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In vivo Microdialysis and Liquid Chromatography/Thermospray Mass Spectrometry of the Novel Anticonvulsant 2,3 : 4,5-Bis-*O*-(1-methylethylidene)-β-D-fructopyranose Sulfamate (Topiramate) in Rat Brain Fluid

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The concentration of a novel anticonvulsant, 2,3 : 4,5-bis-*O*-(1-methylethylidene)-β-D-fructopyranose sulfamate (topiramate), was determined in the extracellular fluid of rat brain by *in vivo* microdialysis combined off-line with liquid chromatography/thermospray mass spectrometry. A microdialysis probe was stereotaxically implanted in the nucleus accumbens region of the rat brain. The maximum concentration of topiramate in the brain dialysate for a dose of 50 mg kg^{-1} i.v. was ~10 μM and occurred 45 min post-injection. The detection limit of topiramate in the extracellular fluid of rat brain was in the 0.1 μM range using selected ion monitoring techniques. The base peak, which was the ammonium adduct ion $[M + NH_4]^+$, was used for detection. An internal standard of d_{12}-labeled topiramate was utilized for quantitation by isotope dilution analysis. © **1998 John Wiley & Sons, Ltd.**

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KEYWORDS: anticonvulsant; topiramate; *in vivo* microdialysis; mass spectrometry

INTRODUCTION

Microdialysis sampling is a flexible technique for the study of *in vivo* pharmacokinetics of the extracellular fluid in the brain.[1–4] In recent years, microdialysis with mass spectral detection has emerged as an important tool in biochemical research.[5–20] Interest in 2,3 : 4,5-bis-*O*-(1-methylethylidene)-β-D-fructopyranose sulfamate (topiramate) as a potent anticonvulsant prompted us to study the extracellular fluid of rat brain by *in vivo* microdialysis combined off-line with liquid chromatography/thermospray mass spectrometry (LC/TSP-MS).[21] Topiramate and its metabolites are subject to thermal decomposition during gas chromatographic/mass spectrometric analyses[22] and, since these compounds have no significant UV absorption or fluorescence, LC/TSP-MS analysis is the preferred choice. Electron ionization, thermospray ionization and tandem mass spectrometric studies of topiramate have been reported previously.[23–25]

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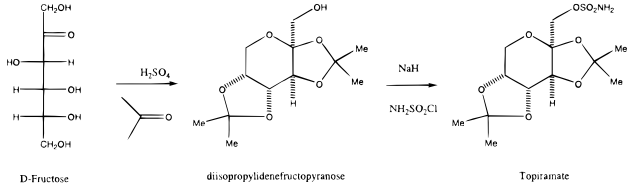
EXPERIMENTAL

Materials

The carbohydrate 2,3 : 4,5-bis-*O*-(1-methylethylidene)-β-D-fructopyranose sulfamate (topiramate, $C_{12}H_{21}O_8NS$, M_r 339) (Fig. 1) and the internal standard 2,3 : 4,5-bis-*O*-(1-methylethylidene)-d_{12}-D-fructopyranose sulfamate (topiramate-d_{12}, $C_{12}H_9D_{12}O_8NS$, M_r 351) were synthesized as reported previously.[21] Briefly, D-fructose and acetone were reacted under acidic conditions to yield diisopropylidenefructopyranose. To a cold solution of diisopropylidenefructopyranose was added sodium hydride. After the mixture had been stirred, sulfamoyl chloride was added to yield topiramate. Topiramate-d_{12} was synthesized by using acetone-d_6 in place of acetone. All solvents were of HPLC grade and used as purchased. Triply distilled water adjusted to pH 7.4 with NaOH was used as the dialysate solution.



D-Fructose diisopropylidenefructopyranose Topiramate

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Animals and microdialysis procedure

Male Wistar rats were purchased (Charles River) and maintained for at least 1 week at 21–23 °C) on a 12 h–12 h day–night light cycle. The rats had access to rat chow and water *ad libitum*. Prior to the experiment, rats at a body mass of 280–300 g were fasted overnight. Each rat was anesthetized by administering a 1% aqueous chloral hydrate solution (4 ml kg^{-1} i.p.). A polyethylene catheter was inserted into the jugular vein for later administration of topiramate. The rat was placed in a stereotaxic apparatus for later placement of the microdialysis probe. The chloral hydrate solution was administered as needed to maintain the desired level of anasthesia (typically 1 ml kg^{-1} at 1 h intervals). The skull was exposed and the location for placement of the microdialysis probe was identified (from Bregma: +2.0 AP, −1.0 ML) and a small burr hole was created. The microdialysis probe (Carnegie Medicin CMA 12; tip diameter 0.5 mm, length 3.0 mm) was stereotaxically implanted in the nucleus accumbens (8.5 mm from the dura). These procedures were reviewed and approved by the institutional IACUC and adhered to the USPHS Guide for the Care of Laboratory Animals.

Topiramate was administered i.v. at 50 mg kg^{-1} in ~1 ml of water. A CMA 100 pump (1 ml syringe) was used to deliver triply distilled water (pH 7.4). The flow-rate was maintained very low (2 µl min^{-1}) to minimize effects of the dialysis fluid on the ionic strength of the extracellular fluid bathing the tip of the probe. Dialysate fractions were collected for 15 min periods for 5 h. Dialysate samples were analyzed without further sample preparation. The efficiency of the recovery of the analyte was determined by removing the microdialysis probe from the brain and immersing it in a beaker containing standard solutions of 1 and 2 µM concentrations of topiramate. The ratios of the LC/TSP-MS signal intensity of the dialysate *vs.* the standard solution were determined. These ratios (10.3 and 7.4%, respectively) were used to estimate the average recovery (8.9%) of the probe. Stock solutions of topiramate in the range 0.07–3 µM were used to generate a calibration graph. The ratio of the stock solution to the internal standard produced a straight line with $r = 0.9986$.

LC/TSP-MS conditions

Thermospray analyses were performed using a Finnigan MAT TSQ-70 mass spectrometer with a TSP-2 thermospray ion source. The following conditions were used: source temperature, 218 °C; vaporizer temperature, 112 °C; mass range, 356–372 Da; multiplier, 2000 V; and scan rate, 0.5 s per scan. A Varian Model 9010 liquid chromatographic pump in series with a Chromegabond MC8 column (15 cm × 4.6 mm i.d., 5 µm film thickness) was used to deliver the mobile phase (30:70 MeOH–0.05 M ammonium acetate) at a flow-rate of 1.5 ml min^{-1}. A 40 µl sample, which included 20 µl of dialysate and 20 µl (4 ng) of the internal standard, was injected. The retention time for topiramate in the combined samples was 1.6 min.

RESULTS AND DISCUSSION

A typical thermospray mass spectrum of a 0.45 µM topiramate dialysate sample is shown in Fig. 1. This mass spectrum corresponds to a 40 µl sample containing 6 ng

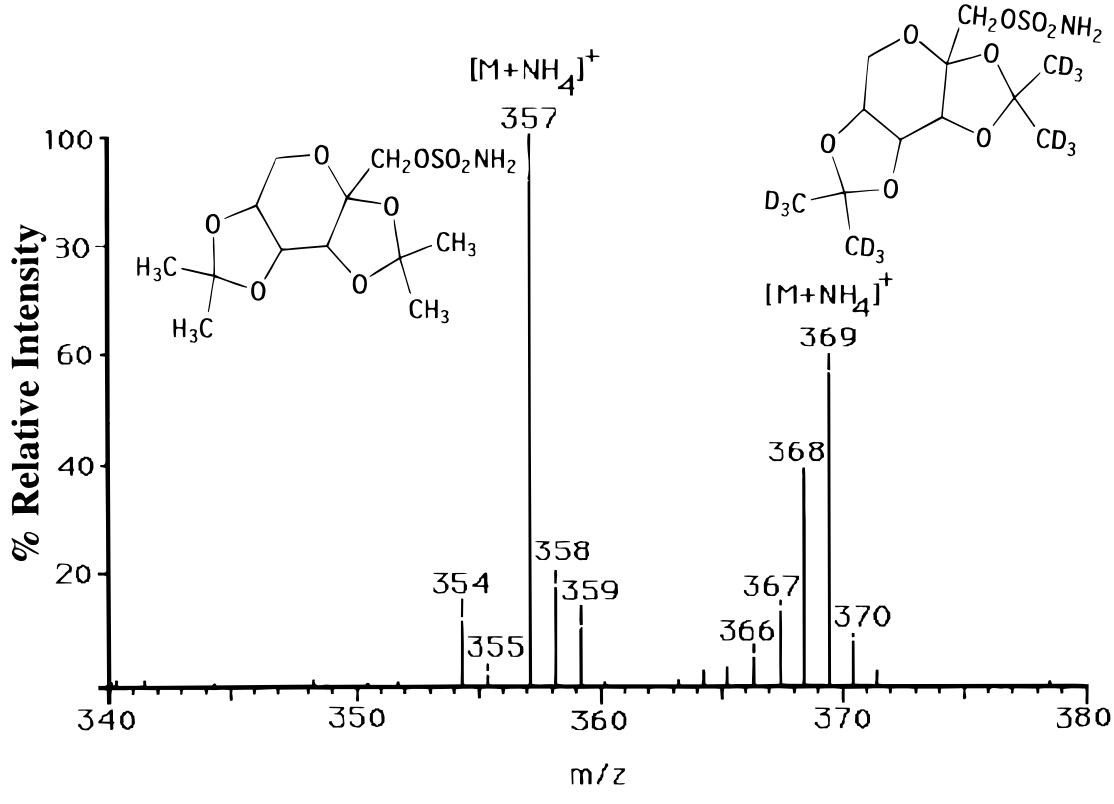

Figure 1. Thermospray mass spectrum and structure of topiramate ($[M + NH_4]^+$, *m/z* 357) and topiramate-d_{12} ($[M + NH_4]^+$, *m/z* 369).

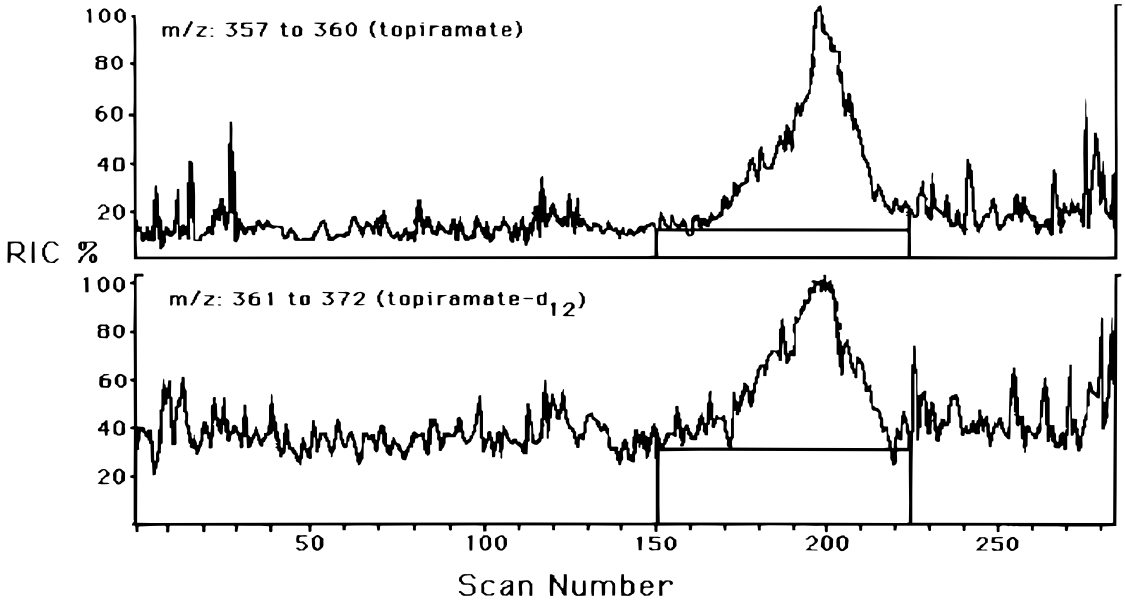

Figure 2. Selected ion chromatogram of topiramate cations of *m/z* 357–360 and the internal standard topiramate-d_{12} cations of *m/z* 361–372.

of topiramate and 4 ng of topiramate-d_{12}. The peaks at *m/z* 357 and 369 are the ammonium adduct cations ($[M + NH_4]^+$) of the non-deuterated ($M_r = 339$) and deuterated ($M_r = 351$) topiramate species. Fragmentation of the non-deuterated and deuterated topiramate was minimal, and was ideal for quantification studies. The peak at *m/z* 354 was determined to be a background cation since it appeared throughout the LC/TSP-MS analysis. This impurity is possibly trioctylamine. The peaks at *m/z* 358, 359, 370 and 371 are the carbon and sulfur isotopic ions of non-deuterated and deuterated topiramate. The peaks between *m/z* 361 and 368 represent various deuterated species of the internal standard. These cations arose from the incomplete labeling of the topiramate-d_{12} internal standard. These cations did not interfere with the assay since they are well separated from topiramate.

The peaks at *m/z* 357–360 were used for quantification of topiramate and peaks at *m/z* 361–372 were used for the internal standard. A selected ion chromatogram representing these regions is shown in Fig. 2. Co-elution of topiramate and the internal standard occurred

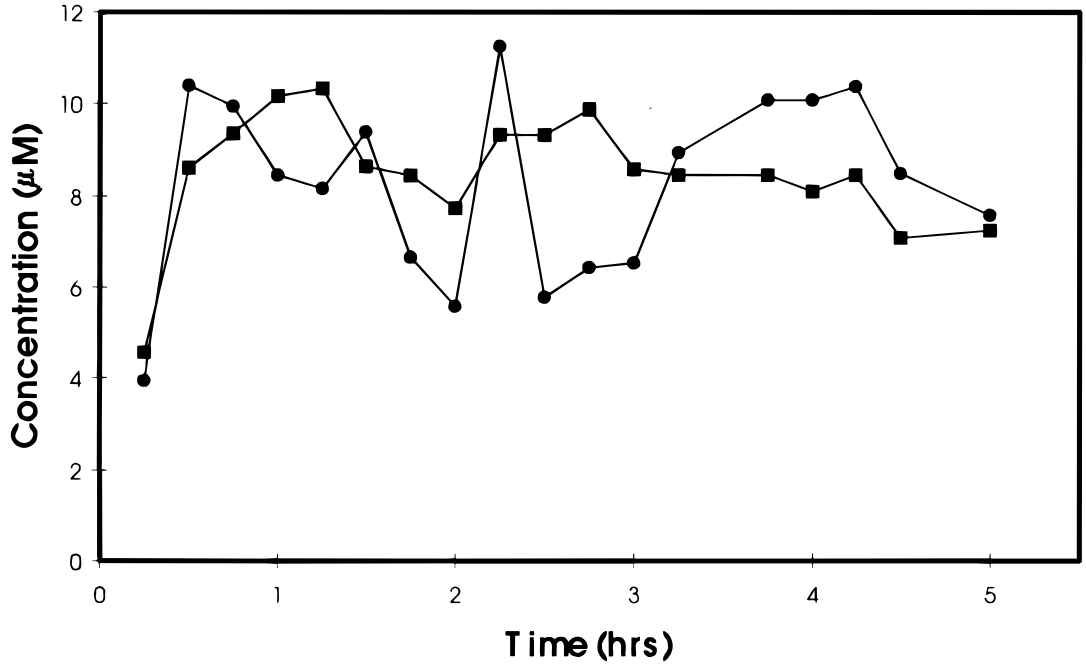

Figure 3. Concentration profile of extracellular rat brain fluid levels of topiramate dosed at 50 mg kg^{-1} i.v. over a 5 h collection period for two rats.

between scan numbers 150 and 225. The maximum signal strength occurred at scan number 200 or a retention time of 1.6 min. The detection limit of topiramate in the LC/TSP-MS analysis was ~0.1 μM (20 μl injected) using selected ion monitoring. This corresponded to a detection limit of 700 pg with a signal-to-noise ratio 5:1. The sensitivity obtained was sufficient for studying the time course of the topiramate concentration in rat brain fluid when dosed at 50 mg kg^{-1}.

The results of the LC/TSP-MS analysis of dialysates obtained from two rats as a function of time are shown in Fig. 3. The concentration axis was corrected for probe recovery losses. The extracellular concentration of topiramate peaked at ~10 μM at ~45 min after administration and ranged between 6 and 10 μM during the 5 h collection period. These data indicate that topiramate equilibrates quickly across the rat blood–brain barrier and brain fluid levels in the micromolar range are maintained over an extended period.

It is of importance to note that in this study we utilized triply distilled water as the perfusion medium instead of an artificial cerebrospinal fluid (CSF) (130 mM NaCl, 2.65 mM KCl, 1.4 mM $CaCl_2$, 2.0 mM $MgCl_2$ and 300 μM ascorbic acid in distilled water). We used triply distilled water instead of CSF to avoid sample clean-up prior to LC/TSP-MS analyses. If the salt concentration balance in the extracellular space proximal to the microdialysis probe is compromised, it is possible to alter the analytical data. We maintained a very low flat-rate (2 μl min^{-1}) to minimize effects of the dialysis fluid on the ionic strength of the extracellular fluid bathing the tip of the probe. The absolute bioavailability of a single oral 30 mg kg^{-1} gavage dose of topiramate is ~100% in male rats.[26] Recently, tissue distribution studies of [^{14}C]topiramate in male rats, administered oral solution doses of 20 mg kg^{-1}, have been completed.[27] At 1 h following dosing, the mean concentration ($n = 4$) of radioactive topiramate in the brain was 11 μM and at 6 h it was 4 μM. From our work, the mean topiramate concentration ($n = 2$) utilizing microdialysis coupled with LC/TSP-MS at 1 h was 9 μM and at 5 h it was 7 μM. These results suggest that our microdialysis sampling procedure, at least for topiramate, was not significantly compromised by the use of triply distilled water as the perfusion medium.

REFERENCES

1. B. H. C. Westerink, G. Damsma, H. Rollema, J. B. De Vries and A. S. Horn, *Life Sci*. **41**, 1763 (1987).
2. W. H. Church and J. B. Justice, Jr, *Anal. Chem*. **59**, 712 (1987).
3. T. E. Robinson and I. Q. Whishaw, *Brain Res*. **450**, 209 (1988).
4. A. P. Newton and J. B. Justice, Jr, *Anal. Chem*. **66**, 1468 (1994).
5. O. Ben-Yoseph, D. M. Camp, T. E. Robinson and B. D. Ross, *J. Neurochem*. **64**, 1336 (1995).
6. P. E. Andren, S.-N. Lin and R. M. Caprioli, in *Mass Spectrometry*, edited by D. M. Desiderio, Vol. 2, p. 237. Plenum Press, New York (1994).
7. K. Dix, L. J. Detering, K. B. Tomer and L. T. Burka, *J. Pharm. Sci*. **83**, 1622 (1994).
8. P. Michelsen and G. Pettersson, *Rapid Commun. Mass Spectrom*. **8**, 517 (1994).
9. M. Kajita, N. Mitsuharu, T. Niwa, W. Maruyama, D. Nakahara, N. Takeda, H. Yoshizumi, A. Tatematsu, K. Watanabe, M. Naoi, *et al*., *J. Chromatogr. B* **654**, 263 (1994).
10. D. H. Callaghan, J. A. Yergey, P. Rousseau and P. Masson, *Pulmon. Pharmacol*. **7**, 35 (1994).
11. R. M. Caprioli and M. J. F. Suter, *Int. J. Mass Spectrom. Ion Processes* **118–119**, 449 (1992).
12. L. J. Deterding, K. Dix, L. T. Burka and K. B. Tomer, *Anal. Chem*. **64**, 2636 (1992).
13. M. C. Linhares and P. T. Kissinger, *Anal. Chem*. **64**, 2831 (1992).
14. T. Niwa, W. Maruyama, D. Nakahara, N. Takeda, H. Yoshizumi, A. Tatematsu, A. Takahashi, P. Dostert, M. Naoi and T. Nagatsu, *J. Chromatogr*. **578**, 109 (1992).
15. S. Y. Chang, T. A. Moore, L. L. Devaud, L. C. E. Taylor and E. B. Hollingsworth, *J. Chromatogr*. **562**, 111 (1991).
16. M. R. Marien and J. W. Richard, *J. Neurochem*. **54**, 2016 (1990).
17. R. M. Caprioli, *Anal. Chem*. **62**, 477A (1990).
18. S. D. Menacherry and J. B. Justice, Jr, *Anal. Chem*. **62**, 597 (1990).
19. R. M. Caprioli and S.-N. Lin, *Proc. Natl. Acad. Sci. USA* **87**, 240 (1990).
20. L. C. Nicolaysen, H. T. Pan and J. B. Justice, Jr, *Brain Res*. **456**, 317 (1988).
21. B. E. Maryanoff, S. O. Nortey, J. F. Gardocki, R. P. Shank and P. Dodgson, *J. Med. Chem*. **30**, 880 (1987).
22. J. A. Masucci and G. W. Caldwell, in *Modern Practice of Gas Chromatography*, edited by R. L. Grob, p. 323. Wiley, New York (1995).
23. G. W. Caldwell, K. L. Sorgi, L. Scott, B. E. Maryanoff, C. A. Maryanoff, J. A. Masucci, S. O. Nortey, W. R. Sisco, A. Micheel and C.-Y. Ko, *Org. Mass Spectra* **24**, 1051 (1989).
24. J. A. Masucci, W. J. Jones and G. W. Caldwell, in *Proceedings of the 40th ASMS Conference on Mass Spectrometry and Allied Topics*, Washington, DC, 1992, p. 1869.
25. J. A. Masucci, M. E. Ortegon, W. J. Jones, R. P. Shank and G. W. Caldwell, in *Proceedings of the 41th ASMS Conference on Mass Spectrometry and Allied Topics*, San Francisco, CA, 1993, p. 832a.
26. A. J. Streeter, P. L. Stahle, J. F. Hills, P. N. Lucas, L. A. McKown, S. L. Nese, D. M. Desai, M. L. Holland, J. F. Pritchard and A. R. Takacs, *Pharm. Res*. **11**, S-372 (1994).
27. A. J. Streeter, personal communication.