Regulatory Toxicology and Pharmacology 73 (2015) 401–412

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Carcinogenicity testing of eliglustat in mice and rats

Rafif Dagher [a, 1], Malene Watzinger [b, 2], Guillaume Chevalier [b], Catherine Thirion-Delalande [b], Frederic Gervais [b], Roy Forster [b, *]

[a] Genzyme, Waltham, MA 02451, USA
[b] CiToxLAB France, 27005 Evreux, France

ARTICLE INFO

Article history:
Received 21 May 2015
Received in revised form
27 July 2015
Accepted 28 July 2015
Available online 30 July 2015

Keywords:
Eliglustat
Cerdelga
Carcinogenicity
Glucosylceramide synthase inhibitor
Gaucher disease
Lysosomal storage disease

ABSTRACT

Eliglustat is a novel glucosylceramide synthase inhibitor for long-term oral treatment of type 1 Gaucher disease (GD1), an inherited metabolic disorder. The carcinogenic potential of this drug has been evaluated in lifetime carcinogenicity bioassays in mice and rats. Administration of eliglustat to Swiss CD-1 mice at 0, 10, 25 or 75 mg/kg/day for 104 weeks by dietary admixture did not influence survival or bodyweight evolution, or produce any clinical indication of poor condition. At histopathology, no increases in tumor incidence for any tumor type were attributed to treatment with eliglustat. Systemic exposure to eliglustat was confirmed by a reduction in circulating levels of glucosylceramide. Administration of eliglustat to Sprague–Dawley rats by oral gavage for 105 weeks at 0, 10, 25 or 75 mg/kg/day (males) or 103 weeks at 0, 5, 15 or 50 mg/kg/day (females) did not affect survival rates, but resulted in reduced bodyweight evolution in male rats (−18% at high dose), indicating that the MTD had been achieved. At histopathology, no increases in tumor incidence were attributed to treatment with eliglustat. Systemic exposure was confirmed by toxicokinetic analyses. In conclusion, eliglustat was not carcinogenic to mice or rats in standard lifetime bioassays.

1. Introduction

Eliglustat (Fig. 1; synonym eliglustat tartrate, previously Genz-112638), commercialized under the name Cerdelga, is an orally available glucosylceramide synthase inhibitor for the treatment of Gaucher disease by substrate reduction therapy (SRT). Gaucher disease is an inherited lysosomal storage disease that results from a deficiency of acid-(β)-glucosidase, for which glucosylceramide (GL-1) is the major substrate. In patients with Gaucher disease type 1 (GD1), the reduced ability to hydrolyze GL-1 results in lysosomal accumulation in liver, spleen, and bone marrow with resulting characteristic pathology. The goal of SRT is to reduce the synthesis of glucosylceramide to better match the impaired rate of catabolism, thereby preventing glucosylceramide accumulation and decreasing the accumulated glucosylceramide.

A partial schematic of the glycosphingolipid synthetic pathway is shown in Fig. 2. GL-1 synthesis is the first rate-limiting step in ganglioside and neutral glycosphingolipid biosynthesis, with the more complex glycosphingolipids synthesized by sequential addition of carbohydrate moieties to the GL-1 base. Specific catabolic enzymes within the lysosome catalyze the breakdown of glycosphingolipids such that a low concentration of each glycosphingolipid normally exists within the lysosome. Lysosomal storage diseases are caused in most cases by defects in the genes that encode these catabolic enzymes, resulting in decreased catabolic activity and substrate accumulation within the lysosome. As GL-1 synthesis is the first, rate-limiting step in the GL-1 based biosynthetic pathway, SRT reduces the synthesis of GL-1 and the more complex gangliosides and neutral glycosphingolipids to restore metabolic balance [Shayman, 2010].

As part of the regulatory preclinical safety package for eliglustat, a wide range of studies have been performed to evaluate its general toxicity, pharmacokinetics, reproductive toxicity and other potentially harmful properties. Since the drug is intended for chronic use, an assessment of carcinogenic potential is also required. We

Abbreviations: FDA CAC, Food & Drug Administration Executive Carcinogenicity Assessment Committee; GD1, type 1 Gaucher disease; GL-1, glucosylceramide; MTD, Maximum Tolerated Dose; SRT, substrate reduction therapy.

* Corresponding author. CiToxLAB France, PO Box 563, 27005 Evreux cedex, France.
E-mail addresses: rdagher@catabasis.com (R. Dagher), malene.watzinger@glpg.com (M. Watzinger), guillaume.chevalier@fr.citoxlab.com (G. Chevalier), catherine.thirion-delalande@fr.citoxlab.com (C. Thirion-Delalande), frederic.gervais@fr.citoxlab.com (F. Gervais), roy.forster@fr.citoxlab.com (R. Forster).
[1] Present address: Catabasis, Cambridge, MA 02139, USA.
[2] Present address: Galapagos, 93230 Romainville, France.

http://dx.doi.org/10.1016/j.yrtph.2015.07.024
0273-2300/

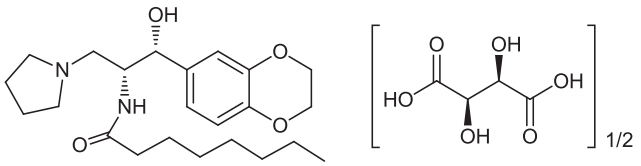

Fig. 1. Molecular structure of Eliglustat tartrate.

present here the results of rodent lifetime carcinogenicity bioassays of eliglustat performed in rats and mice.

2. Materials and methods

2.1. Test material

Eliglustat tartrate (batch number T1136, tartrate salt form with purity >99%) was supplied by Genzyme Corporation together with analytical certification.

2.2. Administration of eliglustat

2.2.1. Dietary administration

Eliglustat has a short plasma half-life in mice (between 18 and 30 min when administered orally) and for this reason the dietary route was selected for the mouse study. Dietary admixtures of eliglustat in SAFE A04C P2.5 diet (SAFE, Augy, France) were prepared on a weekly basis and were stored in closed bags at room temperature and protected from light prior to use. The stability of eliglustat in dietary mixtures under these conditions was confirmed. Initial concentrations of eliglustat for administration in the dietary admixtures were estimated on the basis of previous studies. During the first 3 months of the study, the concentrations of dietary admixtures were adjusted weekly according to body weight and food consumption. Thereafter, the concentrations in the diet were adjusted on a monthly basis.

Achieved dose-levels were calculated for each treated group as follows:

$$D = C \times (FC \div BW)$$

whereby:

D = achieved dosage (mg/kg/day),
C = nominal concentration (ppm: mg of test item per kg of diet),
FC = mean food consumption (g/animal/day),
BW = mean body weight (g)

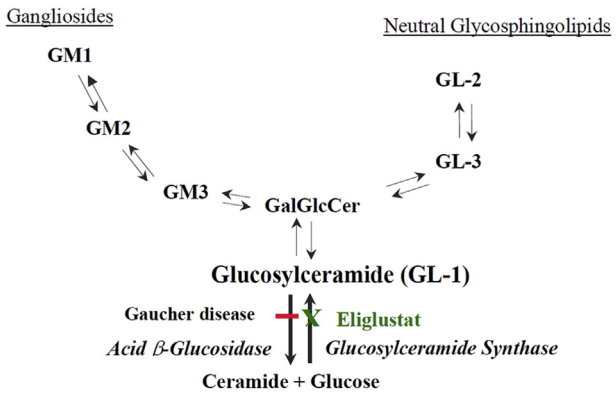

Fig. 2. Glycosphingolipid synthetic pathway.

2.2.2. Administration by oral gavage

Rats were treated with eliglustat by oral gavage. Eliglustat was prepared as an aqueous solution in reverse osmosis-purified drinking water (Elix 5 RO, Millipore SA). The quantity of dose formulation or vehicle administered to each animal was adjusted according to the most recently recorded body weight. A constant dosage-volume of 5 mL/kg/day was used.

The concentration of formulations was regularly confirmed by chemical analysis over the course of the study. Aqueous solutions of eliglustat at 100 mg/mL have been found to be stable for >2 months at room temperature.

2.3. Animals and husbandry

2.3.1. Mice

CD-1® IGS, *Caesarian Obtained*, *Barrier Sustained-Virus Antibody Free* (COBS-VAF®) mice, were obtained from Charles River Laboratories (l'Arbresle, France). Mice were approximately 6 weeks old on the first day of drug treatment (male mean body weight: 32.5 g, range: 25.5 g–38.6 g, and female mean body weight: 24.8 g, range: 20.4 g–29.6 g).

The mice were housed in individual polycarbonate cages (24.0 cm × 13.5 cm × 13.0 cm) containing autoclaved sawdust (SICSA, Alfortville, France). The study room conditions were: temperature: 22 ± 2 °C, relative humidity: 50 ± 20%, light/dark cycle: 12 h/12 h. The cages were moved clockwise around the room every 5–9 weeks in order to avoid bias caused by placement. The animals were provided with environmental enrichment (Nestpack and/or Aspen Cube). All mice had free access to treated or untreated diet and to drinking water (filtered tap water).

2.3.2. Rats

Sprague–Dawley, Crl CD® (SD) IGS BR, *Caesarian Obtained*, *Barrier Sustained-Virus Antibody Free* (COBS-VAF®) rats were obtained from Charles River Laboratories Italia (Lecco, Italy). On the first day of treatment, the animals were 6 weeks old; male mean body weight was 207 g (range: 176 g–238 g) and female mean body weight was 160 g (range: 135 g–187 g).

The rats were housed in a barriered rodent unit (temperature: 22 ± 2 °C, relative humidity: 50 ± 20%, light/dark cycle: 12 h/12 h) in suspended wire-mesh cages (43.0 cm × 21.5 cm × 18.0 cm). Each cage contained two rats of the same sex and group. The cages were placed in numerical order on the racks, and every 5–7 weeks, all the racks were moved clockwise around the room to avoid bias caused by placement in the room.

All rats had free access to R/M-H pelleted maintenance diet (SSNIFF Spezialdiäten GmbH, Soest, Germany), which was distributed weekly. The animals had free access to bottles containing tap water (filtered with a 0.22 μm filter).

2.4. Carcinogenicity studies

2.4.1. Mice

The study design of the mouse study is shown in Table 1a. Swiss CD-1 mice received eliglustat by dietary admixture over the course of the study at target dose-levels of 10, 25 and 75 mg/kg/day. Two control groups of 50 males and 50 females received control diet only. The dose-levels were based on the results of a 13-week dietary preliminary study, and were confirmed in consultation with the FDA CAC. The intended duration of the study was at least 104 weeks. Supplementary (satellite) mice were included in the study for toxicokinetics sampling, and for GL-1 analysis.

2.4.2. Rats

The study design of the rat study is shown in Table 1b.