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TERATOGENIC EFFECTS OF LAMOTRIGINE ON RAT FETAL BRAIN

A morphometric study

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ABSTRACT - A study of the teratogenic activity of an antiepileptic drug - lamotrigine – was carried out in the brain of fetuses of rats who had received the drug. The dosage levels studied corresponded to four times the median effective dose (ED50) in rats. The drug was administered during the organogenesis period. Rats were sacrificed one day prior to term and fetuses were macroscopically examined, weighted and cephalic segments sectioned (Wilson technique), for histological study by stereological analysis, using Merz's grid for drawing and point counts. Cortex, subcortex, ependyma and lateral ventricles were analyzed. The same methodology was applied to the control group; data were compared with by the non-parametric Mann-Whitney statistical analysis test. Results showed that fetuses of the experimental group had reduced body weight at birth, increased volume and diameter of the cerebral structure, increased density of the subcortical layer, and ventricle dilatation .Possible mechanisms of this teratogenicity were discussed.

KEY WORDS: teratogenesis, lamotrigine.

Efeitos teratogênicos da lamotrigina em cérebro de fetos de ratos: estudos morfométrico

RESUMO – Foi realizado estudo da atividade teratogênica de uma droga antiepiléptica - lamotrigina - em cérebro de fetos de ratas que receberam a droga. Estudamos dose que corresponde a 4 vezes a dose efetiva mediana (ED50) em ratos. A droga foi administrada durante o período de organogênese, as ratas foram sacrificadas 1 dia antes do termo e os fetos foram examinados macroscópicamente, pesados e foram realizados cortes no segmento cefálico (técnica de Wilson) e feito preparo histológico para análise estereológica (utilizado grade de Merz para desenho e contagem dos pontos). Foram analisados: córtex, subcórtex, epêndima e ventrículos laterais. A mesma metodologia foi aplicada ao grupo controle e os dados foram submetidos a análise estatística por teste não paramétrico de Mann-Whitney. Os resultados mostraram nos fetos do grupo tratado: redução do peso ao nascimento, aumento do diâmetro e volume da estrutura cerebral, aumento da densidade da camada subcortical e dilatação ventricular. Possíveis mecanismos de teratogenicidade foram discutidos.

PALAVRAS-CHAVES: teratogênese, lamotrigina.

There is no completely satisfactory definition for epilepsy, generally a chronic condition comprising a group of illnesses having in common, recurrent epileptic seizures, occurring in the absence of toxic, metabolic, or febrile situations. According to the World Health Organization, epilepsy has a prevalence of around 5% of the total population[1]. Therapeutic and surgical practices allow 80% of the patients to have a normal life, with little interference of epileptic seizures in it[2]. Although in the past, reproduction was discouraged in epileptic women, over 90% of pregnancies in such patients currently have an uneventful outcome when appropriately managed[3]. In approximately one third of pregnant epileptic women an increase of seizure frequency relative to the period prior to pregnancy occurs and among other factors, has been attributed to increased estrogen and reduced AED (antiepileptic drug) serum levels[2].

The first systematic study of AEDs teratogenicity, conducted by Jans and Fuchs in 1964[4], demonstrated an average of 2.2% malformations in 262 children exposed intra-uterus to AED, a value not significantly greater than that of the general population. However other authors - Lindhout and Omtizigt[5] in 1992 - found an absolute risk factor of 7 to 10%, i.e., 3 to

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Table 1. Results.

	Controls	Treated	Significance (α)
Weight	2.731 ± 0.335	2.503 ± 0.304	0.05*
Volume density	0.6563 ± 0.1698	0.8466 ± 0.2324	0.05*
Surface density	3.8 ± 0.3	4.0 ± 0.6	> 0.05^{ns}
Mean diameter	3072.5 ± 630.9	3732.0 ± 676.2	0.01*
Cortex thickness	1871.87 ± 251.15	2007.69 ± 416.04	> 0.05^{ns}
Subcortex density	10896 ± 0.1867	12896 ± 0.2825	0.05*
Ependyma	305.5 ± 74	367 ± 92	> 0.05^{ns}
Lateral ventricles	88 ± 53	146 ± 68	0.05*

Results expressed as averages ± standard deviation: ^{ns}, not statistically significant; *, statistically significant result.

5% higher than that in the general population. The number and doses of AEDs utilized can also interfere with the final result , high doses and polytherapy increasing this risk[6,7].

Anomalies and malformations commonly found in association with the use of AED are: a) neural tube defects – "spina bifida", mostly related to the use of sodium valproate[8] and carbamazepine[9]; b) facial cleft - labial or palatal[10] and, c) congenital cardiac defects (atrial septal defect, Fallot's tetralogy, ventricular septal defect, aortic coarctation, pulmonary stenosis and persistence of the arterial channel)[11]. The majority of studies on fetal malformations and AEDs, have been made in patients treated with the five leading AEDs - phenytoin (DPH), carbamazepine (CBZ), phenobarbital (PB), primidone (PR), and sodium valproate (VPA)[4-11]. In general, investigators agree that the risk of fetal malformations due to AEDs is about twofold above normal, and is enhanced by polytherapy and high AED serum levels.

Lamotrigine (LTG) is a recent drug considered to be effective against partial tonico-clonic seizures, secondarily generalized. The drug's mechanism of action is related to blockade of voltage dependent sodium channels which stabilize pre-synaptic membranes and inhibit excitatory neurotransmitter release, in special of glutamate and aspartate[12]. Analysis of 42 pregnancies in 1993, did not reveal clear-cut evidence of a relationship between LTG and teratogenesis[13]; however, information on LTG's teratogenicity remains insufficient.

METHOD

In this study, a dose of 1.5 mg of LTG, corresponding to four times the effective mean dose (ED50), was administered to white rats by gastric intubation on days 9, 10 and 11 of pregnancy (corresponding to the organogenesis period). Animals were sacrificed one day prior to term (treated rats = 04; litters = 51 fetuses / control rats = 04; litters = 39 fetuses). Fetuses were examined macroscopically, weighted, and portions of their cephalic segments dissected by Wilson's technique as follows: palate surface down, 3 coronal sections made immediately frontal to the eyes, through the eyes and retro-occularly passing the lateral ventricles, the last section was utilized in the present study[14]. Histological preparations from the cortex, subcortex, ependyma, and lateral ventricles from aleatorily chosen 30% of the offspring were prepared by inclusion in paraffin, sectiong at intervals of 2mm with a 6 micrometer thickness, and dyeing with Hematoxilin – Eosin. Nine histological sections from each fetus were prepared for stereological analysis using Merz's grid for drawing and point counts, maintaining a space of 56 microns per cut. The same methodology was applied to the control group. Data were statistically analyzed by the non-parametric Mann-Whitney test[15].

RESULTS

As demonstrated in the Table 1, in the treated group we found lowered body weight at birth, increased structural diameter, increased volume density, increased lateral ventricles and increased subcortical density. Other items analyzed did not show significant alterations.

DISCUSSION

Teratogenicity can be expressed by interference in proliferation, migration or differentiation at the cellular level. The basis for recognition of teratogenicity is a reproducible repetition and association of a given agent with a recognizable pattern of malformation, growth delay, mutagenesis and embryo or fetal death. Due to the fact that certain agents

have the same metabolic pattern, they are also associated with similar patterns of malformation, resulting in a recognizable syndrome. Some good examples of these effects are the AEDs[16].

In this research we observed that alterations like low birth weight, ventricle dilatation, subcortical density enhancement with consequent increase in cerebral volume and diameter, were associated with the use of lamotrigine during the organogenesis period. Genetic-molecular susceptibly to teratogenesis is probably heterogeneous. The equilibrium between metabolic activation and detoxification determines the levels of reactive intermediates; besides this, not only inhibition of detoxification by drug interaction, but also genetically determined deficiencies of enzymes for detoxification are potential factors for teratogenesis[5].

Nau in 1995[17], showed that AED therapy can have a significant effect on endogenous retinoid metabolism; due to the importance of retinoids in signalizing crucial biological events during embryonic development, alterations in their metabolism can be important factors for AED teratogenesis.

Wells et al. in 1997[18], argued that bioactivation of cytochrome P450, prostaglandin H synthetase, lipooxygenation, and/or free radical reactivation, contribute to the oxidation of macromolecules like DNA, proteins and lipids which can determine intrauterine death or teratogenesis.

Animal studies are limited by inter-species variability and by the fact that in many studies doses used were much higher than those utilized in humans. These characteristics decrease the reliability of the results of studies on animals, on human teratogenesis evaluation, although these studies may aid in the localization of events related to biological plausibility[19]. The occurrence of embryopathy associated with talidomide, preceded the erroneous belief that human teratogenicity cannot be predicted on the base of animal studies. Drugs that have been found to be teratogenic in man have caused similar effects in animals[20].

Our research concludes that lamotrigine has teratogenic effects on the brain of rats. Further research must be carried out to corroborate these findings and establish their applicability to humans. One of the major purposes of teratology is to anticipate risks before they materialize[16].

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