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use in the 1950s as a sedative; because of its teratogenic effects (malformation of the extremities), however, it was withdrawn from the market.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 optimum 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-
which increased from 38.6 and 10.5 μmol/L Fe to 54.9 and 19.5, respectively, on the addition of ascorbate; the latter results were similar to those obtained on the Hitachi 717. In contrast, Precipath and Precinorm control sera (Boehringer Mannheim, Sydney, Australia) gave iron results with the Unimate 7 kit (17.1 and 22.0 μmol/L Fe) that were similar to the Hitachi 717 method (15.5 and 20.5, respectively) and were unaffected by increased reaction/reduction times or by the addition of ascorbate.

We compared iron results with the Unimate 7 kit, the Hitachi 717 method, and an earlier Roche kit, which involves ascorbate as a reducing agent (Unimate 5, cat. no. 2023752), with the Reference Method proposed in 1990 by the International Committee for Standardisation in Haematology (ICSH) (2). The samples were sera from renal dialysis patients and four external QC samples from the Australian Quality Assurance Program (AQAP). The results from this study, shown in Fig. 1, indicate a good correlation between the ICH and Hitachi 717 and Unimate 5 methods, with means of the latter two methods slightly lower than that of the ICHS method. In contrast, the Unimate 7 method gave poor correlation and considerably lower means than the ICHS method. These differences were particularly accentuated with the QC material and two patients' specimens, thus re-emphasizing the unreliability of hydroxylamine reduction with certain specimens (see Fig. 1). The Unimate 5, Hitachi 717, and ICHS methods, but not the Unimate 7 method, gave values close to the AQAP target values shown in Fig. 1. Since our preparation of this letter, the Unimate 7 kit has been removed from the Australian market.

Because the discrepant results with the hydroxylamine method may have been due to pharmacologic iron dextran in serum, we prepared iron dextran in water at iron concentrations of 71.6, 35.8, 17.9, and 9.0 μmol/L (Ferrum H.; Hausman Labs, St. Gallon, Switzerland). The Hitachi 717 and Unimate 5 and 7 kits gave undetectable iron results at these concentrations. The ICHS method gave 23 and 3 μmol/L for the 71.6 and 35.8 μmol/L samples, respectively; the other concentrations of iron dextran were undetectable by the ICHS method. These results indicate that the three kit methods do not detect iron in the form of a dextran complex, whereas the ICHS method detects iron as its dextran complex when concentrations exceed 35 μmol/L.

We conclude that hydroxylamine is not an efficient reducing agent for iron analysis by the ferrozine method. Why certain sera cannot be analyzed correctly with hydroxylamine reduction is not known but may be related to dialysis producing lower amounts of endogenous antioxidants/reductants in the serum (3). We recommend that new methods developed for estimating iron be compared with the ICHS method over a wide range of diagnostic specimens except where the patients have recently been receiving iron dextran therapy.

References

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Two representatives from Hoffmann-La Roche reply:

To the Editor:

The diagnostically relevant form of serum iron that is available for anabolic metabolism, e.g., erythropoiesis, is that portion bound to its carrier protein, transferrin. To measure this fraction, the iron must be specifically dissociated from transferrin before it can bind with iron-chelating reagents. Iron from other sources in serum, e.g., porphyrin systems and iron–sulfur clusters present in other proteins, should not be measured and may not be indicative of the individual’s iron status. When specific iron chelators such as ferrozine are used that give color reactions with ferrous iron only, an agent to reduce the ferri iron liberated from transferrin is also required.

Commercially available diagnostic kits for automated serum iron analy-