Stability of Thalidomide in Human Plasma

To the Editor:

Thalidomide was initially introduced into medical use in the 1950s as a sedative; because of its teratogenic effects (malformation of the extremities), however, it was withdrawn from the market. Chronic use of thalidomide was also associated with peripheral neuritis. Lately, interest in thalidomide has renewed after demonstration of its activity in various inflammatory and autoimmune disorders, including oropharyngeal ulcerations, Behçet syndrome, lepra reactions, and graft-vs-host disease after bone marrow transplantation (1, 2). The mechanism of action of thalidomide in these diseases is not known. Only limited information is available on the pharmacokinetics of the drug and its optimal dosage in these new potential indications. Especially when used in severely ill patients with advanced changes of the gastrointestinal function, e.g., the treatment of complicated forms of graft-vs-host disease after bone marrow transplantation, the absorption of thalidomide may be unpredictable. According to animal studies, a minimal plasma thalidomide concentration of 5 mg/L is necessary to produce a maximal response (3). To improve the efficacy and safety of thalidomide therapy in humans will require further studies in target patient populations.

Thalidomide is almost insoluble in water and only fairly soluble in most organic solvents. In aqueous solutions, it undergoes spontaneous hydrolysis at physiological and alkaline pH (4). Hydrolysis of thalidomide has also been noted in plasma of several animal species, including monkeys, such that only ∼80% of the added drug is found unchanged after 60 min incubation at 37 °C (5). However, the occurrence of hydrolysis in human plasma has been questioned; in an earlier study, no statistically significant change in plasma thalidomide concentration was noted in samples stored for 1 h at room temperature (6).

We evaluated the stability of thalidomide in human plasma and the specificity and reproducibility of its determination by using the HPLC method of Chen et al. (6). A Merck-Hitachi L6200 pump apparatus (Hitachi, Tokyo, Japan) was used with a LiChroCART 100 RP-8 column (250 × 4 mm) and precolumn (4 × 4 mm; E. Merck, Darmstadt, Germany). The mobile phase was set up from eluent A (methanol:potassium phosphate, 0.01 mmol/L, pH 6.2, 10:90, by vol) and eluent B (methanol), going from 20% to 80% eluent B over 12 min. All reagents were of analytical grade (E. Merck). The flow rate was 1.5 mL/min. The intraassay CV of plasma thalidomide determinations was 6.7% at 1.5 mg/L and 1.5% at 4 mg/L (n = 8). To test the stability of thalidomide in human plasma, we supplemented plasma samples with the drug to contain a nominal concentration of 5 mg/L thalidomide. After 1 and 2 h at room temperature, the thalidomide concentration had decreased by 6% and 14%, respectively (n = 4 at each time point, P < 0.001 in comparison with samples extracted immediately; ANOVA followed by Tukey procedure). On the other hand, when the samples were kept in an ice bath, no drug loss occurred, the measured mean ± SD thalidomide concentrations being 4.8 ± 0.1, 4.9 ± 0.1, and 4.8 ± 0.1 mg/L (n = 4 at each time point) in the samples processed immediately or after 1 and 2 h, respectively (P = 0.36, ANOVA).

These findings suggest that when analyzing plasma thalidomide concentrations in pharmacokinetic studies and in patients' samples, the blood samples must be handled refrigerated and the plasma separated promptly to avoid decomposition of thalidomide.

References

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Underestimation of Serum Iron with Automated Methods

To the Editor:

Consistent with the paper by Tietz et al. on the unsatisfactory status of serum iron measurements (1), we report that serum iron is grossly underestimated in some patients' specimens and quality-control (QC) samples in a ferrozine method.

We measured serum iron with a Roche kit method (Unimate 7, cat. no. 2023408) on a Cobas Bio analyzer (Roche Diagnostics, Nutley, NJ) which uses a 400-s reduction step with hydroxylamine (R1), followed by complexation with ferrozine for 140 s (R2) and absorbance measurement at 550 nm. Serum iron in a dialysis patient measured by this method was zero, whereas in recent analyses it had averaged ∼10 μmol/L (reference range 8–30 μmol/L). This patient had a normal blood film and hematological indices. When assayed on an Hitachi 717 by an alternative ferrozine method (cat. no. 1040880; Boehringer Mannheim, Mannheim, Germany), the iron concentration was 8 μmol/L. The main difference between the two procedures is that the Hitachi 717 includes a 300-s reduction with ascorbate instead of hydroxylamine and a 300-s reaction with ferrozine.

Increasing the reduction time to 800 s and the ferrozine reaction time to 350 s failed to affect the low iron result obtained in this dialysis patient with the Unimate 7 kit. How-