Application Note

Cells: human cardiomyocytes
Tools: CardioExcyte 96

Assessing cardiotoxic risk of anti-cancer agents on Nanion's CardioExcyte 96

University of Göttingen and Nanion Technologies GmbH, Munich.
Human stem cell-derived cardiomyocytes kindly provided by Ncardia.

Ncardia Stem cell experts

MAX-PLANCK-GESELLSCHAFT

Summary

New anticancer agents have led to higher life expectancy for patients surviving cancer. However, treatment-related morbidity factors such as cardiac toxicity have become important issues for long-term cancer survivors[1-3]. Cardiotoxic side effects such as arrhythmia, thromboembolism and myocardial ischemia are common with anti-cancer drugs such as the anthracyclines[1-3]. This has led to the need for a sub-speciality of medicine, cardio-oncology or oncocardiology, to promote cardiovascular health whilst providing the best therapy to fight cancer.

It is important to be able to assess the cardiotoxic risk of new and existing cancer therapies in order to facilitate effective cardiovascular health during chemotherapy. In addition, advances in human stem cell derived cardiomyocytes (hiPSC-CMs) and, indeed, patient-derived hiPSC-CMs offers new possibilities for personalized medicine, being able to assess a patient's risk of developing cardiovascular complications based on their own cells, thus taking into account their own genetic factors[4].

Using the measurement of electrical impedance coupled with human stem cell-derived cardiomyocytes (hSC-CMs) we could confirm the cardiotoxic effects of paclitaxel, also known as Taxol, a microtuble stabilizing drug approved for the treatment of breast, ovarian and lung cancer[5]. In addition, we investigated different combinations of cylophosphamide (CP), doxorubicin (DOX) and 5-Fluorouracil (5F) and found that any combination which included DOX was highly cardiotoxic.

Results

The impedance signal recorded on the CardioExcyte 96 changes as a result of alterations in confluency, cell contact (morphological shape) and conductivity of adherent cells and thereby provides a measure of toxicity. In addition, effects on contractility can also be measured by monitoring the mean beat (MB). Base impedance of hSC-CMs grown on the NSP-96 plates for the CardioExcyte 96 was monitored over 170 hours after treatment with paclitaxel (Figure 1A & B).

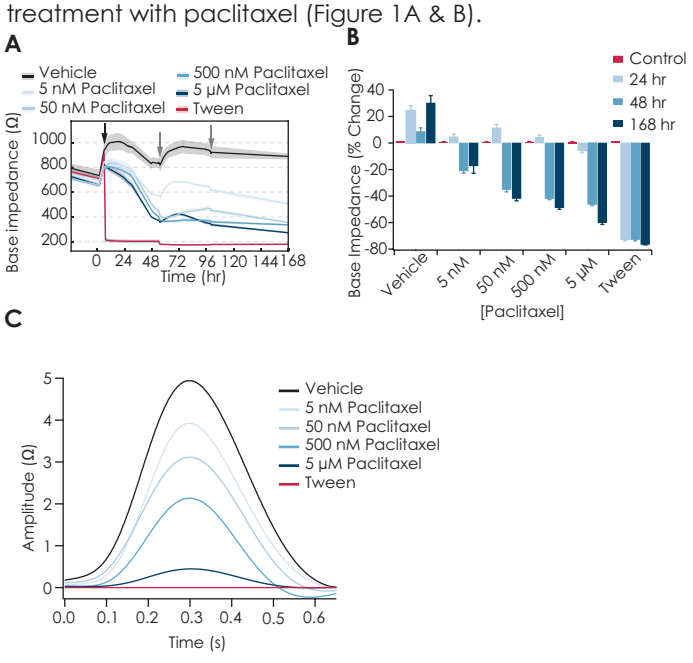

Figure 1: A Increasing concentrations of paclitaxel induce a decrease in base impedance of Cor.4U cells which can be monitored continuously. Black arrow indicates addition of compound, grey arrows indicate wash with fresh media without compound. Tween (2%) induced 100% cell death and was used as a positive control. **B** Paclitaxel causes a concentration- and time-dependent decrease in base impedance and decrease in amplitude of the mean beat (**C**).

nanion

Application Note

Paclitaxel has been approved for the treatment of ovarian, breast and lung cancer since the 1990's[5]. However, it is also known that paclitaxel is associated with adverse side effects including cardiotoxicity[6]. Paclitaxel showed a concentration- and time-dependent effect on base impedance of hSC-CMs (Figure 1A & B), consistent with a change in cell health. The amplitude of the mean beat was also reduced indicating an effect on contractility (Figure 1C).

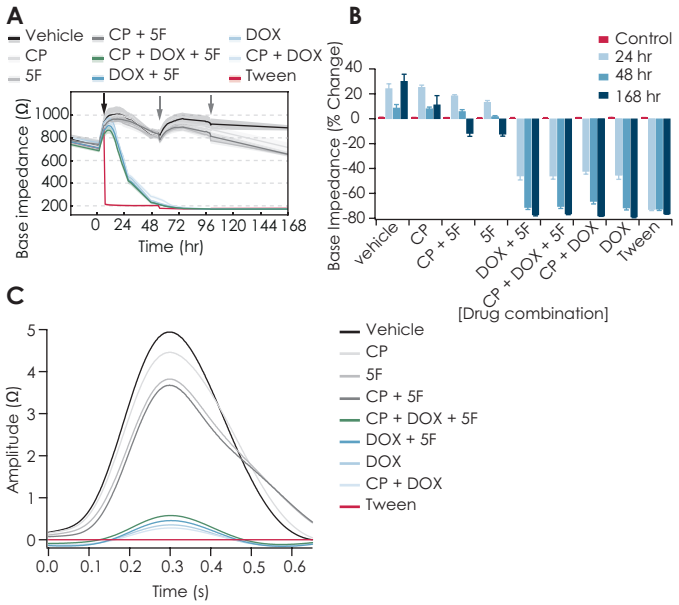

Figure 2: A Various combinations of therapeutic agents cyclophosphamide (CP; 0.64 µg/ml), doxorubicin (DOX; 0.032 µg/ml) and 5-Fluorouracil (5F; 0.64 µg/ml) caused different effects on base impedance. In all combinations where DOX was present, a cardiotoxic effect was observed. Tween (2%) induced 100% cell death and was used as a positive control. **B** In all combinations containing DOX, a time-dependent decrease in base impedance and decrease in amplitude of the mean beat (**C**) was observed, whereas only minimal effects were seen where DOX was not present.

The combination therapy of CP, DOX and 5F is commonly used to treat recurring breast cancer[7]. However, anthracycline antibiotics such as doxorubicin are known to cause cardiotoxic side effects, which can be potentially fatal[8]. We could confirm the cardiotoxic effects of DOX as a decrease in base impedance was observed in all combinations containing DOX, whereas minimal effects on base impedance were observed when using CP or 5F, alone or in combination (Figure 2A & B). DOX also caused a decrease in amplitude of the mean beat indicating an effect on contractility.

In summary, the CardioExcyte 96 can be used to investigate the cardiotoxic risk of new and existing cancer therapies. Indeed, with the use of patient-specific hiPSC-CMs, this offers the potential to personalize the risk factors of cardiotoxicity of cancer therapeutics based on genetic and sex variabilities of individual patients. The impedance signal of the CardioExcyte 96 is a reliable, non-invasive and accurate measurement for testing cardiotoxicity of cancer therapeutics.

References

1. Bovelli, D., *et al*. 2010. Annals of Oncology 21 (Suppl. 5): v277–v282
2. Cubbon, R.M., & Lyon, A.R. 2016. IHJ 168: s77 – s85
3. Lenneman, C.G., & Sawyer, D.B. 2016. Circ. Res. 118: 1008-1020
4. Sheng, C.C., et al. 2016. J. Am. Coll. Cardiol. Basic Trans. Science. 1: 386–98
5. Weaver, B.A. 2014. MBoC. 25: 2677-2681
6. Marupudi, N.I., et al. 2007. Expert Opin. Drug Saf. 6(5): 609-621 7.
7. Chatterjee, K., *et al.*, 2010. Cardiology. 115:155–162
8. Fukuda, M, *et al.*, 1999. Oncology. 7 (Suppl. 3): 77-81

Methods

Cells

We thank Ncardia for providing the hSC-CMs (Cor.4U) and Oliver Reinhardt and Dr. Frauke Alves from the University of Göttingen for performing the experiments.

Impedance measurements

Impedance measurements were conducted according to Nanion's standard procedures for the CardioExcyte 96. Cor.4U cells were pre-plated on NSP-96 plates and the base impedance signal allowed to stabilize before compound added. Compounds were applied in the external media. About 2 hours before drug application the medium was completely removed from the wells and 200 µl fresh medium was added. All signals were normalized to a group of control measurements (n=5-11) on the same plate. Cardiomyocytes were incubated in the compound for 48 hours after which the media was replaced with fresh media without compound. Impedance signal was monitored for a total of 190 hours.

Nanion Technologies GmbH
Ganghoferstr. 70A
80339 Munich, Germany

phone +49 89 219 095-0
fax +49 89 218997960
www.nanion.de • info@nanion.de