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Safety and Health Research

Dual Effect of Clonidine on QT Interval Duration and Body Temperature in Cynomolgus Monkeys: QT Correction Formula for Changes in Core Body Temperature

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INTRODUCTION

QT interval lengthening produced by a drug candidate may be related, in part, to the inhibition of hERG current, decreased heart rate (HR) and/or decreased core body temperature (BT)[1,2]. In the safety evaluation of drugs acting on the central nervous system, which are usually tested in monkeys and known for their potential effect on BT, it is important to assess the origin of any QT lengthening.
To discriminate between a possible effect of HR and/or BT on QT lengthening in conscious non-restrained cynomolgus monkeys, we recently proposed a QT-interval correction method for the influence of core BT changes resulting from circadian variations, based on QT Bazett (QTcB) and QT Fredericia (QTcF) formulas[3]. Parameters were recorded during dark and light periods over 26 hours in 21 male and 19 female cynomolgus monkeys. During this period, BT ranged from 35.6 to 39.4°C, with a mean ± SD of 37.9 ± 0.6°C[3].
To evaluate the performance of these formulas, we investigated the effect of Clonidine, an α2-adrenergic receptor agonist, on BT, HR, QT, QTcB, QTcF, QTcBcT (=QTcB-13.8(38-BT)) and QTcFcT (=QTcF-17(38-BT)) in non-restrained telemetered cynomolgus monkeys. In addition, the effect of Clonidine was evaluated on hERG potassium current (IKr) in stably transfected HEK 293 cells using the whole-cell patch-clamp technique.
We highlighted a dual action of Clonidine on QT lengthening by acting simultaneously on body temperature and hERG current. Under our experimental conditions (associating Clonidine administration with large decreases in HR), QTcBcT performed slightly better than QTcFcT in correcting QT for changes in body temperature.

MATERIALS AND METHODS

***In vivo* investigation:** Telemetered cynomolgus monkeys

One group of 4 cynomolgus monkeys (2 males and 2 females) that had been previously implanted with telemetry transmitters (Data Science International) for continuous measurements of body temperature and cardiovascular parameters, including blood pressure (data not shown) and ECG parameters (lead II), especially QT interval duration, were used in the present investigation.
Animals were group housed in a controlled environment (temperature 19-24°C and light/dark cycle: 12h/12h), except during the telemetry recordings, and acclimated to the telemetry room for a minimum period of two weeks prior to the telemetry recordings. Blood samples were taken from each animal for analysis of hematology and biochemistry parameters to confirm their clinical status prior to the telemetry recordings. During the acclimation period, the quality of ECG and arrhythmias was checked for each animal over a 1 hour ECG recording. Blood pressure and ECG analysis were performed to check that cardiovascular parameters were within the normal range prior to the beginning of the study.
Animals were treated by intramuscular injection of either the vehicle (0.9% NaCl) or Clonidine (100 µg/kg) following a cross-over design, with a 4-day washout period between treatments. Data were recorded continuously over a 26-hour period (2 hours before treatment and 24 hours after treatment) and analysed. ECG recordings began no less than 2 hours before dosing and ended no sooner than 24 hours afterwards. Core BT and ECG parameters [PQ, QRS, QT, QTcB (corrected QT for HR according to the Bazett formula), QTcF (corrected QT for HR according to the Fridericia formula), QTcB corrected for body temperature QTcBcT =QTcB-13.8(38-BT) and QTcF corrected for body temperature QTcFcT =QTcF-17(38-BT)] were analysed before (mean of measurements obtained at -90, -75 and -60 min) and approximately 0.16, 0.33, 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 20 and 24 hours after treatment.

***In vitro* investigation:** whole cell Patch-Clamp

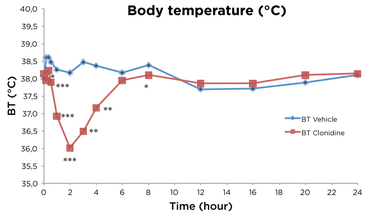
The effect of Clonidine was evaluated from 3 to 300 µM on the hERG potassium current (IKr) in stably transfected HEK 293 cells (3 cells/concentration) using the whole-cell patch-clamp technique. The cells were cultured in a medium of Minimum Essential Medium Eagle (MEM), supplemented with 10% heat-inactivated fetal bovine serum (FBS), 50 units/mL penicillin, 50 µg/mL streptomycin and 2 mM L-glutamine. Cells were plated in 35 mm petri dishes 12 to 36 hours before the patch clamp whole-cell experiments. IKr currents were recorded using a step-ramp protocol which gave a conditioning pre-pulse at +40 mV for 1 s and then repolarized to –80 mV with a test ramp (0.6 mV/s) repeated at 5 s intervals. The whole-cell patch-clamp results were given as reduction of current amplitude in ionic IKr currents with measurement of inhibition percentage after a steady-state in the presence of drug treatment. Currents were recorded with an Axopatch 200 amplifier (Molecular Devices, Sunnyvale, CA), and series resistance was >60% compensated. Command pulses were generated, and currents were acquired using a Pentium-based computer running pCLAMP software (version 10.5) equipped with a DigiData 1440 AD converter (Molecular Devices). Currents were filtered at 5 kHz. All recordings were performed at near-physiology temperature (32–35°C).
Clonidine stock was prepared in DMSO at 1 mM. The stock was diluted with the appropriate volume of Tyrode's solution to obtain the desired concentrations at 3 µM, 10 µM, 30 µM, 100 µM and 300 µM, with a concentration of DMSO not exceeding 0.3% (v/v).

RESULTS

***In vivo* investigation:** Telemetered cynomolgus monkeys

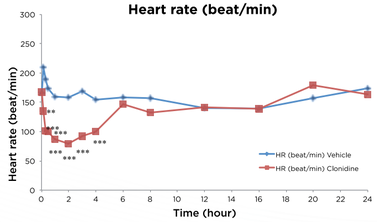
Intramuscular injection of Clonidine (100 µg/kg) produced statistically significant decreases in HR and core BT. The greatest changes in BT and QT interval were simultaneously observed around 2 hours after injection of Clonidine. Statistically significant decreases in BT (36.0 ± 0.6 vs 38.2 ± 0.6°C, p<0.001; Figure 1) and HR (79 ± 10 vs 158 ± 9 beat/min, p<0.01, Figure 2) were observed, along with statistically significant increases in QT (385 ± 54 vs 217 ± 15 ms, p<0.001, Figure 3), QTcB (433 ± 18 vs 350 ± 11 ms, p<0.001, Figure 4), QTcF (416 ± 24 vs 298 ± 11 ms, p<0.001, Figure 5), QTcBcT (406 ± 19 vs 352 ± 12 ms, p<0.001, Figure 6) and QTcFcT (382 ± 16 vs 301 ± 10 ms, p<0.001, Figure 7).

Figure 1: Effect of single intramuscular administration of Clonidine (100 µg/kg) on core body temperature in telemetered non-restrained cynomolgus monkeys



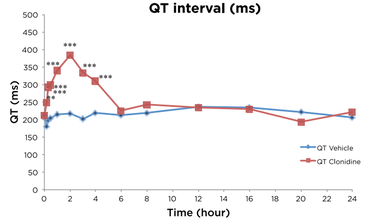
*, p<0.05; **, p<0.01; ***, p<0.001.

Figure 2: Effect of single intramuscular administration of Clonidine (100 µg/kg) on heart rate in telemetered non-restrained cynomolgus monkeys



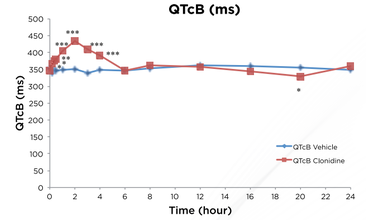
**, p<0.01; ***, p<0.001.

Figure 3: Effect of single intramuscular administration of Clonidine (100 µg/kg) on QT interval duration in telemetered non-restrained cynomolgus monkeys



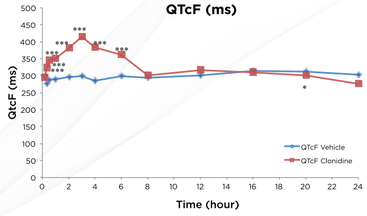
**, p<0.01; ***, p<0.001.

Figure 4: Effect of single intramuscular administration of Clonidine (100 µg/kg) on QTcB in telemetered non-restrained cynomolgus monkeys



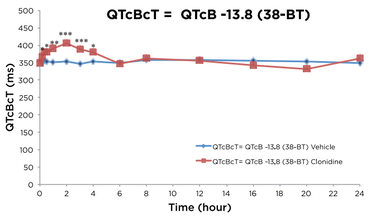
*, p<0.05; **, p<0.01; ***, p<0.001.

Figure 5: Effect of single intramuscular administration of Clonidine (100 µg/kg) on QTcF in telemetered non-restrained cynomolgus monkeys



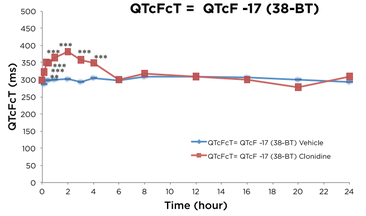
*, p<0.05; **, p<0.01; ***, p<0.001.

Figure 6: Effect of single intramuscular administration of Clonidine (100 µg/kg) on QTcBcT in telemetered non-restrained cynomolgus monkeys



*, p<0.05; **, p<0.01; ***, p<0.001.

Figure 7: Effect of single intramuscular administration of Clonidine (100 µg/kg) on QTcFcT in telemetered non-restrained cynomolgus monkeys

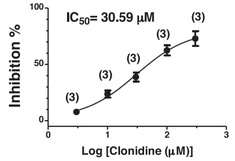


**, p<0.01; ***, p<0.001.

***In vitro investigation*:** whole cell patch-clamp

Clonidine inhibited IKr currents by 7.78% at 3 µM, 23.75 % at 10 µM, 38.78 % at 30 µM, 62.49 µM at 100 µM and 72.92 at 300 µM. Based on the inhibitory profile, a concentration-dependent curve was obtained to generate an IC50 value of 30.59 µM (Figure 8).

Figure 8: Concentration-response curve of Clonidine on hERG current in in HEK-293 cells



IC_{50} of Clonidine on hERG was calculated from the concentration-response curve, which was used to evaluate the relationship of Log [Clonidine (µM)] vs. the inhibitory response over a concentration range of 3 µM to 300 µM.

REFERENCES

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(2) Abdel-Ilah El Amrani, Francine El Amrani-Callens, Stéphane Loriot, Pramila Singh, Roy Forster (2016). QT interval correction for drug-induced changes in body temperature during integrated cardiovascular safety assessment in regulatory toxicology studies in dogs: A case study. *Journal of Pharmacological and Toxicological Methods*: 81: 136–143.

(3) Abdel-Ilah El Amrani, Francine El Amrani-Callens, Stéphane Loriot, Pramila Singh and Roy Forster. Citoxlab, B.P. 563, Evreux, France. Proposed QT-interval correction method for the influence of changes in core body temperature based on circadian variations in conscious non-restrained cynomolgus monkeys. *Abstract SPS Annual Meeting, September, 2017.*

DISCUSSION AND CONCLUSION

Based on physiological changes in QT interval duration and BT during dark and light periods over 26 hours in 40 non-restrained telemetered cynomolgus monkeys, we have suggested QT correction formulas for changes in core body temperature, QTcFcT and QTcBcT based on QTcB and QTcF, respectively. We have highlighted a dual action of Clonidine on QT lengthening and that Clonidine acts simultaneously on body temperature and hERG current. Under our experimental conditions, where decreased HR and hERG inhibition were observed after Clonidine administration, QTcBcT performed slightly better than QTcFcT in correcting QT for changes in body temperature. The incomplete QT correction for changes in core body temperature observed after Clonidine administration was probably related, in part, to the slight inhibitory effect of hERG current.