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Original article

A non-human primate model for investigating drug-induced risk of orthostatic hypotension and sympathetic dysfunction: Preclinical correlate to a clinical test

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ABSTRACT

Introduction: Drug induced orthostatic hypotension (OH) is an important clinical concern and can be an unexpected hurdle during drug development. OH is defined as an abnormal decrease in blood pressure (BP) triggered by a rapid postural change. The sympathetic nervous system is critical for controlling normal cardiovascular function and compensatory responses to changes in posture. Thus, OH can also serve as a surrogate indicator of sympathetic dysfunction. However, preclinical conscious models for investigating risk of OH and/or sympathetic dysfunction are lacking. Herein, we describe a conscious nonhuman primate (NHP) model which mimics the widely used clinical tilt table test for OH.

Methods: Male, Cynomolgus NHPs (n = 7–8) implanted with radio-telemetry transmitters were placed in modified tilt chairs in a supine position. Subsequently, a 90° head up tilt was performed for 3 min followed by return to the supine position. BP and heart rate were continuously monitored. Test compounds were administered either intravenously or via oral gavage in a crossover design, with blood samples collected at the end of the each tilt to assess total drug concentrations.

Results: Tilt responses were assessed following treatment with positive control compounds that cause sympathetic dysfunction; hexamethonium (ganglionic blocker) and prazosin (alpha-1 adrenergic receptor antagonist). Both compounds induced marked OH as evidenced by robust and sustained BP reduction in response to a head up tilt (decrease of 25–35 mm Hg for hexamethonium, decrease of 21–44 mm Hg for prazosin). OH incidence rates increased in a dose-dependent manner. OH incidences following treatment with minoxidil (vasodilator) were markedly lower to those observed with hexamethonium and prazosin indicating the role of sympathetic dysfunction in causing OH.

Discussion: These data demonstrate that the NHP tilt test is a valuable model for investigating OH risk. This model fills an important preclinical gap for assessing such a safety concern and can be applied to programs where a sympathetic deficit and/or OH are anticipated or clinically observed.

1. Introduction

Adverse effects of novel drug molecules on the cardiovascular (CV) system are a major focus of safety pharmacology core battery assays, as described in the ICH S7A guidance. Current in vivo preclinical safety pharmacology models are well positioned to study effects on ambulatory cardiovascular parameters under resting conditions. However, effects manifested during stress on the CV system or effects on specific regulatory mechanisms, such as the sympathetic system, may not be detected with the typical battery of assays used. One daily physical stressor which requires the full capabilities of the cardiovascular system is acute postural change, i.e. supine/sitting to standing position (Stewart, 2012). A normal compensatory response driven primarily by the sympathetic system prevents sustained hypotension in response to postural change. Abnormalities in these compensatory pathways result in orthostatic hypotension (OH). Clinically, OH is defined as a sustained reduction of systolic blood pressure of at least 20 mm Hg or diastolic blood pressure of 10 mm Hg within 3 min of standing or head-up tilt to at least 60° on a tilt table (Freeman et al., 2011). OH results in decreased brain perfusion and is symptomatically manifested as lightheadedness, dizziness or fainting (syncope) upon standing up.

Drug induced orthostatic hypotension (OH) is an important clinical adverse effect and may pose an unexpected hurdle during development. There is a relative paucity of preclinical models, particularly in conscious animals, to identify OH and/or sympathetic dysfunction risks (Picard et al., 2011). Consequently, such a risk is often detected relatively late in the drug development cycle when risk mitigation options are limited. Drugs of several modalities such as anti-psychotics, alpha-1 adrenergic

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Table 1
Summary of total incidences of OH observed during the study.

Compound	Dose (mg/kg)	Pre-tilt SBP, change from baseline (or vehicle)	OH response rate* (% responders)
Hexamethonium (IV)	0.075	−5 mm Hg	0/8 (0%)
	0.25	−14 mm Hg	4/8 (50%)
	0.75	−42 mm Hg	8/8 (100%)
Prazosin (IV)	0.005	−9 mm Hg	5/8 (63%)
	0.015	−20 mm Hg	6/8 (75%)
	0.05	−38 mm Hg	8/8 (100%)
Minoxidil (oral)	8	−35 mm Hg	2/7 (29%)

* OH defined as a reduction in systolic blood pressure of ≥20 mm Hg at 90 s following head up tilt. SBP — Systolic blood pressure, OH — Orthostatic hypotension.

antagonists for benign prostate hypertrophy, vasodilators and other anti-hypertensive medications have been reported to increase the risk of OH (Arnold & Shibao, 2013; Fine & Ginsberg, 2008; Leung, Barr, Procyshyn, Honer, & Pang, 2012). This risk is even greater in the elderly, who are more vulnerable to OH and syncope. As an example, Kamaruzzaman and colleagues have reported that use of beta blockers and three or more antihypertensives were independently associated with OH in women aged 60–80 years (Kamaruzzaman, Watt, Carson, & Ebrahim, 2010). The authors also suggest that OH or its symptoms are one of the major reasons for withdrawal of antihypertensive medication in this population.

In the clinic, a diagnosis of OH is generally made by bedside assessments of blood pressure and heart rate in a supine position and after 1 and 3 min of standing (Shibao, Lipsitz, & Biaggioni, 2013). Alternately, a tilt table test may be employed for OH assessments (Lanier, Mote, & Clay, 2011). Despite well-established clinical tests, similar tests in animal models which can serve as preclinical correlates are lacking. Some of the more widely used models utilize anesthetized rodents for OH assessments (Guillaume, Herve, Picard, & Lacroix, 2008). However, due to the lack of upright posture in rodents, it is unclear whether the compensatory mechanisms in rodents are identical to humans. The same applies to other non-rodent species such as dogs. Furthermore, anesthesia has been shown to influence the autonomic nervous system which could further confound the results (Shimokawa, Kunitake, Takasaki, & Kannan, 1998; Watkins & Maixner, 1991). Non-human primates (NHPs), given their upright posture, may be a more relevant species for assessing an OH risk. One previous study has reported OH assessments in conscious NHPs using tilt (Pals & Orley, 1983). However, blood pressure recordings were performed using exteriorized arterial catheters which require additional surgery. Susceptibility to OH in NHPs has also been studied using lower body negative pressure (Murphy, Renninger, & Ju, 2006). But since this technique is not widely used in humans, translation and physiological relevance of the data may be unclear. Radio-telemetry implanted NHPs are routinely used in cardiovascular safety pharmacology assays. Thus, a standardized and sensitive OH assay in these radio-telemetry implanted NHPs would be a valuable addition to the safety pharmacology toolbox and could easily serve as an add-on to standard cardiovascular safety studies. The present paper describes the development and validation of such a NHP model for assessment of OH.

2. Materials and methods

2.1. Animals

All experiments involving animals were conducted as per the guidelines and study protocols reviewed and approved by Pfizer Institutional Animal Care and Use Committee. Male, Cynomolgus NHPs (SNBL, Mauritius origin), aged 5–6 years, were used in the study. The NHPs were part of the Pfizer safety pharmacology colony, and had undergone adequate washout from any previous studies prior to use in the present studies. The NHPs were surgically implanted with TL11M2-D70-PCT radiotelemetry transmitters from Data Sciences International (St. Paul, MN). The tip of the pressure catheter of the transmitter was placed in the descending aorta.

2.2. Tilt chairs and procedure

For this study, we utilized rhesus primate chairs from Plas Labs, Inc. (Lansing, MI) that were customized in-house as described. Large circular metal hoops were attached to the sides of the Plexiglas seat box and seated in v-groove wheels that were attached to the base. This modification allowed the entire chair to be smoothly rotated at a 90° angle from a horizontal supine position to a vertical head-up position (Fig. 1C). Seat extensions for back support and a plastic headrest were also added to provide additional comfort and security for the animal.

A tilt paradigm mimicking the clinical tilt table test was used for evaluation of an orthostatic hypotension response. Briefly, each primate was placed in the modified primate chair described above and a tilt was induced as follows: step 1) the chair was adjusted so that the NHP was positioned horizontally (supine) for at least 10 min to achieve a stable hemodynamic baseline; step 2) the chair was tilted (approximately within 2–3 s) so as to induce a 90° head-up, or vertical, position; and step 3) the tilt was concluded by returning the monkey back to the horizontal/supine position (typically 3 min after step 2). A shorter head up period (~2 min) was used when the blood pressure decrease exceeded 50 mm Hg in a limited numbers of incidences. Blood pressure waveforms were continuously collected by telemetry over the entire tilt session using an acquisition rate of 500 Hz, and 1 s mean values of the systolic pressure and heart rate were derived (Ponemah v 5.0, DSI). Diastolic blood pressure data was recorded but is not reported in this paper.

All NHPs used on the study underwent multiple (5–6) training sessions to acclimate them to the tilt chairs as well as to the tilt procedure.

2.3. Experimental design

A cross-over experimental paradigm was used to investigate the effects of test drugs, so each NHP received both vehicle and test compound under identical testing conditions. Hexamethonium bromide (H0879), prazosin hydrochloride (P7791) and minoxidil (M4145) were obtained from Sigma Aldrich Inc. (St. Louis, MO). Hexamethonium and prazosin were administered to NHPs (N = 8) intravenously via a temporary venous catheter placed in the saphenous vein on the study day. An additional temporary catheter was placed in the contralateral saphenous vein for blood collection. Three doses each of hexamethonium (0.075, 0.25 and 0.75 mg/kg) and prazosin (0.005, 0.015 and 0.05 mg/kg) prepared as solution in sterile water for injection were used. These doses were administered on the same treatment day, in an escalating dose fashion as indicated in Fig. 1. A baseline tilt response was assessed prior to compound administration and approximately 10 min after each dose. Minoxidil formulation (8 mg/kg, suspension in 0.1% methylcellulose) was not amenable to parenteral administration and so was administered to NHPs (N = 7) via an oral gavage. Tilt responses were assessed at 3 and 6 h following minoxidil or vehicle dosing. These time points were selected since it was anticipated that the minoxidil plasma concentrations would be close to the Cmax (3 hpd) and close to below limits of quantification (6 hpd). A washout period of at least 48 h was allowed between treatments with different compounds or vehicle. Blood samples were collected in K_2EDTA coated tubes at the end of each tilt for assessing plasma concentrations.

2.4. Data analysis

Data are represented as a change from pre-tilt supine levels. The pre-tilt supine values were obtained by averaging the blood pressure and heart rate values 1 min prior to head up tilt. Additionally, absolute pre-tilt blood pressure values are reported to quantify the level of