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Assessment of toxicity and tolerability of a combination vehicle; 5% Pharmasolve, 45% Propylene glycol and 50% Polyethylene glycol 400 in rats following repeated intravenous administration

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ABSTRACT

The selection of a suitable vehicle for administration of NCEs in non-clinical studies is always a challenge for poorly soluble compounds. Challenge is increased if the dose formulation is intended for *intravenous (i.v.)* administration where isotonic, biologically compatible pH and solution form is an absolute requirement. Vehicle toxicity and tolerability data are not readily available for a number of combination vehicles therefore, an *i.v.* tolerability studies was planned in rats with 5% v/v Pharmasolve (NMP), 45% v/v Propylene glycol (PG) and 50% v/v Polyethylene glycol (PEG) 400 combination, at dose volume of 0.5, 1, 2 and 5 mL/kg body weight for 28 days. The vehicle combination was administered via lateral tail vein and effects on clinical signs, body weights, feed consumption, clinical pathology and histopathology were evaluated. Clinical signs of toxicity like tremors, convulsions and death were noticed at 5 mL/kg during the course of the study. At 2 mL/kg, injection site injury without systemic toxicity was noticed. In conclusion, 1 mL/kg of a combination vehicle of 5% NMP, 45% PG and 55% PEG 400 can be administered intravenously once-a-day up to 28 days without any discomfort or injury to rats.

1. Introduction

A suitable dose formulation is vital to characterize any chemical entity intended to be a potential drug. A key factor determining the route of administration in preclinical species is based on planned clinical dosage form; however in early phase of discovery, to characterize the pharmacokinetic profile, especially for calculation of bioavailability, intravenous administration is indispensable to each molecule. Substances administered intravenously must be freely soluble in vehicle. Solubility of any chemical depends on its physicochemical properties. Based on intrinsic properties, chemicals are classified into four biopharmaceutical classes; from class I being highly soluble and highly permeable to class IV being poorly soluble and poorly permeable; other two falls in between in term of solubility and permeability (FDA, 2009). Formulation of poorly soluble chemicals is of great concern in preclinical studies for adequate assessment and characterization of chemicals. Each route of administration has limits of volumes to be administered. With the limited volume and poor solubility of chemicals, it is very challenging to select a suitable vehicle to administer desired amount of dose to animals especially for intravenous dose formulation where isotonic, biologically compatible pH and solution form (free from any particulate matter) are desired characters (Bittner et al., 2002).

For preclinical studies, a suitable vehicle should be feasible for administration, should not have undesirable side effects; should be compatible with bioanalytical methods and represents clinical formulations. In order to overcome the solubility issues, most of the poorly soluble compounds were formulated with combination of aqueous solvents and a co-solvent either employing water soluble/miscible organic solvents. Co solvency, pH adjustment, surfactant addition and complexation are the most commonly employed pharmaceutical approaches for solubilizing drug candidates with low aqueous solubility (Sathesh Babu et al., 2007). Among them, use of co solvent (i.e., co solvency) is one of the most popular approaches for improving the solubility of poorly aqueous soluble drugs in pharmaceutical liquid formulations (Amin et al., 2004). Co-solvents are the mixtures of miscible solvents with water, which can dramatically change the solubility of poorly aqueous soluble drugs (Yalkowsky and Roseman, 1981). The advantage of co solvent technology enhancing drug solubility in a liquid-based formulation includes

Abbreviations: ALP, Alkaline Phosphatase; EDTA, Ethylene diamino tetra acetic acid; *i.v*, Intravenous; IVC, Individually ventilated cages; Kg, Kilograms; MCV, Mean corpuscular volume; NCE, New chemical entity; RBC, Red blood cells

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(Yeh et al., 2009) convenience, removing the need for mixing solvent before administration; safety, avoiding contamination in the dispensing process; inexpensive, and no need for expensive pharmaceutical technology for formulation of dosage form. The most frequently used low-toxicity co solvents for parenteral use are propylene glycol, ethanol, glycerin, polyethylene glycol (PEG) 400, dimethylsulfoxide (DMSO) and dimethylacetoamide (DMA) (Avdeef, 2007; Rowe, 2009; Strickley, 2004; Gad et al., 2006).The water-soluble organic solvents and surfactants used in commercially available injectable formulations include propylene glycol, ethanol, polyethylene glycol 400, glycerin, dimethylacetamide (DMA), N-methyl-2-pyrrolidone (Pharmasolve), Dimethyl Sulfoxide (DMSO), Solutol HS 15, Cremophor EL, Cremophor RH 60, and polysorbate 80.

Non availability or sparse reporting over organic solvent's tolerability in combination use gave us an idea to design a study to evaluate the toxicity of combination solvents following repeated administration. We selected combination of 3 co-solvents viz. 5% Pharmasolve, 45% Propylene glycol and 50% Polyethylene glycol PEG 400 for evaluation of adverse clinical effects along with routine toxicity parameters in our study through intra venous route dosed over a period of 28 days. This combination of co-solvents was choosen since it has been shown to solubilize most of our in-house discovered chemicals which are otherwise insoluble in aqueous solvents. All the three solvents chosen have strong solubilizing properties and tolerability individually. Our intend to perform this study was to establish toxicity and tolerability of this combination and use as vehicle in repeated dose preclinical toxicity studies of NCEs which precipitate in aqueous vehicles.

N-methyl-2-pyrrolidone (Pharmasolve™), one of the main pharmaceutical co solvent, acts as strong solubilizing excipient with low viscosity in parenteral preparations (Avdeef, 2007; Strickley, 2004). With the wide usage in pharmaceutical industry reported LD_{50} of Pharmasolve by *i.v* route is 2.2 mL/kg in rats (Bartsch et al., 1976). Propylene glycol (PG) is a USFDA listed commonly used pharmaceutical excipient. PG is commonly used as a solvent in preclinical studies and the reported NOAEL for rat is 2 g/kg and used as a co-solvent in parenteral formulations in the range of 10–60% (EMA, 2014). Polyethylene glycol 400 (PEG 400) is generally considered to be among the safest organic co-solvents and are very commonly used in preclinical *in vivo* pharmacokinetic and efficacy studies due to their solubilizing capabilities and tolerability. The reported tolerated dose of PEG 400 for rat by oral and *i.v* route is 5 mL/kg and 0.5 mL/kg, respectively (Hermansky et al., 1995). All of these have been reported to be well tolerated individually but no literature is available for combination of all three solvents.

2. Materials and methods

2.1. Test formulation (vehicle)

N-methyl-2-pyrrolidone (Pharmasolve™), Propylene glycol (PG) and polyethylene glycol 400 (PEG 400) were purchased from sigma Aldrich and mixed in ratio of 5% v/v + 45% v/v and 50% v/v respectively. Physiological saline was purchased from local medical supplier (Manufactured by Aculife Healthcare Pvt. Ltd).

2.2. Animals, housing, accommodation and diet

In house bred Wistar rats of Suven Life Sciences Limited, with a weight range of 134–170 g for males and 128–158 g for females were used in the study. All rats were group housed (two to three per cage) in sterilized solid bottom polysulfone cages (IVC system) with stainless steel grill top facilitates for feed and water bottle and bedding of clean corncob. The cages were suspended on stainless steel rack in air conditioned rooms with 10–15 air changes per hour and 12 h light/dark cycle with temperature between 21 ± 3 °C and relative humidity between 30 and 70%. The rats had free access to pelleted nutrimix feed and water. Animal handling and experimental procedure were approved by the Institutional Animal Ethical Committee, and the experiments were performed in accordance with the “Guide for the care and use of laboratory animal” (National Research Council, 1985) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.3. Acclimatization, dosing and dosing scheme

All the rats were acclimatized for experimental conditions five days prior to commencement of dosing. During acclimatization all the animals were observed cage side twice daily for any clinical signs and mortality. Animal were randomized by body weight stratifications into five groups. Animals of group I were dosed at 5 mL/kg of physiological saline solution and rest of groups dosed with combination of 5% Pharmasolve™, 45% Propylene glycol (PG) and 50% Polyethylene glycol (PEG 400) at dose volume of 0.5, 1, 2 and 5 mL/kg for 28 days by lateral tail vein (dose levels of 5 mL/kg and 2 mL/kg were terminated on day 1 and day 17 of treatment respectively because of severe toxicity). Group allocation is represented in Table 1. For *i.v.* administration, rats were physically restrained in plastic restrainer fabricated by local vendor (B.I.K Industries, Mumbai, India). To facilitate easy administration of test formulations, tail was rubbed by cotton soaked with warm water to dilate the vein. The test formulations were administered through lateral tail vein with 26 gauge needle. The administration of requisite volume of formulation was completed within 1–2 min; the maximum volume administered to individual animal was 1.01 mL.

2.4. Investigations

The animals were observed at least twice a day for any mortality, treatment related clinical signs or changes in behavior. Body weights and feed consumption were recorded weekly. Routine laboratory investigations including hematology and clinical chemistry were undertaken at scheduled termination except for 2 mL/kg group where investigations were performed on day 17. For hematology following parameters were measured on whole blood taken in tubes containing 10% EDTA as anticoagulant: Hematocrit, hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, platelet count, total RBC count, total WBC count, reticulocyte count, basophils, eosinophils, lymphocytes, monocytes and neutrophils.

For clinical chemistry the following parameters were assayed on plasma separated from whole blood taken into tubes containing lithium heparin as anticoagulant: Alanine amino transferase, albumin, albumin/globulin ratio, alkaline phosphatase, aspartate aminotransferase, blood urea nitrogen, calcium, chloride, creatinine kinase, creatinine, globulin, glucose, phosphorous, potassium, sodium, total bilirubin, total cholesterol, total protein and triglycerides.

On completion of 28- day dosing period, the surviving rats were sacrificed under isoflurane anesthesia. Macroscopic examination was carried out on all the animals sacrificed at terminal/pre-terminal or found dead. Organs/tissues were collected and preserved for microscopic examination for rats those received multiple doses of test formulation. Most of tissues were fixed in 10% neutral buffered formalin except testes which were fixed in Modified Davidson's fixative. Organ weights were also measured for selective organs; adrenals, brain, epididymis, heart, kidneys, liver, ovaries, spleen, thymus, testes and uterus. Histopathological investigations were carried out on all major organs, injection site included.

3. Statistics

Descriptive statistics (Mean ± SD) were calculated for all parameters. One way ANOVA followed by post hoc Dunnet's test was applied for continuous variable of toxicological end points. Owing to early termination from control, clinical pathology and organ weight data of