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RO4938581, a $GABA_A\alpha5$ modulator, displays strong CYP1A2 autoinduction properties in rats

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ABSTRACT

Autoinduction in drug metabolism is a known phenomenon observed when a drug induces the enzymes responsible for its own metabolism. The potency, rate and extent of autoinduction following a given treatment paradigm may have therapeutic implications in clinic as well as for in vivo pharmacological assessments in animals. RO4938581, an imidazo-triazolo-benzodiazepine, is a novel $GABA_A\alpha5$ negative modulator recently pursued for the treatment of cognitive dysfunctions. As circulating plasma levels of RO4938581 were shown to decrease rapidly after repeated dosing in rats, with CYP1A2 being involved in the metabolism of the compound, we examined the potential role of RO4938581-mediated autoinduction of CYP1A2. Incubation of rat hepatocytes with RO4938581 revealed potent CYP1A2 induction with significant increase in enzymatic activity at concentrations of 0.1 nM and RO4938581 was shown to be 700-fold more potent than β-napththoflavone. Ex vivo studies revealed a 7-fold increase in metabolic CYP1A2 activity in liver microsomes prepared from rats administered with 0.1 mg/kg of RO4938581 24 h before. This induction profile was reflected in vivo in pharmacokinetic studies in rats where an 8-fold reduction in plasma exposure was observed after a second dose. The reduction in plasma exposures due to CYP1A2 autoinduction were confirmed functionally in contextual fear conditioning paradigm in rats, where a positive pharmacological effect observed after acute drug administration disappeared completely after sub-chronic dosing. Together, these findings suggest that RO4938581 possesses potent CYP1A2 autoinductive properties in rats and may serve as a tool for mechanistic metabolism or drug–drug interaction studies encircling this enzyme in rats.

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1. Introduction

The cytochrome P450 (CYP) enzymes play a central role in the biotransformation of drugs in the liver, and in many instances CYP mediated biochemical processes are responsible for the major clearance pathway of most drugs currently on the market [1]. Regulation and expression of the different CYP enzymes may be affected following substrate recognition and interactions with xenobiotics. In vivo, the CYP enzyme levels and activities vary depending on whether the enzymes are at basal or induced levels. CYP induction caused by drug molecules is a well-known phenomenon which may influence the pharmacokinetics of compounds metabolised by the induced enzyme [2]. If a compound causes induction of the CYP enzymes responsible for its own metabolism, it may increase its own metabolic clearance. This will lower the levels of circulating drug and potentially reduce pharmacological efficacy. This phenomenon is referred to as metabolic autoinduction and has been observed in both animals and humans with drugs such as barbiturates, rifampicin, and phenytoin [3,4]. The time course and magnitude of the observed autoinduction is thought to depend on the chain of events required for protein synthesis, including specific transcriptional activation, turnover rate of the induced enzyme, and the concentration of the inducing compound at the site of induction [5,6]. Thus, the extent of autoinduction and its temporal profile in vivo is both drug- and CYP-dependent.

In animals, most induction studies have adopted multiple-dose exposure paradigm to inducers over several days on the assumption that a lag phase for the transcriptional and translational process is necessary to yield an induction at the functional level. However it has been demonstrated that autoinduction may occur rapidly in rats, at least within the CYP1A family as shown for a wide variety of compounds. Thus, for the investigational anti-angiogenic agent TSU-68, CYP1A1 and CYP1A2 activities have been reported to be increased 4-fold within 24 h after a single high dose in rats and, as a result of autoinduction, a concomitant 4-fold decrease in

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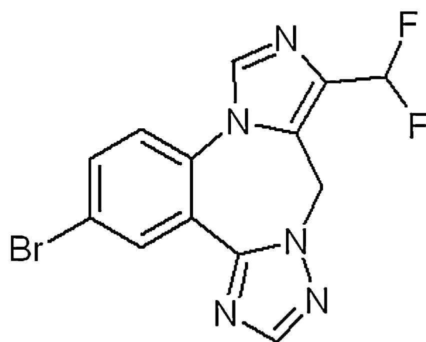

Fig. 1. Chemical structure of RO4938581 ((3-Bromo-10-difluoromethyl-9H-imidazo[1,5-a][1,2,4]triazolo[1,5-d] [1,4]benzodiazepine).

circulating plasma levels after a second dose within this time frame [7,8]. Also, for compounds like methoxy-nitroanilines and the polychlorinated toxin dioxin (TCDD), more than 5-fold enhancement in CYP1A2 activity and protein expression have been reported to occur within 24 h after exposure to a single dose in rats [9,10]. During in vivo pharmacological animal studies applying short-term repeated dosing designs with compounds metabolised by CYP1A enzymes, autoinduction may thus potentially influence the pharmacokinetics and hence eradicate the pharmacological response.

RO4938581 (3-Bromo-10-difluoromethyl-9H-imidazo[1,5-a][1,2,4]triazolo[1,5-d] [1,4]benzodiazepine) (Fig. 1) is a novel $GABA_A\alpha5$ receptor inverse agonist or negative allosteric modulator [11]. This mode of action is hypothesised to provide a pro-cognitive potential [12,13,14] As shown in the present study, CYP1A2 plays a major role in the hepatic metabolism of this compound in rats. In addition, as opposed to acute dosing in rats, sub-chronic treatment proved to be pharmacologically ineffective as a result of diminished systemic exposure. We therefore set out to investigate the potential role of CYP1A2 autoinduction for RO4938581 in rats, and its functional consequence on pharmacokinetics and drug evoked pharmacological read-out using a contextual fear conditioning paradigm after acute and repeated drug administration.

2. Materials and methods

2.1. Materials

RO4938581 (3-Bromo-10-difluoromethyl-9H-imidazo[1,5-a][1,2,4]triazolo[1,5-d] [1,4]benzodiazepine) was synthesised at H. Lundbeck A/S. Ethoxyresorufin, β-naphthoflavone and furafylline were obtained from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals were of reagent grade or of the highest purity available commercially. Rat liver microsomes were purchased from XenoTech (Lenexa, KS, USA). Cryopreserved rat and human hepatocytes were obtained from Biopredic International (Rennes, France).

2.2. Animals

Male Lister hooded rats (220–250 g, 7–8 weeks old) obtained from Charles River (Germany) were used throughout all ex vivo and in vivo experiments. Animals were housed pairwise under controlled conditions (12 h of light starting at 0600 h, 20 ± 2 °C, 30–70% humidity). The animals had access to food and water ad libitum. All animal experiments were performed in accordance with the European Communities Council Directive #86/609 and the directives of the Danish National Committee on Animal Research Ethics.

2.3. In vitro CYP1A2 induction

Cryopreserved rat or human hepatocytes were cultured in a sandwich culture system for 48 h prior to a 48 h treatment with 9 concentrations of RO4938581 (0.001–300 nM for rat hepatocytes; 1 nM to 10 μM for human hepatocytes). β-naphthoflavone was used as comparator inducer for CYP1A2. At the end of the treatment, cell medium was removed and the culture washed with fresh culture medium to remove the test items. The CYP1A2 probe substrate ethoxyresorufin was added to the cells and incubated for 30 min. The supernatants were then sampled for LC–MS analysis of the metabolite resorufin. The induction was calculated as fold increase in enzyme activity relative to vehicle (0.1% DMSO).

2.4. Ex vivo CYP1A2 induction

Groups of 3 rats were orally treated with either RO4938581 (0.1 mg/kg) or vehicle (20% HP-β-cyclodextrin, pH 4) for one or two days (once-daily). Livers from dosed rats and a control group were excised 24 h after the last treatment and used to prepare microsomes. Intrinsic clearances of CYP1A2 probe ethoxyresorufin were then determined in the microsomes at a final concentration of 5 μM according to the procedures described in Section 2.5.

2.5. Microsomal stability determination

The rat liver microsomal intrinsic clearance of RO4938581 was determined by assessing the elimination of test compound over the incubation time using a previously described standard protocol [15]. Briefly, the test compound was incubated at a final concentration of 1 μM with rat liver microsomes for 60 min, using NADPH as cofactor. Aliquots (100 μl) were withdrawn at different time-points and reactions were stopped by adding 100 μl of acetonitrile. Following centrifugation, the supernatants were analysed by LC–MS/MS.

To assess the metabolic role of CYP1A2 in the biotransformation of RO4938581, an additional stability study was performed where rat microsomes were co-incubated with the known CYP1A2 selective inhibitor furafylline at a concentration of 10 μM and otherwise treated as described above.

2.6. In vivo pharmacokinetics

The pharmacokinetics of RO4938581 was assessed in rats following once-daily dosing regiments for three consecutive days of dosing. Each group of animals ($n = 6$) received either intravenous (iv) (0.1 mg/kg) or oral (po) doses (0.1 or 0.02 mg/kg) each day (24 h apart) and serial blood samples (100 μl) were taken from the tail vein in each animal from 0 to 6 h after each dose on all three days. Drug solutions were prepared in 20% HP-β-cyclodextrin (pH 4) for both iv and po administrations and dosed in volumes of 1 and 2 ml/kg, respectively. The same drug solution was used for each group over the three consecutive days of dosing. The solutions were stable at room temperature for at least 10 days.

2.7. Plasma bioanalysis

RO4938581 was analysed in rat plasma by means of Ultra-Performance LC® (UPLC®) chromatography followed by tandem-MS (MS/MS) detection. 150 μl of internal standard in acetonitrile (5 ng/ml) with 0.1% ammoniumhydroxide was added to 25 μl of calibration standards, QC samples and test samples using a Biomek robot (Beckman Coulter Inc., Brea, CA, USA). After centrifugation ($6200 \times g$, 4 °C, 20 min) 100 μl supernatant from each sample was transferred to a new plate and mixed with 100 μl water with 0.1% ammoniumhydroxide. After a quick centrifugation ($6200 \times g$, 4 °C,

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