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One should avoid retro-orbital pharmacokinetic sample collections for intranasal dosing in rats: Illustration of spurious pharmacokinetics generated for anti-migraine drugs zolmitriptan and eletriptan

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ABSTRACT

Because of the avoidance of first pass metabolic effects due to direct and rapid absorption with improved permeability, intranasal route represents a good alternative for extravascular drug administration. The aim of the study was to investigate the intranasal pharmacokinetics of two anti-migraine drugs (zolmitriptan and eletriptan), using retro-orbital sinus and jugular vein sites sampling. In a parallel study design, healthy male Sprague–Dawley (SD) rats aged between 8 and 12 weeks were divided into groups (n = 4 or 5/group). The animals of individual groups were dosed intranasal (~1.0 mg/kg) and oral doses of 2.1 mg/kg of either zolmitriptan or eletriptan. Serial blood sampling was performed from jugular vein or retro-orbital site and plasma samples were analyzed for drug concentrations using LC-MS/MS assay. Standard pharmacokinetics parameters such as T_{max}, C_{max}, AUC_{last}, $AUC_{0\text{-}inf}$ and $T_{1/2}$ were calculated and statistics of derived parameters was performed using unpaired t-test. After intranasal dosing, the mean pharmacokinetic parameters C_{max} and AUC_{inf} of zolmitriptan/eletriptan showed about 17-fold and 3-5-fold higher values for retro-orbital sampling as compared to the jugular vein sampling site. Whereas after oral administration such parameters derived for both drugs were largely comparable between the two sampling sites and statistically non-significant. In conclusion, the assessment of plasma levels after intranasal administration with retro-orbital sampling would result in spurious and misleading pharmacokinetics.

1. Introduction

In a drug discovery set up, rodents comprising of mice and rats are extensively used for the generation of pharmacokinetic parameters for the early lead compounds. Such pharmacokinetic parameters are used in the decision of establishing suitability and druggability of the chemical structures or scaffolds employed by the medicinal chemists. Furthermore, the structures may be modified based on other in vitro derived parameters such as metabolic stability, cytochrome P450 liability, protein binding etc. Since delineation of initial pharmacokinetic parameters obtained in rodents are used to enable key decisions including clinical candidate nomination, we were interested to explore the relevance of site specific pharmacokinetic sample collections particularly in rodent studies.

Earlier work that examined clinical chemistry measurements in Fischer rats (serum enzymes, cholesterol, triglycerides etc.) has suggested that sampling site could be a major contributor for the variability in the parameter values and therefore had recommended careful selection of the sampling site (Neptun et al., 1985). In this work, the various sampling sites that were evaluated for clinical chemistry measurements included right ventricle, aorta, vena cava, retroorbital sinus, and tail vein (Neptun et al., 1985). Similarly, the follow-up work of Smith et al. (1986) with similar sampling sites suggested the existence of variability in the clinical haematology parameters in Fischer rats (red blood cell count, hemoglobin, white blood cell count, haematocrit etc.) (Smith et al., 1986).

Although there appeared to be differences in the clinical chemistry or heamatology parameters, the work of Hui et al. (2007) suggested

Abbreviations: AAALAC, Association for the Assessment and Accreditation of Laboratory Animal Care International; $AUC_{0\text{-}inf}$, area under the concentration time curve extrapolated to infinity; AUC_{last}, area under the concentration time curve until the last measured time point; C_{max}, maximum plasma concentration; HPLC, High Performance Liquid Chromatography; LC-MS/MS, Liquid chromatography-mass spectrometry; PK, pharmacokinetics; T_{max}, time to achieve peak plasma concentration; $T_{1/2}$, terminal elimination half-life

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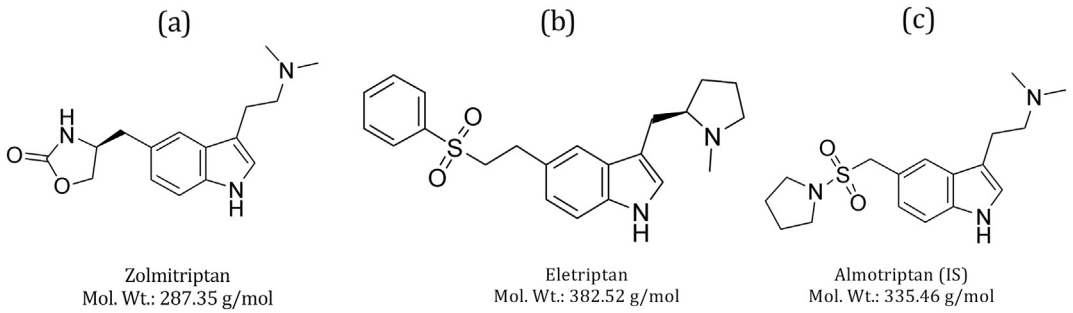

Fig. 1. Chemical structure representation of (a) zolmitriptan (b) eletriptan (c) almotriptan (IS).

that different sampling sites for estimation of pharmacokinetic parameters did not produce appreciable differences in either peak concentration (C_{max}) or area under the concentration vs. time cure (AUC) for several marketed drugs such as clonidine, fluoxetine, gemfibrozil, glipizide, methotrexate and pentoxifylline that were evaluated in this investigation (Hui et al., 2007). The three pharmacokinetic sampling sites evaluated in the study were tail vein, retro-orbital sinus, and femoral artery (Hui et al., 2007). It was important to note that the study evaluated diversified drug structures in various therapeutic classes; however, only oral dosing of the six marketed drugs was tested (Hui et al., 2007). We were interested to explore the appropriateness of sampling sites to determine the pharmacokinetic parameters after intranasal dosing. Therefore, the present study aimed to investigate the intranasal pharmacokinetics of two anti-migraine drugs, zolmitriptan and eletriptan, (Fig. 1) using retro-orbital sinus and jugular vein sites for pharmacokinetic sample collection.

2. Material and methods

2.1. Reagent and chemicals

Eletriptan hydrobromide, purity, 99% *w*/w (Vicenza, Italy), zolmitriptan hydrochloride, purity, 99.5% *w*/w (Hyderabad, India) almotriptan maleate, purity 99.91% (internal standard, Fig. 1) was purchased from Clearsynth Labs Ltd. (Mumbai, India). HPLC grade methanol, acetonitrile and formic acid were purchased from Merck (Mumbai, India), water purification has been performed in house and derived from purification system Elix Millipore (Bangalore, India), the pharmaceutical grade excipients such as polyethylene glycol 400 (BASF, Germany), butylated hydroxytoluene (Sigma, India), polyethylene glycol, polysorbate 80, butylated hydroxyanisole, disodium edetate dehydrate, benzyl alcohol, citric acid monohydrate, disodium phosphate dodecahydrate (Merck, Germany) were purchased from the respective vendors. Sodium-heparin was purchased from Biological E limited (Haridwar, India) and normal saline was purchased from Aculife (Sachana, India). Animal SD rats were bred in-house and supplied by animal research facility, Zydus Research Centre (Ahmedabad, India). All other chemicals/reagents were of analytical grade and used without further purification.

2.2. Instruments and equipment

A HPLC system (Shimadzu, Kyoto, Japan) comprising of LC-20AD pump, a vacuum degasser DGU-20A3, CTO-20AC column oven, SIL HTc auto injector with sample cooler. API 3200/API4000 triple quadrupole mass detector with turbo ion spray source (MDS SCIEX, Concord Ontario, Canada), thermostated centrifugation system was from Eppendorf (Hamburg, Germany). Automatic sample transfer pipettes were purchased from Eppendorf (Hamburg, Germany). Vortex-mixture was from Spinix (Mumbai, India). The analytical column ACE CN 50 ∗ 4.6 mm, 5 µm was procured from Advanced Chromatography Technologies ltd. (Aberdeen, Scotland).

2.3. Formulation preparation

A solution formulation of zolmitriptan hydrochloride was prepared in pharmaceutically acceptable excipients comprising of polyethylene glycol 400, polyethylene glycol, polysorbate 80, butylated hydroxyanisole, butylated hydroxytoluene, disodium edetate dehydrate, benzyl alcohol, citric acid monohydrate, disodium phosphate dodecahydrate and eletriptan hydrobromide solution was prepared in purified water. Both the solutions were suitable for the intranasal or per oral dose administration in the animals as well as in humans. The solution formulation was prepared at 25 mg/mL strength to meet dose volume administration for intranasal (i.e., > 35 µL/animal). The suspension formulation was prepared in 0.5% sodium CMC (carboxyl methylcellulose) in purified water for per oral administration. The strength of oral formulations was kept at 0.84 mg/mL to meet the dose volume requirement of 2.5 mL/kg.

2.4. Animal experiment design

Male SD (Sprague–Dawley) rats were breed in-house and animals of 8–12 weeks of age were received from Animal Research Facility, Zydus Research Centre, Ahmedabad, India. Prior to the study, animals were housed and acclimatized for about 2–3 days under controlled experimental conditions of 12 h-light/dark cycle with free access of feed and purified water. The experimental designs including the animal usage in the study were approved by the independent Institutional Animal Ethic Committee (IAEC) of Zydus Research Centre, Ahmedabad India. The study protocol for experimentation on animal (ZRC/DMPK/BP/005/05-2K16) was approved by IAEC. The study facility was approved by AAALAC International agency.

The animal welfare, care and use were managed by professionally well trained technical personnel with animal husbandry as an educational background. The technical personnel involved were also well trained in blood draws via retro-orbital sinus such that it imparts minimal pain to the animals. The animal studies performed for this research work were single dose and terminal studies. The retro-orbital blood sampling technique employed in our study is being widely used across the pre-clinical drug research to support pharmacokinetic studies. After completion of the study, these animals were not used in any other studies and humanely sacrificed using CO_2 asphyxiation. Because the primary objective of the study was to measure the drug levels in the blood but not a pharmacodynamic outcome, retroorbital or any other blood collection technique is not expected to interfere in the pharmacokinetic outcome. Generally, physiological changes occurring during retro-orbital sinus blood draws were expected to have negligible influence on drug pharmacokinetics.

The experiment used parallel study design (Fig. 2), the animals were divided into 8 groups each of having 4 or 5 animals. Jugular cannulation was performed in animals of 4 out 8 groups for collection of blood sampling from jugular vein. The animals of 4 groups (cannulated or non-cannulated) were remained non-fasted and used for the intranasal dose administration and other 4 groups were kept overnight fasting