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Original article

Pharmacokinetic–pharmacodynamic modeling of QRS-prolongation by flecainide: Heart rate-dependent effects during sinus rhythm in conscious telemetered dogs

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ABSTRACT

Introduction: The duration of the QRS interval is determined by the ion currents involved in cardiac depolarization. Class I antiarrhythmic drugs reduce cardiac excitability and conduction by inhibiting Nav1.5 channels responsible for I_{Na}, thus increasing the QRS interval. Previous studies in humans as well as in animal models have demonstrated a more pronounced effect on QRS-prolongation during higher heart rates. In the present study, the effects of the Nav1.5 inhibitor flecainide on cardiovascular parameters, were studied in the telemetered beagle dog under normal autonomic control. The heart rate dependency of QRS prolongation was characterized using pharmacokinetic–pharmacodynamic (PKPD) modeling. **Methods:** Four male telemetered beagle dogs were administered placebo or flecainide (100, 150 and 200 mg) in a Latin square design. The QRS interval and heart rate were recorded, and blood samples were taken. Plasma concentrations of flecainide were fitted to a one compartment oral model and the intrapolated plasma concentrations were fitted to QRS and heart rate data sampled during 5 h after dosing. **Results:** Flecainide increased the QRS interval in all dogs, whereas there were no effects on heart rate. Using the PKPD model, a statistically significant heart rate-dependent QRS prolongation was linked to individual concentration–time profiles of flecainide. **Discussion:** PKPD analysis of QRS interval data from unrestrained dogs with sinus rhythm can elucidate mechanisms previously only described during controlled heart rhythm. Specific questions can therefore be addressed in generically designed cardiovascular telemetry safety studies and different types of relationships between parameters can be uncovered. In addition, the present approach can be used to better characterize drug-induced QRS effects in cardiovascular dog models.

1. Introduction

The duration of the QRS interval is determined by the ion currents involved in cardiac depolarization. Class I antiarrhythmic drugs reduce cardiac excitability and conduction by the inhibition of Nav1.5 channels responsible for I_{Na}, thus increasing the QRS interval. The drugs are subdivided into classes a–c based on the characteristics of the blockage they create. Although these drugs are clinically useful as antiarrhythmics, Nav1.5 inhibition also has the potential to produce arrhythmias under certain circumstances (CAST, 1989; Starmer, Grant, & Colatsky, 2003). A link between QT prolongation and the risk of developing fatal disturbances in the cardiac rhythm is well described. Therefore, possible drug-induced alterations in the QT interval are carefully monitored according to the relatively specific ICH S7B guidelines, whereas assessment of changes in the QRS interval is described only in broad terms. However, given the possible arrhythmogenic potential of Nav1.5 inhibition, and since a number of registered non-cardiology drugs have been found to inhibit Nav1.5, the interest in a more thorough safety pharmacological evaluation of QRS changes has increased (Erdemli et al., 2012; Harmer, Valentin, & Pollard, 2011; Heath et al., 2011). Flecainide is a class 1c antiarrhythmic that displays a use-dependent blockage by preferential affinity to the open state of the Nav1.5 channel. Compared to class 1b drugs, flecainide dissociates slowly, consequently producing a constant block during the cardiac cycle (Anno & Hondeghem, 1990; Ramos & O'Leary, 2004). In line with flecainide's electrophysiological properties, previous studies in humans (Takanaka, Lee, Nonokawa, Sugiyama, & Yame, 1994) as well as in animal models (Cros, Skinner, Moors, Lainee, & Valentin, 2012; Heath et al., 2011) have demonstrated a more pronounced effect on QRS prolongation during higher heart rates. Experimental

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and mathematical models indicate that use-dependent Nav1.5 inhibitors with slowly dissociating kinetics can prolong the vulnerable period of the cardiac cycle thus promoting arrhythmias, in particular during higher heart rates (Starmer, Lancaster, Lastra, & Grant, 1992; Starmer et al., 2003). Earlier studies on rate-dependent effects on the QRS interval, has usually altered the heart rate through different types of pacing which is not commonly used in safety pharmacological studies. Pharmacokinetic–pharmacodynamic (PKPD) modeling strives to describe concentration–response relationships in various experimental models and studies. Modeling is not routinely used in safety pharmacological studies and is not described in the ICH S7A or S7B guidelines but has big potential since it is possible to extract more information from studies conducted at early stages of drug development which facilitates the selection of drug candidates and decision making for further development (Cavero, 2007). In the present study, the effects of flecainide on cardiovascular parameters were investigated in the telemetered beagle dog during autonomic heart rate regulation, and, by using PKPD modeling, the frequency dependency of Nav1.5 inhibition is described.

2. Materials and methods

2.1. Animals

Four male beagle dogs (9–11 kg body weight, 23–30 months; Kennel Rååhöjden, Örkelljunga, Sweden) were used. They had previously been used in other studies, but at least two months had passed since their last drug administration. Before and throughout the study they were fed a daily ration of Specific CXD (A/S Arovit Petfood, Esbjerg, Denmark). All experiments were performed in accordance with the NIH guidelines for the care and use of laboratory animals and approved by the animal care and use committee for southern Stockholm.

2.2. Surgical procedure

A telemetric transmitter (TL11M3-D70-PCTP; DSI, St. Paul, MN) was implanted during anesthesia in the abdominal muscle. The arterial pressure catheter was advanced into the abdominal aorta via the femoral artery, whereas the left ventricular pressure (LVP) catheter was introduced into the heart through the apex. The ECG leads were placed in the thorax, with the negative lead cranial to the heart, and the positive lead onto the heart apex. After surgery, the animals were allowed at least four weeks of recovery before they were included in the study.

2.3. Study protocol

In the morning (app. 09.00 h), the animals received fixed doses of flecainide or placebo according to a Latin square crossover design during four separate study days, at intervals of at least three days (Table 1). The fixed doses correspond to approximately 10, 15 and 20 mg/kg (24, 36 and 48 μmol/kg) of the parent form of flecainide. Cardiovascular data was recorded in individual pens by telemetry 1 h before dosing and 24 h post-dose. The dogs were temporarily moved from the pens for dosing and blood sampling. Furthermore, 4 h after dosing, the dogs were given their once-daily food ration and allowed outdoor access (30 min) and 7 h after dosing, they had a shorter break (10–20 min) with outdoor access. The dark cycle occurred between 18.00 and 06.00 i.e. 9–21 h after dosing. Blood samples were taken from the jugular vein before dosing and 0.5, 1, 2, 4, 7 and 24 h post-dose. After the last blood sample, the animals were released and kept group housed until the next dose was administered.

Table 1
Study design and dosing overview. The doses are given in milligrams.

Experimental day	Dog 1	Dog 2	Dog 3	Dog 4
1	0	100	150	200
4	150	200	0	100
8	200	150	100	0
11	100	0	200	150

2.4. Drugs and formulations

Flecainide acetate tablets (100 mg tablets, whole and/or half; Meda AB, Solna, Sweden) were placed in gelatin capsules according to the selected dose levels. As placebo, empty gelatin capsules were used. As a reference for plasma analysis, flecainide acetate from Sigma-Aldrich, St. Louis, MO was used.

2.5. Analysis of plasma samples

Blood was sampled in K_2-EDTA tubes that were immediately placed on ice. Plasma was prepared in a cooled centrifuge and kept at −20 °C until analysis. The plasma concentration of flecainide was analyzed at Global DMPK, AstraZeneca, Södertälje, Sweden by protein precipitation and liquid chromatography followed by mass spectrometric detection (LC–MS/MS). The lower limit of quantification of the assay was 5 nmol/L and the calibration range was 5 to 12,500 nmol/L. Unbound flecainide concentrations were estimated in reference to reported protein binding data in humans (41%) (Zordan, Padrini, Bernini, Piovan, & Ferrari, 1993) and in dogs (63%) (Heath et al., 2011).

2.6. Telemetric data acquisition and analysis

Telemetric cardiovascular data was continuously sampled using Dataquest Open ART (DSI, St. Paul, MN) and IOX2 v2.4.2.6 software (Emka technologies, Paris, France) from 1 h pre-dose until 24 h post-dose, except when the dogs were moved from their individual housing for outdoor breaks and when taking blood samples. QRS, PQ and QT were automatically analyzed according to user-defined reference waveforms using ECG-auto v2.5.1.18a (Emka technologies, Paris, France) during four 1-min periods before dosing (baseline) and during 1 min in relation to every blood sample. Continuous 20-s averages of QRS, blood pressure and heart rate were created from 60 min pre-dose until 500 min post-dose. Among these, every third 20-s average calculated above was used, i.e. one data point every minute, for filtering and PKPD modeling described below. Furthermore, continuous 5-min averages were created for data visualization using R version 2.11.1 (R Foundation for Statistical Computing, Vienna, Austria). In addition, QT was corrected for changes in heart rate (QTcR) using the individual correction formula previously described (Ollerstam et al., 2007).

2.7. Pharmacokinetic modeling

The pharmacokinetic data was fitted individually for each dog using data on all doses simultaneously to a one-compartment oral dosing model (Phoenix 6.2, Pharsight, Sunnyvale, CA) that described the observed data well and allowed interpolations of plasma concentrations to each sampled QRS value.

2.8. Filtering of QRS data according to heart rate

In order to visualize the heart rate dependency without PKPD modeling, the dataset was filtered using Phoenix 6.2 (Pharsight, Sunnyvale, CA). QRS values with a concomitant heart rate below 100 were sorted in one matrix and values above 160 were sorted in another. Data between these values was excluded from analysis. This data was