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Original article

Abuse liability assessment in preclinical drug development: Predictivity of a translational approach for abuse liability testing using methylphenidate in four standardized preclinical study models

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ABSTRACT

Objectives: Preclinical abuse liability assessment of novel clinical CNS-active candidates involves several tests, addressing different aspects characteristic for abuse potential, which are considered predictive for substance abuse of these candidates, thus ensuring an appropriate translational approach. To demonstrate how such a strategy could work, a known drug of abuse, methylphenidate was evaluated in a full rodent test battery, comprising four test models, and in accordance with the requirements of the FDA, ICH and EMA guidelines.
Methods: Methylphenidate was tested orally at 2.5, 5 or 10 mg/kg for its physical dependence potential in a repeated dose non-precipitated withdrawal test, for its drug profiling in a drug discrimination learning procedure (single escalating doses), and for its reinforcing properties in a conditioned place preference test (alternate dosing days) and an intravenous self-administration procedure (0.05 to 1 mg/kg/IV infusion during 5 daily 1-h test sessions). The stimulant D-amphetamine served as positive control and was administered subcutaneously at 0.8 mg/kg in the first three test models. In the intravenous self-administration procedure rats were habituated to intravenously self-administer D-amphetamine at 0.06 mg/kg/IV infusion prior to methylphenidate substitution.
Results: Cessation of subchronic dosing up to 10 mg/kg methylphenidate led to sustained or even exacerbated effects on locomotion and behavior, body temperature, body weight, food consumption, and alteration of the diurnal rhythm during withdrawal. Clear generalization to D-amphetamine was obtained in the drug discrimination test at 5 and 10 mg/kg. Distinct reinforcing properties were present in the conditioned place preference test at 10 mg/kg and in the intravenous self-administration study from 0.05 mg/kg/IV infusion onwards. The maximum plasma exposure after oral administration of methylphenidate over the dose ranges tested in the present rat studies covered at least 1.9-fold to 18.9-fold the recommended human therapeutic exposure of 10 ng/ml, a plasma level that is considered representative of the human efficacious methylphenidate dose. The ratio C_{max} Hu/rat calculated from the intravenous self-administration data ranged from 14.9 to 576.5. Consequently the regulatory requirements, stating that preclinical drug abuse liability studies should include high doses that produce plasma levels that are multiples of the therapeutic dose were fulfilled (FDA, EMA, ICH).
Discussion: The presented preclinical models, implemented within a drug development environment, were considered highly predictive to assess the abuse potential of methylphenidate, and in accordance with the regulatory requirements of drug licensing authorities in terms of appropriate methods, dose selection and subsequent plasma exposure.

1. Introduction

The investigation of abuse potential of new CNS-active molecular entities has become a critical topic in drug development since the release of the EMA guideline (EMA, 2006), the ICH guideline (ICH, 2009), and recently the FDA draft guidance (FDA, 2010) and the subsequent FDA's decision tree (Bonson & Sun, 2011). In particular within the preclinical safety evaluation area the need of drug abuse liability testing has become a major issue as all compounds in development exerting an activity in the brain (regardless of therapeutic area) are subject to these regulations.

In our lab four preclinical study models, investigating various aspects of abuse potential, i.e. physical dependence potential, discriminative properties and measures of direct and indirect reinforcing properties, were adapted from the known models in literature (Ator & Griffiths, 2003; Bardo, 2000; CPDD, 2006; Cunningham, Gremel, & Groblewski,

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2006; Koob, 2000; Shippenberg & Koob, 2002), standardized, made compliant with the GLP regulations and implemented according to the regulatory requirements for testing CNS-active compounds in drug development. To date these adapted models are the basis of our preclinical abuse liability assessments and are evaluated in this article for their predictive validity using methylphenidate, a known drug of abuse. Methylphenidate (MPH), used to treat attention deficit–hyperactivity disorder (ADHD), has been scheduled for its abuse potential as CII in the US (FDA–DEA, 2012) and worldwide (Convention on Psychotropic Substances, 1971). It is structurally related to amphetamine and increases the extracellular dopamine in the striatum and the nucleus accumbens by inhibiting the dopamine transporter (Challman & Lipsky, 2000). Subsequently D-amphetamine-sulfate (AMP) was selected as a positive control in the present preclinical studies, based upon the comparable pharmacological action of both compounds. The D-form of amphetamine is a central nervous system stimulant and sympathomimetic with multiple mechanisms of action including inhibition of adrenergic and dopaminergic uptake, release of monamines, and inhibition of monoamine oxidase (Shire Canada Inc., 2011). AMP has been prescribed in the treatment of narcolepsy, attention deficit disorders and hyperactivity in children. It has been scheduled for its abuse potential as CII in the US (FDA–DEA, 2012) and internationally (Convention on Psychotropic Substances, 1971).

The preclinical abuse liability assessment as described here for MPH is a critical component in the development of CNS-active drugs supporting the safety evaluation with regard to abuse potential and possible scheduling. The translational approach of this example with MPH will be discussed based upon the outcome of the four presented standardized preclinical tests, conducted in male Sprague-Dawley rats, and in view of the known neuropharmacology and clinical findings of MPH. The question whether abuse liability studies performed in ADHD diseased rats versus common Sprague-Dawley rats could add to a higher predictivity of the abuse potential of MPH will be discussed briefly. Finally the proper selection of an efficacious dose range, needed to comply with the regulatory requirements in terms of preclinically testing a several-fold of the human efficacious dose (C_{eff} Hu) and the biological relevance of the various test results within this dose range will be considered.

2. Material and methods

2.1. Animals and husbandry

All tests were performed in an AAALAC-accredited (Association for Assessment and Accreditation of Laboratory Animal Care) testing facility. An ethical protocol was written and approved by the Ethics Committee for each of the four studies.

Housing and experiments were conducted in accordance with the European (European Convention, 1986, 2007) and Belgian guidelines (Belgian Law, 1991), and with the principles of euthanasia as stated in the report of the American Veterinary Medical Association Panel (AVMA, 2001).

Young (6 to 9 weeks old upon arrival) specific pathogen free (SPF) male Sprague-Dawley (Crl: CD®) rats were used in the four studies. All animals had an acclimatization period of at least 5 days before being used in any experimental procedure.

Rats were housed separately in transparent polysulfone cages (floor area: 940 cm^2) with a wire-mesh lid suspended in wheeled racks. Bedding material (corn cob size 12, Eurocob, France) and cage enrichment (wooden blocks, Sizzle nest and/or transparent polycarbonate tunnels) were provided. There was a 12/12 light/dark cycle and illumination did not exceed 700 lx. The test rooms were air-conditioned with their own supply of filtered fresh air. The standardized test conditions for temperature (20–23 °C), relative humidity (40–70%) and illumination (700 lx) were regularly controlled and recorded.

Rats were given free access to water. Food [R/M-H pelleted maintenance diet, Ssniff (Soest, Germany)] was provided ad libitum in the withdrawal (WD) and the conditioned place preference (CPP) tests but was restricted to 20 g per day in the self-administration (IV SA) test to maintain a stable body weight. In the drug discrimination learning (DDL) test daily food supply was presented according to the following schedule: training days (Mondays to Thursdays): 9 g available in cage after training; test days (Fridays): 18 g available in cage after test sessions; weekends (no training or test sessions): 16 g on Saturdays; and 13 g on Sundays. In this latter test food rewarding during training and during the test sessions was offered via sugar pellets (45 mg dustless precision pellets, Bioserv, US) (1 pellet per 10 lever presses).

Rats were checked at least once daily for general health, abnormal behavior or unusual appearance, untoward clinical signs, toxic or pharmacological response, and moribund state or mortality.

2.2. Equipment

A Plexx thermometer (temperature reader (DAS 7007S) with implantable and programmed temperature transponders (IPTT-300)) was used in the WD test.

For the DDL test operant conditioning chambers (Skinner boxes: modular test chamber ENV-007, Med Associates Inc.; floor area: 750 cm^2) with 2 response levers and equipped with a pellet dispenser (ENV-203-45, Med Associates Inc.) were used. The test chambers were placed in a steel soundproof box with light and ventilation.

The conditioned place preference apparatus (Med Associates Inc.) consisted of a test box divided into two separate compartments [21 × 21 × 68 cm (width × height × length)] interconnected by a short gray tunnel section (so-called neutral compartment). The compartments had different colored sidewalls (black and white) with differently textured floors (smooth metal horizontal rods or wire mesh) in order to provide both tactile and visual environmental cues. The test box was housed in a soundproof box to prevent any audible cues disrupting the conditioning.

For the IV SA test operant conditioning chambers (Skinner boxes: modular test chamber ENV-007, Med Associates Inc.) with 2 respond levers (of which the left one was made inactive) were used. The chambers were put in a wooden box with light and ventilation. An automated syringe pump system (model PHM-100-3.3, MED-PC, USA) was configured to intravenously deliver the appropriate drug amounts via short infusions, with each infusion lasting between two and five seconds.

2.3. Drugs and dose rationale

2.3.1. Methylphenidate-HCl (MPH)

Methylphenidate is a piperidine derivative (methyl 2-phenyl-2-(piperidin-2-yl) acetate). The drug substance consists of a racemic mixture of two stereoisomers, (±)-threo-enantiomers with stereodescriptors R,R and S,S, respectively. The correct stereochemistry was established by utilizing the (±)-threo-ritalinic acid as the starting material (oral communication Noramco, Athens, GA, US). The oral LD_{50} of MPH in the rat is 350 mg/kg (Sigma-Aldrich, 2012) whereas the IV LD_{50} of MPH is 48 to 50 mg/kg (Matthey & Smith, 2006; Separham, Eghbal, Tamizi, & Jouyban, 2011).

MPH is commercially available as a hydrochloride salt (Concerta®, Ritalin®) for the treatment of ADHD. An overview of the maximum oral human dose of MPH and the subsequent maximum exposures (C_{max}) at peak time (T_{max}) is given in Table 1 (Concerta® (methylphenidate HCl), 2007; Ritalin, 2001), as well as intravenous dosages which are however rarely used in the clinic (Janowsky et al., 1978; Kerenyi, Koranyi, & Sarwer-Foner, 1959).

In the present rat studies, oral dosages of 2.5, 5 and 10 mg/kg MPH were selected (WD, DDL, CPP) based upon literature data (Botly, Burton, Rizos, & Fletcher, 2008; Kollins, MacDonald, & Rush, 2001; Wooters, Walton, & Bardo, 2011). Methylphenidate hydrochloride