Standardised in vitro Electrophysiologic Measurements Using Isolated Perfused Porcine Hearts – Assessment of QT Interval Alterations

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Summary
During the last decade there have been many clinical case reports on severe arrhythmias following drug administration. These potentially fatal heart rhythm disturbances are often associated with a QT interval prolongation in the ECG. The aim of the present study was to evaluate, whether isolated hemoperfused porcine hearts from slaughter pigs are suitable to detect a drug effect on the ECG. In the absence of test substances heart rate, QT or QRS interval remained fairly constant during 4 hours of perfusion. In contrast, after application of the positive control sotalol a reproducible, concentration-dependent QT interval prolongation as well as adverse effects such as described in clinical case reports were observed. Lidocaine, which served as a negative control, did not change QT interval duration. As a complex model the isolated hemoperfused porcine heart is a versatile system for monitoring drug effects on heart electrophysiology. Therefore, the model might reduce or even replace certain in vivo dog experiments in heart toxicology and safety pharmacology.

Keywords: QT prolongation, isolated heart, swine, hemoperfusion, in vitro, ECG

1 Introduction
Porcine hearts as well as hearts of small laboratory animals (rats, rabbits) have previously been used for isolated perfusion (Henze et al., 1989; Kimose et al., 1990; Budrikis et al., 1998; Chinchoy et al., 2000; Zabel et al., 1995; Miller et al., 1998; Liu et al., 1999). In contrast to the present study, animal experiments were not avoided in most studies since the pigs were sacrificed only for the surgical excision of hearts after regular anesthesia (Chinchoy et al., 2000 and Budrikis et al. 1998). Henze et al., (1989) and Kimose et al., (1990) used hearts from slaughter pigs and achieved a maximal perfusion time of 120 minutes compared to a maximal perfusion time of 240 min in the study presented here.

With a perfusion time of 4 hours the isolated perfused porcine heart exceeds the perfusion time of isolated hearts of small laboratory animals remarkably (Zabel et al., 1995; Miller et al., 1998; Liu et al., 1999), which can be perfused maximally for 2 hours (Döring, 1996; Budrikis et al., 1998). Moreover, the pig has been mentioned as a suitable species for detecting drug impact with a highly predictive potential (CPMP, 1997; Hammond et al., 2001; Health-Canada, 2001).

During the last decade there have been many case reports of severe arrhythmias following drug administration (Monahan et al., 1990; Farrar et al., 1993; Brandriss et al., 1994). These specific, potentially fatal polymorphic ventricular tachycardias are termed torsade de pointes (TdP) due to their characteristic appearance of continuously twisting QRS complexes in the 12 lead electrocardiogram (ECG). The QT interval prolongation in the ECG is often – though not always – indicative for TdP (Haverkamp et al., 2000).

Drugs known to have proarrhythmic effects belong to a wide range of substance
classes such as antiarrhythmics, antihistamines, antibiotics, antimalarials or psychotropic agents (Eckardt et al., 1998; Haverkamp et al., 2000). Regulatory authorities together with experts in the field started to develop new strategies suitable to identify the proarrhythmic potential of drugs (CPMP, 1997; CPMP, 2000; Health-Canada, 2001). Currently there is no consensus about the most predictive in vitro and in vivo assay. In 1999 a survey was conducted in the pharmaceutical industry with the objective of assessing approaches, practices and methodologies currently used to investigate the proarrhythmic potential of drugs (Hammond et al., 2001). 54 companies involved in the survey obtained in vivo ECG data using different species. The living dog was used in 56% of these in vivo studies. Pigs were rarely used both in in vivo and in vitro studies although the porcine heart is anatomically and physiologically close to the human heart (Horstick et al., 1997; Piper et al., 1998).

The aim of the present study was to evaluate, whether the isolated porcine heart as used by Mediport Biotechnik is suitable to detect a drug effect on the QT interval in the ECG and on heart rhythm. As porcine hearts from slaughter pigs were used, this isolated heart model might reduce or even replace complex in vivo testings.

2 Animals, material and methods

2.1 Preparation of porcine hearts
Hearts (300 - 500 g) of German Landrace Pigs weighting 70 - 100 kg were obtained at selected local abattoirs from the routine slaughtering procedure except that the animals were not treated with hot water after bleeding. The animals were anesthetised via electric shock (250 J for 8 sec) and killed by bleeding through the cranial vena cava. Hearts were excised within 5 minutes through a paramedian transection of the thorax. Coronary arteries were immediately perfused with cardioplegic solution (4°C) according to Modersohn et al. (2001) supplemented with 2000 LU./l Heparin (Liquemin*N 25.000, Hoffmann-La-Roche). Hearts were transported to the laboratory in cardioplegic solution at 4°C. Autologous blood was drawn off the Vena cava cranialis and collected in a stainless steel jar containing 50 ml of 3.8% sodium citrate solution. Blood was transported on ice in 11 plastic bottles containing 10,000 I.U. heparin.

To connect the hearts to the perfusion system the right coronary artery and the left anterior descending branch (LAD) as well as the circumflex coronary artery (CX) of the left coronary artery were cannulated using 3 flexible silicon catheters with a 3 cm teflon tip and an inner diameter of 3 mm. For fixation in the perfusion system, a T connector (3/4", Cole-Parmer) was inserted and fixed in the aorta.

2.2 Perfusion system

The perfusion system (Fig.1) was developed by Mediport Biotechnik based on the principles published recently (Grosse-Siestrup and Modersohn, 1997; Modersohn et al., 1997; von Baeyer et al., 1997). For perfusion of hearts a blood-based perfusate with a hemoglobin concentration of 80 g/l was used. Auto-

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Fig. 1: Schematic drawing of the perfusion system
alogous blood was diluted with dialysate buffer according to Modersohn et al., (2001) and supplemented with 4.0% bovine serum albumin (Albumin Fraction V, Roth) and 2000 I.U./l Heparin (Liquemin® 25,000, Hoffmann-LaRoche). The perfusate was oxygenated to reach an oxygen saturation (SO₂) of 100%, warmed up to 38°C and dialysed using a dialysis cartridge (Hemoflow F7 low flux, Fresenius) against 31 of dialysate solution (Fig. 1).

Key parameters to be controlled during perfusion were as follows:
- Coronary perfusion pressure (CPP): 70 - 80 mmHg
- Organ flow (CBF/Loo g): 60 - 120 ml/min·(100 g organ weight)⁻¹
- Heart rate (HR): Autonomous sinus rate at 100 - 140 min⁻¹ or when sinus rhythm was below 100 min⁻¹ paced (EDP 20/A, Biotronik) at 100-
- pH in the arterial perfusate: 7.38 - 7.42
- SO₂ in the arterial perfusate: 100%
- Ca²⁺ concentration in the arterial perfusate: 2.2 - 2.9 mM

During perfusion total coronary blood flow (CBF), CPP and perfusate as well as dialysate temperature were monitored and adjusted appropriately. Blood gas analysis (ABL555, Radiometer) of both perfusate and dialysate as well as oximetry (OSM3, Radiometer) of the perfusate was performed prior to start of perfusion and then every 30 minutes during the experiment.

2.3 Electrocardiogram (ECG)
ECG electrodes were placed in the perfusate close to the reservoir wall simulating the Wilson and Einthoven configuration with the perfusate serving as electrolyte. A 12-lead ECG (Corina, Marquette Hellige) and a commercial analysis software (Cardiosoft V4.1, Marquette) for leading, visualising and analysing the ECG leads were used. For electrophysiological characterisation and collection of basic data 10 hearts were perfused for 4 hours in the absence of test substances.

2.4 Drug testing
After connection to the perfusion system hearts were allowed to equilibrate during an adaptation period. Experiments were started when a CPP between 70 and 80 mmHg and an CBF_Loo g of 60 to 120 ml/min·(100 g organ weight)⁻¹ was reached.

Basic values were registered over a 30 min time period. Afterwards the test substance was administered at the lowest concentration as indicated in the figure legends. 10 hearts were treated with either sotalol (Rentibloc®, Fuisz Pharma, Laupheim) or lidocaine (Lidocain 0.5%, Steigerwald Arzneimittelwerk GmbH, Darmstadt). The antiarrhythmic sotalol was used as positive control due to its known proarrhythmic potential (Roden, 1993; Eckardt et al., 1998; Haverkamp et al., 2000). Because there is no clinical report on TdP following lidocaine administration, the antiarrhythmic lidocaine served as negative control. Initially the lowest dose of the particular drug was chosen according to the known human therapeutic plasma levels. ECG leads were recorded for 30 min after each drug administration. A second dose at higher concentrations was applied as indicated.

3 Results
3.1 Standardised ECG leads
ECG recording from the isolated porcine heart rendered ECG cycles with a de-

**Tab. 1: ECG parameters in the absence of test substances**
10 hearts were perfused for a total of 4 hours and ECG parameters analysed after 0.5, 1, 2, 3 and 4 hours of perfusion. Values are means ± SD.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Perfusion time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (min⁻¹)</td>
<td>0.5 1 2 3 4</td>
</tr>
<tr>
<td>120 ± 11</td>
<td>118 ± 9</td>
</tr>
<tr>
<td>QRS (ms)</td>
<td>128 ± 12</td>
</tr>
<tr>
<td>QT (ms)</td>
<td>337 ± 31</td>
</tr>
</tbody>
</table>

![Fig. 2: Original print-out of a detail of an ECG lead recorded from an isolated porcine heart](image-url)

The markers are set by the ECG analysis software indicating the different ECG portions specified in the following legend:
P: P wave (atrial depolarisation); PQ: PQ interval (time from the beginning of atrial excitation until the beginning of ventricular depolarisation); QRS: QRS complex (ventricular depolarisation); T: T wave (ventricular repolarisation); QT: QT interval (ventricular excitation from the beginning of depolarisation to the end of repolarisation)
tailed appearance comparable to a human ECG lead (Fig. 2).

To determine HR, QRS and QT interval 10 hearts were perfused for 4 hours (Tab. 1). Values for HR, QRS and QT interval remained nearly unchanged during the 4 hours of perfusion. Heart rate varied between 116 min⁻¹ and 120 min⁻¹, QRS between 127 ms and 132 ms and QT between 331 ms and 338 ms, respectively. These changes were in the range of the biological variation. Because of the high reproducibility of the heart perfusion system it could be used to estimate a potential drug impact on heart function.

3.2 Drug impact on ECG

After application of the positive control sotalol a concentration-dependent QT interval prolongation was observed in all 5 individual experiments. Whereas 4 μg/ml sotalol, which is in the range of the human therapeutic plasma concentration, increased the QT interval by 7.4 ± 3.2% (MW ± SEM) compared to the QT interval without sotalol, 8 μg/ml increased the QT interval by 13.4 ± 5.5%. The negative control lidocaine did not change basal QT interval duration (Tab. 2).

After addition of 4 μg/ml sotalol multiple effects on heart rhythm were observed in a single experiment (Fig. 3): 14 min after addition of sotalol the ECG was asystolic but recovered spontaneously exhibiting a normal sinus rhythm (Fig. 3B). 30 sec later ventricular tachycardia was observed (Fig. 3 C), which another 30 sec later changed into a bradyarrhythmia (Fig. 3 C). Bradyarrhythmia after sotalol application was observed in additional 2 hearts.

Tab. 2: QT prolongation following sotalol or lidocaine administration

Test substances were applied after registration of basic values; influence on QT interval is expressed as maximal percentage of prolongation over basic values within 30 min after administration of test substance. Values are means ± SEM from the indicated number of individual experiments.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration</th>
<th>% QT</th>
<th>Human therapeutic plasma concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(μg/ml)</td>
<td>n</td>
<td>(MW±SEM) (μg/ml)</td>
</tr>
<tr>
<td>Sotalol</td>
<td>4</td>
<td>5</td>
<td>7.4 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>5</td>
<td>13.4 ± 5.5</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>6</td>
<td>3</td>
<td>0.7 ± 0.5</td>
</tr>
</tbody>
</table>

*according to: Pharmazie 1997; 52(12), 895-911

4 Discussion

4.1 Assessment of QT interval alterations using isolated perfused porcine hearts

In the absence of test substances heart rate, QT or QRS interval remained fairly constant without significant variations during 4 hours of perfusion. In agreement with literature data a reproducible, concentration dependent QT interval prolongation was observed after application of the positive control sotalol. The delay of heart depolarisation is the mechanism of sotalol action and marked QT interval prolongation was seen in approximately 5% of patients receiving d,L-sotalol. The incidence of TdP ranged between 1.8 and 4.8% (Haverkamp et al., 2000). Adverse effects as described in clinical case reports - such as bradyarrhythmia, tachyarrhythmia or TdP-like arrhythmias (Schmid et al., 1989; Eckardt et al., 1998; Haverkamp et al., 2000) were observed in 3 out of 5 individual experiments. In our study, lidocaine did not cause a change in QT interval duration. According to the literature lidocaine was found to produce a slight shortening in QT interval duration on dog Purkinje fibres (Roden, 1993). These findings support the isolated porcine heart as a suitable method for the monitoring of heart electrophysiology.

Mediport Biotechnik has established an ample data base comprising results from the testings of positive and negative control drugs from different substances.
classes like antipsychotics, antibiotics, antimalariais, antihistaminics etc. This warrants that the model of the perfused porcine heart serves as a well characterised system for the assessment of drug impact on heart electrophysiology. Along this line experts and regulatory authorities agree that the simultaneous conduction of experiments with positive control substances is mandatory in a study design for the assessment of a drug's impact on cardiac electrophysiology (Hammond et al., 2001; Health-Canada, 2001). Since the QT interval duration depends on heart rate, the use of QT correction formulas is a common practice in clinical (Moss, 1993; Zareba et al., 1997) and experimental studies (Salata et al., 1995; Ohtura et al., 1999), although there is controversy, whether and which formula should be utilised (Funck-Brentano and Jaillon, 1993; Haverkamp et al., 2000; Health-Canada, 2001). Hammond and co-workers (Hammond et al., 2001) recommend that the QT interval duration should be corrected, when a statistically significant change in heart rate is observed. The percentage of QT interval prolongation shown in this study is based on values where no significant change in heart rate was observed and hence no QT interval correction for heart rate was necessary.

4.2 QT alterations in patients following sotalol administration

Woosley and co-workers (Woosley et al., 1990) have conducted a clinical trial in patients with non-sustained ventricular arrhythmia where they correlated sotalol plasma concentrations with the change in QTc. Sotalol plasma concentrations associated with significant increases in QTc were 2.55 μg/ml reached at a mean oral dosage of 969 mg/day. Changes in electrophysiologic parameters measured at a dosage of 800 mg/day of d-sotalol were 13 ± 9% prolongation of QTc. In the studies presented here the QT prolongation was 7.4 ± 3.2% after administration of 4 μg/ml sotalol and 13.4 ± 5.5% following 8 μg/ml sotalol. These findings are in line with those achieved in the clinical trial. However, it has to be taken into account that the patients involved in the clinical study were treated with sotalol due to their arrhythmias, making a differentiation between treatment and a possible drug effect in healthy patients difficult.

4.3 Perspectives for a test scheme comprising the isolated porcine heart

The isolated perfused porcine heart is a sophisticated model generating a data pool, which reflects the complex cardiac molecular architecture as a whole. As such it provides valuable information comparable to that gained in the in vivo dog model. Thus, it allows for more concise conclusions as can be drawn from isolated cell systems.

Since the regulatory requirement for testing the impact of new drugs on heart electrophysiology and proarrhythmic potential demands a highly predictive value of a chosen method, the isolated porcine heart might offer an alternative in vitro-model avoiding animal testing to a certain extend.

A feasible test strategy for the assessment of the potential of new drugs to prolong repolarisation has been proposed by Haverkamp and co-workers (Haverkamp et al., 2000): The critical evaluation of the expected clinical value of a new compound has top priority before the start of studies. Similarities to compounds known to prolong QT interval has to be taken into account. For a first estimation of a possible effect on QT, in vitro tests in transgenic cells with cloned channels or isolated cells / tissues were suggested. These assays could be used as a screening to confine the number of new compounds, while compounds affecting action potential duration (APD) or ion currents involved in the cardiac repolarisation of the heart should be excluded from or re-evaluated in preclinical development, respectively. New compounds showing no depolarisation-prolonging potential in the screening phase should be evaluated further. At this stage, complex models are postulated, the in vivo dog model being the preferred model in toxicology and safety pharmacology (Hammond et al., 2001). As the isolated perfused porcine heart is a close to in vivo model generating continuous ECG data under standardized conditions, it might be suitable to reduce or in the future even replace s dog experiments.

Abbreviations

CFB: coronary blood flow
CFB, organ flow
CPP: coronary perfusion pressure
ECG: electrocardiogram
HR: heart rate
QRS: QRS interval
QT: QT interval
QTc: QT interval corrected for heart rate
SO2: oxygen saturation
Tdp: Torsades des Pointes

References


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