Title: Local transdermal therapy to the breast for breast cancer prevention and DCIS therapy: Preclinical and Clinical evaluation.

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Supplementary Materials

Method description of quantitation methods by liquid chromatography-tandem mass spectroscopy (LC-MS/MS)

Study 1: (Z) tamoxifen and metabolites [(Z) NDT, (E) 4-OHT, and (Z) 4-OHT, (Z) endoxifen] were determined using LC-MS/MS (API 3000; AB SCIEX, Foster City, CA). A 100 µL aliquot of plasma was mixed with 200 µL of acetonitrile containing 1 ng each of the deuterated analogs of the analytes (TRC, Toronto, Canada). After vortex-mixing for five minutes, the sample was centrifuged at 4°C and 7000 RPM for 10 minutes, the supernatant transferred to an autosampler vial and diluted with 200 µL of water before analysis. Rat mammary tissue specimens were finely cut with surgical scalpels and extracted with 0.2 mL of 50% methanol in water (v/v), 2 mL of saline and 4 mL of acetonitrile containing 20 ng of each of the internal standards. The sample tubes were capped and shaken in a mechanical shaker for 2 h. The resulting extract was centrifuged as above and a 300 µL aliquot diluted with 200 µL of water for analysis.

Chromatographic separation was achieved with a Kinetex PFP 2.6µ column, 50×2.1 mm (Phenomenex, Torrance, CA). The mobile phase was A: 0.1% formic acid in water (v/v) and B: 0.1% formic acid in acetonitrile (v/v). After injection, initial conditions with A at 60% were held for 2 min, decreased to 30% in 4 min and then to 5% (step gradient) for 3 min, returning to initial conditions for 4 min of re-equilibration time. The flow rate was 0.3 ml/min at 25 °C. Retention times for (Z) tamoxifen, (Z) NDT, (E) 4-OHT, (Z) 4-OHT and (Z) endoxifen were 8.6, 7.9, 5.2, 5.7 and 5.6 min, respectively. Total run time was 13 min. A turbo ion spray interface was used as the ion source operating in positive mode. Acquisition was performed in multiple reaction monitoring mode using m/z 372.2 → 72.1, 358.2 → 58.2, 388.2 → 72.1 and 374.2 → 58.2 at low resolution for tamoxifen, NDT, 4-OHT and endoxifen, respectively.

Study 2: Telapristone, and its main metabolite (d-telapristone) were determined using LC-MS/MS (API 3000; AB SCIEX, Foster City, CA). A 100 µL aliquot of rat plasma was mixed with 200 µL of acetonitrile containing 25 ng of the mifepristone (used as internal standard, Sigma-Aldrich, St. Louis, MO). After vortex-mixing for one minute, the sample was centrifuged at 4°C and 7000 RPM for 10 minutes, the supernatant transferred to an autosampler vial and diluted with 400 µL of water before analysis. Rat mammary tissue specimens were finely cut with surgical scalpels and homogenized with acetonitrile using a 1:2 (w/v) ratio. A 200 µL aliquot of the resulting homogenate was extracted with 0.3 mL of water and 1 mL of methyl ter-butyl ether containing 20 ng of mifepristone (RU486). After
vortex-mixing for 5 min, the samples were centrifuged to remove precipitated proteins, stored in a freezer at approximately -70°C for at least an hour, and then the top layer decanted into a clean tube and evaporated under nitrogen flow. The residue was reconstituted in 250 μL of 40% acetonitrile in water, vortex-mixed for ten minutes, and centrifuged again; the resulting supernatant was transferred to an autosampler vial for instrumental analysis.

Chromatographic separation was achieved with a Kinetex C18 2.6 μm column, 50 mm x 2.1 mm (Phenomenex, Torrance, CA). The mobile phase was A: 0.1% formic acid in water (v/v) and B: 0.1% formic acid in acetonitrile (v/v). After injection, initial conditions with A at 70% were held for 0.5 min, decreased to 5% in 2.5 min, held for 3 min at the same conditions and returned to initial conditions in one min for 3 min of re-equilibration time. The flow rate was 0.3 ml/min at 35 °C. Retention times for telapristone and d-telapristone were 2.1 and 1.5 min, respectively. Total run time was 10 min. A turbo ion spray interface was used as the ion source operating in positive mode.

Acquisition was performed in multiple reaction monitoring mode using m/z 506.4 → 134.3 and 492.2 → 120.2 at low resolution for telapristone and d-telapristone, respectively.

**Study 3:** Diclofenac was determined by LC-MS/MS (API 3000; AB SCIEX, Foster City, CA). A 100 μL aliquot of plasma was mixed with 200 μL of acetonitrile containing 5 ng of diclofenac-d4 (internal standard; CDN Isotopes, Quebec, Canada). After vortex-mixing for five minutes, the sample was centrifuged at 4°C and 7000 RPM for 10 minutes and the supernatant transferred to an autosampler vial for analysis. For breast tissue analysis, approximately 100 mg of material was mixed with 400 μL of saline and 1.5 mL of acetonitrile containing 25 ng of internal standard and shaken for 2 h. The resulting extract was centrifuged as above and an aliquot submitted for analysis.

Chromatographic separation was achieved with a BETASIL Phenyl-Hexyl 3μm column, 30×2.1 mm (Thermo Fisher Scientific Inc., Waltham, MA). The mobile phase was A: 0.1% formic acid in water (v/v) and B: 0.1% formic acid in acetonitrile (v/v). After injection, initial conditions with A at 50% were held for 0.5 min, decreased to 5% in 0.5 min and held for 3 min, returning to initial conditions in 0.5 min for 3.5 min of re-equilibration time. The flow rate was 0.3 ml/min at 25 °C. Retention time for diclofenac was 1.4. Total run time was 8 min. A turbo ion spray interface was used as the ion source operating in positive mode. Acquisition was performed in multiple reaction monitoring mode using m/z 296.0 → 214.0 and 298.0 → 216.0.