THALIDOMIDE IS DISTRIBUTED INTO HUMAN SEMEN AFTER ORAL DOSING

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ABSTRACT:

As part of a double-blind placebo-controlled study of the effect of thalidomide on body weight and the viral load of human immunodeficiency virus-seropositive patients, plasma and semen samples were analyzed for the presence of thalidomide. Patients were orally dosed with 100 mg of thalidomide/day for 8 weeks. Blood samples were obtained at baseline and weeks 4, 8, and 12, and semen was obtained at baseline and weeks 4 and 8. Samples were extracted with solid-phase cartridges and analyzed by liquid chromatography/tandem mass spectrometry using atmospheric pressure chemical ionization in the negative ion mode. Two of four patients taking thalidomide were able to provide semen samples. Both had detectable levels of thalidomide in their plasma (10–350 ng/ml) and semen (10–250 ng/g) at weeks 4 and 8. There was an apparent correlation between plasma and semen levels. Semen levels could be significantly greater for therapeutic doses of more than 100 mg/day. Since the threshold dose for birth defects and thalidomide exposure is not known, male patients are advised to use barrier contraception.

Thalidomide [α-(N-phthalimido)glutarimide] was withdrawn from Europe in 1961 when its teratogenic effects in humans became evident. It was being prescribed as a nonbarbiturate sedative hypnotic and anti-emetic (Powell, 1999). Thalidomide is the Food and Drug Administration-approved commercial formulation of thalidomide currently indicated for the treatment of erythema nodosum leprosum, an acute inflammatory reaction of lepromatous leprosy (Calabrese and Resztak, 1998). Thalidomide has both anti-inflammatory and antiangiogenic activities (D’Amato et al., 1994; Radomsky and Levine, 2001).

There are currently over 100 thalidomide clinical trials for various inflammatory and oncologic conditions (Calabrese and Resztak, 1998; Celgene internal document, Warren, NJ). Recent findings, particularly in refractory multiple myeloma and metastatic colorectal cancer, have been encouraging (Singhal et al., 1999; Govindarajan et al., 2000). To ensure the safe dispensing and use of the drug, Celgene instituted the mandatory System for Prescribing Safety (STEPS) (Zeldis et al., 1999). Its requirements include the registration of physicians, patients, and pharmacists and pregnancy testing in females. Male patients are cautioned on the use of contraception because it is not known whether thalidomide is distributed into semen or sperm. Registered STEPS users are evenly distributed between the sexes. With increasing thalidomide use, the risk for exposure of naive individuals and fetuses through transmission in bodily fluids, such as milk and semen, is a safety concern. The present communication confirms the presence of thalidomide in the semen of HIV-seropositive patients treated with thalidomide at 100 mg/day for 8 weeks.

Materials and Methods

Thalidomide (Thalomid) is a racemic mixture of (+)-R and (−)-S enantiomers. It is synthesized by Chemsyn (Lenexa, KS) with a purity greater than 99%, as determined by HPLC (Teo et al., 2001a). Thalidomide is formulated as 50-mg gelatin capsules. The thalidomide analog CC-4047 (99% purity) was used as the internal standard. All other chemicals were reagent or HPLC grade and obtained from commercial sources.

A double-blind placebo-controlled study was performed on eight HIV-seropositive patients (seven male one female) to determine the effect of thalidomide on body weight and viral load. All patients were receiving highly active antiretroviral therapy. Patients were administered 100 mg of thalidomide/day or a matching placebo at bedtime (7–12 PM) for 8 weeks. As part of the study, blood and semen samples were taken within 13 h (10 AM–1 PM) after dosing at baseline and weeks 4, 8, and 12 and at baseline and weeks 4 and 8, respectively, to determine thalidomide exposure levels. Samples were placed on ice before processing. Plasma was prepared by centrifuging at 15,000 rpm. Plasma and semen samples were stabilized within 30 min of collection by adding an equal amount (v/v and w/w, respectively) of 0.025 M Sorensen’s citrate buffer, pH 1.5, and stored at −70°C, as described previously (Teo et al., 1999). Validated LC-MS/MS assays were developed for human plasma and semen (S. Teo, manuscript in preparation).
FIG. 1. LC-MS/MS elution profiles of thalidomide in semen for two patients at weeks 4 and 8.

<table>
<thead>
<tr>
<th>Time</th>
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<tr>
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<td>NS</td>
<td>29</td>
<td>45</td>
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NS, patient unable or unwilling to provide sample.

a Below lower limit of quantification.

TABLE 1
Plasma and semen levels of thalidomide in HIV seropositive patients during 100 mg of thalidomide/day over 8 weeks
preparation. Frozen buffered semen and plasma samples were allowed to thaw to room temperature, vortexed, and centrifuged at 10°C for 2 min at 13,500 rpm for semen and 5 min at 3000 rpm for plasma. Aliquots of 100 μl of semen and 1 ml of plasma were taken. The internal standard was spiked to a concentration of 250 and 2500 ng/ml for semen and plasma, respectively (S. Teo, manuscript in preparation).

Retention times for thalidomide and the internal standard were 1.7 and 1.9 min, respectively. Assays of thalidomide in plasma and semen were validated for 2 to 250 ng/ml of plasma and 2 to 250 ng/g of semen. Lower limits of quantification for plasma and semen were 2 ng/ml and 2 ng/g, respectively with linearity demonstrable to 250 ng/ml and 250 ng/g, respectively. Limits of quantification for plasma and semen were 2 ng/ml and 2 ng/g, respectively. No thalidomide was present in the plasma and semen of healthy volunteers. All samples were then passed through primed Oasis HLB (Waters, Milford, MA) solid-phase extraction cartridges and washed with 1 ml of solid-phase extraction wash consisting of water, methanol, and formic acid (70:30:0.5 v/v/v). Cartridges were then washed with 1 ml of water and eluted with 1 ml of methanol. Eluates were evaporated to dryness with nitrogen. Residues were redissolved in 200 μl of water, acetonitrile, and acetic acid (90:10:0.1 v/v/v), vialled, and analyzed by LC-MS/MS.

Semen and plasma samples were analyzed by atmospheric pressure chemical ionization negative ion mode using a Hewlett Packard 1100 HPLC coupled to a Micromass Quattro LC (Beverly, MA) mass spectrometer. An ODS Summit (Crawford Scientific, Lanarkshire, UK) column (3.5 cm × 3.2 mm; 3 μm) connected to a Phenomenex C18 (4 mm) precolumn (Phenomenex, Cheshire, UK) was used for the separation. The mobile phase used was a mixture of water, acetonitrile, and acetic acid (75:25:0.1 v/v/v) pumped at 0.5 ml/min. Negative ion mass spectra were then acquired by injecting 75-μl aliquots. Samples were desolvated at a source temperature of 150°C. The collision gas argon was set at 1 × 10⁻³ millibars. Cone voltage and collision energy were set at 15 V and 12 eV, respectively. Retention times for thalidomide and the internal standard were 1.7 and 1.9 min, respectively.

Results and Discussion

Of the four patients dosed with thalidomide, two were able to provide semen samples. They were both Caucasian, 49 years of age, in good health, and weighed 83 and 70 kg during screening and 88 and 74 kg at week 8. They had stable viral loads of <400 copies of HIV RNA/ml and were on multiple nucleoside analogs and protease inhibitors (patient 15, ritonavir, stavudine, dideoxinosine, saquinavir; patient 18, zidovudine, didanosine, saquinavir, indinavir) as part of their highly active antiretroviral therapy along with trimethoprim for pneumocystis carinii pneumonia prophylaxis. Both had detectable thalidomide levels at weeks 4 and 8 (Table 1; Fig. 1). These levels correlated with thalidomide plasma levels. No thalidomide was present in the plasma and semen of placebo-dosed patients. The decrease in plasma and semen levels at week 8 could be due to thalidomide inducing its own metabolism. Previous studies have shown that thalidomide increased hepatic cytochrome P-450 in the rat (Tsambaos et al., 1994). The threshold dose for human thalidomide exposure and teratogenicity is unknown; however, thalidomide at 25 mg/day for 2 to 3 days and at 50 mg/day for 1 day has been shown to produce characteristic birth defects in humans (Newman et al., 1993). Unfortunately plasma levels were not determined in these cases. Therefore, it is not possible to determine the theoretical threshold semen levels for the development of human birth defects because of the lack of correlative data between plasma and semen. In healthy and HIV-seropositive humans, thalidomide exhibits first-order absorption and elimination pharmacokinetics, which are independent of dose from 50 to 400 mg (Noormohamed et al., 1999; Teo et al., 1999; 2001b).

Exposure to thalidomide, as determined by the area under the curve and Cmax was proportional to dose over the same range. Therefore, semen levels could increase proportionally with oral dose. Current investigational use, however, has gone beyond this dose range. For instance, multiple myeloma patients are started at 200 mg/day, rising over time to 800 to 1200 mg/day before being titrated downwards to an acceptable side-effects dose level (Singhal et al., 1999; Govindarajan et al., 2000). These maintenance doses are usually significantly greater than the 100 mg/day used in this study. Semen levels could therefore be greater than those obtained here. The present study did not identify a “safe” period after thalidomide discontinuation. Male patients taking thalidomide are therefore advised to use appropriate barrier contraception.

References


