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The effects of taurine on Vigabatrin, high light intensity and mydriasis induced retinal toxicity in the pigmented rat

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ABSTRACT

The overall purpose of this study was to establish a model that may be used for examining the effect of Vigabatrin-induced retinal toxicity in pigmented rats, and subsequently examine the possible effects of taurine on the retinal toxicity. In the first part of the study, pigmented Long Evans rats were subjected to combinations of induced mydriasis, low/high light intensities (40/2000 lx) and oral administration of near-MTD (Maximum Tolerated Dose) doses (200 mg/kg/day) of Vigabatrin for up to 6 weeks. The combination of mydriasis and high light intensity applied to Long Evans rats resulted in retinal damage that was increased by the administration of Vigabatrin.

In the second part of the study Long Evans rats were subjected to combinations of induced mydriasis and high/low light intensity (40/2000 lx) while being orally administered low (30 mg/kg/day) or high (200 mg/kg/day) doses of Vigabatrin for up to 6 weeks. In addition, selected groups of animals were administered taurine via the drinking water (20 mg/ml), resulting in systemic taurine concentrations of approximately threefold the endogenous concentration.

The combined results of the studies demonstrate that retinal damage can be induced in pigmented animals when combining mydriasis and high light intensity. Retinal damage was functionally evaluated by electroretinography (ERG), then confirmed by histopathology. While depending on mydriasis and high light intensity, administration of Vigabatrin increased the retinal toxicity and resulted in the formation of rosette-like structures in the retina in a dose-related manner. Administration of taurine did not alleviate the Vigabatrin-induced retinal toxicity, as demonstrated either functionally by ERG or morphologically, although systemic concentrations of 3-fold the endogenous levels were reached, and it was thus not possible to demonstrate a protective effect of taurine in these pigmented animals.

1. Introduction

Vigabatrin is a neuromodulator resembling the neurotransmitter gamma-amino butyric acid (GABA), the main inhibitory neurotransmitter of the central nervous system (CNS), as its only structural difference is the addition of a vinyl ($-CH=CH_2$) group. This addition creates a molecule that covalently binds and selectively inhibits GABA transaminase (GABA-T), thus leading to an increase in GABA concentration in the synaptic cleft (Ben-Menachem, 2011). When approved in 1994 (NDA 20-427) the safety profile of Vigabatrin was rather benign, showing a relatively low degree of toxicity, with dosages of 200 mg/kg/day being well tolerated in dogs for 1 year without clinical signs.

Following the use of Vigabatrin in patients with epileptic conditions, reports started to appear on a possible deleterious effect of Vigabatrin on the nervous system, reported as peripheral visual field defects. It is now well documented that treatment with Vigabatrin may result in retinopathy characterised by irreversible, bilateral concentric peripheral visual field constriction (Plant and Sergott, 2011), and there appears to be a strong relationship

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between the visual field defects, the duration of treatment and total dose of Vigabatrin (Malmgren et al., 2001). Several case studies have, however, also suggested that visual field constrictions occur in epilepsy patients treated with other GABAergic compounds and even in untreated patients (Plant and Sergott, 2011).

The retinal effects of Vigabatrin have also been examined in non-clinical studies and most of these have employed non-pigmented animal models. The use of non-pigmented animals for assessment of possible ocular damage of pharmaceutical products in humans is somewhat questionable. The albino animal lacks melanin in the eyes and it is generally agreed that pigment (melanin) in the eyes protect the retina against the detrimental effects of ambient light. Rapp and Williams (1980) suggested that the inherent susceptibility of the retina to damage by light was the same for both pigmented (Long Evans) as well as non-pigmented (Sprague-Dawley) rats, but that pigmentation caused considerable resistance to light damage. The lack of pigment not only causes the eyes to appear red (due to the blood in the eye capillaries being visible) but also modifies the general mechanism of how the eye processes vision when compared to pigmented animals. When comparing several rat strains Prusky et al. (2002) found that albino rats had consistent impaired visual acuity. This may be caused by light scattering inside the albino rat eye as well as gradual retinal degeneration caused by exposure to ambient light, and was not recorded in the pigmented Long Evans rat.

The original data package used for registration of Vigabatrin included several studies in both dogs and monkeys (NDA 20-427). No retinal damage was identified in these studies although dogs were administered Vigabatrin at dosages up to 200 mg/kg/day for 12 months and cynomolgus monkeys were administered Vigabatrin at dosages up to 300 mg/kg/day for up to 6 years. Both species are pigmented animals and were kept under standard housing conditions, i.e. at indoor light intensities. To support the NDA submitted by Ovation Pharmaceuticals to the FDA in 2006, eye sections from these original studies had been re-examined and it was confirmed that no treatment-related changes were present, thus supporting the theory that pigmentation protects the eye from light-induced damage. Interestingly, a reassessment of the 18-month mouse study (in CD-1, albino), that was initially considered to be without retinal findings, showed that treatment with 50, 150 or 200 mg/kg/day Vigabatrin resulted in retinal toxicity in this non-pigmented species, although without a clear dose-dependency. Rapp and Williams (1980) found that a light intensity of 10–1200 lx caused retinopathy in non-pigmented rats, while no noticeable effects were seen after 16 days of exposure to 1200 lx in pigmented rats. This corresponds to the findings of Jammoul et al. (2009), where retinopathy was induced in non-pigmented rats exposed to 125–130 lx for 45 or 65 days.

Previous studies have demonstrated that Vigabatrin affects retinal and vitreous concentrations of GABA, aspartate, glutamate and taurine (e.g. Neal et al., 1989). In the study by Jammoul et al. (2009) treatment with Vigabatrin lowered the plasma concentrations of all amino acids measured in the study, including taurine, in Wistar rats and that taurine supplementation in the drinking water reduced the retinal lesions. Taurine is an endogenous molecule and it has been suggested that it offers neuroprotection by pathways not fully elucidated (Ripps and Shen, 2012). This possible effect of taurine was reviewed by Plant and Sergott (2011), who also offered possible explanations for such an effect (for example the possibility that taurine promotes the generation of rod photoreceptor cells from retinal progenitor cells), although no conclusions were drawn. The authors suggested that patients taking Vigabatrin would benefit from reduced light exposure and consumption of taurine-rich foods.

The present study examined the possible protective effect of taurine on Vigabatrin-induced retinal toxicity in a pigmented animal model.

2. Materials and methods

The study consisted of two parts that were run consecutively at WIL Research Europe-Lyon, formerly Ricerca Biosciences SAS, France, in August and September 2010 (first part) and December 2011 to January 2012 (second part). The study was performed in male Long Evans (LE) rats of the strain RjOrl: LE and in male Sprague-Dawley (SD) rats of the RjHan: SD strain. All animals were purchased from CERJ Centre d'Elevage R. Janvier, France. The animals were approximately 11 weeks of age when allocated to the study and weighed 300 g or more. The animals were fed with rat pelleted complete diet ad libitum (Diet reference Safe A04C-10) and had unrestricted access to water. The overall study design is shown in Table 1. All animal procedures were conducted according to national and local animal welfare legislation as well as to the animal welfare policy of H. Lundbeck A/S. Animal use was minimised by varying the number of animals in each group to provide sufficient data for meaningful assessment while keeping the number of control animals as low as possible. Bioanalysis of taurine was performed at H. Lundbeck, Denmark, and bioanalysis of Vigabatrin was performed at Sequani Limited, UK.

The objective of the first part of the study was to demonstrate the Vigabatrin-induced retinal effect in pigmented rats using a combination of light exposure and induced mydriasis. A single group of Sprague-Dawley rats (group 4) was included as controls for development of retinal toxicity. Groups 2 and 5 were administered 200 mg/kg/day Vigabatrin (Patheon Pharma, Batch number 110032) by oral gavage from day 0 to day 7. Selection of this dose level of Vigabatrin was based on the work by Jammoul et al. (2009), where an intraperitoneal dose level of 200 mg/kg/day was demonstrated to cause clear retinopathy in non-pigmented animals. No toxicokinetic data was available for intraperitoneal administration of Vigabatrin, but previous studies have demonstrated that Vigabatrin administered orally is nearly 100% absorbed. An oral gavage dose of 200 mg/kg/day was thus expected to be equivalent to the same dose administered by the intraperitoneal route. The initial dose level of 200 mg/kg/day was reduced to 150 mg/kg/day from the second week of treatment, due to larger than expected body weight changes at the initial dose level.

In the second part of the study the objective was to investigate in the pigmented rats if the retinal damage induced by a 6-week oral administration of Vigabatrin may be counteracted by administration of taurine. High and low dosages of Vigabatrin were 150 and 30 mg/kg/day, respectively. Plasma samples for analysis of systemic exposure to Vigabatrin were taken at the end of weeks 1, 3 and 6 from both study parts. Samples were taken at the estimated time of maximal plasma concentration (1 h post dosing) and plasma was analysed by LC–MS/MS following solid phase extraction.

Animals were subjected to standard room light in the animal facility (approximately 40 lx) or to high intensity light of approximately 2000 lx using a 12 h dark/12 h light regime. Based on data by Rapp and Williams (1980) and Jammoul et al. (2009), a light intensity above 1600 lx was considered necessary to achieve results in the present study, and as 2000 lx corresponds to average day light this light intensity was consequently used.

Mydriasis was induced by ocular instillation of 1% atropine in both eyes at least twice weekly. The eyes of all animals were hydrated with daily instillations of 0.9% NaCl eye drops, up to 5 times per day. Animals were observed at least twice daily for mortality/morbidity and clinical signs. Body weights were recorded twice weekly.