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23.07.2003

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USt /VAT ID-NO
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Gerichtsstand Reutlingen

Docket No. 02D-0232
ICH S7B
Safety Pharmacology Studies foe Assessing the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals

To Whom it May Concern:

Multi Channel Systems MCS GmbH is a company focussing it´s business on automated electrophysiological techniques. One of the classical bottlenecks in drug development is the electrophysiology in safety pharmacology. We developed a novel approach to screen drug candidates for effects on cardiac repolarization properties.

We feel, that our data should be known by FDA representatives and the scientific community and propose a set of data for discussion by authorities andscientists.

The Micro Electrode Array technology enables the user to acquire data from up to 60 channels simultaneously. A 96 channel layout is under development. This increases the throughput dramatically. The second advantage is these technique is based on extracellular recording – this means a non invasive recording.

We have prepared a short outline of the experiments we have done concerning ventricular repolarization which is attached. All the reference substances tested behave in the same way as they do in accepted QT in-vitro models.

02D-0232

C6

Systems

Our main concern is to emphasize three points :

1. Extracellular recording of cardiac action potentials reveals field potential data, which allow to reveal the same information as retrieved by transmembrane recording
2. Embryonic cell culture with spontaneous activity enables us to access ventricular repolarization data **and** changes in endogenic rhythm. Thus it allows a certain predictivity on proarrhythmogenic effects of a drug.
3. Non Mammalian cell preparations seem to be of similar high predictivity as accepted mammalian models. The effects of all tested drugs were in the embryonic chicken culture very comparable with mammalian / human results.

Supporting point 2 & 3 we would like to add, that the chick analogue of hERG is expressed in embryonic and early postnatal chicken ventricle at a mostly unchanged level during embryonic development. (real time RT-PCR data)

Even so we are aware, that the extracellular recording technique is a rather novel technique for cardiac applications, it is well studied in neurobiology. The interpretation of these data is as easy and reliable as it is from transmembrane recordings. The main advantage of this technology is, that it is easy to automate. This allows to test a large number of drug candidates in an early stage of development. Thus it can save substantial cost for the developing company and increase drug safety.

We would like to thank you for considering this novel technology. It is a pleasure to share our data with you and we would be glad to receive some constructive feedback on our data.

Sincerely

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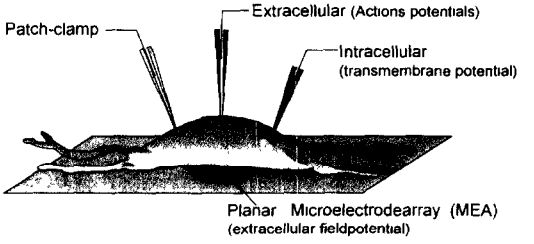
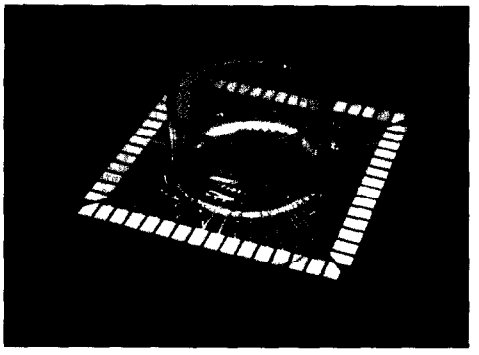
Micro Electrode Arrays in cardiac safety pharmacology

1.) Abstract & Summary

The screening of large numbers of drug candidates for possible QT prolongation in the electrocardiogram by standard electrophysiological methods is one of the major bottlenecks in current safety pharmacology.



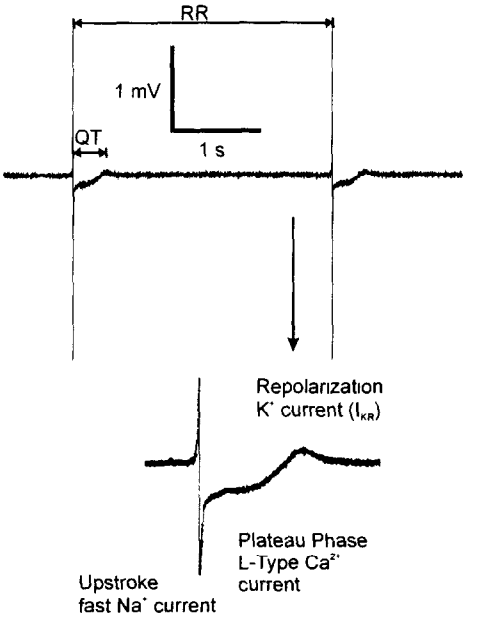
Micro Electrode Arrays (MEA) represent an innovative way to reveal action potential data from cardiac myocytes, cultivated directly on the chip. MEAs allow cultivation and recording



on the same device. 60 Electrode of 30 µm diameter are positioned in a 8 x 8 grid. A MEA is shown in the illustration on the left.

Advantages of this system are higher throughput, easier automation and standardization. In contrast to standard electrophysiological there is no need to attach the cell with a patch pipette. This means, that there is not even an optical control required. Cells are cultivated and measured on the same device with up to 64 channels simultaneously.

Due to the extracellular ablation of the action potential (AP), the so called field potential (fAP) the shape of the signal looks different. However all components of a classical cardiac AP can be detected and pharmacological characterized. For the purpose of QT interval measurement the ventricular AP duration (APD) is the parameter of interest. This is measured as fAPD as shown in the illustration.

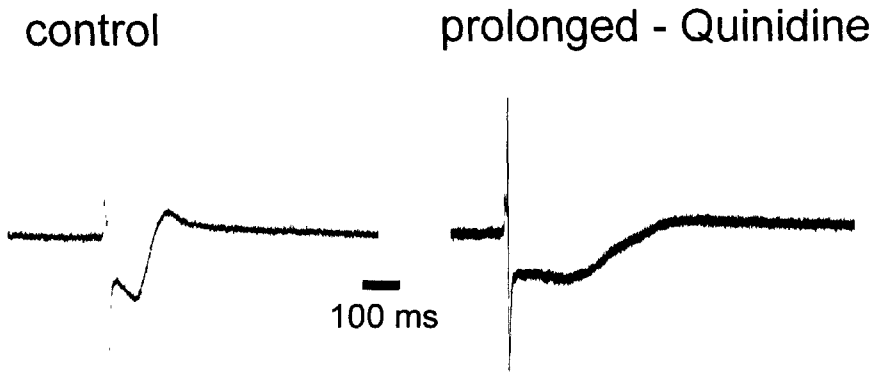


A selection of drugs with a known effect on the ventricular action potential have been tested. In general the results are in line with the literature / data obtained in established systems. In the appendix dose response curves are shown. Drugs tested include :

1. Antiarhythmics
- Quinidine – fAPD prolonged by more than 200 %
- Sotalole – significant fAPD prolongation (100%)
- Amiodarone – minor fAPD prolongation

2. Antihistaminics
- Terfenadine – fAPD prolongation in nanomolar concentration range
- Astemizol – fAPD prolongation in nanomolar concentration range
- Fexofenadine – 1000fold higher concentrations required Terfenadine !

3. E4031 as HERG blocker – nanomolar concentrations cause fAPD prolongation
4. Verapamil as false positive of a HERG assay – no effect
5. Cisapride as a drug removed from market due to QT prolongation – subnanomolar concentrations cause effective fAPD prolongation.



In a molecular biological approach the existence of the chicken analogue of HERG channels was shown by real time RT-PCR. Data are shown in the following part.

The following description is based on (Egert & Meyer, 2003)

2.) Introduction

Analyses of cardiac electrical potentials in-vivo, such as in the electrocardiogram, are well known to reveal information about system properties of the heart, arrhythmia, indications of conduction failures etc. While such recordings are absolutely indispensable, the spatial resolution and the opportunities for manipulation with this approach are limited. In-vitro investigations of isolated organs, e.g. Purkinje fibers, papillary muscle, Langendorff heart or patches of cardiac, have long been used to study the mechanisms of the generation and propagation of cardiac potentials at higher spatial resolution with either intra- or extracellular recording. Cultures of cardiac myocytes, e.g. harvested after enzymatic digestion from cardiac tissue, on the other hand, offer the opportunity of single cell or aggregate analyses, e.g. for developmental (Banach et al. 2003), pharmacological and biophysical studies. Although these cultures do not maintain the structure of cardiac tissue, the functional properties of action potential generation and propagation, contractility and, depending to some extent on the culture system, the ion channel composition of the original cells are conserved or reestablished. The ease of production and the simple structure of these cell and tissue culture systems thus allow the researcher to address question not easily accessible otherwise in organs or animal preparations. The motion of the cells in most of these preparations, however, hinders studies with conventional electrodes, in particular intracellular or patch-clamp recording, and in optical recordings with voltage sensitive dyes.
Extracellular recording of field potentials (FP) from contracting myocyte cultures is, however, facilitated considerably when the cells are grown in culture dishes with integrated microelectrode arrays (MEA, Fig. 1). Cells grown on MEAs will adhere tightly to the substrate and contract isometrically, avoiding the motion artifacts that usually deteriorate the signal-to-noise ratio (SNR). These devices enable non-invasive simultaneous, multi-site, extracellular recordings from myocytes, excluding the mechanical stimulation of the cell that can hardly be avoided with conventional recording techniques.

MEAs with 60-70 electrodes are produced with thin-film photolithographic techniques and have become commercially available. Full recording systems are available currently as the MEA60 - System from Multi Channel Systems (MCS, Reutlingen, Germany)[1]

[1] www.multichannelsystems.com

3.) System description:

The MEAs we use have 60 microelectrodes, with a diameter of 30 µm, positioned on an 8x8 grid with 200 µm spacing (Fig. 1). The recording area thus covers 1.4 x 1.4 mm^2. The electrodes themselves are flat, with a rough surface of TiN, 80-200 MΩ impedance (at 1 kHz) and recessed into the substrate by max. 1 µm. The culture chamber is formed by a 2 cm diameter glass ring with 6 mm height glued to the MEA base plate, resulting in a chamber volume of ca. 1.8 ml.
In addition to these standard layout, there are modifications in the electrode material (like gold or indiumtinoxide ITO) and size to optimize the MEA layout for specific applications.

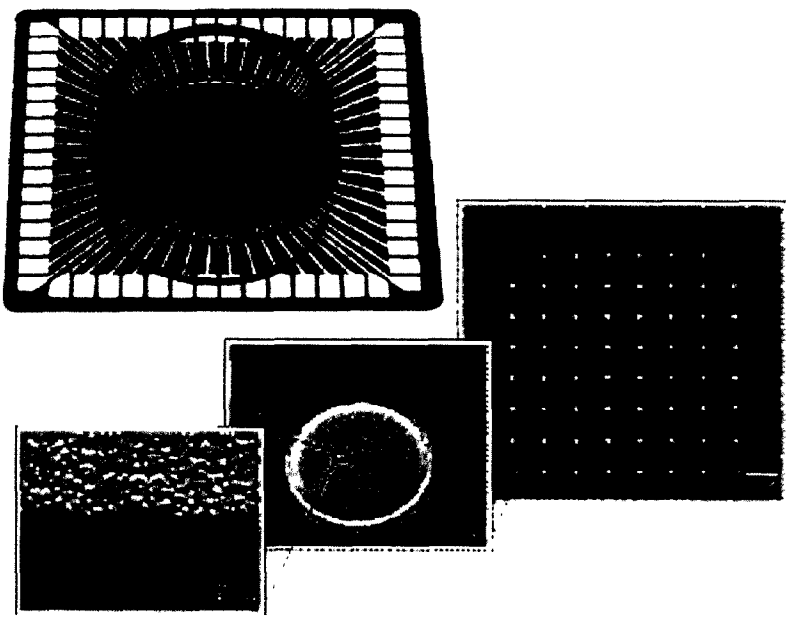


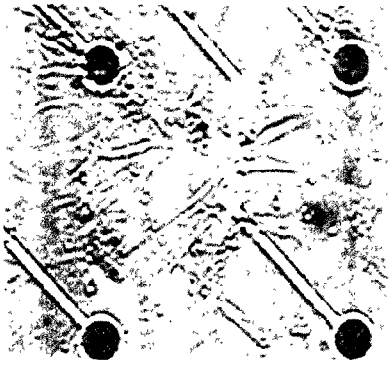
Figure 1 :
MEA Layout : In the upper panel a complete MEA is shown. The electrode grid is in the center surrounded by a culture ring, mimicking a culture dish. The squares surrounding this area are the contact pads, were contact pins contact the MEA to the amplifier. In the lower panel a zoom from the 8x8 grid, to a single electrode, to the TiN surface of an electrode is shown (from right to left).

The recording system has amplifiers with fixed bandwidth (we use 0.1, 1 or 10 Hz-3.2 kHz) and amplification (we use 1000x or 1200x) (customization possible). It allows the continuous or triggered-window recording of all or selected channels simultaneously with sampling rates up to 50 kHz/channel (12 bit analog-to-digital conversion, mapping the input voltage range to 4096 steps). Various analysis and display functions are provided for on-line monitoring of an experiment. Further analyses are carried out off-line, using tools written for MATLAB (The Mathworks, Natick, USA).

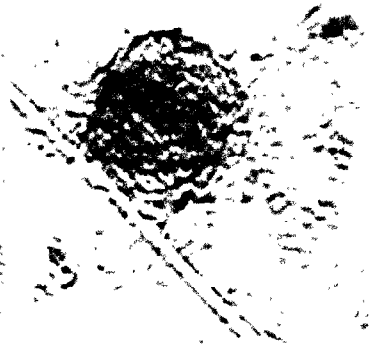
4.) Tissue Culture

Cardiac tissue culture was taken from embryonic chicken ventricles. The heart of chicken embryos was removed after 10 – 12 days in the incubator. The ventricle was

isolated, minced and digested with 0,05 % trypsine (original activity 10400 U/mg) in DMEM medium (all Chemicals: Sigma, Deisenhofen, Germany). After 8 min of digestion the supernatant was removed and discarded. 3,5 mL of trypsine solution were added. Every 8 min the supernatant was collected in ice cold DMEM Medium with 10% fetal bovine serum and replaced by fresh trypsine solution. After 4– 5 digestion cycles the heart was completely digested. Cells were pelleted and plated in high density on the electrode field of the MEAs. Recording was done 2– 5 days after the preparation.



Proliferating ventricular myocytes on MEA



Ventricular myocyte aggregation with single cells spreading out. Cultivation on MEA

Figure 2 :
Ventricular myocytes and electrodes are shown in the left image, whereas an aggregation of cardiac myocytes is shown in the right image. These two form represent the most frequently used preparations.

Even so the tissue culture as described above represents the basis of all data shown here. Other established tissue cultures on the MEA include neonatal rat and mouse cultures and cardiac myocytes derived from embryonic stem cells (Kathrin Banach, Loyola University Chicago; Prof. Jürgen Hescheler, University of Cologne; Ralf Kettenhofen, Axiogenesis AG Cologne; Milena Angelova, Medigene Munich).

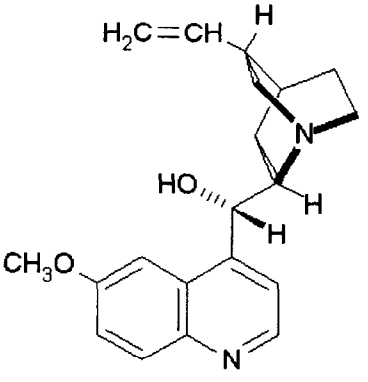
5.) Results

5.1.) Pharmacological Validation

The major goal of the pharmacological validation of the system was to challenge cultivated cardiac myocytes on the MEA with a selection of well described drugs and compare the results with data obtained by other, established assay systems.
The pharmacological tools used to validate the system have been selected in order to cover various therapeutically classes (e.g. Antiarrhythmics , Antihistaminics). The second group of drugs selected included drugs that are known to reveal false positive results in other assays (eg. Verapamil). Or drugs, that are specific inhibitors of ionic channel classes (E4031) or are approved drugs, taken from the Market due to known QT prolonging effects (Cisapride).

5.1.1 Antiarrhythmic drugs

5.1.1.1 Quinidine



Quinidine is an approved class IA antiarrhythmic drug. Various authors describe a QT prolonging effect of Quinidine in therapeutic doses (lower micromolar range). These effects vary by gender. Whereas already in the 1970s QT prolongation of this drug could be shown, newer publications demonstrate an inhibition of HERG channels. Our data show a substantial prolongation of the fAPD. Data were acquired from 1 µM up to 150 µM. However as spontaneous activity stopped between 50 µM and 150 µM not all experiments allow acquisition of the last data point.

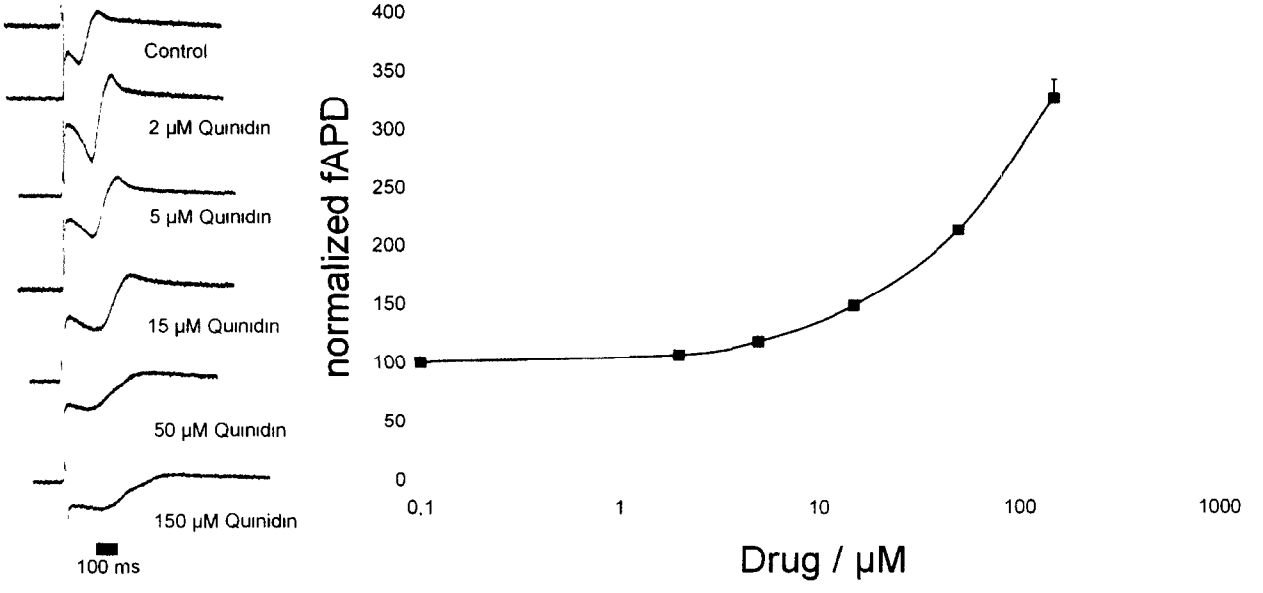


Figure 3 :

In the left panel sample fAP recording under the influence of various Quindine doses are shown. The right panels displays the dose response data of Quinidine. Concentration is shown on the x- axis in a logarithmic scale, whereas the linear y- axis shows normalized fAPD (normalized on control conditions in absence of drug)

Even so unphysiological high concentrations of Quinidine are required to retrieve a maximal prolongation of the fAPD, a 20% prolongation is observed already in the range of 10 µM. It should be considered, that other drugs causing a 20% QT prolongation are considered as harmful.
The conclusion here is, that the required concentrations are higher than those acquired from other in-vitro test systems. However the concentrations are in the same magnitude.

Quinidine References :

Fieldman A, Beebe RD, Sing Sum Chow M.
The effect of quinidine sulfate on QRS duration and QT and systolic time intervals in man.
J Clin Pharmacol. 1977 Feb-Mar;17(2-3):134-9.

Jenzer HR, Hagemeijer F.
Quinidine syncope: torsade de pointes with low quinidine plasma concentrations.
Eur J Cardiol. 1976 DEC;4(4):447-5

Campbell WB.
EKG of the month: QT prolongation induced by quinidine in therapeutic doses
J Tenn Med Assoc. 1982 May;75(5):340-1

Paul AA, Witchel HJ, Hancox JC.
Inhibition of the current of heterologously expressed HERG potassium channels by flecainide and comparison with quinidine, propafenone and lignocaine.
Br J Pharmacol. 2002 Jul;136(5):717-29

5.1.1.2 Sotalol

Sotalol is another antiarhythmic drug with an accepted QT prolongation as side effect. Beside the QT prolongation ventricular fibrillation and Torsades occur in patients treated with Sotalol. The torsade risk for female patients is significantly higher than for male patients.

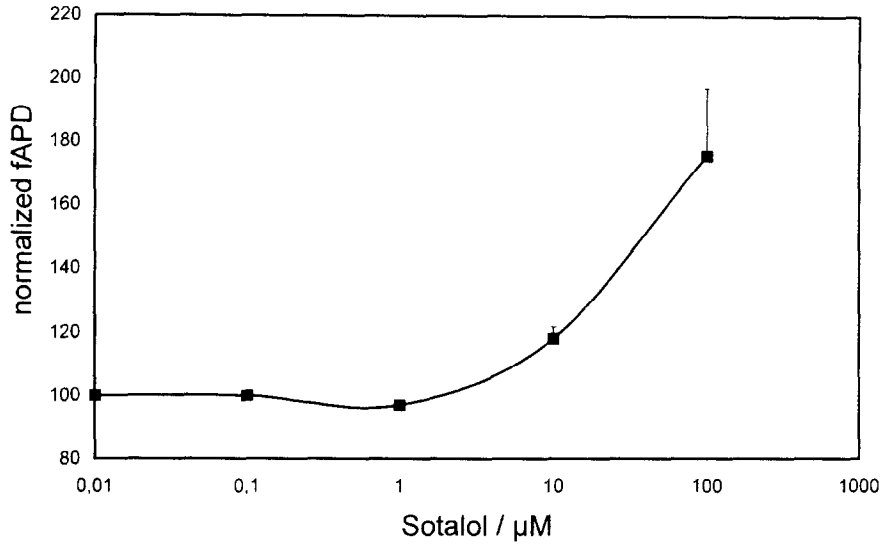


Figure 4 : Dose response curve for sotalol

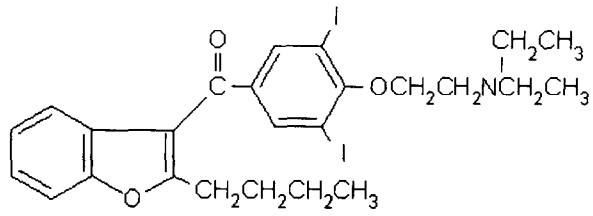
Sotalol References :

Farkas A, Lepran I, Papp JG.
Proarrhythmic effects of intravenous quinidine, amiodarone, D-sotalol, and almokalant in the anesthetized rabbit model of torsade de pointes.
J Cardiovasc Pharmacol. 2002 Feb;39(2):287-97

Cammu G, Geelen P, Baetens P, De Vos J, Demeyer I.
Two cases of torsades de pointes caused by sotalol therapy.
Resuscitation. 1999 Jan;40(1):49-51.

Lehmann MH, Hardy S, Archibald D, quart B, MacNeil DJ.
Sex difference in risk of torsade de pointes with d,l-sotalol.
Circulation. 1996 Nov 15;94(10):2535-41

5.1.1.3 Amiodarone



Amiodarone is an antiarhythmic, which is primarily used in the treatment of atrial fibrillation.

One of the major aims of amiodarone therapy is to avoid the reoccurence of supraventricular arhythmia. It is well known, that amiodarone does cause a minor QT prolongation. However, reports of ventricular arhythmia like torsade de pointe are very rare. Clinical data show, that QT prolongation occurs only after several days of Amiodarone therapy (pers. comm. OA Dr. Brandts, MH Herne).

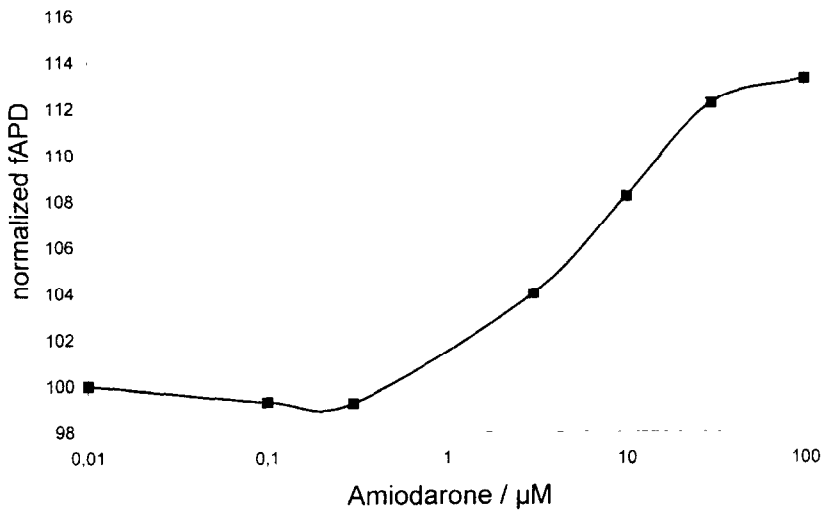


Figure 5 : Dose – response curve for amiodarone

Amiodarone References :

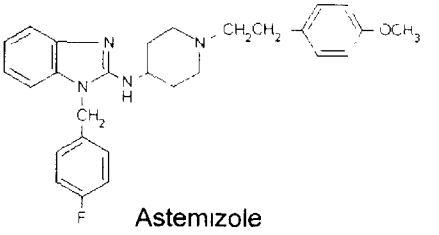
van Opstal JM, Schoenmakers M, Verduyn SC, de Groot SH, Leunissen JD, van Der Hulst FF, Molenschot MM, Wellens HJ, Vos MA.
Chronic amiodarone evokes no torsade de pointes arrhythmias despite QT lengthening in an animal model of acquired long-QT syndrome.
Circulation. 2001 Nov 27;104(22):2722-7

Bicer S, Patchell JS, Hamlin DM, Hamlin RL.
Acute effects of escalating doses of amiodarone in isolated guinea pig hearts.
J Vet Pharmacol Ther. 2002 Jun;25(3):221-6

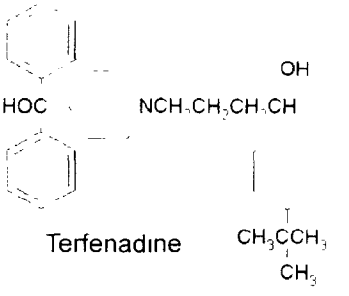
Nkomo VT, Shen WK.
Amiodarone-induced long QT and polymorphic ventricular tachycardia.
Am J Emerg Med. 2001 May;19(3):246-8.

5.1.2 Antihistaminics

The studied anthistaminic drugs (H1 receptor antagonists) include Terfenadine, Astemizole and Fexofenadine. These drugs are used in the symptomatic therapy of allergic diseases (rhinoconjunctivitis, allergic skin diseases). In rare cases an proarrhythomgenic effect of Terfenadine and Astemizol was observed. This was in some cases transitory, whereas in other cases lethal form of ventricular fibrillation occurred. The mechanism how Terfenadine causes this disease is well studied and seems to be comparable for Astemizole.



Astemizole



Terfenadine

Terfenadine is in the liver methabolized into Fexofenadine and a second metabolite by the CYP3A4 enzyme of the Cytochrome-P-450 system. If this system is not working sufficiently, the degradation of Terfenadine does not take place sufficiently fast, so that Terfenadine accumulates in the plasma. As unmetabolized Terfenadine can cause ventricular repolarization disturbances, QT prolongation and in rare cases Torsade des Pointes might be the consequences. However Fexofenadine, the control substance should not evoke fAPD prolongation. Depending on the assay system published IC50 values vary. Most authors find IC50 values in the range of 10-50 nM for Astemizol and 50 – 150 nM for Terfenadine.

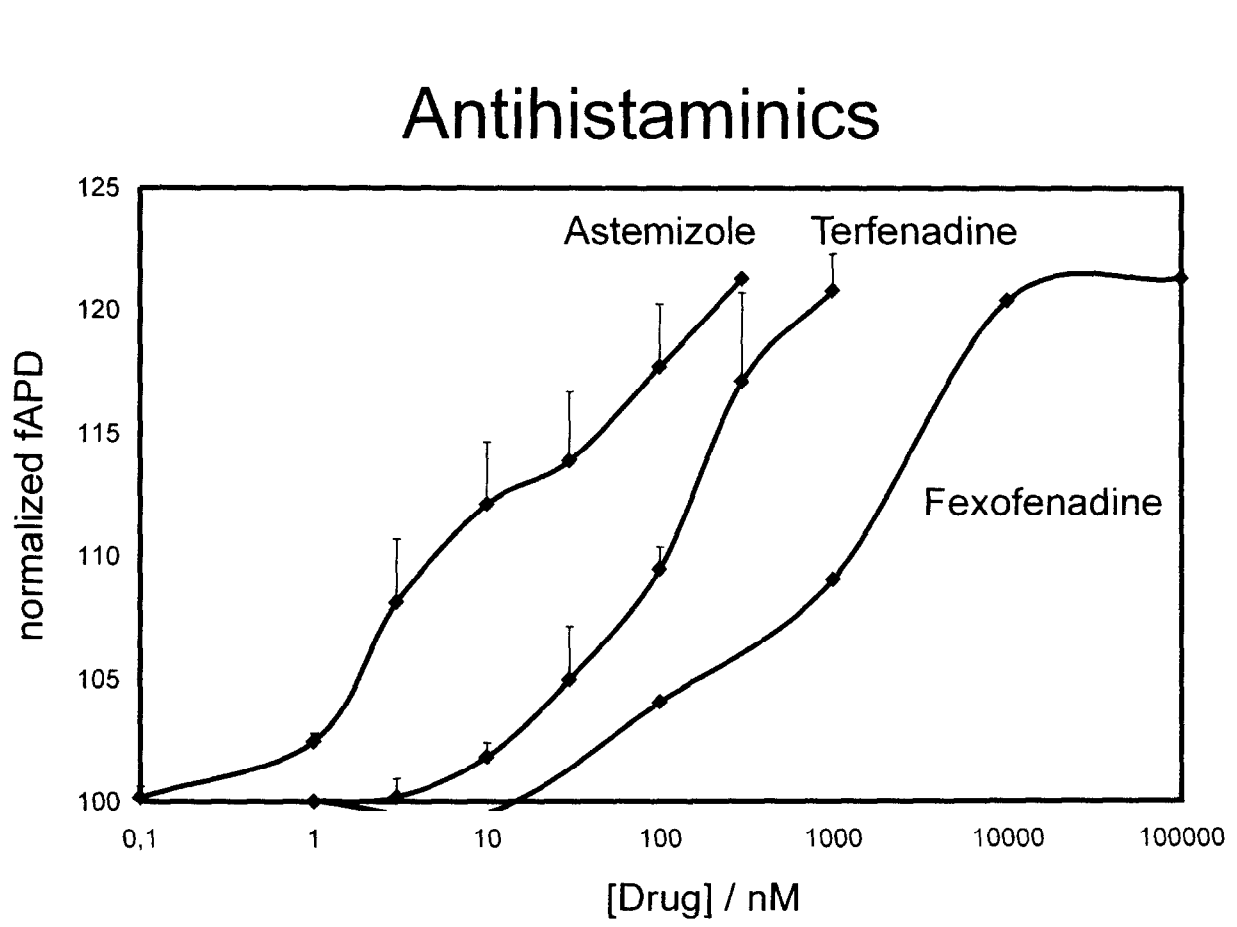


Figure 6 :

Dose response curves for the antihistaminic drugs Tefenadine, Astemizol and fexofenadine

Our data are in the same range. However for reasons not yet understood the negative control (Fexofenadine) caused in high concentrations also an fAPD prolongation. Whereas spontaneous activity stopped at concentrations of more than 30 nM Astemizole resp. 100 nM Terfenadine, there was still spontaneous activity at 100 µM of Fexofenadine. This clearly implies a much lower cardiotoxicity of Fexofenadine compared to Astemizol and Terfenadine.

Antihistaminic Drugs References:

Suessbrich H, Waldegger S, Lang F, and Busch AE, Blockade of HERG channels expressed in Xenopus oocytes by the histamine-receptor antagonists terfenadine and astemizole, FEBS Letters 385: 77-80 (1996)

Taglialatela M, Pannaccione A, Castaldo P, et al., Molecular basis for the lack of HERG K+ channel block-related cardiotoxicity by the H1 receptor-blocker cetirizine compared with other second-generation antihistamines, Mol Pharmacol 54: 113-121 (1998)

Zhou Z, Vorperian VR, Gong Q, Zhang S, and January CT, Block of HERG potassium channels by the antihistamine astemizole and its metabolites desmethylastemizole and norastemizole, J Cardiovasc Electrophysiol 10: 836-843 (1999)

Salata JJ, Jurkiewicz NK, Wallace AA, Stupienski RF, Guinosso PJ, and Lynch JJ, Cardiac electrophysiological actions of the histamine H1-receptor antagonists astemizole and terfenadine compared with chlorpheniramine and pyrilamine, Circ Res 76(1): 110-119 (1995)

Carmeliet E, Effects of cetirizine on the delayed K+ currents in cardiac cells: comparison with terfenadine, Br J Pharmacol 124: 663-668 (1998)

Lacerda AE, Kramer J, Shen, K-Z, Thomas D, and Brown AM, Comparison of block among cloned cardiac potassium channels by non-antiarrhythmic drugs, Eur Heart J Supplements 3 (suppl K): K23-K30 (2001).

Woosley RL, Chen Y, Freiman JP, and Gillis RA, Mechanism of the cardiotoxic actions of terfenadine, JAMA 269(12): 1532-1536 (1993)

5.1.3 E-4031

E-4031 is a methanesulfonanilide, class III antiarrhythmic drug, that was initially reported to prolong cardiac action potential duration and block I_{KR} in ventricular cells at submicromolar concentrations.

Later publications have shown that E-4031 is a specific blocker of HERG channels. This specificity and the high potency of this drug make it a standard reference. Our data are in line with various other authors, that E-4031.

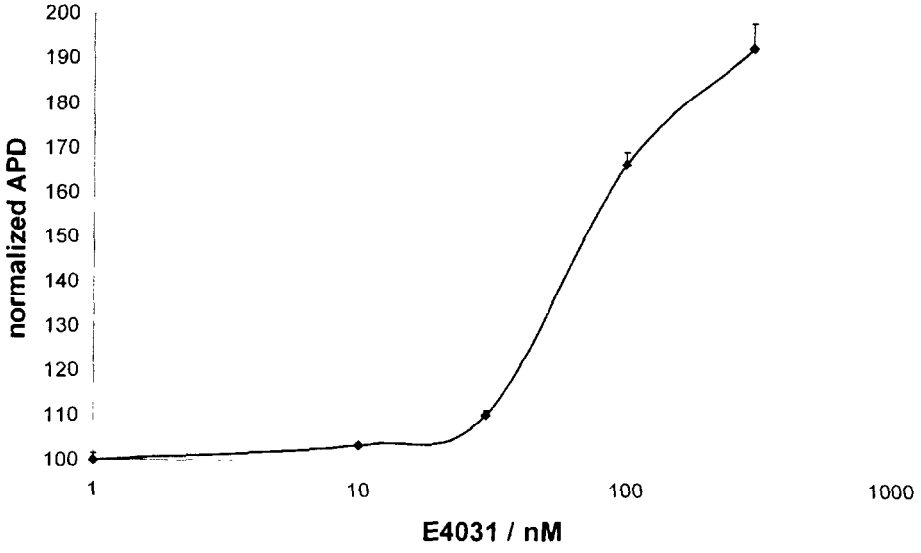


Figure 7 :Dose response curve for E-4031

As our tissue culture model involves cells which are able to generate spontaneous activity, and as these cells are coupled forming a functional syncytium, it is possible to observe primitive forms of arrhythmia.

These include early afterdepolarization (EAD) evoked action potentials, but can go as far as a kind of Tachycardia, "Torsade de Pointes". We know about the problems of transferability, however we would like to share the observations. Similar effects have been observed for Quinidine and Sotalol. Colleagues reported about EADs when cells were challenged with Cisapride.

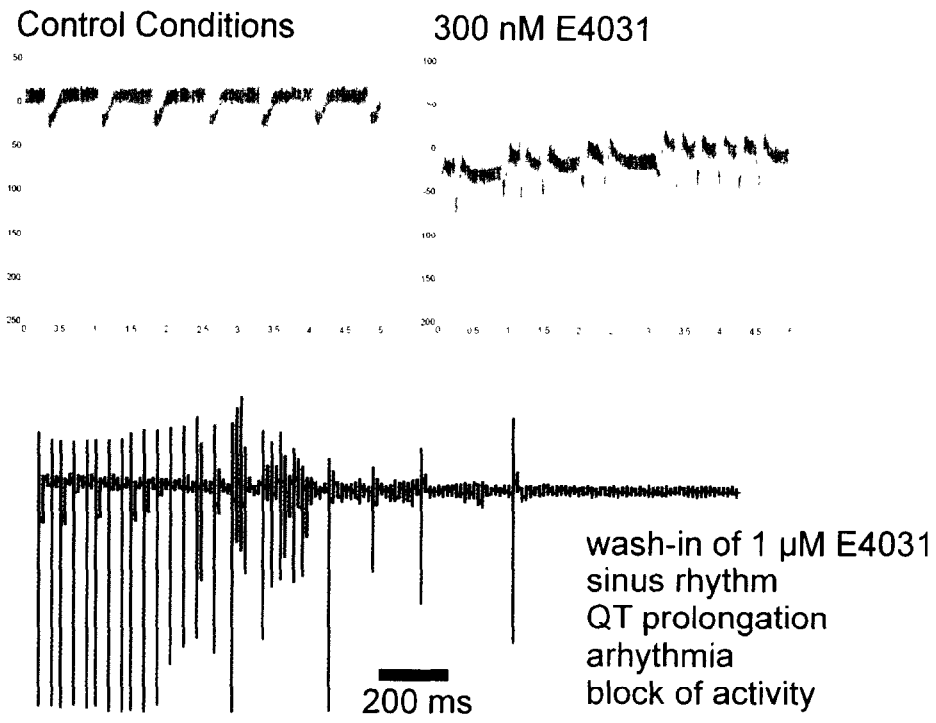


Figure 8 : Arrhythmia on the MEA

E-4031 References:

Liu S, Rasmusson RL, Campbell DL, Eanf S, and Strauss HC, Activation and inactivation kinetics of an E-4031-sensitive current from single ferret atrial myocytes, Biophys J 70: 2704-2715 (1996)

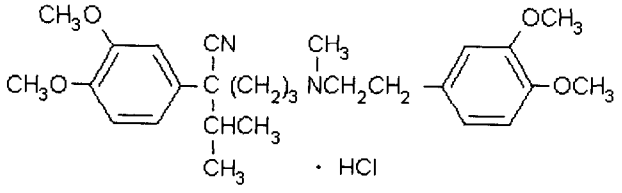
Abbott GF, Sesti F, Splawski I, et al., MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia. Cell 97: 175-187 (1999)

Zhou Z, Gong Q, Ye B, et al., Properties of HERG channels stably expressed in HEK-293 cells studied at physiologic temperature, Biophysic J 74: 230-241 (1998)

Sanguinetti MC and Jurkiewicz NK, Two components of cardiac delayed-rectifier K+ current: Differential sensitivity to block by class III antiarrhythmic agents, J Gen Physiol 96: 195-215 (1990)

5.1.4 Verapamil

Verapamil (5-[N-(3,4-Dimethoxyphenylethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile hydrochloride) is known as antagonist of the L-Type Calcium channel. The IC50 for L-Type Ca channels is in range of 100 nM. However at 150 nM there is the IC50 for a potent inhibition of HERG Channels. Verapamil is a notable example of a false positive: it blocks human ether-a-go-go-related (HERG) K(+) channels, but is reported to have little potential to trigger Torsade de Pointes (De Ponti et al.,2002). The dual effect of Verapamil on L-Type Calcium Channels and HERG channel even abolishes early afterdepolarisations, which represent the cause of Torsades (January CT. et al., 1989).



In a pure HERG assay Verapamil shows inhibition of HERG currents, whereas action potential duration in native cells is not affected. MEA recordings show only a minor shortening of action potential duration on a dose scale from 1 nM up to 3 µM. However positive controls show significant action potential prolongations (Quinidine, E4031, Sotalol as shown above).

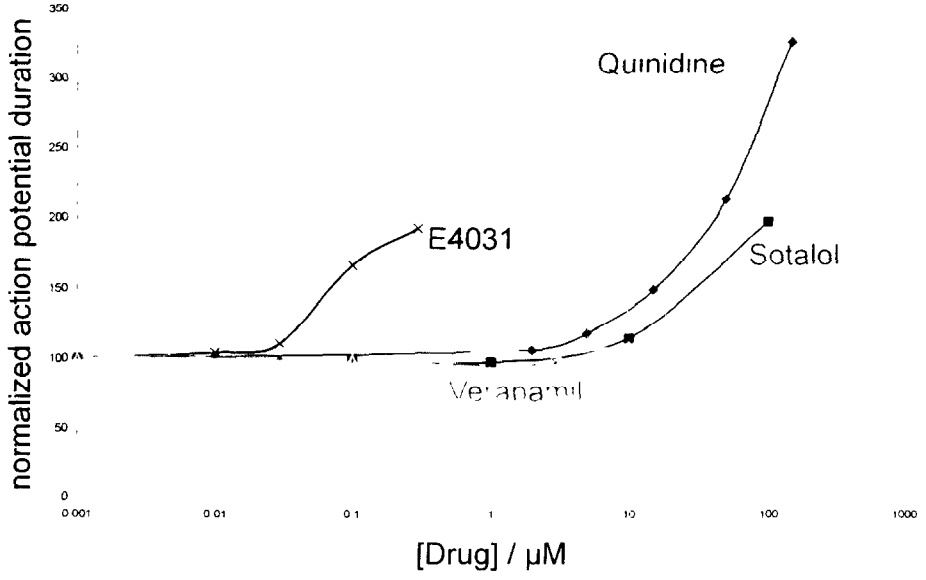


Figure 9:

Dose response curves of various drugs. It is remarkable, that Verapamil does not cause any fAPD prolongation, until cells stop spontaneous activity (due to either L-Type Ca Channel block, or HERG block)

The conclusion of these data is, that one – channel – assays do not reflect the complex regulatory mechanisms, which underlie the cardiac action potential. There is a significant risk, to lose potential drug candidates as false positive results of a safety pharmacological test based solely on heterogeneously expressed HERG channels. Cardiac safety requires cardiac cells.

Verapamil References:

De Ponti F, Poluzzi E, Cavalli A, Recanatini M, Montanaro N.
Safety of non-antiarrhythmic drugs that prolong the QT interval or induce torsade de pointes. an overview
Drug Saf. 2002;25(4):263-86.

January CT, Riddle JM.
Early afterdepolarizations: mechanism of induction and block. A role for L-type Ca2+ current.
Circ Res. 1989 May;64(5):977-90

Yang T, Snyders D, Roden DM
Drug block of I(kr): model systems and relevance to human arrhythmias.
J Cardiovasc Pharmacol. 2001 Nov;38(5):737-44.

5.1.5 Cisapride

Cisapride ((±)-cis-4-amino-5-chloro-N-[1-[3-(4-fluorophenoxy) propyl]-3-methoxy-4-piperidinyl]-2-methoxybenzamide monohydrate) is a prescription drug treatment approved only for severe nighttime heartburn experienced by adult patients with gastroesophageal reflux disease (GERD) that does not adequately respond to other therapies.
As of December 31, 1999, use of cisapride has been associated with 341 reports of heart rhythm abnormalities including 80 reports of deaths. Most of these adverse events occurred in patients who were taking other medications or suffering from underlying conditions known to increase risk of cardiac arrhythmia associated with Cisapride.
Even so the clinical data suggest a rather complex pharmacology, Cisapride was proven as a highly potent inhibitor of HERG channels in all in-vitro models studied. Depending on the model system studied IC50 values from subnanomolar ranges up to 15 nM were obtained. Our data show a first prolongation in subnanomolar ranges, whereas a half maximal was obtained at about 1 nM. However, concentrations of 30 nM and above lead to a loss of spontaneous activity.

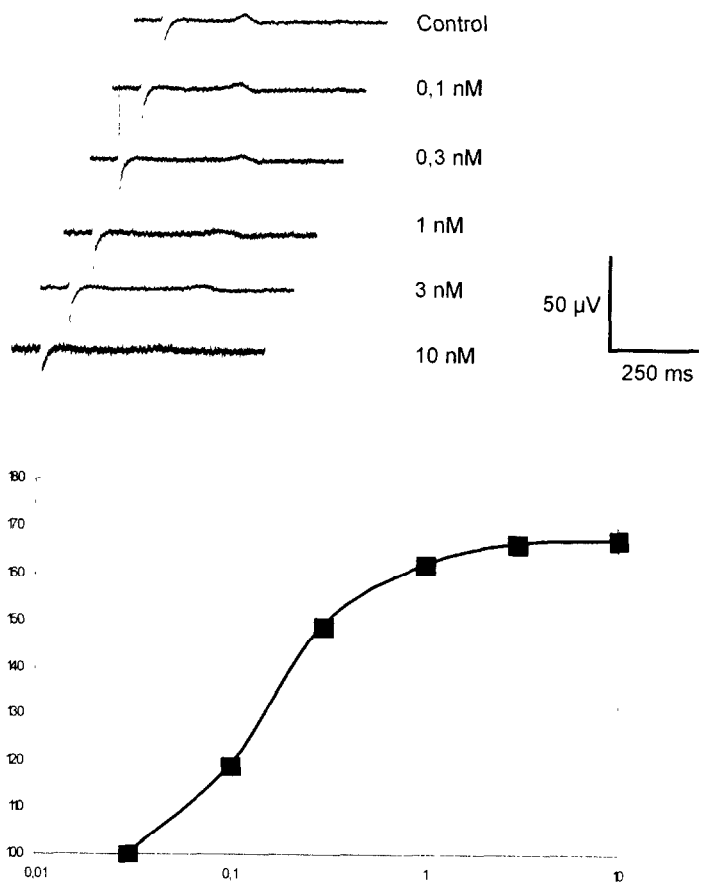


Figure 10:
Cisapride evokes a fAPD prolongation in subnanomolar concentrations. The upper panel shows averaged traces at various cisapride concentrations. The lower panel shows the dose response curve.

Cisapride References :

Lacerda AE, Kramer J, Shen, K-Z, Thomas D, and Brown AM, Comparison of block among cloned cardiac potassium channels by non-antiarrhythmic drugs, Eur Heart J Supplements 3 (suppl K): K23-K30 (2001)

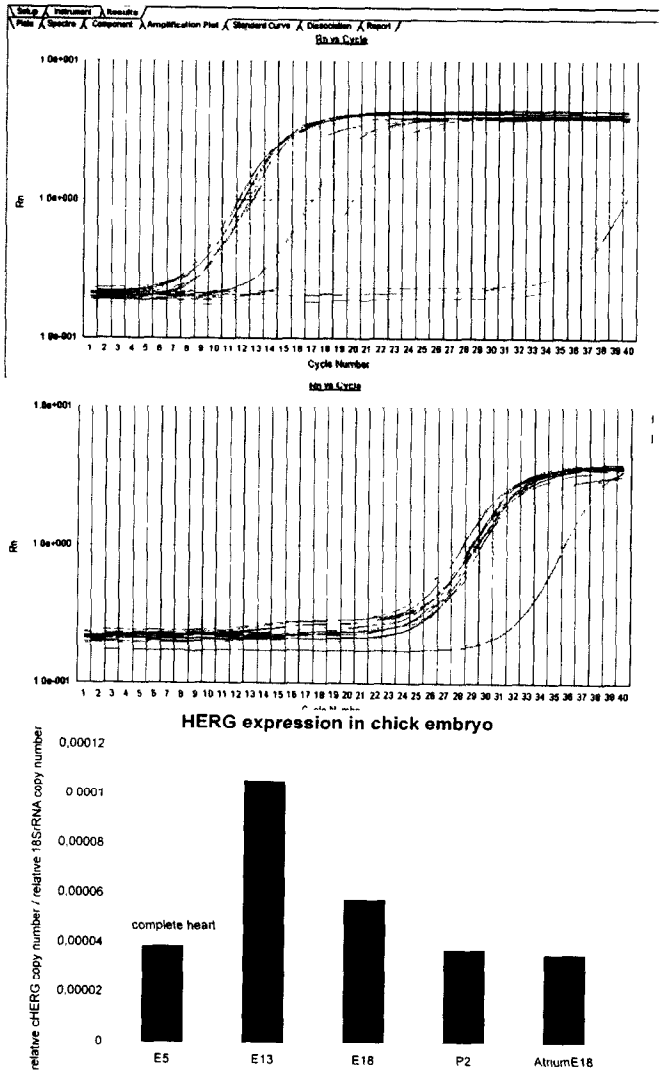
Mohammad S, Zhou Z, Gong Q, and January CT, Blockage of the HERG human cardiac K+ channel by the gastrointestinal prokinetic agent cisapride, Am J Physiol 273: H2534-H2538 (1997)

Walker BD, Singleton CB, Bursill JA, et al., Inhibition of the human ether-a-go-go-related gene (HERG) potassium channel by cisapride: affinity for open and inactivated states, Br J Pharmacol 128: 444-450 (1999)

Drolet B, Khalifa M, Daleau P, Hamelin BA, and Turgeon J, Block of the rapid component of the delayed-rectifier potassium current by the prokinetic agent cisapride underlies drug-related lengthening of the QT interval, Circulation 97: 204-210 (1998)

5. 2 Molecular biological evaluation of the system

Even so all data from the experiments described above clearly suggest, that the tissue culture system described above is a suitable system to validate the influence of various drugs on cardiac repolarization. It is a new, not established system. In order to proof, that the chicken analogue of the human HERG channel is expressed in cardiac myocytes isolated from embryonic chicken ventricle.



raw 18SrRNA positive control

raw chicken HERG

analyzed chickenHERG / 18SrRNA

Figure 11:
data from a real time RT-PCR experiment. Upper panel shows positve control 18SrRNA in various dilutions with NTC controls. The middle panel shows the same set of data for chickenHERG. The lower panel summarizes data for the different developmental stages as indicated.

However, one has to be aware, that real time RT-PCR only allows to retrieving information about the mRNA level for a specific gene. There is a certain correlation of mRNA to the protein level, but there is no 1:1 transferability of the data to protein level. Nevertheless our data show two things: On the one hand side there is a clear difference between the chickenHERG mRNA level and the non template controls, indicating that there is chickenHERG translation taking place. On the other hand side, there is in no way any evidence in the data, that during embryonal development chickenHERG expression is going to increase.
In the figure above mRNA levels of chickenHERG, normalized to 18SrRNA are shown. Please take particular note, that the E5 sample originated from a whole heart (due to the small size of the embryo at this development stage a separation of atrium and ventricle was not possible) whereas E13, E18 and P2 are ventricular preparations. The last bar codes for an atrial preparation at E18 stage.

References Molecular Biology :

GGA271210 1581 bp mRNA linear VRT 11-AUG-2000
Gallus gallus partial mRNA for HERG protein (erg gene).
ACCESSION AJ271210
Crociani,O., Cherubini,A., Piccini,E., Polvani,S., Costa,L., Fontana,L., Hofmann,G., Rosati,B., Wanke,E., Olivotto,M. and Arcangeli,A.
erg gene(s) expression during development of the nervous and muscular system of quail embryos
Mech. Dev. 95 (1-2), 239-243 (2000)

6. Conclusion

The data shown above allow to postulate following points :

a.) extracellular recorded field potentials allow the user to retrieve a comparable set of information about potential duration and repolarization properties as intracellular recordings do.
b.) pharmacological data obtained with well studied reference substances reveal comparable effects for all substances studied.
c.) molecular biology shows that the expression of the chicken analogue of the HERG channel is translated already during early embryonic development.
d.) MEA recording allows to retrieve data from up to 60 channels simultaneously and thus increases throughput drastically.

Even so further validation and investigation are essential, the data obtained till now make the MEA system a promising alternative to increase throughput in cardiac safety pharmacology. An increased throughput allows an early access to cardiac safety minimizing the risk for the patient and the cost for the developer.

7. References

A reference section is added in the respective paragraph. References here are publications concerning MEA and cardiac applications.

Banach K, Halbach MD, Hu P, Hescheler J, Egert U.
Development of electrical activity in cardiac myocyte aggregates derived from mouse embryonic stem cells.
Am J Physiol Heart Circ Physiol. 2003 Jun;284(6):H2114-23

Feld Y, Melamed-Frank M, Kehat I, Tal D, Marom S, Gepstein L.
Electrophysiological modulation of cardiomyocytic tissue by transfected fibroblasts expressing potassium channels: a novel strategy to manipulate excitability.
Circulation. 2002 Jan 29;105(4):522-9

Kehat I, Gepstein A, Spira A, Itskovitz-Eldor J, Gepstein L. Abstract High-resolution electrophysiological assessment of human embryonic stem cell-derived cardiomyocytes: a novel in vitro model for the study of conduction.
Circ Res. 2002 Oct 18;91(8):659-61.

Meiry G, Reisner Y, Feld Y, Goldberg S, Rosen M, Ziv N, Binah O.
Evolution of action potential propagation and repolarization in cultured neonatal rat ventricular myocytes.
J Cardiovasc Electrophysiol. 2001 Nov;12(11):1269-77.

Ulrich Egert, Thomas Meyer
Heart on a Chip – Extracellular multielectrode recordings from cardiac myocytes in vitro - a chapter in "Methods in Cardiovascular Research", S. Dhein and M. Delmar (eds.)
in press