INTRODUCTION

Ketamine is a well-known agent used in anesthesiology since 1960s. The analgesic properties of ketamine were discovered in 1990s. Surprisingly, quite recently this agent was also found to display unique antidepressive properties. Researchers from Celon Pharma S.A. have conducted comparative analyses of antidepressive effects exerted by R- and S-enantiomers of ketamine and its metabolites in dry powder inhalation (DPI) as a delivery method.

AIM

The aim of this study was to develop and validate LC-MS/MS assays for stereoselective quantification of R,S-ketamine and its metabolites, R,S-norketamine, (2S,6S)-hydroxynorketamine, (2R,6R)-hydroxynorketamine and R,S-dehydroxynorketamine in rat plasma and brain samples enabling the characterization of the ketamine pharmacokinetics after administration of a low intravenous (i.v.) or intratracheal (i.t.) dose.

KETAMINE METABOLISM

Six metabolites of R- and S-ketamine were selected and synthesized to observe antipressant effect of ketamine racemate after dry powder inhalation. Mass spectrometer parameters were optimized for (R)-norketamine (R-NOR), (S)-norketamine (S-NOR), (2S,6S)-hydroxynorketamine (S-HNK), (2R,6R)-hydroxynorketamine (R-HNK), (R)-dehydroxynorketamine (R-DHNK) and (S)-dehydroxynorketamine (S-DHNK).

MATERIALS AND METHODS

Samples (rat plasma and brain) were purified by protein precipitation and centrifugation. Analyses separation was achieved on a chiral Lux® 3 µm Amlose-2 column with a mobile phase consisting of 10mM ammonium acetate, acetonitrile and 2-propanol. The flow rate was set at 0.5 mL/min. Mass spectrometric detection was performed with use of Agilent 6460 mass spectrometer in positive ionization mode. Ketamine-d4 was used as an internal standard (IS).

RESULTS

Due to very low volume (50µL) of samples collected during preclinical studies only protein precipitation could be used as a sample preparation technique. As a result lower limit of quantitation (LLOQ) for all of compounds was determined to be 12.5 ng/mL. Analytes were separated in one 45 minutes run. Quantitation was performed using at least two MRM transitions per analyte and IS. The quadratic calibration curves for all analytes were obtained over the concentration range 12.5–2500 ng/mL. The intra- and inter-day precisions were <7.9% and the accuracy (relative error) was within 15.3%.

CONCLUSION

Method was successfully used for analysis of plasma and brain samples from pharmacokinetic studies. The assay will help to characterize the stereoselective metabolic pattern of ketamine after low dose DPI administration. Dry powder inhalation administration provided successful systemic and brain tissue exposure with the 85% bioavailability for racemate dosing.

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