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Non-aqueous capillary electrophoresis of tamoxifen and its acid hydrolysis products

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Abstract

Tamoxifen and its acid hydrolysis products were separated and tentatively identified by non-aqueous capillary electrophoresis with thermooptical absorbance and electrospray ionization mass spectrometry. Acid hydrolysis is a convenient method of generating tamoxifen degradation products. The parent compound and seven hydrolysis products were separated in 9 min.

Keywords: Non-aqueous capillary electrophoresis; Thermooptical absorbance detection; Detection, electrophoresis; Tamoxifen

1. Introduction

Tamoxifen [*trans*-1-(4-β-dimethylaminoethoxyphenyl)-1,2-diphenylbut-1-ene], Fig. 1, is a non-steroid antiestrogen that has been widely used for treatment of breast cancer over the last 25 years [1,2]. Tamoxifen has recently been approved by the Food and Drug Administration (FDA) of the USA for reducing breast cancer risk [3]. However, it may increase the risk of endometrial cancer and blood clots that could lead to a stroke [4–6]. Therefore, monitoring of tamoxifen and its metabolites is important. Tamoxifen and some of its metabolites are described in Table 1. The parent compound and metabolites X and B are the main species in serum and saliva.

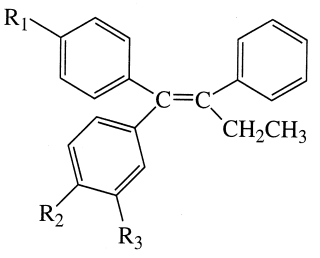

Fig. 1. Structure of tamoxifen. See Table 1 for identity of substituents.

Qualitative and quantitative analysis of small amounts of tamoxifen and metabolites in complex

Table 1
Structural information and molecular masses of tamoxifen and four metabolites (see Fig. 1)

Compound	R_1	R_2	R_3	M_r
Tamoxifen	$(CH_3)_2N(CH_2)_2O$	H	H	371
Metabolite B	$(CH_3)_2N(CH_2)_2O$	OH	H	387
Metabolite D	$(CH_3)_2N(CH_2)_2O$	OH	OH	403
Metabolite E	HO	H	H	300
Metabolite X	$CH_3N(CH_2)_2O$	H	H	357

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biological matrices requires highly selective and sensitive analytical techniques. Tamoxifen and its analogs extracted from biological tissues are conventionally analyzed by high-performance liquid chromatography (HPLC). The HPLC effluent is subsequently UV irradiated to convert the compounds to their phenanthrenes, and finally measured by fluorescence detection [7–9]. This technique is sensitive but time-consuming (over 50 min) and uses large volumes of organic solvent. It is therefore not practical for routine tests in the clinical laboratory. Ng et al. [10] reported a 9-min non-aqueous CE separation of tamoxifen and three metabolites. The HPLC and CE methods do not give molecular information and are useful only when standards are available. Capillary gas chromatography with mass spectrometry (GC–MS) has also been used for analysis of tamoxifen [11–13]. However, GC–MS requires chemical derivatization before analysis, which may complicate identification of new metabolites. Lu et al. [14] reported an on-line CE–electrospray ionization mass spectrometry (ESI-MS) method for analysis of tamoxifen and six metabolites. However, this technique uses surfactants to achieve separation, resulting in reduced ionization efficiency during electrospray and generating significant contamination of the vacuum chamber.

We report an analytical method to separate structural isomers of tamoxifen and its hydroxylation products using non-aqueous CE. Because tamoxifen metabolites are not commercially available, we developed a simple procedure to produce hydroxylated tamoxifen metabolites by incubating tamoxifen with 0.1 *M* HCl. Not all of these hydroxylated products may be produced in vivo. We refer to the compounds as degradation products to distinguish them from the product of metabolic action.

We used non-aqueous CE with both thermooptical absorbance detection (TOAD) and ESI-MS detection to study the tamoxifen degradation products. Although surfactant based separation has been used for tamoxifen, this study is the first example of non-aqueous CE separation of tamoxifen degradation products, and is by far the simplest CE–ESI-MS analytical method for these compounds. Non-aqueous CE provides high separation efficiency, selectivity, and requires only small sample volumes for analysis, which is desirable in routine analysis of tamoxifen and degradation products in patients [15].

2. Experimental

2.1. Chemicals and materials

Tamoxifen was purchased from Sigma (St. Louis, MO, USA). A stock solution of tamoxifen (500 μg/ml) was prepared in 100% methanol and working solutions were prepared by dilution with methanol. Ammonium acetate and hydrochloric acid at analytical grade were obtained from ACP Chemicals (Montreal, Canada). HPLC-grade methanol and acetonitrile were purchased from BDH (Toronto, Canada). The fused-silica capillary was purchased from Polymicro Technologies (Phoenix, AZ, USA).

2.2. Instrumentation

The CE–TOAD system has been described in detail elsewhere [16,17]. A 45-cm (effective length 39 cm)×50 μm I.D.×180 μm O.D. was used. The running buffer was composed of 20 m*M* ammonium acetate in methanol–acetonitrile (7:3, v/v) for the separation. The applied voltage across the capillary was 12 kV. Sample injection was performed at 5 kV for 5 s.

The CE–ESI-MS system consisted of a laboratory-made CE instrument directly interfaced to a PE Sciex API 100 single quadrupole mass spectrometer (Concord, Canada) through an ion spray unit [18]. The CE capillary was inserted into the electrospray tube with about 0.5–1.0 mm extending outside of the spray tip. The coaxial sheath flow liquid was 100% methanol and was introduced by a syringe pump (Harvard Apparatus, Southnatick, MA, USA) at a flow-rate of 2 μl/min. Capillaries for both CE and sheath flow were 55 cm long with the same dimensions as those described above. CE separation was carried out with the applied voltage of 20 kV at the injection end. Electrospray ionization conditions were optimized as described in the previous study [18]. The electrospray conditions included ion spray voltage (IS) 3500 V, orifice voltage (OR) 10 V, ring voltage (RNG) 260 V, and nebulizer gas flow (NEB) 1 equivalent to 0.17 l/min. Total ion electropherograms were acquired using the scan mode (200–800 amu) at scan speed 1.21 s, step size 1 amu, and dwell time 1.5 ms. The mass spectrometer was operated under vacuum at $0.9 \cdot 10^{-5}$ Torr (1 Torr=133.322 Pa). The ion detector voltage was constant at 2.3 kV.

2.3. Incubation of tamoxifen in 0.1 M HCl and sample preparation

Acid-mediated tamoxifen degradation experiments were carried out by incubation in 0.1 *M* HCl. Tamoxifen solutions at 0, 1, 5, 10, and 12.5 μg/ml were prepared by adding appropriate amounts of 500 μg/ml stock methanol solution into 2 ml of 0.1 *M* HCl. Another set of tamoxifen solutions was prepared in water as a control. All of these solutions were kept in an incubator at 37°C. Aliquots of 50 μl were periodically withdrawn from each solution and quickly dried using vacuum centrifugation. The dried residues were kept at 4°C until analysis. For non-aqueous CE, ESI-MS and CE–ESI-MS analysis, the dried residue was redissolved in 50 μl of methanol.

3. Results and discussion

Hydroxylated tamoxifen degradation products were generated by incubation of tamoxifen with 0.1 *M* hydrochloric acid. This acid mixture mimics stomach acidity and provides a useful source of degradation products, particularly those that are not commercially available. As we show below, a set of seven degradation products was generated in addition to the parent compound.

To investigate the separation efficiency of tamoxifen and its degradation products using non-aqueous CE, we optimized the concentration of ammonium acetate and the ratio of acetonitrile to methanol in the CE running buffer. We found that a CE buffer composed of 20 m*M* ammonium acetate and acetonitrile–methanol (3:7, v/v) provided baseline separation of tamoxifen and its degradation products without the use of a surfactant. A typical non-aqueous CE separation of the tamoxifen samples detected by TOAD and ESI-MS is shown in Fig. 2. Non-aqueous CE–TOAD detected tamoxifen and five degradation products (Fig. 2a). When we analyzed the same sample using the on-line non-aqueous CE–ESI-MS technique, tamoxifen and seven products were separated and detected (Fig. 2b). In Fig. 2a, peaks 3 and 7 were not detected by TOAD. In Fig. 2b, peaks 3, 6 and 7 were barely detected in the total ion electropherogram; selected ion monitoring revealed the components.

The tamoxifen standard comigrated with the first

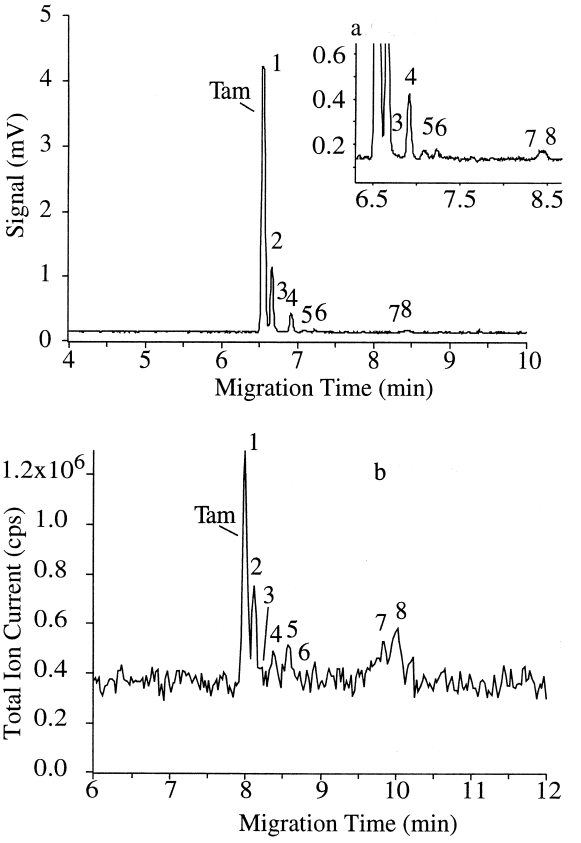

Fig. 2. Analysis of a methanol extract from a tamoxifen sample (5 μg/ml) after incubation with 0.1 *M* HCl at 37°C for 72 days by (a) CE–TOAD and (b) CE–ESI-MS, presented as a TIC trace. The inset shows an expanded view of the TOAD data.

peak from the sample. A simple one-point normalization procedure was used to normalize the two electropherograms based on the tamoxifen migration time [19]. The corrected migration times of the acid degradation products from CE–TOAD matched those from CE–ESI-MS.

CE–TOAD was used to monitor the changes of tamoxifen in acid at 37°C. We observed that peak 2 slowly increased as incubation time progressed (data not shown). Peaks 3 and 7 were not detected. Peak 4 was detected only after 7 days incubation.

We confirmed the purity of the peaks using CE–ESI-MS by examining the mass spectra across the entire peak. Fig. 3 shows the ESI mass spectra of tamoxifen and the other product peaks. Structural and molecular mass information of some of these compounds are described in Table 1. Peak 1 gave the

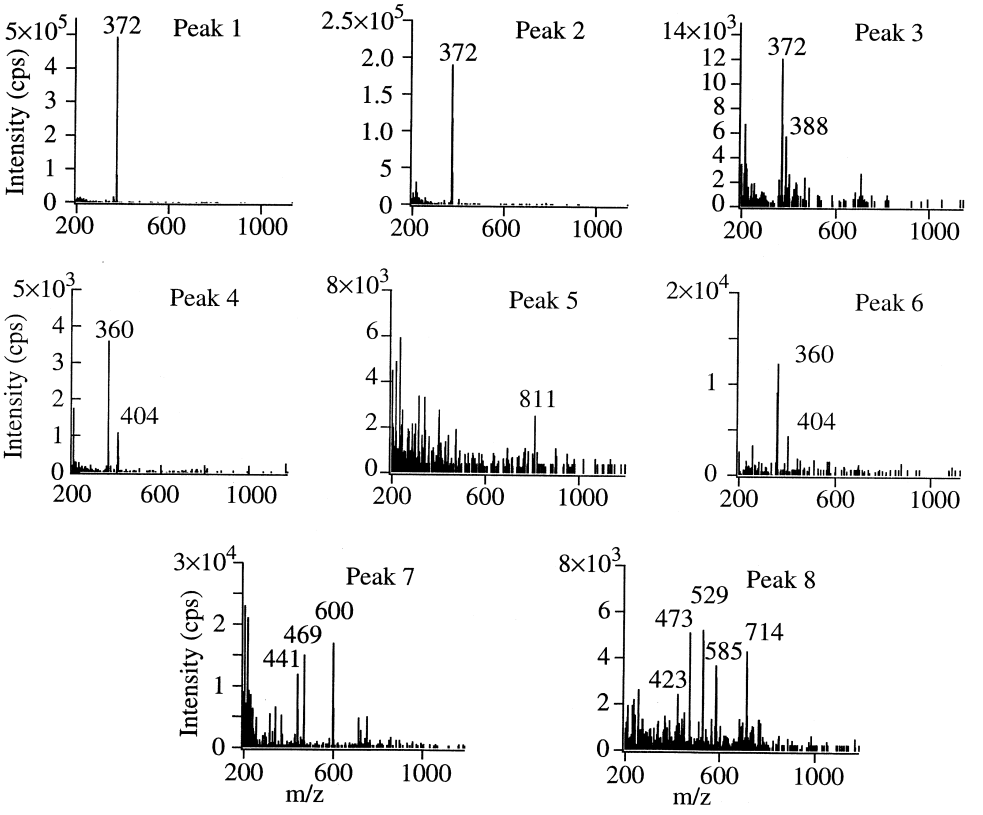

Fig. 3. Mass spectra of the eight peaks (from Fig. 2b) detected by CE–ESI-MS.

molecular ion at 372 $(M+1)^+$ with no fragment ions under these conditions. Peak 1 is the parent compound (tamoxifen). Peak 2 had an identical mass spectrum to that of tamoxifen, suggesting that it may be a structural isomer. The mass spectrum (*m*/*z* 388 and 372) of peak 3 suggests that it might be monohydroxytamoxifen. Peak 4 contained the major mass ions at *m*/*z* 404 $(M+1)^+$and 360 $(M+H-44)^+$, which supports tentative identification of peak 4 as dihydroxytamoxifen. The fragment ion *m*/*z* 360 may be due to loss of $N(CH_3)_2$. The mass spectrum did not allow us to locate the positions of the two hydroxy groups. Peak 5 did not give a useful spectrum to indicate whether this peak is a tamoxifen degradation product or a contaminant. The spectrum of peak 6 was similar to that of peak 4, suggesting that they are structural isomers of dihydroxytamoxifen. Peaks 7 and 8 have molecular ions $(M+1)^+$ at *m*/*z* 600 and 714, respectively, and they are tentatively identified as dimers of metabolite E (M_r 300) and metabolite X (M_r 357), respectively. Comparing intensities of peaks showed that the major acid hydrolysis products were the tamoxifen isomer (possible E form, peak 2) and dihydroxytamoxifen (peak 4). This nonenzymatic process is different from the enzymatic transformation where the major components are tamoxifen and 4-hydroxytamoxifen [20].

4. Conclusions

We have demonstrated that non-aqueous CE can separate tamoxifen and some of its degradation products without the use of surfactants, which facilitates coupling of CE with ESI-MS. On-line CE–MS gives both the migration time and a mass spectrum, which aids in the identification of the degradation products.

Acknowledgements

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References

[1] B.E. Henderson, R.K. Ross, M.C. Pike, Science 259 (1993) 633.
[2] V.C. Jordan, Oncology 11 (2 Suppl. 1) (1997) 7.
[3] V.C. Jordan, J. Nat. Cancer Inst. 90 (1998) 967.
[4] V.C. Jordan, Hospital Pract. 33 (1998) 59.
[5] V.J. Assikis, P. Neven, V.C. Jordan, I. Vergote, Eur. J. Cancer 32A (1996) 1464.
[6] L.M. Sargent, Y.P. Dragan, C. Sattler, N. Bahnub, G. Sattler, P. Martine, A. Cisneros, J. Mann, S. Thorgeirsson, V.C. Jordan, H.C. Pitot, Carcinogenesis 17 (1996) 1051.
[7] R.R. Brown, R. Bain, V.C. Jordan, J. Chromatogr. 272 (1983) 351.
[8] M. Nieder, H. Jaeger, J. Chromatogr. 413 (1987) 207.
[9] S. Chamart, M. Hanocq, M. Helson, J. Chromatogr. B 496 (1989) 365.
[10] C.L. Ng, H.K. Lee, S.F.Y. Li, J. Liq. Chromatogr. 17 (1994) 3847.
[11] C.P. Daniel, S.J. Gaskell, H. Bishop, R.I. Nicholson, J. Endocrinol. 83 (1979) 401.
[12] P. Daniel, S.J. Gaskell, H. Bishop, C. Campbell, R.I. Nicholson, Eur. J. Cancer Clin. Oncol. 17 (1981) 1183.
[13] J. Girault, B. Istin, J.B. Fourtillan, Biol. Mass Spectr. 22 (1993) 395.
[14] W. Lu, G.K. Poon, P.L. Carmichael, R.B. Cole, Anal. Chem. 68 (1996) 668.
[15] J.M. Sanders, L.T. Burka, M.D. Shelby, R.R. Newbold, M.L. Cunningham, J. Chromatogr. B 695 (1997) 181.
[16] K.C. Waldron, N.J. Dovichi, Anal. Chem. 64 (1992) 1396.
[17] X.-F. Li, C.-S. Liu, P. Ross, E.B. Hansen Jr., C.E. Cerniglia, N.J. Dovichi, Electrophoresis 19 (1998) 3178.
[18] C.-S. Liu, X.-F. Li, D. Pinto, E.B. Hansen Jr., C.E. Cerniglia, N.J. Dovichi, Electrophoresis 19 (1998) 3183.
[19] X.F. Li, H. Ren, X. Le, M. Qi, I.D. Ireland, N.J. Dovichi, J. Chromatogr. A 869 (2000) 375.
[20] B.J.A. Furr, V.C. Jordan, Pharmacol. Ther. 25 (1984) 127.