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Single jugular vein cannulated rats may not be suitable for intravenous pharmacokinetic screening of high logP compounds

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ABSTRACT

Rat is commonly used for pharmacokinetic screening during pharmaceutical lead optimization. To handle the large number of compounds, rats with a single jugular vein cannulation are commonly utilized for intravenous pharmacokinetic studies, where the same cannula is used both for dose administration and blood sampling. We demonstrate that the single cannula method is not suitable for all compounds, especially for high logP compounds. We propose an alternative dual cannulation technique in which two cannulas are placed in the same jugular vein, thus avoiding an additional surgery. Compounds were administered orally or via intravenous infusion to compare PK parameters, including bioavailability, using both procedures. For itraconazole and amiodarone, known to bind to the cannula, the measured plasma exposures were substantially higher in the single cannulated rats than those from dual cannulated rats. Area under the plasma concentration time curve differed by 79% and 74% for itraconazole and amiodarone, respectively. When compared to the single cannulation approach, clearance, volume of distribution and bioavailability determined by dual cannulation were 39%, 60% and 38% higher for itraconazole, and 46%, 34% and 42% higher for amiodarone, respectively. In contrast, all pharmacokinetic parameters were similar between single and dual-cannulated rats for the hydrophilic compound atenolol. Based on these results, we recommend the use of dual cannulated rats for intravenous pharmacokinetic studies when testing a series of hydrophobic compounds that may be prone to non-specific binding to the cannula. If single cannulated model is selected for pharmacokinetic screening, we recommend a bridging study with dual cannulated rats with representative compounds of a given chemical series.

1. Introduction

Pharmacokinetic evaluation in animals is an integral part of the drug discovery workflow during lead optimization to select compounds with desirable pharmacokinetic properties. Rat is one of the most commonly used rodent species for pharmacokinetic studies because of its ease of handling, small size, ease of dose administration and opportunity to sample blood serially from the same animal. To enable serial blood sampling, different blood vessels are cannulated, e.g. jugular vein, femoral vein, carotid artery, femoral artery, tail vein, etc. (Hui et al., 2007; Johannessen et al., 1982; Korfmacher et al., 2015). To improve throughput and to avoid two surgical interventions, single vein cannulated rats are commonly used for both oral and intravenous pharmacokinetic studies (Chen et al., 1992; Holenarsipur et al., 2015; Iwaki et al., 1996; Keiser et al., 2009; Lin et al., 2002; Mukkavilli et al., 2014; Toyn et al., 2014; Usach et al., 2014; Wang et al., 2013). In case of oral studies, drug formulation is administered by oral gavage and the blood samples are collected from the venous cannula. In the case of intravenous studies, drug formulation is administered through the cannula by infusion followed by serial blood sample collection through the same cannula at various time points. Multiple laboratories follow this procedure for routine pharmacokinetic screening for most compounds, since intravenous dose administration is generally found not to influence the drug concentration in subsequent blood samples collected from the same site (Upton, 1975). Overestimation of midazolam concentrations in a rat pharmacokinetic study is reported after dosing and sampling through the same cannula (Kotegawa et al., 1998). Couple of other investigators did not observe higher concentrations when the same cannula was used for dosing and sampling, as catheter binding depends on multiple factors (Boudinot and Jusko, 1986; Upton, 1975). Nirogi et al., demonstrated that compounds can non-specifically bind to tubing and probe membranes, thereby affecting the brain microdialysis results (Nirogi et al., 2012). The single jugular vein method is commonly used owing to its convenience by teams conducting large number of rat pharmacokinetic studies, e.g. new chemical entity pharmacokinetic

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screening in a drug discovery setting. However, there are only limited reports on the impact of using this method versus dual cannulation on primary pharmacokinetic parameters like clearance and volume of distribution of compounds. In this study, we investigated relevance of physicochemical properties of compounds, such as hydrophobicity or hydrophilicity, relating to non-specific binding of the test compounds to the cannula and demonstrated limitations of the single cannulated rat model as it leads to overestimation of plasma concentrations.

To address this issue, a dual cannulation approach was evaluated where-in compound administration and blood sample collection processes are separated thereby addressing the limitations of single cannulated rat. When two blood vessels need to be cannulated e.g. for compounds showing non-specific binding, generally combinations such as jugular vein/carotid artery (Burt et al., 1980; Feng et al., 2015), jugular vein/femoral vein (Moslen et al., 1988) or femoral vein/femoral artery are well reported. Here we report a novel surgical procedure wherein two cannulas are implanted in single jugular vein each of which was used for dose administration and blood sample collection, respectively. This is an innovative procedure from the perspective of 3R (Replacement, Reduction, Refinement) principles of animal experiments, where the cannulation technique is *refined* to avoid a second surgery at a second site on the rat. Refinement refers to improvements to scientific procedures and husbandry which minimize actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable (https://www.nc3rs.org.uk). The proposed novel dual cannulation model decreases potential pain to the animal as surgery of the second vessel is avoided.

Herewith we present results comparing single and dual cannulated rat models using three marketed compounds that have different physicochemical properties. Itraconazole, which is used for the treatment of variety of fungal infections, is a hydrophobic drug with a log partition coefficient of 7.13 (Riccardi et al., 2015). Amiodarone, an antiarrhythmic agent, is a highly insoluble compound with log partition coefficient 7.8 (Riccardi et al., 2015). Atenolol, used for the treatment of high blood pressure, is a polar hydrophilic compound with a log partition coefficient of 0.23. The overall objective of the current work was a) to demonstrate the limitations of a single vein cannulated rat to generate intravenous pharmacokinetic data, and b) to propose and demonstrate the utility/advantage of a novel dual cannulation technique for such studies.

2. Materials and methods

2.1. Materials

Itraconazole, amiodarone, atenolol, fluconazole, timolol, ritonavir and *N*,*N*-dimethylacetamide were purchased from Sigma-Aldrich (St. Louis, MO). Polyethylene glycol 400 (PEG-400) was purchased from Affymetrix (Cleveland, OH). EDTA dipotassium salt (K_2EDTA) was from Merck Specialties Pvt. Ltd. (India). Acetonitrile and formic acid was purchased from Merck and Fluka, respectively. Milli-Q water was prepared using a Millipore water purification system. Polyethylene (PE) and silicone catheters were purchased from Instech Laboratories, Inc. (PA, USA). All other chemicals used were of reagent grade.

2.2. Animals

All animal experiments were performed in accordance with protocols approved by the Institutional Animal Ethics Committee (Syngene International Ltd., Bangalore) and in a facility accredited by the association for assessment and accreditation of laboratory animal care international (AAALAC). Male Sprague–Dawley rats (280 to 320 g, 9 to 10 weeks of age) were purchased from Vivo Bio Tech Ltd., India (breeders were from Taconic). Animals were housed at 21 ± 3 °C and relative humidity 50 ± 20%. Animals were maintained in 12 h light and 12 h dark cycle and were given free access to food and water.

2.3. Preparation of catheters and surgical procedure for jugular vein cannulation

The procedure for jugular vein cannulation is similar to the reported procedure (Thrivikraman et al., 2002), with a few modifications. The two catheters to be used in dual cannulation procedure were designed based on anatomical relevance of the jugular vein. For the first catheter PE (polyethylene) 50 was bonded with 3 cm of silicone tubing at the tip and for second catheter the PE50 catheter was bonded with 3 cm of PE10 catheter. Specifications of the catheters are shown in Fig. 1. Each catheter was 55 cm in length which was suitable for metabolic cages. All procedures were performed aseptically. In brief the dual cannulation procedure involved, placing rat under general anesthesia with 3.5% isoflurane. The dorsal and ventral neck areas were shaved and wetted with povidone/iodine. A longitudinal incision of 1 to 1.5 cm was made anterior to the right scapular region. The right jugular vein was located and bluntly dissected free from the surrounding tissues and a small flat stainless steel support was placed below the isolated jugular vein. A pair of 4–0 silk sutures was passed under the vein. A small cut was made on the jugular vein using a sharp iris scissor and the proximal end with silicone tip of the first catheter, attached to a 1 cm^3 syringe filled with the heparinized saline, was inserted and advanced to the atrium. Then the second catheter with the PE 10 catheter was inserted adjacent to the first catheter up to the same length and both were tied with the help of the silk suture. Precaution was taken such that no blood came out through the incised vein. Upon checking for patency, both catheters were exteriorized subcutaneously on the nape with the help of a trocar

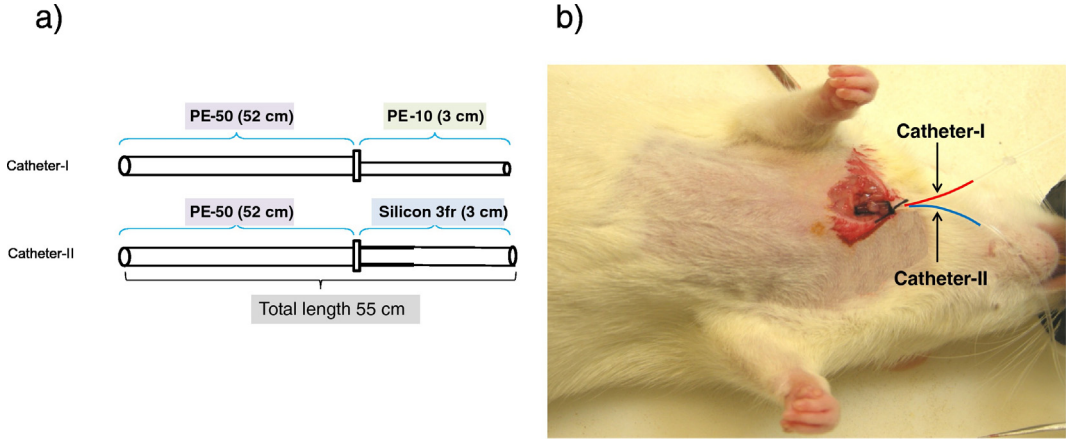

Fig. 1. a. Specifications of different catheters used for cannulation of jugular vein in rat. b. Sprague-Dawley rat with two catheters in the same jugular vein.