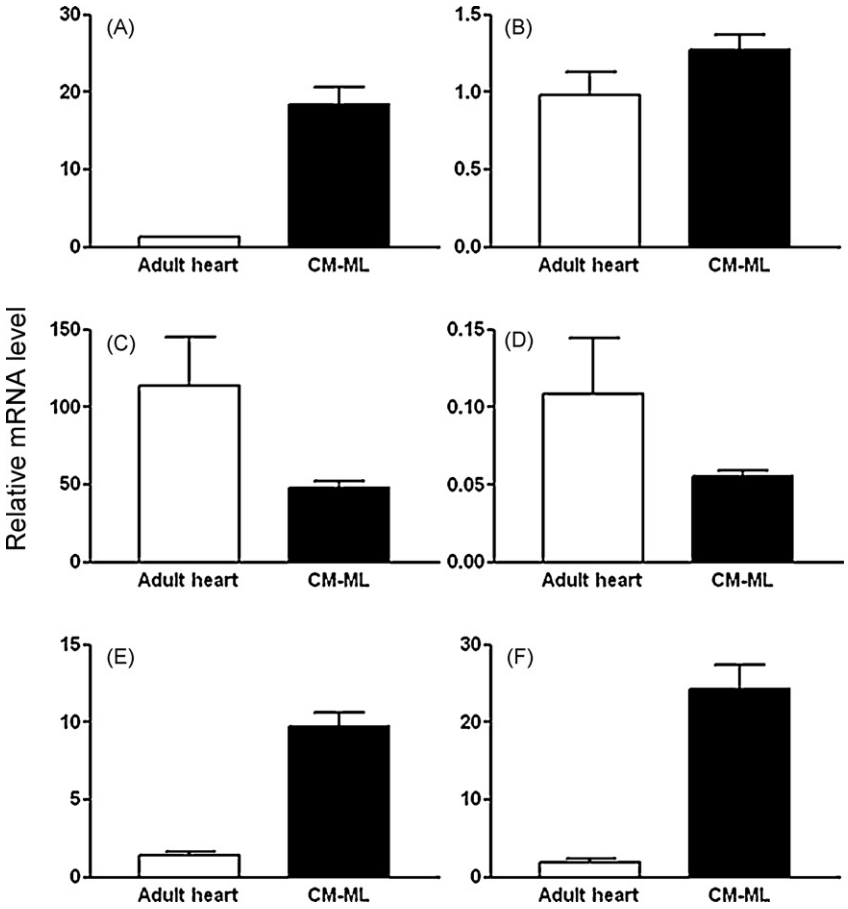

Fig. 1. Gene expression in hESC-derived cardiomyocyte monolayer (CM-ML) in comparison to human heart tissue. The relative mRNA levels of cardiomyocyte markers and ion channels were determined by real-time qPCR. The bar graphs show the mean values and the error bars indicate the positive value of the SEM. (A) NKX2.5; (B) TNNT2; (C) MYH6; (D) MYH7; (E) KCNH2; (F) HCN4 ($n = 3$ adult heart, $n = 18$ CM-ML).

2.6. Immunochemiluminescence assay

The cTnT levels were measured using an automated immunochemiluminescence instrument (Elecsys 2010, Roche) and the Elecsys Troponin T STAT assay reagent (Roche) according to the manufacturer's recommendations (Forest et al., 1998). A biotinylated mouse monoclonal antibody [M11.7] linked to streptavidin-coated paramagnetic beads (Roche) was used for antigen capture. A mouse monoclonal antibody [M7] labeled with ruthenium trisbypyridyl complex (Roche) was used for detection.

2.7. Statistical analysis

The data from gene expression analyses and toxicity testing are presented as means ± SEM.

3. Results and discussion

3.1. Characteristics of the hESC-derived cardiomyocytes

Spontaneously contracting clusters of cells were identified by visual inspection in cultures of differentiating hESCs, and were harvested by microdissection. The cardiomyocyte clusters typically constitute multilayer aggregates of cells and as such may not represent the optimal format for drug accessibility and biomarker release. Thus, in order to prepare the cells in an assay-ready format suitable for drug exposure and read-out, hESC-derived cardiomyocyte clusters were collected and dissociated into single cell suspensions and subsequently seeded as monolayers into 96-well plates. In order to verify the cardiac phenotype of these monolayer cultures, the cells of individual wells were harvested for real-time qPCR analysis of cardiac marker genes (Fig. 1). Human adult heart tissue was used as reference material. The mRNA levels of NKX2.5, KCNH2 (hERG), and HCN4 (funny channel) were several folds higher in the hESC-derived cardiomyocyte monolayers compared to adult heart tissue. This is, however, not expected since NKX2.5 and HCN4 are associated with the early cardiac maturation stage, and high levels of KCNH2 mRNA have been reported in hESC-derived cardiomyocytes (Améen et al., 2008; Steel et al., 2009). On the other hand, MYH6 and MYH7, encoding structural proteins, were expressed at slightly higher levels in the human tissue compared to the monolayer cell cultures. Of particular note, and of importance for this study, the mRNA levels of TNNT2 were almost identical between the heart tissue samples and the hESC-derived cardiomyocytes supporting the similarity between the *in vitro* differentiated stem cells and the tissue biopsies. In order to verify the protein expression of troponin in the monolayer cultures, immunohistochemical stainings of the differentiated cell populations were performed. Fig. 2 shows strong positive troponin staining in the majority of the cells, further underscoring the utility of hESC-derived cardiomyocytes as model system to study drug-induced cardiotoxicity by biomarker analysis.

3.2. Evaluation of antibodies for surface plasmon resonance analysis

Two monoclonal antibodies against cTnT and one against FABP3 were evaluated for their usefulness as ligands in an SPR-based assay

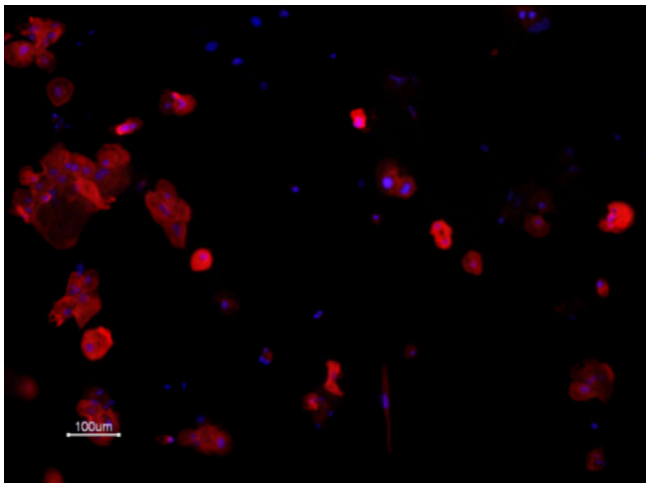

Fig. 2. Immunohistochemical staining of hESC-derived cardiomyocytes for troponin expression. Monolayers of hESC-derived cardiomyocytes (CM-ML) were dissociated and seeded at a density suitable to identify individual cells. The presence of cardiomyocytes is indicated by cells staining positive for cardiac troponin I (red), whereas blue indicates cell nuclei (DAPI). Bar = 100 μM (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

for testing drug-induced cardiotoxicity. The cTnT [1F11] antibody binds to a conserved region of cTnT (amino acids 146–160), and therefore recognizes cTnT from several different species, including human. The cTnT [7G7] antibody recognizes a more variable region of cTnT (aa 60–70), and is human-specific. Each of the three antibodies was immobilized in separate flow channels on an SPR sensor chip. The amount of immobilized antibody ligand per channel was between 9000 and 10,000 RU. A fourth flow channel on the same chip was first amine-activated and then deactivated with ethanolamine and used as reference surface.

The hESC-derived cardiomyocytes maintained in 96-well plates were exposed to 80 μM doxorubicin for a period of 24 h. Conditioned cell culture medium was collected from the wells and analyzed in the SPR instrument. Representative resulting sensorgrams (overlay) are shown in Fig. 3. The negative control, i.e. the

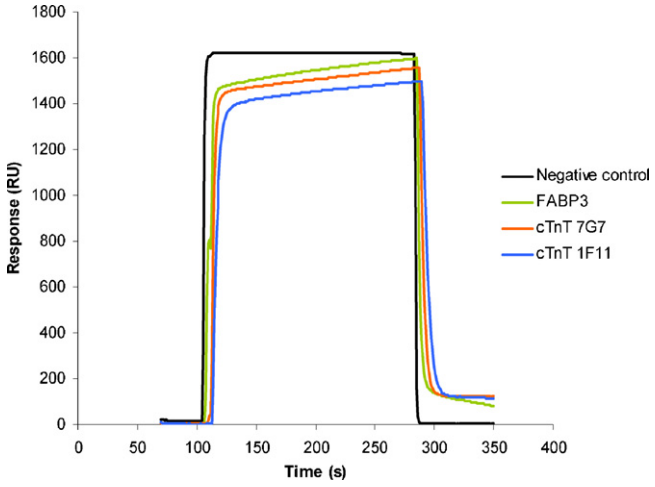

Fig. 3. Two monoclonal antibodies against cTnT, 1F11 and 7G7, and one against FABP3 were evaluated for their usefulness as ligands in an SPR-based assay for testing drug-induced cardiotoxicity. Each of the three antibodies was immobilized in separate flow channels on an SPR sensor chip. The fourth flow channel was amine-activated and then deactivated with ethanolamine and used as reference surface. hESC-derived cardiomyocyte monolayers were exposed to 80 μM doxorubicin for 24 h. Cell culture medium was collected and analyzed on the SPR instrument.

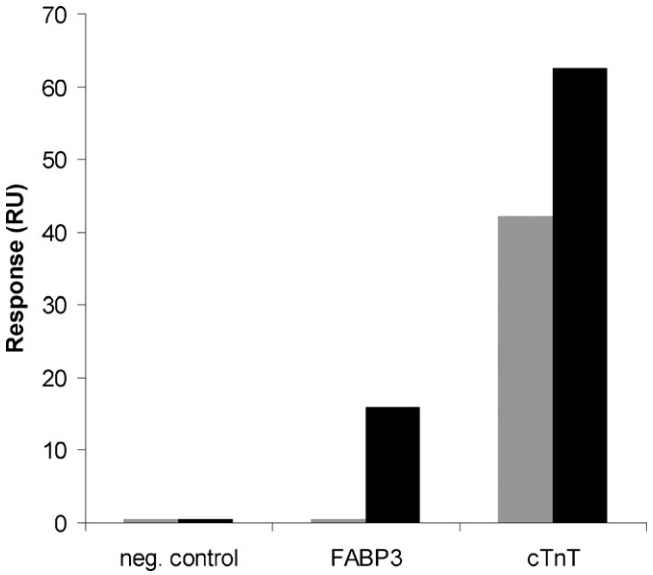

Fig. 4. Background effects of cell culture medium on different antibodies. Cell culture medium was incubated without cells (grey bars) or with cells (black bars) for 24 h. The medium samples were analyzed on the SPR instrument where the 7G7 antibody against cTnT and the antibody against FABP3 were immobilized in separate flow channels on an SPR sensor chip. One flow channel was amine-activated and then deactivated with ethanolamine and used as a negative control reference surface.

channel without antibody, showed an effect related to the sample medium *per se* (bulk effect), and resulted in a constant signal shift during the injection period. After the injection, the signal returned to the baseline. The flow channels with antibodies against cTnT or FABP3 showed the same constant bulk effect shift with a superimposed increasing signal due to the capture of target proteins by the antibody ligands. After the injection period, the bulk effect disappeared and the signal leveled out at a higher value, corresponding to the amount of protein remaining bound on the surface. All three antibodies performed well. Of the two cTnT antibodies, 7G7 gave the strongest response and was chosen for all further experiments.

To investigate the influence of other surface interactions or effects in the medium which were not caused by the assayed biomarkers we compared culture media incubated in plates with or without contracting cardiomyocytes. After 24 h of incubation the media was collected and analyzed in the SPR instrument (Fig. 4). The non-cellular effects, i.e. where cardiomyocytes had not influenced the medium samples, were referred to as the background signal in the assay. Neither of the media gave any such background signal when exposed to flow channel with the deactivated control surface. The flow channel with the FABP3 antibody ligand gave no background signal from medium without cardiomyocytes, while medium which had experienced contracting cardiomyocytes gave an SPR-signal of 16 RU. The cTnT [7G7] antibody ligand gave rise to a more significant background effect from both media. It appeared that certain media components, e.g. serum proteins, may have interacted with this particular ligand.

3.3. *In vitro testing – release of troponin T after doxorubicin exposure*

3.3.1. *SPR measurement*

The hESC-derived cardiomyocytes in the culture plates were exposed to doxorubicin for 24 h in the concentration range 0.625–320 μM. The amount of cTnT released into the culture medium was analyzed on the SPR instrument (Fig. 5). Cardiomyocytes exposed to ≤10 μM doxorubicin showed no release of

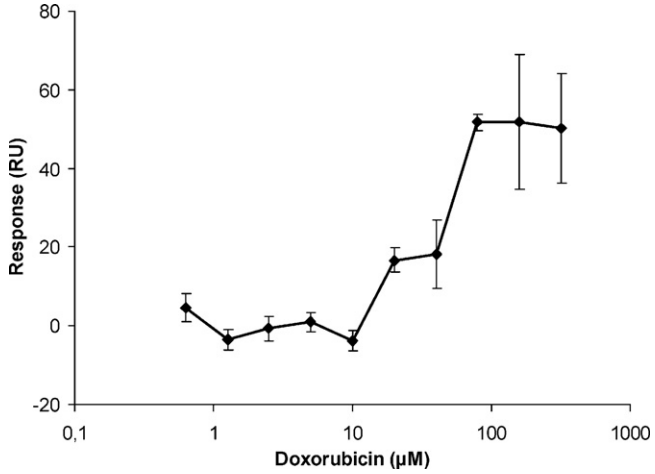

Fig. 5. Cardiac troponin T release from hESC-CM after exposure to doxorubicin. hESC-derived cardiomyocytes were exposed to doxorubicin for 24 h in the concentration range 0.625–320 μM. Cell culture medium was collected and the amount of cTnT was analyzed on a the SPR instrument. The data are shown as mean values and the error bars indicate the SEM (n = 4–7).

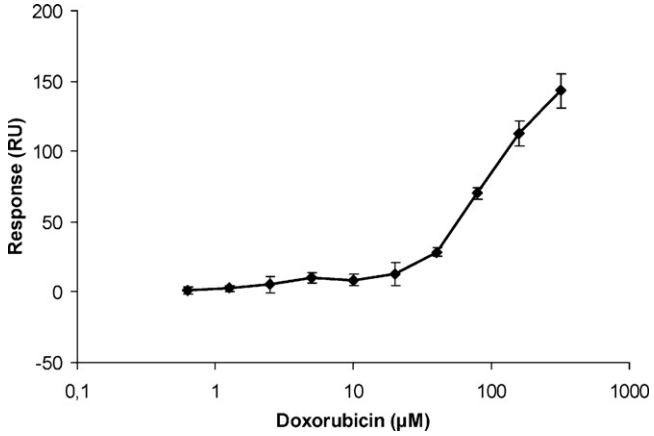

Fig. 7. FABP3 release from hESC-CM after exposure to doxorubicin measured on SPR. hESC-derived cardiomyocytes were exposed to doxorubicin for 24 h in the concentration range 0.625–320 μM. The amount of cardiac fatty acid binding protein (FABP3) released into the cell culture medium was analyzed on an SPR instrument. The data are shown as mean values and the error bars indicate the SEM (n = 4–7).

cTnT, above that of non-drug controls. Cardiomyocytes exposed to ≥20 μM showed an increasing amount of cTnT in the culture medium, with a maximum response of about 50 RU at 80 μM doxorubicin.

3.3.2. Immunochemiluminescence quantitation

The SPR method was compared with an immunochemiluminescence reference method routinely used in the clinic to analyze cTnT levels in human plasma to diagnose acute myocardial infarction (Forest et al., 1998; Klein et al., 2010). The hESC-derived cardiomyocytes were exposed to doxorubicin for 24 h in the concentration range 0.625–320 μM. The amount of cTnT released into the culture medium was analyzed with the immunochemiluminescence method (Fig. 6). Cardiomyocytes exposed to ≤20 μM doxorubicin showed no release of cTnT above that of non-drug controls, while cardiomyocytes exposed to ≥ 40 μM showed an increasing amount of cTnT in the culture medium, reaching about 60 ng/mL at 320 μM doxorubicin.

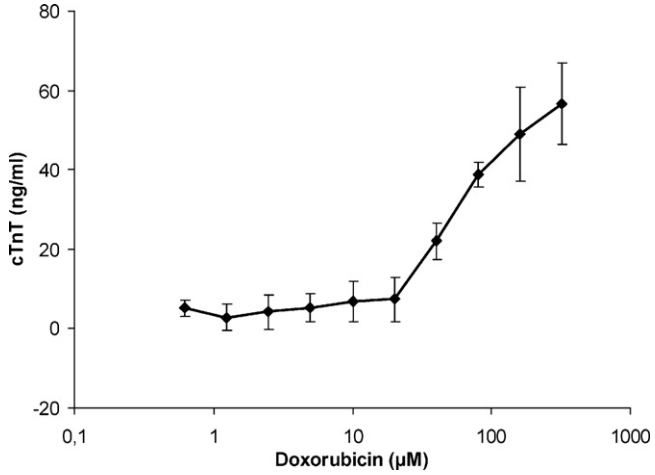

Fig. 6. Cardiac troponin T release from hESC-CM after exposure to doxorubicin measured by a immunochemiluminescence method. hESC-derived cardiomyocytes were exposed to doxorubicin for 24 h in the concentration range 0.625–320 μM. The amount of cTnT released into the cell culture medium was analyzed. The data are shown as mean values and the error bars indicate the SEM (n = 4–7).

3.4. Release of FABP3 after doxorubicin exposure

The SPR assay was also used to assay cardiac fatty acid binding protein (FABP3) as an alternative to other biosensor methods for quantification of this cardiac biomarker (Key et al., 1999a,b; O'Regan et al., 2002; Chan et al., 2005). Furthermore, it was possible to analyze the FABP3 simultaneously with cTnT on the biochip in the same sample from the hESC-derived cardiomyocytes exposed to doxorubicin. The cells were exposed for 24 h in the concentration range 0.625–320 μM. The amount of FABP3 released into the cell culture medium is shown in Fig. 7. The amount of FABP3 in the medium increased gradually with increasing dose of doxorubicin up to 10 μM. Cardiomyocytes exposed to ≥ 20 μM doxorubicin showed a strongly increasing amount of FABP3 in the culture medium, with a maximum response of about 140 RU at 320 μM of doxorubicin.

Compared to the above cited biosensor methods the SPR biosensor showed a higher detection limit; the SPR chip we used had a detection limit of 30 ng/mL while a screen-printed carbon electrode reported a detection limit of 4 ng/mL (O'Regan et al., 2002) and an amperometric immunoelectrode 10 ng/mL (Key et al., 1999a,b). However, the SPR biosensor has advantages in being easier to handle and automate.

3.5. Comparison of cTnT and FABP3 assays for drug-induced cardiotoxicity

A comparison of the FABP3- and cTnT assays in response to doxorubicin is shown in Fig. 8. The magnitude of response for cTnT was similar when measured using immunochemiluminescence and SPR. However, FABP3 gave a response that was 2.5-fold higher. Also, at lower concentrations of doxorubicin, FABP3, in contrast to cTnT, showed a steadily increasing signal (insert, Fig. 8). This suggests that FABP3 is a more sensitive biomarker for cardiomyocyte cell injury, at least *in vitro*, than the more commonly used cTnT marker.

The clinical assay based on immunochemiluminescence had a sensitivity for TnT that was significantly higher (Forest et al., 1998) and had a range of detection that by far covers the investigated range. However, in this range the biosensor showed sufficient sensitivity for sensitive detection of both analytes.

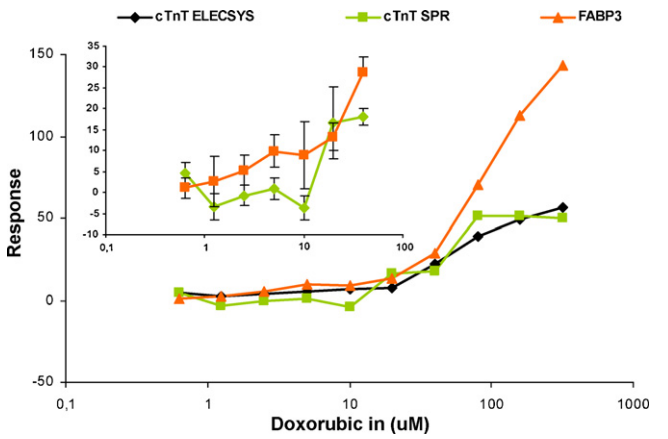

Fig. 8. Comparison of FABP3 and cTnT assays. The hESC-derived cardiomyocytes were exposed to doxorubicin for 24 h in the concentration range 0.625–320 μM. The amount of cTnT (green) and FABP3 (red) released into the cell culture medium was analyzed on the SPR instrument. The amount of cTnT was also measured by immunochemiluminescence (black). The insert shows an enlargement of the SPR assays for cTnT and FABP3 in the doxorubicin range 0.625–40 μM. The data are shown as mean values and the error bars indicate the SEM (n = 4–7) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

3.6. *Effect of cell numbers – increased throughput with fewer cells*

Miniaturization of assay formats is an important means for increasing throughput and lowering analysis costs per sample. Therefore the effect of cell numbers on the toxicity response was examined. The hESC-derived cardiomyocytes were seeded in 96-well plates at different cell densities, ranging from 15,000 to 58,000 cells per well. The cardiomyocytes were exposed to 80 μM doxorubicin for 24 h and the amount of FABP3 released into the culture medium was analyzed in the SPR instrument (Fig. 9). In this range, there was a linear relationship between the number of cells and the amount of FABP3 released into the medium. This indicates that the experiment can be scaled-down to 15,000 cells per well or less, making it possible to perform the assay with a higher throughput.

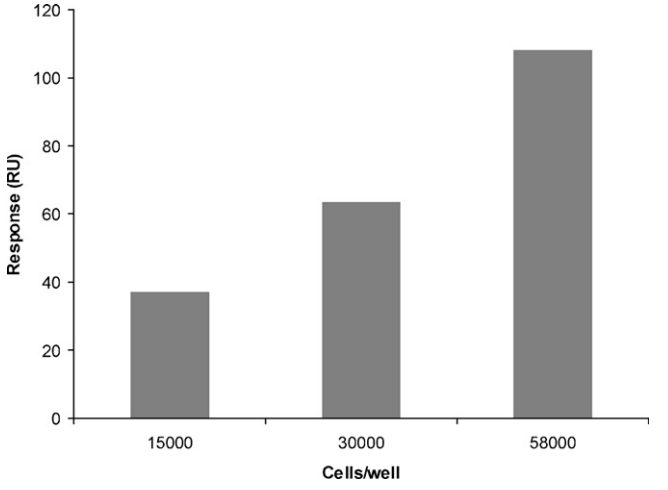

Fig. 9. The effect of cell numbers on toxicity response. hESC-derived cardiomyocytes were seeded in 96-well plates in different cell densities, ranging from 15,000 to 58,000 cells per well. The cells were exposed to 80 μM doxorubicin for 24 h and the amount of FABP3 released into the cell culture medium was analyzed on an SPR instrument. In this range there was a linear relationship between the number of cells and the amount of FABP3 in the medium, with R^2 = 0.9995.

4. Conclusions

The purpose of this study was to investigate the potential of hESC-derived cardiomyocytes in combination with the SPR technique for drug-induced cardiotoxicity testing. The hESC-derived cardiomyocytes dissociated into monolayers were characterized, the usefulness of three different antibodies against two cardiac biomarkers for SPR was analyzed, and a novel method for cardiotoxicity based on these findings is presented. The hESC-derived cardiomyocytes appears to be well suited for *in vitro* testing. The human origin of the cells gives the assay clinical relevance benefits over animal models. Moreover, since they are non-dividing, the cardiotoxic effect of for example antineoplastic drugs such as doxorubicin is more similar to cardiomyocytes *in vivo* than that from *in vitro* assays based on transformed cell lines.

The combination of hESC-derived cardiomyocytes with cTnT and FABP3 biomarkers is an interesting novel alternative for *in vitro* cardiotoxicity. The SPR instrument used in this study had four flow cells, allowing parallel analysis of up to three biomarkers including one reference surface as negative control. Other commercially available SPR instruments allow parallel analysis of more than 30 biomarkers and may thus allow even greater resolution and diversity of the measurements in cellular response towards cardiotoxic agents (Safsten et al., 2006).

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References

Altena, R., Perik, P.J., van Veldhuisen, D.J., de Vries, E.G., Gietema, J.A., 2009. Cardiovascular toxicity caused by cancer treatment: strategies for early detection. Lancet Oncol. 10, 391–399.

Améen, C., Strehl, R., Björquist, P., Lindahl, A., Hyllner, J., Sartipy, P., 2008. Human embryonic stem cells: current technologies and emerging industrial applications. Crit. Rev. Oncol. Hematol. 65, 54–80.

Andersson, H., Kågedal, B., Mandenius, C.F., in press. Monitoring of troponin release from cardiomyocytes during exposure to toxic substances using surface plasmon resonance. Anal. Bioanal. Chem.

Caspi, O., Itzhaki, I., Arbel, G., Kehat, I., Gepstien, A., Huber, I., Satin, J., Gepstein, L., 2009. In vitro electrophysiological drug testing using human embryonic stem cell derived cardiomyocytes. Stem Cells Dev. 18, 161–171.

Chan, C.P.Y., Wan, T.S.M., Watkins, K.L., Pelsers, M.M.A.L., Van der Voort, D., Tang, F.P.W., Lam, K.H.K., Mill, J., Yuan, Y., Lehmann, M., Hempel, A., Sanderson, J.F., Glatz, J.F.C., Renneberg, R., 2005. Rapid analysis of fatty acid-binding proteins with immunosensors and immunotests for early monitoring of tissue injury. Biosens. Bioelectron. 20, 2566–2580.

Dmitriev, D.A., Massino, Y.S., Segal, O.L., 2003. Kinetic analysis of interactions between bispecific monoclonal antibodies and immobilized antigens using a resonant mirror biosensor. J. Immunol. Methods 280, 183–202.

Dolci, A., Dominici, R., Cardinale, D., Sandri, M.T., Panteghini, M., 2008. Biochemical markers for prediction of chemotherapy-induced cardiotoxicity: systematic review of the literature and recommendations for use. Am. J. Clin. Pathol. 130, 688–695.

Forest, J.C., Massé, J., Lane, A., 1998. Evaluation of the analytical performance of the Boehringer Mannheim Elecsys 2010® immunoanalyzer. Clin. Biochem. 31, 81–88.

He, J.Q., Ma, Y., Lee, Y., Thomson, J.A., Kamp, T.J., 2003. Human embryonic stem cells develop into multiple types of cardiac myocytes: action potential characterization. Circ. Res. 93, 32–39.

Karlsson, R., Michaelsson, A., Mattsson, L., 1991. Kinetic analysis of monoclonal antibody–antigen interactions with a new biosensor based analytical system. J. Immunol. Methods 145, 229–240.

Key, G., Schreiber, A., Feldbrügge, R., McNeil, C.J., Jørgenson, P., Pelsers, M.M.A.L., 1999a. Multicenter evaluation of an amperometric immunosensor for plasma fatty acid binding protein, an early marker for acute myocardial infarction. Clin. Biochem. 32, 229–231.

Key, G., Schreiber, A., Feldbrügge, R., McNeil, C.J., Joergensen, P., Pelsers, M.A.L., Glantz, J.F.C., Spener, F., 1999b. Multicenter evaluation of an amperometric immunosensor for plasma fatty acid-binding protein: an early marker for acute myocardial infarction. Clin. Biochem. 32, 229–231.

Klein, G., Kampmann, M., Baum, H., Rauscher, T., Vukovic, T., Hallermayer, K., Rehner, H., Muller-Bardorff, M., Katus, H.A., 1998. Clinical performance of the new cardiac markers troponin T and CK-MB on the Elecsys 2010. A multicentre evaluation. Wien Klin. Wochenschr. 110 (Suppl. 3), 40–51.

Kunz, U., Katerlamp, A., Renneberg, R., Spener, F., Cammann, K., 1996. Sensing fatty acid bind protein with planar and fiber-optical surface plasmon resonance spectroscopy devices. Sens. Actuators 32, 129–155.

Liedberg, B., Nylander, C., Lundström, I., 1983. Surface plasmon resonance for gas detection and biosensing. Sens. Actuators 4, 299–304.

Marks, R.S., et al., 2007. In: Marks, R.S., Cullen, D.C., Karube, I., Lowe, C.R., Weetall, H.H. (Eds.), Handbook of biosensors and biochips. John Wiley & Sons, Ltd, ISBN:978-0-470r-r01905-4.

Nakata, T., Hashimoto, A., Hase, M., Tsuchihashi, K., Shimamoto, K., 2003. Human heart-type fatty acid-binding protein as an early diagnostic and prognostic marker in acute coronary syndrome. Cardiology 99, 96–104.

O'Donoghue, M., de Lemos, J.A., Morrow, D.A., Murphy, S.A., Buros, J.L., Cannon, C.P., Sabatine, M.S., 2006. Prognostic utility of heart-type fatty acid binding protein in patients with acute coronary syndromes. Circulation 114, 550–557.

O'Regan, T., Pravda, M., O'Sullivan, C.K., Guilbault, G.G., 2002. Development of a disposable immunosensor for the detection of human heart fatty-acid binding protein in human whole blood using screen-printed carbon electrodes. Talanta 57, 501–510.

O'Brien, P.J., 2008. Cardiac troponin is the most effective translational safety biomarker for myocardial injury in cardiotoxicity. Toxicology 245, 206–218.

Okamoto, F., Sohmiya, K., Ohkaru, Y., Kawamura, K., Asayama, K., Kimura, H., Nishimura, S., Ishii, H., Sunahara, N., Tanaka, T., 2000. Human heart-type cytoplasmic fatty acid-binding protein (H-FABP) for the diagnosis of acute myocardial infarction. clinical evaluation of H-FABP in comparison with myoglobin and creatine kinase isoenzyme MB. Clin. Chem. Lab. Med. 38, 231–238.

Reppel, M., Pillekamp, F., Brockmeier, K., Matzkies, M., Bekcioglu, A., Lipke, T., Nguemo, F., Bonnemeier, H., Hescheler, J.J., 2005. The electrocardiogram of human embryonic stem cell-derived cardiomyocytes. Electrocardiology 38, 166–170 (Suppl).

Safsten, P., Klakamp, S.L., Drake, A.W., Karlsson, R., Myszka, D.G., 2006. Screening antibody-antigen interactions in parallel using Biacore A100. Anal. Biochem. 353, 181–190.

Sandstedt, J., Jonsson, M., Lindahl, A., Jeppsson, A., Asp, J., 2010. C-kit+ CD45− cells found in the adult human heart represent a population of endothelial progenitor cells. Basic Res. Cardiol. 105, 545–556.

Satin, J., Kehat, I., Caspi, O., Huber, I., Arbel, G., Itzhaki, I., Magyar, J., Schroder, E.A., Perlman, I., Gepstein, L., 2004. Mechanism of spontaneous excitability in human embryonic stem cell derived cardiomyocytes. J. Physiol. 559, 479–496.

Spangler, B.D., Wilkinson, E.A., Murphy, J.T., Tyler, B.J., 2001. Comparison of the Spreeta (R) surface plasmon resonance sensor and a quartz crystal microbalance for detection of *Escherichia coli* heat-labile enterotoxin. Anal. Chim. Acta. 444, 149–161.

Steel, D., Hyllner, J., Sartipy, P., 2009. Cardiomyocytes derived from human embryonic stem cells – characteristics and utility for drug discovery. Curr. Opin. Drug Discov. Dev. 12, 133–140.

Stummann, T.C., Bremer, S., 2008. The possible impact of human embryonic stem cells on safety pharmacological and toxicological assessments in drug discovery and drug development. Curr. Stem Cell. Res. Ther. 3, 118–131.

Stummann, T.C., Beilmann, M., Duker, G., Dumontier, B., Fredriksson, J.M., Jones, R.L., Hasiwa, M., Kang, J., Mandenius, C.F., Mayer, T., Minotti, G., Valentin, Y.J.P., Zünckler, B.J., Bremer, S., 2009. Report and recommendations of the workshop of the European Centre for the Validation of Alternative Methods for drug-induced cardiotoxicity. Cardiovasc. Toxicol. 9, 107–125.

Synnergren, J., Giesler, T.L., Adak, S., Tandon, R., Noaksson, K., Lindahl, A., Nilsson, P., Nelson, D., Olsson, B., Englund, M.C.O., Abbot, S., Sartipy, P., 2007. Differentiating human embryonic stem cells express a unique housekeeping gene signature. Stem Cells 25, 473–480.

Synnergren, J., Akesson, K., Dahlenborg, K., Vidarsson, H., Améen, C., Steel, D., Lindahl, A., Olsson, B., Sartipy, P., 2008. Molecular signature of cardiomyocyte clusters derived from human embryonic stem cells. Stem Cells 26, 1831–1840.