Original Article
Journal of Epilepsy Research
pISSN 2233-6249 / eISSN 2233-6257

Topiramate in an Experimental Model of Epilepsy - Similarity between Generic, Similar and Reference Drugs

George Linard Silva Malveira, MD, MSc[1,2], Sacha Aubrey Alves Rodrigues Santos, PharmD[1], Gerlânia de Oliveira Leite, BSc, PhD[1], Gdayllon Cavalcante Meneses, PharmD, PhD[3], Adriana Rolim Campos, PharmD, PhD[1,2]

[1]Experimental Biology Center, University of Fortaleza, Fortaleza; [2]Graduate Program in Medical Sciences, University of Fortaleza, Fortaleza; [3]Department of Clinical Medicine, Faculty of Medicine, Federal University of Ceará, Fortaleza, Brazil

Background and Purpose: The literature is still controversial in relation to therapeutic differences between innovative, generic, and similar anti-seizures medications (ASM). Topiramate (TPM) is an ASM used in the treatment of various seizure types and in different epileptic syndromes, as well as in other groups of morbidities, and it is available in many generic and similar forms, besides the innovator. The aim of this translational work was to compare different brands of TPM by using animal models of seizures induced by pentylenetetrazole (PTZ).

Methods: Five brands of TPM (one reference, two similar and two generics) were tested in mice. Animals were previously treated with TPM (n=6/brand) and latencies from PTZ injection to onset of manifestations, first seizure and death were measured and compared between groups. Experiment was conducted in two settings: acute seizure model (PTZ 80 mg/kg) and kindling model (PTZ 20, 30, and 40 mg/kg in 8 alternate days).

Results: The experiment did not demonstrate significant differences between the TPM brands regarding the protective effect in the acute seizure and kindling models.

Conclusions: In conclusion, results can be explained by true therapeutic equivalence or insufficiency of the PTZ model to reveal differences among brands. **(2022;11:1-5)**

Key words: Epilepsy, Topiramate, Generic drugs, Pentylenetetrazole

Received November 18, 2021
Revised February 28, 2022
Accepted March 23, 2022

Corresponding author:
Adriana Rolim Campos, PharmD, PhD
Experimental Biology Center, University of Fortaleza, Av. Washington Soares, 1321
CEP 60811-905 - Edson Queiroz, Fortaleza, Ceará 60811-650, Brasil
Tel. +55 85 3477 3846
Fax. +55 85 3477 3056
E-mail; adrirolim@unifor.br

Introduction

Epilepsy is a brain disease according to the International League Against Epilepsy (ILAE), defined by at least two 24-hour unprovoked or reflex epileptic seizures, one unprovoked or reflex epileptic seizure, and a probability of new seizures within 10 years similar to the risk of recurrence after two seizures and the diagnosis of an epileptic syndrome.[1] Anti-seizure medications (ASMs) are the initial step in the treatment of people with epilepsy and their management, not infrequently, evolves throughout life.[2] Topiramate (TPM) is a sulfamate-substituted derivative of the monosaccharide D-fructose and has its antiepileptic effects mediated by modulation of voltage-dependent sodium channels, potentiation of γ-Aminobutyric acid type A receptors currents, blockade of the glutamate receptor, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainate subtype and inhibition of certain carbonic anhydrase isoenzymes.[3]

Along the years, the use of generic drugs in the treatment of epilepsies has been debated due to the risk of unpredictable consequences such as the recurrence of crises or the appearance of adverse effects.[4] Some analyzes have concluded that generic ASMs are safe when starting a new treatment, but also that, when necessary, the exchange between innovative drugs and their respective generics is accompanied by thorough follow-up.[5] However, according to Odi et al.,[6] the safety of switching between generic products of ASMs continues to be a hot topic in epilepsy management. Therefore, in a translational perspective, this study aimed to evaluate the difference between different brands of TPM in a classic experimental model. The purpose was to use, in a comparative design, an *in vivo* experiment without restrictions frequently found in human epilepsy drug treatment studies, such as adherence uncertainty, unmatched groups

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

for epilepsy types and wide genetic heterogeneity. The classic pentylenetetrazole (PTZ) model was chosen for its simplicity, common acceptance, and long history,[7] as well as for its previously demonstrated sensitivity to TPM in terms of latencies to the desired effects.[8]

Methods

Obtaining different brands of TPM

Five brands of TPM were purchased from the conventional trade (drugstore), all in the presentation of packages with 60 coated tablets of 25 mg each. Besides reference (innovative), brands were chosen as the two market sales leaders, both for generic and similar. In the experiments, the drugs were designated as: reference-R (Topamax®; Jansen-Cilag, São Paulo, Brazil), generic 1-G1 (Biossintética, São Paulo, Brazil), generic 2-G2 (Eurofarma, São Paulo, Brazil), similar 1-S1 (Amato®; Eurofarma) and similar 2-S2 (Égide®; Libbs, São Paulo, Brazil).

Animals

Swiss albino mice (20-30 g), aged 45 to 60 days, obtained from the Núcleo de Biologia Experimental of the University of Fortaleza were used. The specific-pathogen-free animals were housed in appropriate cages (individually ventilated cages, Tecniplast [Buguggiate, Varese, Italy]) and kept at room temperature of 22-24°C on a 12:12 hours light:dark cycle. They received standard feed (Purina, São Paulo, Brazil) and water ad libitum. All protocols were in strict compliance with the standards established by Brazil's National Council on Animal Experimentation Control and received approval from the Committee on Animal Research and Ethics of UNIFOR (#6879070220).

Treatments

The TPM-coated tablets were crushed and homogenized, then diluted in 0.9% NaCl in the proportion of 1 tablet of 25 mg to 2.5 mL of 0.9% sodium chloride, producing a concentration of 10 mg/mL. The animals (n=6/group) were treated orally (gavage; *per os*) in a volume of 0.1 mL/10 g of weight, at a dose of TPM of 100 mg/kg. Animals were divided into the following groups: control, 0.9% NaCl (0.1 mL/10 g); reference, generic (G) 1, G2, similar (S) 1, and S2.

Acute PTZ-induced seizure model

Sixty minutes after the administration of TPM or vehicle, all groups received an intraperitoneal injection of PTZ (80 mg/kg; 0.1 mL/10 g of weight) to induce seizures. During the 60 minutes immediately following the administration of PTZ, the animals remained in individual cages and had their behavior recorded on video, using a smartphone device (iPhone®; Apple Inc., Los Altos, CA, USA) for reviewing and time measuring. Primary outcomes were latencies (in seconds) to the onset of manifestations, to first seizure and to death. The manifestations considered in this analysis were those listed on the Racine scale.[9] adapted by Shimada and Yamagata,[7] in which scores from 1 to 5 indicate the intensity of the behavior presented by the animal, with level 1 being the lowest intensity, level 5 of greatest intensity and level 6 death. Events not observed during 60-minute observation were assigned latencies of 3,600 seconds.

Kindling PTZ model

Along 16 consecutive days, animals were daily treated in the regimen described above (2.3). Starting on the second day and every other day thereafter, 60 minutes after the administration of TPM or vehicle, all groups received an intraperitoneal injection of PTZ in incremental doses (20 mg/kg on days 2, 4, 6, and 8; 30 mg/kg on days 10 and 12; 40 mg/kg on days 14 and 16). During the 30 minutes immediately following the administration of PTZ, the animals remained in individual cages and had their behavior recorded on video, using a smartphone device (iPhone®; Apple Inc.) for reviewing and time measuring. Primary outcomes were latencies (in seconds) to the onset of manifestations, to first seizure and to death. The manifestations considered in this analysis were the also those considered in the acute seizure model (2.4). Events not observed during 30-minute observation periods were assigned latencies of 1,800 seconds.

Statistical analysis

All quantitative variables were tested for normal distribution using the Shapiro-Wilk test and the results are presented as median (interquartile interval) for non-normal distribution. The medians of each group (n=6) in seconds to first manifestations, first seizure and death were compared. Statistical analysis was performed using Kruskal-Wallis test followed by Dunn's post-test. For comparisons of three or more paired groups, the Friedman test was used. In addition, the Kaplan-Meier survival analysis was performed to investigate which group presented a relationship with the fastest onset of the studied events. The median time until the event was estimated within the groups, with respective 95% confidence intervals. The log-rank test, a non-parametric test, was used to compare survival curves be-

Copyright ⓒ 2022 Korean Epilepsy Society

tween two or more groups. Data were analyzed using SPSS software for Macintosh, version 23 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 5.01 for Windows (GraphPad Software, La Jolla, CA, USA; www.graphpad.com). In the acute PTZ-induced seizure model, the occurrence of any manifestation, at least one seizure and death, were also analyzed as a categorical variable, and the chi-square test or the likelihood ratio was used. Values of $p<0.05$ were considered statistically significant. Comparisons were performed with groups separately (comparison 1), with grouped G1+G2 and S1+S2 (comparison 2) and with all non-reference grouped (G1+G2+S1+S2; comparison 3).

Table 1. Groups and events evaluated in animals

	Total group (n=36)
Was there a first manifestation?	
No	1.0 (2.8)
Yes	35.0 (97.2)
First manifestation ΔT1 (seconds)	53.5 (48.0-73.0)
Convulsion	
No	2.0 (5.6)
Yes	34.0 (94.4)
Convulsion ΔT2 (seconds)	95.0 (57.5-327.5)
Death	
No	9.0 (25.0)
Yes	27.0 (75.0)
Death ΔT3 (seconds)	433.5 (215.5-2540.5)

Total group quantitative data expressed as median and interquartile range in parentheses.

Results

In the acute PTZ-induced seizure model, 97.2% of the animals presented manifestations, 94.4% had at least one seizure and 75% died within the 60-minute observation period (Table 1). When comparing the reference drug to the two grouped G1+G2 and to the two grouped S1+S2, there was also no statistically significant difference in the latencies for the onset of manifestations ($p=0.129$) nor for the first seizures ($p=0.117$) though the difference in the latencies for death being significant only between the reference drug and the

Table 2. Comparison between times until events (first manifestation, seizure and death) according to the studied groups

	Acute PTZ-induced seizure model					
	First manifestation ΔT1 (seconds)	*p*-value*	First seizure ΔT2 (seconds)	*p*-value*	Death ΔT3 (seconds)	*p*-value*
Drugs		0.307		0.307		0.129
Control	51.0 (37-58)		53.5 (38-59)		145.0 (105-441)	
Reference	68.0 (62-74)		75.5 (69-154)		2,088.5 (426-3,600)	
Generic 1	50.5 (46-65)		126.5 (53-210)		319.0 (204-1,481)	
Generic 2	46.0 (43-55)		151.5 (46-335)		537.0 (172-629)	
Similar 1	60.5 (48-78)		201.0 (66-517)		1,976.0 (235-3,600)	
Similar 2	53.5 (51-84)		193.0 (108-522)		741.5 (256-3,600)	

Values are presented as median and interquartile range in parentheses.
PTZ, pentylenetetrazole.
*Kruskal-Wallis test was used.

Table 3. Kaplan-Meier analysis with comparison of times until the first manifestation, first seizure and death according to the studied groups

	First manifestation	*p*-value*	First seizure	*p*-value*	Death	*p*-value*
Drugs		0.393		0.329		0.184
Control	55 (48.210-61.790)		57 (51.908-62.092)		441 (98.100-783.800)	
Reference	66 (59.305-72.695)		72 (58.421-85.579)		963 (0.000-5926.226)	
Generic 1	53 (41.118-64.882)		72 (51.631-92.369)		414 (329.130-498.870)	
Generic 2	61 (44.026-77.974)		87 (59.841-114.159)		840 (227.200-1,452.700)	
Similar 1	49 (38.816-59.184)		100 (11.730-188.260)		783 (0.000-2,146.000)	
Similar 2	53 (47.342-58.658)		141 (42.550-239.450)		705 (414.700-995.200)	

Values are presented as median and interquartile range in parentheses.
*The log-rank test was used.

control group (p=0.036). And compared to all other groups (G1+G2+S1+S2), so called non-reference, the group treated with the reference drug also did not differ statistically significantly in relation to latencies for the onset of manifestations (p=0.139) nor for the onset of seizures (p=0.096), with only a latency difference for death between the reference and control group animals (p=0.026) (Table 2). Survival analysis found statistically significant difference in the latencies for death (p=0.019), but not for the onset of manifestations or for the first seizure (Table 3).

As an additional finding, when analyzed as categorical variable, there was a statistically significant difference in the frequency of deaths (but not of manifestations or seizures) among the groups (p=0.029) (Table 4). In the kindling experiment, no manifestations were registered in the first 2 of the 8 days of PTZ injection. The subsequent days, designated below as day 1 to day 6, were analyzed. Latencies for first manifestations in the five treated groups were higher than those observed in the control group, but the difference was statistically significant when comparing the reference versus control animals on day 3 (p=0.041) and similar versus control and similar versus reference (p=0.008) on day 6. Latencies for first seizures were not significantly different on any of the experimentation days (Table 5). Regarding latencies for first manifestations, differences were statistically significant on days 3 and 6, but alternating superiority in the protective effect when compared to the control: higher for reference on day 3 (p=0.017), but higher for non-reference on day 6 (p=0.007) (Table 5).

Table 4. Acute pentylenetetrazole-induced seizure model

	Group						p-value
	Control	Reference	Generic 1	Generic 2	Similar 1	Similar 2	
Any manifestation	6 (100.0)	5 (83.3)	6 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)	0.399
At least 1 seizure	6 (100.0)	5 (83.3)	6 (100.0)	6 (100.0)	6 (100.0)	5 (83.3.0)	0.516
Death	6 (100.0)	3 (50.0)	5 (83.3)	6 (100.0)	3 (50.0)	4 (66.7.0)	0.133

Values are presented as number (%), chi-square test or likelihood ratio.

Table 5. Kindling model. Latencies to first manifestation and first seizure

	Group						p-value*
	Control	Reference	Generic 1	Generic 2	Similar 1	Similar 2	
First manifestation							
Day 1	116 (110-1,800)	1,055 (30-1,800)	1,044 (251-1,800)	235 (116-1,800)	164.5 (97-1,800)	184 (120-223)	0.629
Day 2	1,800 (1,800-1,800)	1,800 (1,800-1,800)	1,800 (1,800-1,800)	1,800 (1,800-1,800)	1,800 (1,800-1,800)	1,800 (1,800-1,800)	0.820
Day 3	80 (52-132)	389 (219-1,800)	51.5 (22-106)	296.5 (197-1800)	245 (131-370)	107 (82-259)	0.005†
Day 4	115 (49-147)	118 (87-1,800)	1,108.5 (200-1800)	77 (52-98)	164.5 (102-237)	135.5 (77-273)	0.019‡
Day 5	30.5 (10-73)	103 (88-132)	86.5 (78-161)	108 (33-131)	86.5 (21-140)	75 (51-110)	0.285
Day 6	74 (25-84)	80 (56-97)	95 (77-131)	105.5 (90-153)	127 (105-147)	177 (102-1,800)	0.065
First seizure							
Day 1	1,800 (1,800-1,800)	1,800 (1,800-1,800)	1,800 (1,800-1,800)	1,800 (1,800-1,800)	1,800 (1,800-1,800)	1,800 (1,800-1,800)	1.000
Day 2	1,800 (1,800-1,800)	1,800 (1,800-1,800)	1,800 (1,800-1,800)	1,800 (1,800-1,800)	1,800 (1,800-1,800)	1,800 (1,800-1,800)	1.000
Day 3	975 (128-1,800)	1,800 (473-1,800)	1,800 (567-1,800)	1,800 (1,800-1,800)	1,800 (1,800-1,800)	1,099.5 (347-1,800)	0.499
Day 4	214 (173-1,800)	220 (100-1,800)	1,800 (1,800-1,800)	960 (100-1,800)	319 (102-1,800)	1,800 (1,800-1,800)	0.234
Day 5	1,800 (1,800-1,800)	972.5 (125-1,800)	223.5 (181-1,800)	1,800 (150-1,800)	190.5 (151-1,800)	956.5 (98-1,800)	0.507
Day 6	1,108.5 (238-1,800)	119 (66-424)	164 (118-1,800)	1,800 (160-1,800)	131 (107-592)	1,018.5 (108-1,800)	0.188

Values are presented as median and interquartile range in parentheses.
*Using the Kruskal-Wallis test with Dunn's post-test there was p<0.05.
†Generic 1 vs. reference.
‡Generic 1 vs. generic 2.

Copyright ⓒ 2022 Korean Epilepsy Society

Discussion

Brands of generics and similar were chosen using a commercial performance criterion to enhance the relevance of the findings for the community. The acquisition of the drugs in the conventional trade intended to mimic the exposure of real patients to the variety of brands. Furthermore, manufacturers were not made aware of this experiment to exempt the study form conflicts of interest with any of the tested brands.

In general, the results were compatible with true therapeutic equivalence among the five brands. However, statistical significance in a minority of variables and comparisons suggests that real differences concerning therapeutical properties still exist. A few aspects inherent to the model and the experiment design might also have contributed to the findings. Although the PTZ model in mice has been used in the developmental stages of TPM,[8] it is not clear whether there is an influence of the human formulation on the therapeutic effect observed in this trial. The procedures for administering TPM and PTZ followed a recently published protocol,[7] but variations, such as TPM dose a time interval between doses of TPM and PTZ, may also have influenced the effects observed.

Another possible factor was the reduced group size and consequent limited statistical power. Nevertheless, the animal model proved to be a suitable alternative for comparative studies between brands since it offers some advantages: same morbid condition, genetic homogeneity, matched distribution of age and sex, besides objective, reliable and reproducible outcomes. Despite the limitations, the experiment met the expectation of overcoming the previously mentioned methodological aspects inherent to studies in humans. The findings support the hypothesis of therapeutic equivalence between the reference, generics, and similar forms of TPM. Other outlines of the PTZ model or even other experimental models may offer more accuracy for the purpose of comparing different brands of ASMs in an alternative and translational setting.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Acknowledgements

George Linard Silva Malveira: Investigation, Conceptualization, Methodology, Writing-original draft, review&editing. Sacha Aubrey Alves Rodrigues Santos: Methodology, Writing-original draft, review&editing. Gerlânia de Oliveira Leite: Methodology, Writing-original draft. Adriana Rolim Campos: Conceptualization, Methodology, Writing-original draft, review, editing, Supervision, Project administration, Funding acquisition.

References

1. Fisher RS, Acevedo C, Arzimanoglou A, et al. ILAE official report: a practical clinical definition of epilepsy. *Epilepsia* 2014;55:475-82.
2. Burakgazi E, French JA. Treatment of epilepsy in adults. *Epileptic Disord* 2016;18:228-39.
3. Yacubian EMT, Contreras-Caicedo G, Ríos-Pohl L. *Tratamento Medicamentoso das Epilepsias.* São Paulo: Leitura Médica Ltda, 2014.
4. Yacubian EMT. Medicamentos genéricos no tratamento das epilepsias. Uma Reflexão. *J Epilepsy Clin Neurophysiol* 2007;13:127-30.
5. Holtkamp M, Theodore WH. Generic antiepileptic drugs-Safe or harmful in patients with epilepsy? *Epilepsia* 2018;59:1273-81.
6. Odi R, Franco V, Perucca E, Bialer M. Bioequivalence and switchability of generic antiseizure medications (ASMs): a re-appraisal based on analysis of generic ASM products approved in Europe. *Epilepsia* 2021;62:285-302.
7. Shimada T, Yamagata K. Pentylenetetrazole-induced kindling mouse model. *J Vis Exp* 2018;(136):56573.
8. Shank RP, Gardocki JF, Vaught JL, et al. Topiramate: preclinical evaluation of structurally novel anticonvulsant. *Epilepsia* 1994;35:450-60.
9. Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol* 1972;32:281-94.