Trimethoprim Interference in Methotrexate Assay by an Enzyme Inhibition Assay Kit

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Kitaoka, S., Terasawa, M., Goto, E., Miyaji, T., Tsuchiya, S. and Konno, T. Trimethoprim Interference in Methotrexate Assay by an Enzyme Inhibition Assay Kit. Tohoku J. exp. Med., 1986, 150 (4), 481-482 — Spurious methotrexate (MTX) concentrations in sera and cerebrospinal fluids from leukemia patients who were given trimethoprim (TMP) were estimated using an MTX assay kit which is based on its inhibition of dihydrofolate reductase. The interference of TMP with MTX was confirmed in the assay system. A concentration of 0.17 μg/ml of TMP gave a value for an apparent MTX of 10 μM.

The clinical use of methotrexate (4-amino-4-deoxy-10-methylfolic acid, MTX) requires careful monitoring of its serum and cerebrospinal fluid (CSF) concentrations to facilitate early detection of patients who are at high risk of toxicity (Stoller et al. 1977). Among a number of methods designed to determine the MTX concentrations in biological fluids, a commercially available MTX assay kit is based on its inhibition of dihydrofolate reductase (DHFR) (Wang et al. 1976); bacterial DHFR is conveniently measured by following NADPH consumption spectrometrically. An important pitfall in the use of this method for determination of MTX in body fluids is interference by trimethoprim [2, 4-diamino-5 (3, 4, 5-trimethoxybenzyl) pyrimidine], which is a potent inhibitor of bacterial DHFR (Bock and Pierce 1980; Hande et al. 1980; Cocco et al. 1983). Recently, we had a patient with central nervous system (CNS) leukemia who showed apparent concentrations of MTX in CSF for several weeks after its intrathecal injection, while he was receiving trimethoprim/sulfamethoxazole (T/S) treatment. This experience prompted us to examine the interference of TMP in an assay system for MTX which is based on its inhibition of DHFR (Cocco et al. 1983).

Spurious MTX levels were estimated using an MTX assay Kit (Nippon Ledery, Tokyo) in 7 CSFs and 5 sera from CNS leukemia patients who were on T/S treatment but not on MTX. Out of them, 5 CSFs and 2 sera were subjected simultaneously to TMP determination by spectrofluorometry (Schwartz et al. 1969).

As shown in Fig. 1, spurious MTX concentrations were found to be present in CSFs and sera examined. Such “MTX” levels were correlated with TMP levels (r = 0.96).

To examine the direct interference of TMP with MTX in the assay system used, various concentrations of TMP were added to the assay system and expressed in terms of apparent MTX concentrations. As shown in Fig. 2, a linear relation was obtained between the concentrations of TMP added and apparent MTX estimated. An increment of 0.5 μg/ml
(1.7 x 10^{-6} M) of TMP gave a value for an apparent MTX of 3.0 x 10^{-7} M, which was consistent with that in the assay with body fluids.

These results indicate that the MTX assay system based on DHFR inhibition should be used only in patients not being treated with TMP or its combinations.

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References