Introduction

Assessment of a drug effect on the Central Nervous System (CNS) during the preclinical development of a new chemical entity is part of the ICH S7A guideline on Safety Pharmacology studies for human pharmaceuticals. This is commonly performed in dedicated safety pharmacology studies involving mainly rodents. However, for biotechnology derived products, integrated safety pharmacology assessment in regulatory toxicology studies is required (ICH S6, S9 and M3(R2)). These integrated safety pharmacology studies are generally focused on cardiovascular and respiratory functions, but also on the central nervous system in appropriate animal models. The non-human primate is one of the large animal models commonly used for the development of biologics.

Therefore, the aim of this study was first to validate a functional observational battery (FOB) as a neurobehavioural screening method that could be easily integrated in a 4-week toxicity study or greater. The second objective of the study was to define and validate a tool to help in the interpretation of the results obtained from these tests.

The validation of this model was performed using two reference compounds: D-amphetamine hydrochloride, a sympathomimetic molecule known to increase the CNS activity, and ketamine hydrochloride, an NMDA receptor antagonist known to induce a state referred to as “dissociative anaesthesia”.

Materials and Methods

Test system and Study Design

On the treatment days, the neurobehavioral examinations were performed before administration, and then 1, 2, 4 and 6 hours after treatment. The procedure was adapted from a method described by Gauvin and Baird (2008)(1). These examinations consisted first of a 2-minute observation period of the animals in their home cage. Then a series of tests were performed while restrained in the technician’s hands, such as the evaluation of the reactivity to manipulation recording and stimuli and reflexes. The whole procedure lasted 6 to 7 minutes per animal and per time point, thus allowing possible integration of several time points during the time course of a toxicity study including the pre-treatment period.

Each of the observations was scored using a standardized scoring grid, based on the absence or presence of a sign, or rated on the severity of the effect observed. Then the observations were combined in 3 sub-groups, each of them representing one of the broad domains of the Central Nervous System: neurological, autonomic and behavioural. A mean severity score was then calculated from each of these sub-groups, thus allowing an evaluation of the impact of the compound on each specific function.

Results and discussion

Before treatment, no differences between groups were observed for any of the evaluated parameters. As expected, dose-related neurobehavioral effects were obtained with D-amphetamine and ketamine hydrochloride. D-amphetamine induced mainly hyperactivity, stereotypic behaviour and hyperthermia, with a maximum effect reported 2 hours after treatment (figures 1, 2 and 5). Ketamine induced hypotonia, hypoactivity, ataxia, decreased reflexes and hypothermia, with a maximum effect obtained 1 hour after dosing (figures 3, 4 and 6).

Conclusion and Discussion

This study demonstrated that an adapted functional observational battery of tests in the conscious Cynomolgus monkey may be used as a valuable tool to predict neurobehavioural changes during general toxicology studies. The FOB could easily be performed in all main groups of a regulatory toxicology study to determine dose- and time-dependent effects of test items. Additional tests, such as cardiovascular and respiratory investigations, may also be associated with the FOB in non human primates to cover a full integration of the core battery of safety pharmacology endpoints during toxicological studies for biologics.

References


Use of a functional observational battery in the assessment of safety pharmacology endpoints in general toxicology studies in the conscious Cynomolgus monkey

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