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Original article

Safety pharmacology assessment of drug-induced QT-prolongation in dogs with reduced repolarization reserve

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Abstract

Introduction: Drug-induced QT-prolongation, often based on hERG K^+ current inhibition, has become a major safety concern during drug development. Hence, regulatory guidelines require combined in vitro and in vivo assays to assess the potential of new chemical entities to delay ventricular repolarization. Here, results of a pharmacological validation study with the torsadogenic compound sotalol are presented. **Methods:** Alteration of ECG parameters was investigated in both conscious and anesthetized Beagle dogs (cumulative infusions of D,L-sotalol; $n=6$). The repolarization reserve of the latter was reduced by neurolept anesthesia using the hERG blocker droperidol (0.25 mg/kg/h yielding mean plasma concentrations of 0.5 μM). Furthermore, hERG K^+ current and action potentials (AP; rabbit Purkinje fibers) were measured in vitro. **Results:** The Fridericia corrected QT interval, QTcF, in conscious dogs (control: 254±15 ms), was dose-dependently prolonged by D,L-sotalol (+42 ms at plasma levels of 261 μM; dose 30 mg/kg). In anesthetized dogs, baseline QTcF (337±35 ms) was already prolonged compared to conscious dogs. In addition, QTcF-increase (+90 ms) was more pronounced at lower D,L-sotalol plasma levels (181 μM; dose 10 mg/kg), and proarrhythmic markers T_{peak}–T_{end} and short term variability of QT were increased. These in vivo findings are supported by in vitro data. The hERG K^+ current was blocked by D,L-sotalol (IC_{50} ~1.2 mM, IC_{20} ~250 μM) and droperidol (IC_{50} ~0.1 μM, IC_{20} ~0.02 μM). Purkinje fiber APs were concentration-dependently prolonged by D,L-sotalol (APD_{90}: +60% at 30 μM) and droperidol (APD_{90}: +55% at 1 μM). Low droperidol concentrations increased the sensitivity of Purkinje fibers towards D,L-sotalol-mediated AP prolongation. **Discussion:** In conclusion, the higher sensitivity of anesthetized dogs towards sotalol-induced QT-prolongation is due to a reduced cardiac repolarization reserve caused by the hERG blocker droperidol. Hence, the droperidol-/fentanyl-/N_2O-anesthetized dog is a particularly sensitive animal model for the detection of drug-induced QT-prolongation in safety pharmacology studies.

Keywords: Action potential; Beagle dog; Cardiac repolarization; Droperidol; hERG channel; ICH S7B; Purkinje fiber; QT interval; Reprolarization reserve; Sotalol

1. Introduction

Drug-induced proarrhythmia is a major concern in the development of new therapeutic agents. The prolongation of the cardiac action potential duration (APD), reflected in the electrocardiogram (ECG) as QT interval prolongation, is associated with rare, but potentially lethal, polymorphic ventricular tachyarrhythmia also known as Torsades de Pointes (TdP). The exact relationship between QT interval prolongation and the development of TdP is not yet fully understood (Redfern et al., 2003). Currently no validated in vitro or in vivo model that predicts the potential of drug-induced TdP is available (Belardinelli, Antzelevitch, & Vos, 2003). Therefore, clinicians and regulatory authorities frequently focus on QT interval prolongation as a surrogate endpoint for proarrhythmia (Fermini & Fossa, 2003). While drug treatment-evoked TdP is unlikely to be detected within the context of a normal clinical development program due to low incidence, a carefully designed preclinical study program consisting of complementary in vivo and in vitro studies (ICH Guidance S7B, http://www.ich.org/cache/compo/276-254-1.html) can identify drug-associated QT interval prolongation. The prolongation of APD/QT interval may occur spontaneously in subjects having loss-of-function mutations in cardiac ion channel genes and may also be acquired due to a growing number of antiarrhythmic and also non-cardiovascular drugs, most of which inhibit the rapid component of the delayed rectifier potassium current (I_{Kr}) encoded by the human ether-a-go-go-related gene or hERG. Acquired long

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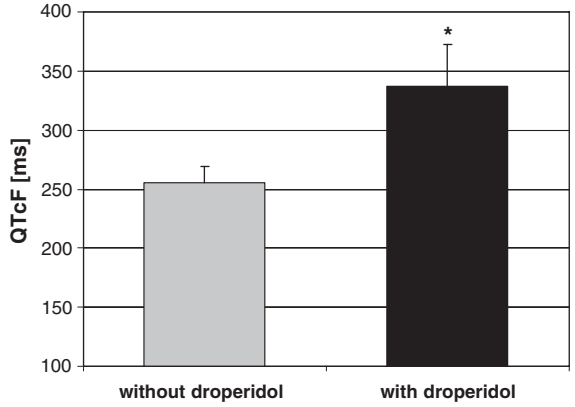

Fig. 1. QTcF interval prior to D,L-sotalol infusions is clearly (*$p<0.001$) prolonged in dogs with neuroleptic anesthesia compared to same dogs without droperidol. At droperidol plasma concentrations of 0.5 μM (185 μg/L) in anesthetized dogs QTcF baseline values were 337±35 ms versus 254±15 ms in conscious dogs.

QT, however, is not restricted to drugs, but includes other factors modulating repolarization like hypokalemia, bradycardia, gender, hypertrophy and/or heart failure (Haverkamp et al., 2000). The magnitude or number of additional factors that reduce repolarization reserve determines the amount of APD/QT interval prolongation which is required to create proarrhythmogenic circumstances (Roden, 1998).

The ICH S7B Guideline recommends to identify preclinical signs of a TdP hazard of new chemical entities (NCE) by an integrative testing approach with evaluations at the cellular, tissue, and intact organism level. At the cellular level, the most logical target is the hERG potassium channel that is usually investigated in a heterologous mammalian expression system by means of the voltage-clamp technique. Since hERG is not the only target for potentially affecting cardiac repolarization, action potential measurements in isolated tissues (e.g. Purkinje fibers) may give useful information on non-hERG-associated repolarization abnormalities. Finally, ECG studies in intact, preferably conscious and unstressed animals, e.g. dogs permit the evaluation of pharmacodynamic, homeostatic compensatory and metabolite effects. Here, an adequate testing practice is described and discussed with regard to sensitivity of hazard detection in drug development. The use of conscious animals is often limited in NCEs with high acute toxicity.

2. Methods

2.1. In vivo electrophysiology studies in Beagle dogs

Six Beagle dogs (3 females, 3 males, age approx. 11 months, body weight 11–16 kg), which were examined for their health status by a veterinary without any findings before the start of the study were used. They were trained standing in a conventional sling apparatus to allow continuous ECG monitoring over a period of 75–90 min. The dogs were fasted the day preceding the experiments. Baseline values were recorded approximately 20 min and averaged 5 min prior to dosing representing predrug values. In a first series of experiments the conscious dogs were brought to a quiet room for ECG monitoring of standard surface limb leads (I, II, III). The dogs received three consecutive intravenous infusions of D,L-sotalol at infusion rates of 0.03, 0.3, and 3 mg/kg/min over 10 min each. After the stop of the highest infusion rate a 30 min washout period followed.

Three to 13 days (median: 8 days) later the same 6 dogs were used in a second series of experiments with general neuroleptic anesthesia. For this purpose after premedication of atropine (0.01 mg/kg i.v.) anesthesia was initiated by intravenous barbital injection (thiopental 20 mg/kg) and sustained by continuous infusion of droperidol (0.25 mg/kg/h) and fentanyl (0.04 mg/kg/h). The non-depolarizing skeletal muscle relaxant alcuronium chloride was infused (0.06 mg/kg/h) and dogs were artificially ventilated with nitrous oxide/oxygen (1:3) at a frequency of 16 breaths/min. Substitution of electrolytes (K^+ 0.25 mmol/L/h, Na^+ 0.8 mmol/L/h, HCO_3^- 0.5 mmol/L/h, Cl^- 0.56 mmol/L/h) was accomplished by continuous infusion of KCl containing

Table 1
Effects of cumulative D,L-sotalol infusions to conscious dogs

Conscious dogs						
Droperidol dose	[mg/kg/h i.v.]	0	0	0	0	0
D,L-sotalol dose	[mg/kg/10 min i.v.]	0	0.3	3	30	Washout
HR	[beats/min]	87±16	79±13	72±10	94±7	78±8
RR	[ms]	710±133	880±181	841±128	640±49	774±77
QT	[ms]	226±23	238±29	253±31	255±20*	264±32*
QTcF	[ms]	254±15	249±16	268±20	296±21**	288±26*
QT STV	[ms]	4.0±2.6	3.2±1.9	4.2±1.6	3.6±1.3	5.6±2.1
T_{peak}–T_{end}	[ms]	39±10	40±10	42±10	46±6	45±5
C [Sot]	[mg/L]	0	0.4±0.1	4.5±0.9	70.8±7.5	22.6±2.2
	[μM]	0	1.5	16.5	261	83
C [Dro]	[μg/L]	0	0	0	0	0
	[μM]	0	0	0	0	0
P [K^+]	[mmol/L]	4.2±0.3				

Given are mean values±standard deviations (SD) prior to infusions start, at the end of each 10 min infusion step, and at the end of the washout period (30 min after last dose). Heart rate (HR), RR interval (RR), QT interval (QT), QT interval corrected for heart rate using Fridericia's formula (QTcF), short term variability of QT interval (QT STV), interval from peak to the end of T wave (T_{peak}–T_{end}), plasma concentration of D,L-sotalol (C [Sot]), plasma concentration of droperidol (C [Dro]), plasma concentration of K^+ (P [K^+]). ECG: *$p<0.05$ vs baseline, **$p<0.01$ vs baseline (Student's paired t-test).