Journal of Pharmacological and Toxicological Methods 82 (2016) 62–67

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

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

Research article

A novel intravenous vehicle for preclinical cardiovascular screening of small molecule drug candidates in rat

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ARTICLE INFO

Article history:
Received 20 April 2016
Received in revised form 9 July 2016
Accepted 14 July 2016
Available online 16 July 2016

Keywords:
Blood pressure
Cardiac contractility
Cardiovascular screening
Formulation
Heart rate
Methods
Safety pharmacology
Solubility
Pharmacokinetic
Vehicle

ABSTRACT

Screening novel, poorly soluble small-molecule candidates for cardiovascular liabilities represents a key challenge in early drug discovery. This report describes a novel vehicle composed of 20% N,N-Dimethylacetamide (DMA)/40% Propylene glycol (PG)/40% Polyethylene Glycol (PEG-400) (DPP) for administration of new chemical entities (NCEs) by slow intravenous (i.v.) infusion in a preclinical anesthetized rat model. The vehicle was designed considering both available excipient safety information and solubilization potential for poorly soluble NCEs. DPP solubilized 11 drugs, 8 of which were insoluble in 5% dextrose in water (D5W), and 5 insoluble in PEG-400 to a target concentration of 30 mg/mL. DPP elicits no adverse cardiovascular responses in the anesthetized rat model despite containing 40% PEG-400, a commonly used organic solvent which elicits hypertension and bradycardia that often confounds interpretation of drug effects. Three compounds demonstrating adequate solubility in both DPP and D5W were screened in the anesthetized rat model. When normalized to plasma exposure, atenolol, sotalol and enalaprilat exhibited comparable mean arterial pressure, heart rate, and cardiac contractility responses regardless of formulation. While the antihypertensive effect of nifedipine was evident with both DPP and PEG-400 formulations, pressor effects from PEG-400 confounded interpretation of the magnitude of nifedipine's response. Plasma concentrations of atenolol and enalaprilat were greater in D5W formulation whereas sotalol exposures were greater when using DPP as a vehicle. These results demonstrate the utility of DPP as an intravenous vehicle for formulating poorly soluble compounds in early preclinical screening for cardiovascular safety studies.

1. Introduction

Early *in vivo* screening plays a significant role in the effort to de-risk systemically available new chemical entities by identifying promising drug candidates with minimal cardiovascular (CV) liabilities. Such assays must enable the researcher to unambiguously differentiate the CV profiles of any formulated NCE from the vehicle(s) employed for solubilization and drug delivery. Consequently formulation plays an important role in early screening efforts for both safety pharmacology and efficacy studies. Ideal formulations must be suitable for the intended route of administration, maintain the stability of the active ingredient and potentially maximize the systemic drug bioavailability (Gad, Cassidy, Aubert, Spainhour, & Robbe, 2006; Gad et al., 2016).

Lack of formulation tolerance data can lead to excessive animal use. In 2016 Gad and colleagues conducted studies examining 25 years of data mining operation of vehicle data, enhancing their earlier 2006 publication. Among the co-solvents used in our DPP study only PEG400 was listed by Gad et al. (2016) in their lists of excipients. Formulation tolerance is an enormous challenge to those involved in early pre-formulation efforts, often requiring experienced formulators for their understanding of the interplay between vehicle components along with a strong mechanistic understanding of drug metabolism and chemical reactions (Li & Zhao, 2007).

It is estimated that approximately 40% of new chemical entities have delivery limitations due to poor solubility or poor bioavailability (Li & Zhao, 2007). Although many vehicles may meet the physical and chemical requirements for pharmaceutical formulation to deliver poorly soluble compounds, wide differences exist with respect to both species and route of administration specific to tolerability for many compositions (Gad, Cassidy, Aubert, Spainhour, & Robbe, 2006).

While the selection and qualification of clinical excipients/vehicles is aided by specific regulatory guidance, sponsors are largely left to their own devices to identify formulations appropriate for their internal *in vivo* models (Lee, Zocharski, & Samas, 2002; Thackaberry et al., 2014).

Abbreviations: CV, cardiovascular; D5W, 5% dextrose in water; dP/dt, cardiac contractility; DMA, N,N-dimethylacetamide; PG, propylene glycol; PEG-400, polyethylene glycol 400; DPP, 20% N,N-dimethylacetamide/40% propylene glycol/40% polyethylene glycol; HR, heart rate; i.v., intravenous; MAP, mean arterial pressure; NCEs, new chemical entities.

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http://dx.doi.org/10.1016/j.vascn.2016.07.002
1056-8719/

Two vehicles commonly used in anesthetized rat CV safety models are 5% dextrose in water (D5W) and 100% polyethylene glycol 400 (PEG-400). While D5W generally exhibits a benign CV profile, it has limited utility for the solubilization of NCE's. Conversely, PEG-400 provides adequate solubilizing power at the expense of a clean CV profile. Intravenous administration of PEG-400 elicits increases in mean arterial pressure in conjunction with decreased heart rate and cardiac contractility that confounds interpretation of drug effects. For early screening efforts, it would be advantageous to identify a primary vehicle capable of formulating most drug candidates for i.v. infusions in anesthetized animals to attain maximal exposures across a range of doses with minimal compound requirements.

A broad panel of vehicles was evaluated for potential application in the anesthetized rat cardiovascular model in our laboratories to identify those with suitable solubilization capacity and minimal hemodynamic side effect profiles. The initial screen included a significant, but not exhaustive, selection of vehicles composed of various combinations of water-miscible cosolvents, surfactants and complexation agents. In all cases, excipients were selected and vehicles composed considering three criteria: solubilization potential, known cardiovascular side effects, and LD_{50} values from vendor information. While several vehicles screened during these processes were benign in the anesthetized rat CV model, DPP offered the greatest opportunity to solubilize candidates from our highly diverse compound library. This result served as the catalyst to further study the hemodynamic characteristics of the solvent system as a platform vehicle for anesthetized CV studies.

DPP is a mixture of three components: N,N-dimethylacetamide (DMA)/propylene glycol (PG) and polyethylene glycol 400 (PEG-400) at a ratio of 20:40:40 by volume, respectively. This work summarizes efforts to characterize the hemodynamic effects of DPP which has proven useful as a general solubilizer for most NCEs studied in our anesthetized rat cardiovascular model. In these studies, we show that DPP is effective in solubilizing 11 model compounds at our typical high working concentration of 30 mg/mL. DPP also elicits minimal cardiovascular effects when administered in the anesthetized rat cardiovascular model. Furthermore, we demonstrate that the hemodynamic effects of four select vasoactive substances (atenolol, enalaprilat, sotalol, and nifedipine) are comparable during i.v. infusion when formulated with DPP, D5W, or PEG-400. These results demonstrate that DPP is a useful alternative for the formulation of poorly soluble compounds in preclinical, anesthetized rat cardiovascular studies.

2. Material and methods

2.1. Materials

Compendial grade excipients were employed for *in vivo* applications whenever possible to minimize the potential for adverse events following administration. Polyethylene glycol 400, USP/NF (Item # PO110) was purchased from Spectrum Chemical (New Brunswick, NJ, USA) while Inactin Hydrate (Item # T133), N,N-dimethylacetamide, 99.8% anhydrous (Item # 271012), propylene glycol (Item # P4347) and all model compounds were acquired through Sigma Aldrich (St. Louis, MO, USA). Inactin and Propylene glycol sourced from Sigma-Aldrich meet USP testing specifications. 5% dextrose in water was purchased from Hospira (Lake Forest, IL, USA). Model compounds studied in this manuscript include atenolol [29122-68-7, R-(+)], carbamazepine [298-46-4], enalaprilat [84680-54-6], hydrocortisone [74050-20-7], itraconazole [84625-61-6], metronidazole [443-48-1], nifedipine [21829-24-4], piroxicam [36322-90-4], sotalol [959-24-0], tenoxicam [59804-37-4], and terfenadine [50679-08-8]. 5% dextrose in water was purchased from Hospira (Lake Forest, IL, USA).

2.2. In vitro solubility studies

Visual solubility for 11 compounds was evaluated at a concentration of 30 mg/mL in D5W, PEG-400, and DPP. 30 mg/mL is generally required to meet the high dose for anesthetized screening studies in rat and is useful to discriminate between potential vehicles in terms of solubilization capability. To assess solubility in 100% D5W and 100% PEG-400, 30 mg of each compound was added to 1 mL of vehicle followed by the application of sonication (VWR Scientific Aquasonic, model 50D) at 37 °F as necessary to increase the rate of dissolution. Solubility assessments in DPP required sequential addition of each vehicle component to the solid API followed by visual inspection for residual solids. Solubility was evaluated by visual inspection only, as is routine in our laboratory for detecting test article precipitation. As an example of the process, to a 30 mg sample of API, 0.2 mL DMA was added with mixing and, in some cases, sonication and/or heat was applied to the sample. Once solubilized in DMA, 0.4 mL of PG was added and mixed until homogenous. Finally, 0.4 mL of PEG-400 was added to create the final DPP formulation after thorough mixing. In some cases, the solubility result of 'yes' or 'no' was determined by direct visualization and confirmed *via* optical microscopy at 3× magnification.

2.3. In vivo anesthetized rat cardiovascular studies

All *in vivo* experiments were conducted under AbbVie Institutional Animal Care and Use Committee and according to the United States Animal Welfare Act. In brief, male Sprague-Dawley rats (325–375 g, Charles River, Indianapolis, IN) were anesthetized with the long-acting barbiturate Inactin (100 mg/Kg i.p.). Body temperature was monitored throughout the experiment and maintained between 37 and 37.5 °C using a glass heating pad. Polyethylene tubing (PE240) was placed in the trachea to keep the airway patent, and rats were allowed to breathe spontaneously. A catheter (PE50) was placed in the femoral artery for measurement of mean arterial blood pressure (MAP) and heart rate (HR) (Fryer et al., 2012; Liu et al., 2007). A 3F Micro-Tip catheter (Millar

Table 1
LC-MS analysis methods for model compounds (Brunner-La Rocca, Weilenmann, Kiowski, Maly, & Follath, 1999).

Drug	Instrument	Column	Additional Information
Sotalol in DPP	API-5000 Mass Spectrometer with Turbo Ion Spray	Imtakt Scherzo SM-C18 (50 × 3 mm, 3 μm)	Samples were run using a gradient of Acetonitrile and 10 mM ammonium acetate mobile phases.
Sotalol in D5W	API-5500 Mass Spectrometer with Turbo Ion Spray	Atlantis HILIC (50 × 4.6 mm, 5 μm)	Samples were run using a gradient of 0.1% formic acid in water and 0.1% formic acid in acetonitrile.
Nifedipine	API-5500 Mass Spectrometer using APCI (Atmospheric Pressure Chemical Ionization)	Fortis C18 (30 × 2.1 mm, 5 μm)	Samples were run using a gradient of 0.1% Formic Acid in Water and 0.1% Formic Acid in Acetonitrile.
Atenolol	API-5500 Mass Spectrometer with Turbo Ion Spray	Atlantis HILIC (50 × 4.6 mm, 5 μm)	Samples were run using a gradient of 0.1% Formic Acid in Water and 0.1% Formic Acid in Acetonitrile.
Enalaprilat	API-5500 Mass Spectrometer with Turbo Ion Spray	Betasil CN (50 × 3 mm, 5 μm)	Samples were run using a gradient of 0.1% Formic Acid in Water and 0.1% Formic Acid in Acetonitrile.

All studies used a standard protein precipitation method (acetonitrile crash). The method also included the addition of a co-eluting internal standard. Concentrations of study samples were calculated by regression analysis of the peak area ratio (parent/internal standard) of the spiked plasma standards *vs.* concentration. Samples were bracketed with standard curves containing eleven standards.