Fluorothalidomide:
A characterization of maternal and developmental toxicity in rabbits and mice

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SUPPLEMENTAL ONLINE MATERIAL

Fig. S1. Thalidomide (TD)-initiated anomalies in New Zealand white (NZW) rabbit fetuses. Gestational day 29 NZW rabbit fetuses exposed in utero to TD (400 mg/kg, buccally) exhibiting [1] gastroschisis, [2] phocomelia, [3] encephalocele, [4] anencephaly and [5] omphalocele. Gastroschisis, often caused by a defect in the abdominal wall, was observed as loops of the small intestine without a covering membrane protruding outside adjacent to the umbilical cord. Omphalocele, a defect caused by failure of the gut loop to return inside the abdomen during development, was seen as the protrusion of amniotic membrane-covered intestines within the umbilical cord. Gastroschisis and omphalocele are in contrast to an umbilical hernia, which was not observed with TD treatment, and usually is observed as a slight, a skin-covered bulge at the naval resulting from a small section of bowel that has herniated through the umbilicus.
Fig. S2. Maternal plasma TD and fluorothalidomide (FTD) concentrations in non-pregnant NZW rabbits. Rabbits (n = 3) were buccally administered a single dose of either TD (150 mg/kg) (open/closed squares) or FTD (160 mg/kg) (open circles). Plasma drug concentrations were quantified by high-performance liquid chromatography coupled with ultraviolet detection (HPLC-UV) at 220 nm.
Fig. S3. *In vitro* formation of TD and FTD hydrolysis products. Disappearance of TD and FTD in potassium phosphate buffer (KH$_2$PO$_4$), pH 6.0 at 37°C, and the formation of their hydrolysis products was followed by plotting peak areas from chromatograms collected using HPLC-UV quantification at 220 nm. Hydrolysis products (HP) were given a numerical designation corresponding to their retention (elution) times relative to the parent compound (i.e. – 0.7, the HP has a peak with a retention time that is 0.7 min earlier than that of TD).
Fig. S4. HPLC-UV chromatograms of *in vitro* TD and FTD hydrolysis. Chromatograms were generated under the following HPLC-UV conditions: mobile phase, acetonitrile and double distilled water (35:65, v/v); flow rate, 0.8 ml/min; column, 5 µm C-18 column (15 cm x 4.6 mm); and UV detection at 220 nm. Representative chromatograms for FTD after a 0 hr and 30 min (**A** and **B**) and TD after a 0 and 1 hr (**C** and **D**) incubation in KH$_2$PO$_4$ buffer, pH 6.0 at 37°C. Hydrolysis products are designated as (HP) and given a numerical designation corresponding to their retention (elution) times relative to the parent compound.
Fig. S5. HPLC-UV chromatograms of *in vitro* TD and FTD breakdown in embryo culture media (ECM). Incubations of TD and FTD in rabbit ECM were carried out at 37°C. Internal standards (IS) for TD and FTD, phenacetin (PHEN) and butylglycine ethyl ester (BGEE) respectively, were added prior drug extraction and used to correct for extraction yield. Chromatograms were generated under the following HPLC-UV conditions: mobile phase, acetonitrile and double distilled water (35:65, v/v); flow rate, 0.8 ml/min; column, 5 µm C-18 column (15 cm x 4.6 mm); and UV detection at 220 nm. Representative chromatograms of blank ECM spiked with TD and FTD pure standards (std) (A and D), TD after 0 and 2 hr incubations (B and C) and FTD after 0 hr and 30 min incubations (E and F).