Journal of Ethnopharmacology 166 (2015) 375–379

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Hypaconitine-induced QT prolongation mediated through inhibition of KCNH2 (hERG) potassium channels in conscious dogs

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ARTICLE INFO

Article history:
Received 12 August 2014
Received in revised form
15 January 2015
Accepted 9 March 2015
Available online 20 March 2015

Keywords:
Hypaconitine
QT prolongation
hERG channels
ECG telemetry
Safety assessment

ABSTRACT

Ethnopharmacological relevance: Hypaconitine is one of the main aconitum alkaloids in traditional Chinese medicines prepared with herbs from the genus *Acotinum*. These herbs are widely used for the treatment of cardiac insufficiency and arrhythmias. However, *Acotinum* alkaloids are known for their toxicity as well as their pharmacological activity, especially cardiotoxicity including QT prolongation, and the mechanism of this toxicity is not clear.

Material and methods: In this study, hypaconitine was administered orally to conscious Beagle dogs, and electrocardiograms were recorded by telemetry. Pharmacokinetic studies (6 h) were conducted to evaluate the relationship between QT prolongation and exposure level. HEK293 cells stably transfected with *KCNH2* (*hERG*) cDNA were used to examine the effects of hypaconitine on the KCNH2 channel by using the manual patch clamp technique.

Results: In the conscious dogs, all doses of hypaconitine induced QTcV (QT interval corrected according to the Van de Water formula) prolongation by more than 23% (67 ms) of control in a dose-dependent manner. The maximum QTcV prolongation was observed at 2 h after dosing. Maximum prolongation percentages were plotted against plasma concentrations of hypaconitine and showed a strong correlation (R^2=0.789). In the in vitro study in HEK293 cells, hypaconitine inhibited the KCNH2 currents in a concentration-dependent manner with an IC_{50} of 8.1 nM.

Conclusion: These data suggest that hypaconitine inhibits KCNH2 potassium channels and this effect might be the molecular mechanism underlying QT prolongation in conscious dogs.

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1. Introduction

Prolongation of the QT in the electrocardiogram can be associated with polymorphic ventricular tachycardia or Torsades de pointes. QT prolongation has increasingly drawn attention from regulatory agencies and the pharmaceutical industry as a potentially serious adverse event (Yap and Camm, 2003). The recent withdrawal of several drugs from the market due to possibly drug-related cardiac arrhythmias has greatly increased concern about drug-induced prolongation of the QT interval (Bouchaud et al., 2009). To avoid QT prolongation and increase the chances for success of new drug candidates, a more rigorous methodology to evaluate the potential for QT prolongation is critical. The International Conference on Harmonization S7B guideline describes a nonclinical testing strategy for assessing the potential of a test substance to delay ventricular repolarization and thereby produce QT prolongation (Food and Drug Administration, 2005). According to the S7B guideline, an in vivo QT assay using conscious, unrestrained animals is one of the most important elements of the testing strategy.

Aconites are the common name of the genus Aconitum L., subordinated to Ranunculaceae (Xiao et al., 2006). Herbal preparations from aconites are well known worldwide for their wide use in traditional medicines of China, Japan, and Korea. These preparations are well known for their toxicity as well, including cardiotoxic and neurotoxic effects. There are quite a few clinical cases of aconite intoxication reported from China Hong Kong, Japan, Germany, and other countries (Chan, 2002; Chen et al., 2012; Poon et al., 2006). Plants of this genus commonly contain diester diterpenoid alkaloids. Alkaloids with an acetyl group at C_8 and a benzoylester group at C_{14} are the potentially toxicalkaloids, including aconitine, mesaconitine, and hypaconitine (Fig. 1) (Hu et al., 2009). Aconitine has been shown to have substantial arrhythmogenic effects (Chen et al., 2013) Early studies indicated that aconitine blocked KCNH2 and kv1.5 potassium channels, which may contributed to action potential duration (APD) prolongation and cardiac arrhythmias (Li et al., 2010).

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http://dx.doi.org/10.1016/j.jep.2015.03.023
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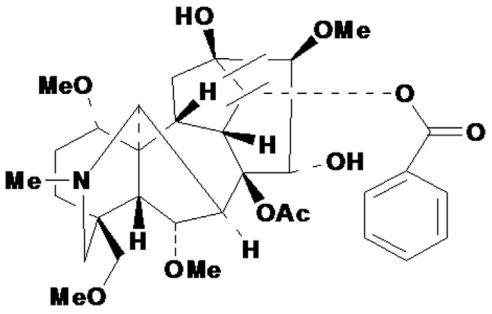

Fig. 1. The chemical structure of hypaconitine.

Hypaconitine has also been reported to induce arrhythmias and even death (Chan, 2012). However, few studies have been conducted to investigate the mechanism of arrhythmia induction by hypaconitine alone. Therefore, the QT prolongation potential of hypaconitine was assayed in conscious dogs. The relationship of plasma concentration to QT prolongation was also investigated. The mechanism underlying QT prolongation was studied through patch clamp tests on KCNH2 channels expressed in mammalian HEK293 cells.

2. Materials and methods

2.1. Chemicals and reagents

Hypaconitine (> 99%), quinidine (> 99%), terfenadine (> 99%) and phenacetin (> 99%) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China); Terfenadine, acetonitrile, ethanol (HPLC grade) and G418 were obtained from Sigma-Aldrich (St. Louis, MO 63178, USA); HEK293 cells purchased from ATCC (Manassas, VA 20110, USA) were stably transfected with hERG cDNA (GenBank accession number NM_000238); DMEM, FBS, pCDNA3.1 (+) and lipofectmine 2000 were provided by Life technologies (Grand Island, NY 14072, USA).

2.2. Animals and treatment

Six beagle dogs (3 males and 3 females, 7.5 ± 0.3 kg, Marshall Biotechnology Co., Ltd (Beijing, China)) were randomly assigned in a Latin Square design. All animals were housed in individual stainless steel cages in a room maintained at 22–26 °C, 60–70% relative humidity and 12 h light/12 h dark cycle. They were fed approximately 300 g of standard laboratory diet daily and had access to water ad libitum. Animals were kept in the fasted state for approximately 12 h pre-dose and 6 h post-dose. All study procedures were reviewed and approved by the Laboratory Animal Care and Use Committee of Zhongshan PharmaSS Corporation, Guangdong, China. Each dog was orally administered three doses of hypaconitine (50, 150, and 450 μg/kg), quinidine (1, 3, and 9 mg/kg), a drug known to prolong the QT interval in animals (Olivier et al., 2003; Testai et al., 2004) and humans (Cubeddu, 2003), as the positive control, and vehicle in the same volume. Each animal received the treatments with a 7-day washout period between doses. The dose-finding study had been conducted according to mice LD_{50} of 5.8 mg/kg (Bisset, 1981). And finally, the present dosage was selected for dog study.

2.3. ECG recording

EMKA non-invasive telemetry for large animals (Emka Technologies, Paris 75015, France) was used for this study. The system consisted of an emkaPACK transmitter with a 5-wire ECG cable to collect 7-lead ECG data with iox2 data acquisition and real-time analysis software (Emka Technologies, Paris 75015, France). All dogs were habituated to the EMKA jacket that was used to carry the non-invasive electrodes and keep them in place for at least 24 h. A seven lead surface ECG configuration (limb I, II, III, aVR, aVL, aVF, and V) was continuously recorded. After ECG signals were stabilized, baseline QT interval corrected using to the Van de Water formula interval was continuously recorded from pre-dose to 6 h post-dose. Data were recorded at 0, 0.5, 1, 2, 4, and 6 h. During the recording, access to the animal room was restricted to the personnel administering the test compounds. At the end of each experiment, the surface ECG electrode patches and jackets were removed and animals were returned to their home cages.

The ECG signals were analyzed using iox2 data analysis software. Baseline values were calculated from the pre-dose data. The QT interval was corrected for heart rate using Van de Water's method:$QT_CV = QT - 0.1087(RR - 1000)$, one of the most common formulae used in conscious dogs (Soloviev et al., 2006; Spence et al., 1998). In the equation, QTcV is the corrected QT internal, QT is the raw QT internal, and RR is the R–R interval.

2.4. Plasma hypaconitine analysis

To evaluate the relationship between plasma hypaconitine and QT prolongation, plasma hypaconitine concentrations were determined. Six-hour pharmacokinetic analyses were carried out to evaluate the relationship between hypaconitine blood levels and QTcV prolongation.

To measure hypaconitine blood levels, 0.5 mL blood samples were drawn at 0, 0.5, 1, 2, 4, and 6 h after dosing. The blood samples were immediately centrifuged at $3000 \times g$ for 10 min, the plasma was collected and then stored at −80 °C until analysis. Before analysis, 80 μL of acetonitrile was added to the 20 μL of plasma to denature the plasma proteins and then the plasma was centrifuged at $14{,}000 \times g$ for 30 min. An aliquot of the supernatant (10 μL) was subjected to liquid chromatography/tandem mass spectroscopy (LC-MS/MS) analysis coupled with Analyst 1.4.2 workstation software. The separation was performed by a CAPCELL PAK C18 column (5 μm, 2.0 mm ID*50 mm) with a flow rate of 0.2 mL/min.

2.5. The effect of hypaconitine on the KCNH2 channel

The hERG gene (KCNH2) was subcloned into pCDNA3.1 (+) vector. Human embryonic kidney 293 (HEK293) cells were cultured at 37 °C (5% CO_2) in DMEM supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA). Cells were transfected with linearized KCNH2 plasmid DNA using lipofectmine 2000 (Invitrogen, Carlsbad, CA). Human KCNH2 stable cell line (HEK293-HuKCNH2) was established using G418 screening and validated using manual patch clamp.

KCNH2 expressing HEK293 cells were subcultured 2–3 times per week in order to maintain optimal cell health. The cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 200 μg/mL of G418. HEK293 cells are plated in 35 mm dishes at least 24 h prior to the day of experiment and maintained at 37 °C/5% CO2. The extracellular solution for the whole-cell patch clamp recordings was composed of (mM): NaCl 145; $MgCl_2$ 1.0; KCl 4.0; Glucose 10; HEPES 10; and $CaCl_2$ 2.0. The pH was adjusted to 7.4 with NaOH. The osmolarity was adjusted to 300 mOsm with sucrose. The intracellular solution for whole-cell patch clamp recordings was composed of (mM): KCl 140; $MgCl_2$ 1; EGTA 5; HEPES 10 and Na_2ATP 4. The pH was adjusted to 7.2 with KOH. The osmolarity was adjusted to 295 mOsm with sucrose.

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