Rapid Determination of 5-Fluorocytosine Levels in Blood

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Levels of 5-fluorocytosine in blood were rapidly calculated by determining the amount of competition in the creatinine iminohydrolase (creatinine deiminase; EC 3.5.4.21) assay for creatinine with the Kodak Ektachem system (Eastman Kodak Co., Rochester, N.Y.). The correlation with bioassay values was extremely high ($r = 0.982$). Standards and samples were highly stable over time.

5-Fluorocytosine (5-FC) is a fluorinated pyrimidine with activity against a number of species of fungi, particularly Candida albicans (Robin) Berkhout and Cryptococcus neoformans (Sanfelice) Vuillemin (8). It is an attractive drug because it can be given orally (9). Dose-related leukopenia has been noted in the past, particularly in patients with renal dysfunction and with serum levels of $>125 \mu g/ml$ (2, 6, 8).

The methods presently used for measuring levels of 5-FC in serum are the microbiological assay (1), high-performance liquid chromatography (11), and the fluorimetric assay (10).

By taking advantage of a limiting factor in the capabilities of the Kodak Ektachem system (Eastman Kodak Co., Rochester, N.Y.), namely, the interference of 5-FC in the determination of creatinine levels in serum, we have been able to find a new, rapid method for the determination of levels of 5-FC in serum.

After noting that 5-FC would falsely increase the levels of creatinine in serum determined with the Kodak Ektachem system, we made standards of 5-FC (Hoffmann-LaRoche Ltd., Vaudrevil, Canada) in both saline and pooled human serum and tested them with a biological assay and with a biochemical assay based on this phenomenon.

The biological assay was done in duplicate with C. albicans ATCC 10231 as the test strain at a concentration of ca. $10^5$ cells per ml of medium. The medium contained yeast nitrogen base (Difco Laboratories, Detroit, Mich.) (6.7 g), L-asparagine (1.5 g), glucose (10.0 g), Noble agar (Difco) (1.5 g), and distilled water (100 ml), added because of its ability to inhibit the diffusion of amphotericin B (5). Plates were incubated for 24 h, and zones were measured with a micrometer.

The biochemical assay was performed by measuring the total creatinine in the standard with the Kodak Ektachem system (3) and also by the Jaffe reaction with a Du Pont automatic clinical analyzer III (DuPont Co., Wilmington, Del.) (4). As the Kodak Ektachem system result represented the contribution from both creatinine and 5-FC, the value obtained for creatinine from the Jaffe reaction was subtracted from the value obtained from the Ektachem system, leaving a balance contributed by 5-FC.

Having noted a striking correlation when four different sets of standards (two sets diluted in pooled human serum and two sets diluted in phosphate-buffered saline) were used, we did the same experiment with an additional 42 serum samples from two patients, both of whom were being treated with combination therapy of amphotericin B and 5-FC. (A total of 60 serum specimens were examined.) The 42 samples tested represented all specimens sent to the chemistry laboratory during hospitalization of the patients; they were not all drawn specifically for 5-FC determination.

To test the stability of standards for the biochemical method, we made fresh standards in serum and also in phosphate-buffered saline and maintained them throughout the experiment at three different temperatures, 4, −20, and −70°C. Samples were tested daily for a total of 21 days.

The biochemical assay was evaluated for interference by other drugs which contain cytosine, including cytosine arabinoside and 2'-fluorouracil aracytosine (supplied by C. McLaren, Bristol Laboratories, Syracuse, N.Y.), and by 5-fluorouracil, which is a degradation product of 5-FC (2). The results were analyzed for Pearson's product moment correlation ($r$) and linear regression by the method of least squares. The biochemical assay was defined as the independent variable, and the biological assay was defined as the dependent variable.

The correlation between the two methods for standards and patient samples is shown in Fig. 1. Although additional data are not shown on the graph, the new assay remained accurate over the range of 0 through 400 $\mu g/ml$, based on standards rather than patient samples. Amphotericin B or elevated creatinine levels in patient serum did not interfere with method correlation. The biochemical assay was not affected by cytosine arabinoside, 5-fluorouracil, or 2'-fluorouracil aracytosine in serum.

The standards were highly stable over the period of 21 days regardless of the diluent or storage temperature (Table 1).

The assay described represents a useful additional test for those institutions which possess or have access to a Kodak Ektachem Analyzer. The additional equipment needed for the determination of creatinine levels in serum by the Jaffe method would not represent an added cost attributable to the measurement of 5-FC levels, as the additional equipment would be necessary because of the interference caused by 5-FC in the creatinine assay.

The principle of the Kodak Ektachem system is that the enzyme creatinine iminohydrolase (creatinine deiminase; EC 3.5.4.21) catalyzes the hydrolysis of creatinine to produce $N$-methylhydantoin and ammonia. The free ammonia then diffuses through a semipermeable membrane to react with an ammonia indicator, bromphenol blue. The method has been extensively tested with a number of substances. The major positive bias of significance has been associated with 5-FC (3). The reason for the interference in the determination of creatinine by the Kodak Ektachem system is that the enzyme creatinine inimohydrolase can cleave the 4-amino-group from 5-FC in a similar way that cytosine deaminase can. This amino group produces ammonia in
concentrations proportional to those of 5-FC, and the ammonia is detected as though it were released from creatinine. This would explain the lack of interference by amphotericin B or 5-fluorouracil, neither of which contains amino groups. Cytosine arabinoside and 2'-fluoroiodoaracytosine do have 4-amino groups, but neither interferes with the assay. The Du Pont ACA III uses a modification of the kinetic Jaffe reaction reported by Larsen. (7) The rate of red chromophore production after the addition of picric acid in an alkaline medium is proportional to the amount of creatinine present. Larsen reported that this method was less susceptible to interference than the unmodified method. Grossly hemolyzed specimens and direct bilirubin levels of >20 mg/dl will interfere with the method (12). Variations of this method can be easily performed in any clinical chemistry laboratory.

The total sample consumed by the test system is ca. 700 μl, and the time required for determinations is ca. 10 min. The stability of standards stored over an extended period of time in either serum or saline at any of the tested temperatures allows the same standards to be used throughout the total course of patient therapy.

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