Stability of 5-Fluorouracil in Whole Blood and Plasma

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We studied the stability of 5-fluorouracil (5-FU) in plasma and whole blood kept at room temperature and on ice for 1 to 24 h. At room temperature, there was a steady loss of 94% of the parent drug over 24 h in whole blood and 52% in plasma. In the presence of an excess of uracil, 5-FU was stable for 24 h, suggesting that the loss of 5-FU is the result of enzymatic degradation. 5-FU is more stable in whole blood and plasma when samples are kept cold. For blood and plasma samples maintained on ice, the loss was only 30% and 10% of the parent drug in the respective samples over 24 h. Frozen plasma samples (−20 °C) were stable for five weeks. Blood specimens collected for quantifying 5-FU should be immediately placed on ice, and the plasma should be separated and frozen as promptly as possible.

Additional Keyphrases: chemotherapy · sample handling, stability · cancer therapy

5-Fluorouracil (5-FU) is an anticancer drug widely used for treating cancer of the gastrointestinal tract, head and neck, and breast. The unique pharmacological characteristics of this drug, such as dose-dependent clearance (1) and variable oral absorption (2), are indications for therapeutic drug monitoring. To define optimal specimen-processing methods for pharmacokinetic studies, we evaluated the stability of this drug in plasma and whole blood.

Materials and Methods

Heparinized 20-mL blood samples were obtained from six healthy volunteers, three men and three women, ages 29 to 36 years. Plasma was separated by centrifugation at 500 × g for 10 min. Aliquots of both plasma and whole blood from each subject were supplemented with a 1 mmol/L aqueous stock solution of 5-FU (Sigma Chemical Co., St. Louis, MO) to give a final concentration of 10 μmol/L. These aliquots were kept either on ice or at room temperature. We also tested a 10 μmol/L solution of 5-FU in phosphate-buffered saline (PBS, NaCl 9 g/L, KH₂PO₄ 210 mg/L, Na₂HPO₄ 725 mg/L, pH 7.2; Biofluids, Rockville, MD) stored under the same conditions. We removed samples for assay at the following times: before the incubation and at 1, 5, and 24 h. After the incubation period, we separated plasma from the whole-blood samples by centrifugation at 500 × g for 10 min, then stored all samples frozen (−20 °C) until assay.

We also evaluated the stability of a pooled plasma sample containing 10 μmol of 5-FU per liter that was stored at −20 °C for five weeks.

5-FU in plasma was measured by reversed-phase "high-pressure" liquid chromatography, with sample extraction as previously described (3).

To separate sets of blood and plasma samples from two healthy donors we added the stock 5-FU solution labeled with ¹⁴C in the 2-position on the ring (ICN Radiochemicals, Irvine, CA) to give a final concentration of 10 μmol/L and incubated these for 24 h at room temperature. Before the incubation plasma and blood were divided into three aliquots: one served as a control; we added NaF (4 g/L) to the second; and to the third we added uracil (Sigma Chemical Co.), 100 μmol/L. After the incubation, we extracted the samples and analyzed them as described above, collecting the eluted fractions each minute and counting their radioactivity with a scintillation counter.

Results and Discussion

The stability of 5-FU in plasma and whole blood, stored on ice and at room temperature for up to 24 h, is shown in Figure 1. The most striking decrease in concentration occurred in whole blood stored at room temperature, with 94% of the drug being lost after 24 h. Under the same conditions, 52% of the drug was no longer detectable in plasma. Blood and plasma samples kept on ice lost respectively 30% and 10% of the original drug concentration after 24 h.

A 10 μmol/L solution of 5-FU in phosphate-buffered saline (pH 7.2), either stored on ice or at room temperature, showed no degradation after 24 h.

Supplemented plasma samples stored at −20 °C for five weeks lost less than 10% of the original drug concentration.

The effect of competitive (uracil) and noncompetitive NaF had little effect on 5FU breakdown. However, an excess of uracil completely blocked degradation after 24 h at room temper.
Mechanism of degradation may involve the erythrocyte.

5-FU is eliminated from the body by enzymatic degradation to inactive metabolites, via the normal catabolic pathway for uracil and thymidine. If loss of 5-FU activity from plasma and blood were a result of catabolism by these enzymes, an excess of uracil would be expected to inhibit the 5-FU breakdown competitively, as we observed.

Our findings suggest that 5-FU is enzymatically degraded in plasma and whole blood, consistent with previous pharmacokinetic studies suggesting extrahepatic drug metabolism (1).

On the basis of these results, we recommend that blood specimens collected for 5-FU assay be placed immediately on ice, and that the plasma be separated as quickly as possible and frozen (−20 °C) until analysis.

References