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Non-clinical safety evaluation and risk assessment to human of aleglitazar, a dual PPAR α/γ agonist, and its major human metabolite

Martin Bopst*, Elke-Astrid Atzpodien

Roche Pharma Research and Early Development, Pharmaceutical Sciences, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd, Grenzacherstrasse 124, 4070 Basel, Switzerland

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ABSTRACT

The non-clinical safety profile of aleglitazar, a peroxisome proliferator activated receptor alpha/gamma agonist, and its major human metabolite M6 was studied in a complete package consisting of drug metabolism and pharmacokinetics characterization, safety pharmacology, genotoxicity, repeat dose toxicity, reproductive toxicity and carcinogenicity studies. These studies identified the following main targets similar to other PPAR agonists: red blood cell parameters, liver, heart, kidney, ovaries, testes, bone marrow, adipose tissue, and fluid accumulation. Additionally, and in the 12-month monkey study only, an increased incidence of generalized hair loss/thinning was observed in all groups including controls. In the rat carcinogenicity study there was no statistically significant increase in tumors. In the mouse carcinogenicity study, there was an increased incidence of angiomatous tumors and there were three males with gallbladder adenoma. No relevant compound-related effects were observed in safety pharmacology, genotoxicity, and a 28-day immunotoxicity rat study. Effects observed in reproductive toxicity studies were similar to those known for other PPARγ agonists. Separate studies with the human metabolite M6 did not reveal findings that would prevent human dosing. Overall, the results from the non-clinical safety studies conducted with aleglitazar and the human metabolite M6 were considered to support the clinical Phase 3 program.

1. Introduction

Here we report the non-clinical safety data that were generated to support the development of aleglitazar, a PPARα/γ dual agonist, with a focus on toxicology aspects and the resulting human risk assessment preceding Phase 3 clinical trials.

Aleglitazar was one of the last, if not the last, PPARα/γ dual agonists in clinical drug development. It is a highly potent and balanced dual PPARα/γ agonist and its structure and molecular profiling were previously published (Dietz et al., 2012). Aleglitazar belongs to the glitazar family and combines the PPARα effects targeting metabolic dyslipidemia with the PPARγ effects targeting peripheral insulin sensitivity and therefore glycemic control, and inflammation. In clinical development, an initial 16-week Phase 2 study in patients with type 2 diabetes mellitus (T2DM) (SYNCHRONY) showed that a 0.15 mg daily dose of aleglitazar significantly improved glycemic and lipid control, while the edema rates were similar to placebo (Henry et al., 2009). The 52-week Phase 2b AleNephro study confirmed the glycemic and lipid data, which were similar to 45 mg pioglitazone with a lower rate of edema (Ruilope et al., 2014). A Phase 3 trial based on the SYNCHRONY study (AleCardio) was started to study the impact of aleglitazar on cardiovascular events in patients with T2DM and a recent acute coronary syndrome (ACS) event. The development of the compound was stopped during this Phase 3 clinical trials due to lack of efficacy in cardiovascular outcomes and presence of PPAR-related class side effects (Lincoff et al., 2014).

In order to support clinical trials an exceptionally large non-clinical safety program, consisting of approximately 100 studies, about 50 in the DMPK area and around 50 toxicity and safety pharmacology studies, was conducted over a period of around 10 years. These included carcinogenicity studies, because of a PPAR-specific requirement from health authorities to complete these studies already before starting large trials with a treatment duration of more than 6 months, i.e. Phase 3 (El-Hage, 2005b; EMA, 2006). An additional factor why the program was larger than is

* Corresponding author.
E-mail addresses: martin.bopst@roche.com (M. Bopst), elke-astrid.atzpodien@roche.com (E.-A. Atzpodien).

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typical for small molecule drug development was that specific non-clinical safety studies with one of the major human metabolites (M6) had to be conducted due to its low exposure levels in animals as compared to humans (Sturm et al., 2012).

2. Materials and methods

2.1. Test item and PPAR activity

Aleglitazar is a potent and balanced dual PPARα/γ agonist and its structure and molecular profiling were previously published (Dietz et al., 2012).

PPAR-dependent reporter gene expression assays showed aleglitazar to be a balanced full agonist for PPARα and PPARγ receptors with a low affinity for PPARδ. As previously reported, aleglitazar activated PPARα and PPARγ-dependent reporter gene transcription requiring 5 and 9 nM concentrations to achieve 50% activation of transcription, whereas 376 nM were required for PPARδ (Dietz et al., 2012; Wright et al., 2014).

In competitive binding assays, the aleglitazar concentrations required for 50% displacement of bound radioligand (IC50) were 35 nM for PPARα and 66 nM for PPARγ, indicating that aleglitazar is a high affinity ligand for these receptors (Sturm et al., 2012). Metabolites M6 and M1 did not appear to activate PPAR receptors (Sturm et al., 2012). Further preclinical pharmacology data were already reported (Bénardeau et al., 2011; Benardeau et al., 2009; Deehan et al., 2012; Dietz et al., 2012; Dzyakanchuk et al., 2010; Grether et al., 2009; Hansen et al., 2009a, 2009b; Werner et al., 2013) demonstrating efficacy including an about 20- to 40-fold lower potency for rodent PPAR alpha activity as compared to the above mentioned human data.

2.2. Study designs and animals

The toxicology and safety pharmacology program of aleglitazar was conducted according to ICH guidelines, with all main safety studies conducted in compliance with Good Laboratory Practice (GLP) regulations. A standard genotoxicity package was done according to ICHS2 and OECD technical guidance documents and consisted of in vitro Ames, chromosome aberration and in vivo micronucleus test in Wistar rat bone marrow. The Wistar rat and cynomolgus monkey (*Macaca fascicularis*) were chosen for general toxicity studies. The cynomolgus monkey was selected as the non-rodent species because of its similarity to human in metabolic patterns. Sprague Dawley, Wistar rats and New Zealand White rabbits were used to assess reproductive effects with the choice for the rat strains being dependend on the availability of historical control data at the relevant laboratory. Wistar rats and CD-1 mice were used for the carcinogenicity studies and Syrian hamster for a dose range finding study. The hamster was initially considered because it showed some exposure of the major human metabolite M6 in contrast to the mouse. Since M6 was spiked in the rat carcinogenicity study, the similarity of the observed toxicity profile in hamster to the other rodent species, and because tumors were observed with other PPAR agonists in rats and mice, rat and mouse were finally chosen following feedback from the US FDA. The care and use of animals was conducted in accordance with the guidelines of the respective national regulations. All in vivo studies were conducted using oral gavage administration. A wide range of dose levels was used due to the progressive nature of some of the observed findings over time, as observed for other compounds containing a PPARγ agonist activity. Consequently, with longer treatment duration findings were seen at lower dose levels than in previous shorter treatment duration studies. Repeat dose toxicity studies and the relevant dose levels are tabulated in Table 1.

Table 1
Overview of repeat-dose toxicity and carcinogenicity studies with aleglitazar.

Species	Treatment duration	Aleglitazar Dose (mg/kg/day)
Rat	18 days	0, 2, 10, 30
	2 weeks	0, 0.2/0.1[a], 0.4/0.3[a], 1, 2
	13 weeks	0, 0.01, 0.1, 1
	26 weeks	Males: 0, 0.03, 0.1, 0.6, 3 Females: 0, 0.03, 0.1, 0.3, 1
	2 years	Males:0, 0, 0.01, 0.06, 0.2, 0.6 [b] Females:0, 0, 0.01, 0.03, 0.1, 0.3 [b]
Mouse	4 weeks	0, 0.1, 1, 4
	13 weeks	0, 0.1, 0.8, 4
	2 years	0, 0, 0.1, 0.3, 1, 3
Hamster	4 weeks	0, 0.1. 1, 4
Monkey	2 weeks (maximum tolerated dose)	200
	2 weeks	0, 0.1, 1, 10, 100
	13 weeks	0, 0.03, 0.1, 0.8/0.4[a], 3
	26 weeks	0, 0.03, 0.1, 0.4/0.2[a]
	52 weeks[c]	0, 0.01, 0.03, 0.1

[a] Dose reduced during study.
[b] 0.3, 1, 3, 10 mg/kg/day M6 were spiked in the compound-treated groups, respectively.
[c] 52 weeks duration instead of the typical 39 weeks duration was requested by regulatory authorities in general for PPAR agonists.

In the 26-week study in monkeys, treatment with the highest dose of 0.4 mg/kg/day was stopped after 9 weeks due to marked subcutaneous edema. After an 8- week treatment-free period, treatment was restarted at a lower dose of 0.2 mg/kg/day for 16 weeks. Dosing of the high dose animals given 3 mg/kg/day was discontinued in Week 4 in the 13-week monkey study due to marked edematous effects. For the next lower dose level, dosing was reduced from 0.8 to 0.4 mg/kg/day after 30 days due to adverse findings in one female. Two-year carcinogenicity studies in rats and mice were conducted using four dose levels (up to 0.6 mg/kg/day in rats and 3 mg/kg/day in mice). The high dose levels were selected according to US FDA criteria for PPAR gamma agonists, i.e. the dose where approximately a 25% increase of heart weight was observed in the 13-week studies (El-Hage, 2005b). In the rat study, the major human metabolite M6 was given up to 10 mg/kg/day together with the parent compound in order to provide sufficient human coverage (approximately 10-fold over the human M6 exposure) in one of the two tested species used for carcinogenicity studies.

The usual set of parameters as required and specified in ICH, OECD or other national guidelines for regulatory toxicology and safety pharmacology studies was evaluated and since the package of studies summarized here is very large these guidelines are not specifically listed. In addition, echocardiography investigations (two-dimensional and M-mode echocardiography) were performed in the 13-week, 6-month, and 12-month monkey studies and evaluated by a trained cardiologist. Specific urinary renal proximal tubule markers were included in the 13-, 26- and 52-week monkey studies and consisted of leucine aminopeptidase, N-acetyl-beta-glucosaminidase (NAG), microalbumine, and microglobulin alpha-1. Systemic exposure levels are provided as Area-Under-the-Curve (AUC) values and are AUC_{0-24h} values.

3. Results

3.1. Exposure multiples in relation to human exposure expected in phase 3

In order to adequately interpret the toxicological findings it is important to put the exposures, at which relevant findings were observed in animals, in context to the relevant human exposure. The non-clinical safety package was completed before start of