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Pharmacokinetics, pharmacodynamics and safety profiling of IS01957, a preclinical candidate possessing dual activity against inflammation and nociception

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ABSTRACT

In spite of unprecedented advances in modern systems of medicine, there is necessity for exploration of traditional plant based secondary metabolites or their semisynthetic derivatives which may results in better therapeutic activity, low toxicity and favourable pharmacokinetics. In this context, computational model based predictions aid medicinal chemists in rational development of new chemical entity having unfavourable pharmacokinetic properties which is a major hurdle for its further development as a drug molecule. Para-coumaric acid (p-CA) and its derivatives found to be have promising antiinflammatory and analgesic activity. IS01957, a p-CA derivative has been identified as dual acting molecule against inflammation and nociception. Therefore, objective of the present study was to investigate pharmacokinetics, efficacy and safety profile based on *in-silico*, *in-vitro* and *in-vivo* model to assess drug likeliness. In the present study, it has excellent pharmacological action in different animal models for inflammation and nociception. Virtual pharmacokinetics related properties of IS01957 have resemblance between envision and experimentation with a few deviations. It has also acceptable safety pharmacological profile in various animal models for central nervous system (CNS), gastro intestinal tract (GIT)/digestive system and cardiovascular system (CVS). Finally, further development of IS01957 is required based on its attractive preclinical profiles.

1. Introduction

Plant based natural products have enormous potential to be developed as new therapeutics for the treatment of inflammatory and painful diseases as a high proportion of marketed drugs have been derived directly or indirectly from natural origin (Daniel et al., 2012). This is quite apparent from the fact that some of the natural products like morphine, salicin, cannabidiol etc. set the new milestone in the arena of analgesic and antiinflammatory drugs (Pragasam et al., 2013). These molecules have distinct edge over its synthetic counterparts for the treatment of inflammatory disorders as the latter having potential side effects (Beg et al., 2011). Even newer approaches such as NO-NSAIDs, dual COX/LOX inhibitors, anti-TNFα therapy etc. have been attempted toward the development of effective antiinflammatory therapy with synthetic drugs which are often associated with lots of undesired toxicity; intolerable and ineffective therapy (Rao and Knaus, 2008; Jain et al., 2014). In spite of the unprecedented advances in modern systems of medicine, there is still necessity for exploration of secondary metabolites present in traditionally used plant or their semisynthetic derivatives which may results in better therapeutic activity, low toxicity

Abbreviations: p-CA, Para coumaric acid; ADME, Absorption, distribution, metabolism and excretion; CH, Chlorpromazine hydrochloride; PS, Pentobarbital sodium; PBS, Phosphate buffer saline, pH 7.4; SGF, Simulated gastric fluid; SIF, Simulated intestinal fluid; P-gp, P-glycoprotein; Rh123, Rhodamine 123; PAMPA, Parallel artificial membrane permeability assay; P_e, Permeability; RED, Rapid equilibrium dialysis; LC-MS/MS, Liquid chromatography tandem mass spectrometry; RLM, Rat liver microsomes; NADPH, Nicotinamide adenine dinucleotide phosphate; $AUC_{0\text{-}t}$, Area under the curve for plasma concentration from zero to the last measurable plasma sample time; $AUC_{0\text{-}\infty}$, Area under the curve for plasma concentration from zero to infinity time; C_{max}, Maximum plasma concentration; T_{max}, Time to reach maximum plasma concentration; $T_{1/2}$, *In-vivo* elimination half-life; V_d, Volume of distribution; Cl, Clearance; LPS, Lippopolysachride; IL, Interleukines; TNF, Tumor necrosis factor; IFN, Interferon; ELISA, Enzyme linked immunosorbent assay; CNS, Central nervous system; GIT, Gastrointestinal tract; CVS, Cardiovascular system; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; SD, Standard deviation; SEM, Standard error of mean

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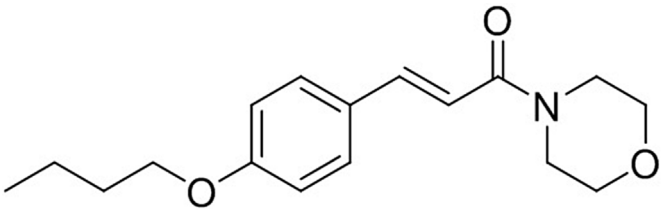

Fig. 1. Chemical structure of IS01957.

and favourable pharmacokinetics. In this context, para-coumaric acid (p-CA), found in variety of fruits, vegetables and plant products have been shown promising antiinflammatory and analgesic activity (Eun et al., 2012; Pei et al., 2016; Ya et al., 2016). p-CA as such has been shown unfavourable pharmacokinetics (Pei et al., 2016; Meng et al., 2006). p-CA conjugate showed improved activity profile in comparison to p-CA as free form. These resultsare encouraging for further investigation on this chemical class [Pei et al., 2016]. In this connection, IS01957, chemically [(E)-3-(4-butoxyphenyl) 1-morpholinoprop-2-ene-1-one)] (Fig. 1) derived from p-CA might be the compound of choice for further investigation due to its natural origin and several favourable pharmacological profiles.

Computational model based predictions acts as virtual filters for drug like properties and assists medicinal chemists for the rational development of drug candidates with acceptable ADME properties, thereby making it as straight forward and swift approach (Bergstrom, 2005). The key problem associated with the development of a new chemical entity into a drug molecule is its poor pharmacokinetics which includes its physicochemical properties. These are in turn closely related to oral bioavailability and subsequently *in-vivo* biological activity. In summary, these critical properties lead to high attrition rate in clinical development (Kerns, 2001). With this background, it has become imperative to investigate on pharmacokinetics, bioefficacy and safety pharmacology have to be performed using *in-silico, in-vitro* and *in-vivo* experimentations and hence taken up for as objectives of the present investigation.

2. Material and methods

2.1. Chemicals and reagents

IS01957 (purity ≥ 99%) was synthesized in Bioorganic Chemistry Division, CSIR-IIIM, Jammu. Diclofenac sodium (purity ≥ 98%), chlorpromazine hydrochloride (CH) (purity ≥ 98%), atropine sulphate (purity ≥ 97%), testosterone (purity ≥ 98%) were purchased from Sigma-Aldrich (St. Louis, USA). Pentobarbital sodium (PS) (purity ≥ 98%) was procured from Loba Chemie (Mumbai, India). All other chemicals/reagents used were of research grade. Water having resistivity of 18.2 MΩ.cm was used throughout the analysis (Millipore water purification system, Millipore, Bedford, USA).

2.2. Animals husbandry, maintenance and ethical prerequisites

All the animal studies were performed following 'Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)' guidelines after obtaining necessary approval from Institutional Animal Ethics Committee of CSIR-Indian Institute of Integrative Medicine, Jammu, India (IAEC approval No: 68/91/8/16). I*n-vivo* experiments were performed using healthy adult BALB/c mice or Wistar rats which were described in individual experiments. Animals were housed in polypropylene cages, kept at standard laboratory conditions (25 ± 2 °C, 50 ± 20% relative humidity, 12 h light/12 h dark cycle), fed with standard pellet diet with water *ad libitum*. Animals were acclimatized for a minimum of one week prior to experimentation.

2.3. Dose formulation for iv-vivo experimentation

Formulation for IS01957 was prepared by using 5% DMSO, 45% PEG-400 and 50% normal saline. Water was used as vehicle to formulate individual formulation of diclofenac sodium, morphine sulphate, atropine sulphate, and chlorpromazine hydrochloride. Pentobarbital sodium and haloperidol was dissolved separately in minimum volume of sodium hydroxide (1N) and hydrochloric acid (0.1M), respectively followed by made up to final volume with water to prepare individual dose formulation. All the drugs were prepared freshly on the day of experiment.

2.4. Pharmacokinetic profile

2.4.1. In-silico prediction of pharmacokinetic and its allied properties

Pharmacokinetic and its allied properties of the molecule can be predicted qualitatively like yes/no or high/intermediate/low and/or quantitatively in the form of absolute number using computational tools for rapid and cost effective drug discovery process (Bergstrom, 2005). Different important physicochemical and pharmacokinetic parameters were predicted using software such as Schrodinger 2015-4, QikProp 4.6, New York, 2015 and ADMET Predictor 8.0, Simulations-Plus, USA. Further experimental data need to be evaluated for better understanding of the virtual results.

2.4.2. Pharmacokinetics allied properties

Several properties of the molecule associated with the pharmacokinetics were evaluated for comprehensive investigation. Solubility in bio-relevant media like phosphate buffer saline pH 7.4 (PBS), simulated gastric fluid pH 1.2 (SGF) and simulated intestinal fluid pH 6.8 (SIF) was measured using miniaturized shake-flask method (Bharate and Vishwakarma, 2015). Lipophilicity was examined based on partition coefficient (logP) of the candidate between octanol/water and distribution coefficient (logD) of the compound between octanol/PBS (Kumar et al., 2016). Chemical stability of the candidate were determined by keeping the sample in PBS, SGF and SIF at 37 °C for 24 h, 2 h and 4 h, respectively followed by comparison with the concentration of IS01957 in freshly prepared individual samples (Mondal et al., 2009).

2.4.3. P-gp induction and inhibition

Ability of the compound to induce or inhibit P-gp was assessed based on intracellular rhodamine 123 (Rh123) accumulations using the Rh123 cell exclusion method. Colorectal LS180 cells were used for these studies due to over expressions of P-gp. For P-gp induction study, cells (2×10^4 cells/well plate) were seeded and allowed to grow for the next 24 h. Cells were further incubated with the test compound and diluted to a final concentration of 5 μM and rifampicin as standard was added to a final concentration of 5 μM in complete media for 48 h. Test compound was removed and cells were incubated with Hank's buffer for 40 min before further incubation with Hank's buffer for 90min containing 10 μM of Rh123 as a P-gp substrate. At the end of Rh123 treatment, cells were washed with PBS and processed for cell lysis using lysis buffer containing 0.1% Triton X 100 and 0.1(N) sodium hydroxide. Lysate (100 μL) was used for reading the fluorescence of rhodamine 123 at 485/529 nm. All samples were normalized by dividing the fluorescence of each sample with total protein present in the lysate. Data were analysed and expressed as mean ± SD or representative of one of three similar experiments unless otherwise indicated (Bharate et al., 2015). Compound was also evaluated for their capability to inhibit P-gp that was performed in the same way as mentioned above except concentration of test compound (50 μM), treatment time (90 min) and elacridar as standard (10 μM) (Mudududdla et al., 2015).

2.4.4. Permeability assay

Parallel artificial membrane permeability assay (PAMPA) was