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Biopharmaceutical Characterization and Oral Efficacy of a New Rapid Acting Antidepressant Ro 25-6981

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ABSTRACT

Ro 25-6981 is a highly potent and selective blocker of *N*-methyl-D-aspartate receptors that has been shown to possess both rapid and sustained antidepressant activity. In the present study, we report the biopharmaceutical characterization of Ro 25-6981 by evaluating gastrointestinal stability, transepithelial permeability, stability in human liver microsomes, and *in silico* metabolic prediction. Moreover, *in vivo* efficacy of Ro 25-6981 after oral administration was evaluated in animal models of depression. When mixed with 5 different simulated gastrointestinal fluids, no loss of parent compound was observed after 6 h, indicating compound stability in the gastrointestinal environment. At the tested concentrations, Ro 25-6981 was shown to have transepithelial permeability with apparent permeability (P_{app}) values comparable to highly permeable drugs. Ro 25-6981 was metabolized within 30 min in human liver microsomes, and the metabolic prediction data showed glucuronidation and sulfation as potential metabolic pathways. The *in vivo* efficacy data suggested that Ro 25-6981, when administered orally at 30 mg/kg, exhibits antidepressant-like activity following oral administration with efficacy comparable to traditional antidepressants that is both dose- and time-dependent. Overall, due to optimal gastrointestinal stability, oral permeability, and oral efficacy, Ro 25-6981 can be a potential therapeutic option for the treatment of depression.

Introduction

The National Institute of Mental Health estimates that around 16.2 million adults in the United States had at least 1 major depressive episode in 2016.[1] Depression in the United States costs society approximately $210 billion per year.[2] The first-line treatment for depression involves the use of antidepressant drugs including selective serotonin reuptake inhibitors (SSRIs), serotonin norepinephrine reuptake inhibitors, tricyclic antidepressants, bupropion, and monoamine oxidase inhibitors.[3] Despite their widespread clinical use, the current antidepressants suffer from demerits including low response rates and delayed therapeutic effects.[4,5] These drawbacks are particularly alarming given that suicide risk is elevated in depressed individuals. Therefore, development of a more efficacious and faster acting antidepressant treatments remains a significant unmet need.

Ro 25-6981 [4-((1*R*,2*S*)-3-(4-benzylpiperidin-1-yl)-1-hydroxy-2-methylpropyl)phenol] (Fig. 1) is a highly potent and selective blocker of *N*-methyl-D-aspartate (NMDA) glutamate receptors containing the *N*-methyl-D-aspartate receptor 2B subunit.[6,7] Like ketamine and other NMDA receptor antagonists, Ro 25-6981 has garnered attention for its rapid (<24 h) antidepressant effects.[8] More recently, we reported that Ro 25-6981 is also an inhibitor of all 3 monoamine transporters and showed rapid and sustained antidepressant activity in animal behavioral models.[9] This finding is in agreement with previously published research by Li et al.,[10] wherein it was reported that Ro 25-6981 rapidly ameliorates anhedonic and anxiogenic behaviors in a rat chronic unpredictable stress model. Therefore, there is substantial evidence that Ro 25-6981 can be developed as a potential first in class rapid- and

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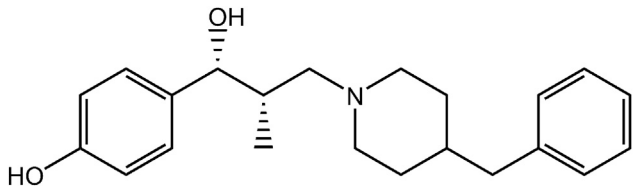

Figure 1. Chemical structure of Ro 25-6981.

sustained-acting antidepressant for the treatment of various forms of depression. In addition to ketamine, L-acetylcarnitine is also currently under investigation for its rapid-acting antidepressant activity.[11]

It is well established in the modern drug discovery paradigm that drugs with optimal biopharmaceutical properties have a higher probability of success in clinical development.[12-14] Furthermore, the oral route of administration is the most patient compliant, widely used, and readily accepted.[15] Therefore, drugs with biopharmaceutical properties allowing oral delivery are most sought after and have higher chances of clinical success. In previous preliminary studies reported, Ro 25-6981 was administered by parenteral route, and there is a gap in understanding the biopharmaceutical properties and their role in the oral delivery of Ro 25-6981.

In the present study, we report the characterization of biopharmaceutical properties of Ro 25-6981 such as stability in simulated gastrointestinal fluids, transepithelial permeability across Caco-2 cell monolayer model, *in vitro* metabolism using pooled human liver microsomes, and *in silico* metabolic prediction. Furthermore, we validated the oral deliverability of Ro 25-6981 by performing *in vivo* efficacy in the animal behavioral model after oral administration of the drug. To the best of our knowledge, we are the first to report biopharmaceutical characterization and oral efficacy of Ro 25-6981.

Materials and Methods

Ro 25-6981 and ifenprodil (internal standard) were obtained from Axon Medchem (Groningen, Netherlands). n-octanol, pepsin, and pancreatin were purchased from Sigma-Aldrich (St. Louis, MO). The biorelevant gastrointestinal media Fasted state simulated gastric fluid (FaSSGF), Fasted state simulated intestinal fluid (FaSSIF), and Fed state simulated intestinal fluid (FeSSIF) were purchased from Biorelevant.com Ltd. (London, UK). Caco-2 cells and the growth medium, Eagle's Minimum Essential Medium with 20% fetal bovine serum were purchased from American Type Culture Collection (Rockville, MD). All other chemicals and reagents used in this study were purchased from VWR (Radnor, PA).

High-Performance Liquid Chromatography

Ro 25-6981 was quantified using a rapid and sensitive HPLC method with UV detection. Ifenprodil was used as an internal standard due to its structural similarity to Ro-25-6981. The HPLC analysis was performed using Waters® 2695 Alliance® instrument equipped with Waters®2996 PDA detector and Empower 3 software (Waters, Milford, MA). The chromatographic separation was carried on a Waters® Atlantis™ C18 column (150 mm × 3.9 mm [i.d.]; particle size, 3 μm), and the mobile phase was acetate buffer (pH 8.0):acetonitrile at 55:45 (v/v) ratio. The flow rate was 0.8 mL/min, and the analytes were monitored at 210 nm detection wavelength. The HPLC method was validated for various parameters including specificity, accuracy and precision, linearity, and robustness.

Calculation and Prediction of Physicochemical Properties

The physicochemical properties of Ro 25-6981 such as p*K*a, log *p*, log D, isoelectric point, solubility, number of hydrogen bond donors, and number of hydrogen bond acceptors were calculated and predicted using Chemicalize® by ChemAxon® Company (Cambridge, MA).

Stability in Simulated Gastrointestinal Fluids

For stability studies, 2 solutions based on United States Pharmacopeia (USP) and 3 solutions based on Biorelevant media were used. The USP-simulated gastric (pH 1.2) and intestinal fluids (pH 6.8) were prepared according to the protocol outlined in USP 37 (Details are provided in Supporting Information). The 3 types of biorelevant media used are FaSSGF (pH 1.6), fasted state simulated intestinal fluid (FaSSIF, pH 6.5), and FeSSIF (pH 5). All 3 biorelevant media were prepared according to the supplier's guidelines. (Details are provided in Supporting Information). For the stability studies, 1 mg of Ro 25-6981 was added to 1 mL each of USP simulated gastric fluid, USP simulated intestinal fluid, FaSSGF, FaSSIF, and FeSSIF in triplicate and incubated in an incubator shaker (VWR, PA) at 37°C at 50 rpm. For all simulated fluids, a 100-μL aliquot was taken at 0, 2, 4, and 6 h and diluted with water to 100 μg/mL concentration and injected into HPLC. The concentration of Ro 25-6981 in all samples was quantified using a linear curve of Ro 25-6981 prepared in each of their respective simulated fluids.

Transepithelial Permeability Studies Using Caco-2 Cell Monolayers

Caco-2 cells (passages 21-28) were grown in American Type Culture Collection-Eagle's Minimum Essential Medium media with 20% fetal bovine serum under a 5% CO_2 atmosphere with 95% relative humidity. The cells were then seeded on 12 well polyester transwells (Corning, Corning, NY) at a density of 2500 cells per well and allowed to grow for 21-29 days before use. On the day of the experiment, the formation of monolayers was confirmed by measuring transepithelial electrical resistance (TEER) across the caco-2 cell monolayers using the EVOM-2 device with chopstick electrodes (Warne Instruments, Sarasota, FL). Transwells with TEER greater than 600 Ω cm^2 were used for the experiment. The TEER was also measured after the experiment was completed (Fig. S1, Supporting Information). Transport medium consisted of Hank's balanced salt solution supplemented with 10 mM *N*-2(2-hydroxyethyl) piperazine-*N*′-poly (amidoamine) (2-ethanesulfonic acid) hemisodium salt buffer (pH 7.4). Transport experiments were carried out after 60 min of incubation with transport buffer prior to treatment with samples. At t = 0 min, the transport buffer was carefully pipetted out without disturbing the monolayers, and the sample was added to the apical side. Ro 25-6981 was added at 0.05 and 0.5 mM concentrations to the buffer. Monolayers were then incubated at 37°C on an orbital shaker at 350 RPM (G76, New Brunswick, NJ). At 0 min and after 90 min, 200 μL aliquots were sampled from the basolateral side and analyzed using HPLC. Fluorescein was used as the cell monolayer integrity marker. Fluorescein permeability assessment was performed immediately after the permeability assay for the test compound. The cell monolayer with a fluorescein permeability of less than 1.5×10^{-6} cm/s was considered intact. The apparent permeability coefficients (P_{app}) of samples were calculated using the following equation: