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Vigabatrin-induced CNS changes in juvenile rats: Induction, progression and recovery of myelin-related changes

Allan D. Rasmussen [a,*], Emily Richmond [b], Karen Malene Wegener [a], Noel Downes [b], Pamela Mullins [b]

[a] Department of Regulatory Toxicology & Safety Assessment, H. Lundbeck A/S, Ottiliavej 9, DK-2500 Valby, Denmark
[b] Sequani Limited, Bromyard Road, Ledbury, Herefordshire HR8 1LH, UK

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ABSTRACT

The purpose of this study was to expand on the knowledge previously published on the central nervous system effects of Vigabatrin in juvenile animals. By employing extended sectioning of the brain and by using four different tissue staining techniques it is demonstrated that oral administration of Vigabatrin to juvenile rats (treatment periods of post-natal day (PND) 4–7, 7–14 or 14–30) will cause histological CNS changes at dose levels of 15 and 50 mg/kg/day, but not at a dose level of 5 mg/kg/day.

No evidence of neuronal degeneration or gliosis was seen at any stage of treatment. Consistent with previous reports microvacuolation, as well as effects on myelination and on oligodendrocytes were recorded. The present study expands on these findings and demonstrates that the variation in the location of the vigabatrin-induced lesions in the juvenile rat brain (both neuropil vacuolation and reduction of myelin) appears to be consistent with the process of myelination: In the youngest animals (PND 4–7) myelination occurs mainly in the hind brain (medulla oblongata and pons) where neuropil vacuolations is recorded. In animals dosed during PNDs 7–14 or during PNDs 14–30, the first changes were found in the thalamus. It seems likely that the earlier stages of myelination are more vulnerable to treatment related effects and the swollen oligodendrocytes seen as the initial change in the thalamus in animals treated during PNDs 4–7 and 7–14 represents an early stage in the development of the myelin lesion which is seen later as neuropil vacuolation.

1. Introduction

Vigabatrin is an oral antiepileptic drug (AED) with the chemical name (±) 4-amino-5-hexemoic acid marketed by H. Lundbeck A/S as tablets under the trade name Sabril as adjunct therapy for adults with refractory complex partial seizures and as a mono therapy for children aged 1 month to 2 years with infantile spasms (Plant and Sergott, 2011). Vigabatrin is an irreversible inhibitor of gamma-aminobutyric acid transaminase (GABA-T), the enzyme responsible for the catabolism of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), and oral treatment with this compound results in increased levels of GABA in the cerebrospinal fluid and brain (Schechter, 1989; Mattson et al., 1994; French, 1999).

Vigabatrin was first registered in the mid-1990s. In animals, vigabatrin had relatively low acute toxicity, and repeated administration for up to one year caused clinical signs, effects on food consumption and body weight and eventually death in a dose- and time-related manner (NDA 20-427, data summarized in Sabers and Gram, 1992). The only consistent histopathological finding was that of intramyelinic edema (microvacuolation or IME) in specific regions of the brain of rats and dogs (see also Gibson et al., 1990). For both species, the finding was at least partially recoverable following cessation of treatment. Intramyelinic edema was not recorded in monkeys after as long as 6 years of treatment, although the absence of findings may have been related to the poorer oral absorption in this species (Gibson et al., 1990). Evoked Potential (EP) and Magnetic Resonance Imaging (MRI) methods have been demonstrated to reliably correlate with histological lesions in animals, while no definitive cases of vigabatrin-induced IME has been identified in patients treated with vigabatrin (Preece et al., 2004; Cohen et al., 2000).

In spite of the fact that histological examination of brain tissue from adult humans treated with vigabatrin has not revealed IME there have been more recent reports on MRI findings in infants treated with vigabatrin. These studies (Desguerre et al., 2008; Pearl et al., 2009) have challenged the conclusion that vigabatrin does

* Corresponding author. Tel.: +45 30 83 41 09.
E-mail address: adar@lundbeck.com (A.D. Rasmussen).

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not cause vacuolar changes in humans, and suggest that the vacuolar changes seen in young animals (Walzer et al., 2011) may also occur in infants treated with vigabatrin. In the paper by Walzer et al. (2011) the key pathological findings in immature rats were identified as vacuolar lesions in the white and gray brain matter. Consistent with the findings in adult rats the vacuoles were confirmed by electron microscopy to be confined to the myelin sheaths and caused a delay but not a cessation of the myelination process. Walzer et al. (2011) found no evidence of damage to neurons and no gliosis.

The composition of the Central Nervous System (CNS) myelin is very similar between rats and humans (Quarles et al., 2006). Nevertheless, there are several differences between the myelination processes occurring in rats and humans. The biology of the human oligodendrocytes (myelin forming cells) is poorly understood and is in effect extrapolated from rodent studies (Bradl and Lassmann, 2010). Two main points are: (i) key regions of the human brain may be underdeveloped in rodents and vice versa, and (ii) the time scale of myelination differs/is different in rodents and humans, and due to the greater complexity of the human brain, myelination takes decades compared to weeks in rodents. Although the new results presented here support and expand on the results from previous studies in rats, the relation toward the human juvenile situation remains uncertain.

Finally, it generally appears that juvenile animals are more susceptible to the toxicological effects of vigabatrin than adult animals, causing effects to be observed at lower dose levels. A review of the NDA (20-427 & 22-006) and NDA (20-427 and 22-006) data shows that clinical signs occur at dosages of 30–50 mg/kg/day in juvenile animals and at 200–300 mg/kg/day in adult animals. Furthermore, effects on body weights are noted at 15 mg/kg/day in juvenile animals while doses of 200–300 mg/kg/day are needed to induce effects in adult animals, and deaths occur at dosages of 30 mg/kg/day in juvenile animals and at 300 mg/kg/day in adult animals. It is not known, however, to what extent the immaturity of the juvenile rat kidney and liver and the corresponding higher systemic exposure to vigabatrin in the juvenile rat contribute to this finding.

In this paper, we expand on the knowledge previously published on the CNS effects of vigabatrin. This was done by employing extended sectioning of the brain and by using 4 different tissue staining techniques. Also, as it is known that the window of opportunity for detecting specific neuronal damage may be narrow, subgroups of animals were necropsied at predetermined time points following the first treatment occasion to allow for detection of onset, duration and recovery of any pathological changes. Our treatment period started at day 4 post-partum, corresponding to below 1 month of age in children, which is the earliest age where vigabatrin-treatment may be started for the indication of infantile spasms.

2. Materials and methods

Two studies were conducted: A preliminary proof of absorption study to measure plasma concentrations and evaluate systemic exposure of the juvenile animals at the selected vigabatrin high dose, and a subsequent investigative brain pathology study testing three dose levels of vigabatrin. The studies were performed in rats from the Crl:CD (SD) IGS strain supplied by Charles River (UK) Limited, Margate, Kent, CT9 4LT, England. The test item used was vigabatrin (Batch No 3070446) supplied by Sanofi-Aventis. The test item was dissolved in USHP water and administered once daily, by oral gavage, using a disposable syringe and plastic or rubber catheter of suitable size depending on the age of the animals. A constant dose volume of 5 mL/kg body weight was used and individual doses were adjusted according to the most recently recorded body weight. Control animals received the vehicle only, following the same regimen as the other groups. All animal procedures were conducted according to national and local animal welfare legislation as well as to the animal welfare policy of the sponsor, H. Lundbeck A/S, Valby, Denmark.

Both studies were performed at Sequani Limited, Bromyard Road, Ledbury, Herefordshire, HR8 1LH, UK. Bioanalysis of plasma samples for vigabatrin content was performed by the Bioanalytical Department of Sequani Limited, using a validated method. Vigabatrin formulation analysis was performed by the Analytical Chemistry Department of Sequani Limited, using a validated method.

2.1. Study 1: Preliminary proof of absorption study (Table 1)

Twenty-four male and 24 female cross-fostered juvenile rats were allocated to the study and divided into 4 groups, each comprising 6 males and 6 females. Animals were administered a single dose of 50 mg/kg on Day 4, 7, 10 or 14 of age. All animals were observed for any visible signs of reaction to treatment. Blood samples for assessment of exposure were taken 1 and 2 h (h) after dosing; all samples were collected terminally, immediately after death. Based on previous studies, the 1 h sampling timepoint was considered the likely T_{max}. Animals were discarded without

Table 1
Overall study design of studies 1 and 2. Overview of group numbers, vigabatrin dose levels, dosing periods and procedures.

Group Nos.	Vigabatrin dose levels (mg/kg/day)	Dosing period (day of age)	Procedure following last treatment of animals
Study 1: Preliminary proof of absorption study			
1	50	4	Each group consisted of 6 males and 6 females. Blood samples were collected from 3 animals of each sex, 1 h and 2 h following a single dose
2	50	7	
3	50	10	
4	50	15	
Study 2: Investigative brain pathology study			
1	0	4–7	Each group consisting of 5 L of 5 male and 5 female cross-fostered pups To provide terminal observations at staggered time points throughout the treatment and treatment-free periods of the study, 1 litter from each group was sacrificed at 8 h (±30 min), 1 day, 3 days, 10 days and 30 days after the first day of dosing A total of 600 pups were dosed
2	5	4–7	
3	15	4–7	
4	50	4–7	
5	0	7–14	
6	5	7–14	
7	15	7–14	
8	50	7–14	
9	0	14–30	
10	5	14–30	
11	15	14–30	
12	50	14–30	