



The role of *CYP2C9* polymorphisms in phenytoin-related cerebellar atrophy

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ABSTRACT

Purpose: Phenytoin is known to be able to induce cerebellar atrophy in patients with epilepsy. It is also known that a *CYP2C9* mutation (*2 or *3) reduces phenytoin metabolism by 25–50% and can increase the risk of phenytoin related side effects. We examined the influence of *CYP2C9* polymorphisms on total cerebellar volume and cerebellar gray and white matter volumes in patients with epilepsy taking phenytoin.

Methods: For the genotyping, 100 adult patients with documented epilepsy who had been taking phenytoin for >1 year were selected. From this group, we randomly selected 19 mutant individuals (MT group; *CYP2C9**2 and *3) for a whole brain volume measurement using MRI and 19 wild type individuals (group WT; *CYP2C9**1) with similar clinical and demographic characteristics to those in the MT group for comparison. Total intracranial volume measurements were used to normalize the acquired volumes, which were separated into gray matter volume, white matter volume, and total volume.

Results: The MT group exhibited a significant reduction in cerebellar white matter volume ($p = 0.002$) but not in total cerebellar volume.

Conclusion: Our study is the first to report evidence linking *CYP2C9* polymorphism and a reduction in cerebellar volume in epileptic users of phenytoin.

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1. Introduction

Genetic variation in the *CYP2C9* gene produces altered phenotypes that can affect metabolism. In individuals with poor *CYP2C9* metabolizer alleles, metabolism of phenytoin can be 25–50% lower than in individuals with wild type alleles depending on the genetic polymorphism the patient carries and drug interactions that affect the cytochrome P450 pathway.¹ Several studies have shown that the most common allelic variants are Arg₁₄₄Cys (*CYP2C9**2) and Ile₃₅₉Leu (*CYP2C9**3), which encode enzymes with decreased turnover rates.² The main route by which phenytoin is metabolized and eliminated is through hepatic oxidation by *CYP2C9* (90%) and *CYP2C19* (10%).³

Cerebellar atrophy has been reported as a common finding in patients suffering from epilepsy.⁴ Although the exact cause is unknown, atrophy is often attributed to either the seizure activity itself or antiepileptic medication,⁵ and phenytoin is by far the most implicated drug.

Since the etiology of cerebellar atrophy in phenytoin users remains unknown, we hypothesize that *CYP2C9* polymorphism may be involved. The aim of this study was to evaluate the relationship between poor phenytoin metabolism and cerebellar atrophy in patients with epilepsy using this medication.

2. Methods

2.1. Subjects

Initial selection. The inclusion criteria were age ≥ 14 years and at least one year of clearly documented use of phenytoin for epilepsy treatment. The study was approved by the Research Ethics Committee at the Hospital de Clínicas, Federal University of Paraná. One hundred subjects were selected for genotyping. Mean age at enrollment was 39.6 ± 10.3 years (17–72 years) and mean age at onset of epilepsy 13.1 ± 12.4 years (1 month–62 years). Mean duration of epilepsy was 26.5 ± 11.9 years (3–48 years) and mean length of phenytoin use was 8.3 ± 6.9 years for all patients, with an average maximum daily dosage of 301.5 ± 78 mg.

2.2. *CYP2C9* genotyping

Crude DNA samples were analyzed using polymerase chain reaction restriction fragment length polymorphism and the

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primers described by Sullivan Klose et al.² to identify the *CYP2C9*2* and *CYP2C9*3* allelic variants of *CYP2C9*. Genotype frequencies of the studied population were 72% for the *1/*1 genotype and 28% for the other genotypes (*1/*2, *2/*2, *1/*3, *2/*3), showing that this population is in Hardy Weinberg equilibrium.⁶

2.3. Study groups

To compare the cerebral and cerebellar volumes of the 28 patients with at least one variant allele (mutant, or MT, group) with the corresponding volumes of the 72 patients with wild type *CYP2C9* (wild type, or WT, group), we randomly selected 19 individuals from MT group. WT group we select 19 individuals that were most matching to each one of the MT group, the selection criteria were number of years of epilepsy and years of phenytoin use.

2.4. Imaging data

The 38 subjects were scanned on a 1.5 T GE Sigma MRI scanner (GE Medical Systems). A 3D volume was acquired for volumetric measurements (TR, 430 ms; TI, 12 ms; flip angle, 180; matrix size, 250 × 250; and FOV, 25 × 25 cm) with continuous 1 mm thick slices. All image records were identified by the MRI number alone, and observers were blinded to subjects' genetic data when measurements were taken.

Volumetric analyses were processed with MIPAV (version 4.3.0 2009 08 03 from Biomedical Imaging Research Services Section, ISL, CIT, NIH).⁷ All images were processed according to a semi automatic standard protocol.⁸

For measurement purposes, we considered brain volume (BV) as the sum of the following volumes of interest (VOIs): cerebral hemispheres, basal ganglia, inter hemispheric fissure, and pons. Cerebellar volume (CV) and BV were calculated excluding cerebrospinal fluid (CSF). Total intracranial volume (TICV) was considered as the sum of CV, BV, and CSF. All VOI measurements were normalized by dividing VOI by TICV (for example, CV/TICV*100 or BV/TICV*100) and recorded as percentages. Normalized CV and BV volumes were analyzed for gray matter (GM) and

white matter (WM) alone or for both (total). Thus, CV GM is the normalized cerebellar gray matter volume. We preferred to use TICV normalized volumes to avoid differences in volumes due to cranial size.

2.5. Reproducibility of measurements

To determine the validity of the volumetric measurements, all examinations were conducted twice by the same observer (intra rater reliability). To evaluate inter rater reliability, 10 randomly selected examinations were measured by another observer, who was blinded to the previous results. The reliability of the whole segmentation procedure, as well as volumetric analysis, was estimated by performing the measurements in all 38 subjects. The relative error in CV was 2.64%, with an estimated measurement error of 0.0019. Pearson's test indicated excellent correlation between samples for CV ($r = 0.96$, $p < 0.0001$). Inter observer reproducibility was analyzed for 10 randomly selected cases. Pearson's correlation test also showed good results for CV ($r = 0.85$, $p = 0.002$).

2.6. Statistical analysis

BVs and CVs for the MT and WT groups were compared by multivariate analysis controlled for age, sex, duration of phenytoin use, epilepsy duration, and monthly mean number of seizures. We used the beta regression model with the Wald test. Pearson's test was used for inter rater and intra rater reliability analysis. For risk factors and comparisons of other characteristics of the groups, we performed a univariate analysis with Student's *t* test. Sex distribution was compared with Fisher's exact test.

3. Results

3.1. Group characteristics

The characteristics of both groups are summarized in Table 1. Although the mean age in the MT group (39.3 years) was higher

Table 1
Characteristics of the MT and WT groups.

Characteristics	MT n = 19	WT n = 19	p value
Age (years)	39.3 ± 8.4	37.9 ± 7.9	0.20
Female (number of individuals)	7	10	0.33 ^a
Phenytoin maintenance dosage (mean/mg)	315 ± 80	305 ± 40	0.55
Duration of phenytoin use (years)	8.4 ± 4.1	9.7 ± 6.4	0.45
Epilepsy duration (years)	25.1 ± 13.0	26.1 ± 12.4	0.79
Seizure frequency (monthly/median)	1.75	1	0.85
<i>Number of patients on polytherapy</i>	13	14	1.0 ^b
Phenobarbital	3	4	
Carbamazepine	3	4	
Lamotrigine	3	2	
Others	4	4	
<i>Reported adverse side effects of phenytoin (number of individuals)</i>			1.0 ^b
Symptoms of cerebellar intoxication	3	2	
Gingival overgrowth	2	2	
<i>EEG (number of individuals)</i>			0.63 ^b
Focal epileptic activity	10	12	
Nonspecific slowing	4	2	
Normal	5	5	
<i>MRI (number of individuals)</i>			0.23 ^b
Mesial temporal sclerosis	4	6	
Normal	15	13	

MT: mutant group; WT: wild or control group; p value calculated with the Student's *t*-test.

^a Fisher exact test.

^b Chi-square test.

than in the WT group (37.9 years) and the number of years of phenytoin use and duration of epilepsy were higher in the latter, no significant statistical differences were observed between the groups. The electroencephalographic findings, brain images, and reported side effects were very similar in both groups, as can be seen in the comparative analysis in Table 1.

3.2. Volume measurements

Multivariate analysis revealed that the normalized volume of cerebellar white matter (CV WM) was the only intracranial volume that showed a significant difference between the groups (Table 2). Analyzing only the white matter, the adjusted cerebellar volume was lower in the mutant *CYP2C9* group (1.8% versus 2.3%; $p = 0.002$). Although total CV (CV WM + CV GM) was lower in the MT group than in the WT group (6.8% and 7.4%, respectively), however this difference was not statistically significant ($p = 0.13$).

4. Discussion

To date, cerebellar volume in patients with epilepsy has been evaluated by visual inspection⁹ and, more recently, quantitative imaging.^{4,10} Both approaches clearly show that patients with epilepsy have smaller cerebellar volumes than normal controls. In fact, cerebellar atrophy has an estimated prevalence of between 16.2% and 30% in these patients.^{5,9}

In *CYP2C9* mutants the maximum metabolic rate for phenytoin can be reduced by 25–54%.¹¹ The poor *CYP2C9* metabolizer genotype has been associated with the development of gingival overgrowth and cutaneous reaction in patients using this drug. In addition, some reports have suggested that use of phenytoin causes cerebellar atrophy.^{4,12} This led us to hypothesize that phenytoin users who carry the *CYP2C9**2 or *3 variants, including those who take normal doses of phenytoin, could have a higher blood phenytoin level and be more predisposed to cerebellar atrophy than wild type *CYP2C9* phenytoin users. In this study, we observed that patients with epilepsy and more than one year of phenytoin use had a reduction in CV WM even if they carried only one variant allele.

The pathogenesis of phenytoin induced cerebellar atrophy is not yet completely understood. Corroborating our findings, Liu et al.¹³ emphasize that seizures or phenytoin itself could have a regional selective effect on cerebellar volume. This selectivity may be restricted to a histological segment such as gray or white matter.

Comparison of both groups in our study revealed a significant reduction in the volume of cerebellar white matter in mutant *CYP2C9* patients. No previous work has shown such specific and localized atrophy of cerebellar white matter in patients with poor metabolism of phenytoin. Even though our results indicate a localized pattern of atrophy of white matter, we believe that the

toxic effect of phenytoin is more diffuse (gray and white matter), as suggested by the trend toward reduction in volume of cerebellar gray matter in our cases and in other studies.^{14–18} A larger study population would allow this hypothesis to be investigated in greater detail.

Pathologic white matter modifications have been implicated in both epileptogenic potential and epilepsy related comorbidities.¹⁹ Nevertheless, there is a dearth of literature on the influence of AEDs on brain white matter in patients with epilepsy. Administration of phenytoin in newborn rats has been shown to lead to a reduction in cerebellar weight and size.²⁰ Furthermore, white matter alterations have been reported in rats after exposure to phenytoin, the main findings being focal axonal swellings.²¹ These effects could be observed even when low doses were used and led to cell morphological alterations and breakage of synaptic connections. Moreover, regional effects could compromise more than just gray matter. Indeed, volumetric studies have shown major changes in brain white matter too.^{15,19} These changes can occur locally and diffusely in both hemispheres, in the fornix, amygdala, cingulate gyrus, thalamus, basal ganglia, temporal lobe and cerebellum.^{14–18} Recently, voxel based morphometry and tensor diffusion studies in patients with epilepsy have shown reductions in both white and gray matter in various parts of the brain, including the cerebellum, compared with normal subjects.²² As in our patients, the reductions were more pronounced in white matter. In other studies using a similar methodological procedure to that we adopted (volume of interest analysis), reductions in white matter were observed.^{19,23} Again, none of the studies referred to above explore the pharmacogenetic aspects or role of phenytoin in these white matter alterations.

The few studies that have been published about AEDs and cerebellar volume are conflicting. Using multiple regression analysis, De Marcos et al. and Luef et al.^{4,12} demonstrated that duration of phenytoin treatment was a principal risk factor for the presence of cerebellar atrophy ($p = 0.001$). Serum phenytoin levels as well as duration of treatment with phenytoin could be related to the development of cerebellar atrophy. In our study we could not evaluate phenytoin serum levels because some patients were not using this medication at the time. However, Luef et al. found no correlation between serum levels of phenytoin and development of cerebellar atrophy.¹²

5. Conclusion

Although several mechanisms have been implicated in the pathogenesis of cerebellar atrophy, it seems likely that it has multifactorial etiologies. Investigation of the pharmacogenetic aspects of phenytoin could explain some unresolved issues regarding the relationship between epilepsy and cerebellar atrophy. However, confirmation of the results described here by replication in a second cohort is required.

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Table 2

Comparison of brain volumes between the MT and WT groups.

	MT (n = 19) % Mean ± SD	WT (n = 19) % Mean ± SD	p value
Total BV	60.3 ± 4.0	60.5 ± 4.4	0.981
Total CV	6.8 ± 1.2	7.4 ± 0.8	0.130
BV-WM	30.1 ± 2.1	31.1 ± 2.9	0.358
CV-WM	1.8 ± 0.3	2.3 ± 0.4	0.002*
BV-GM	29.5 ± 2.5	29.5 ± 2.2	0.318
CV-GM	4.8 ± 0.9	5.1 ± 3.7	0.790

D: Standard deviation; CV: normalized cerebellar volume; BV: normalized brain volume; WM: white matter; GM: gray matter; total: volume of gray matter plus white matter. Beta regression model—Wald test. Multivariate analysis.

* $p < 0.05$.

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