Efavirenz Interference in Estradiol ELISA Assay

To the Editor:

The prognosis of HIV infection has dramatically changed following the introduction of highly active antiretroviral therapy (HAART), a multidrug regimen usually consisting of three to four antiretroviral drugs belonging to the categories of nucleoside and non-nucleoside reverse transcriptase inhibitors (NRTIs and NNRTIs) and of protease inhibitors (PIs). The somewhat remarkable therapeutic properties of these regimens are often associated to some degree of toxicity and side effects, also resulting from drug interactions. These occur both among antiretrovirals and between the latter and unrelated compounds (1), thus implying some restriction in the use of various drugs in several clinical circumstances.

The novel NNRTI efavirenz, a drug exerting an intrinsic antiretroviral activity of the same order of magnitude as PIs (2), was found to increase by 37% the area under the curve of coadministered ethinyl estradiol (3), an effect thought to result from interaction with cytochrome P450.

In a large-scale horizontal screening for metabolic effects of antiretrovirals, we found that the subjects who were administered efavirenz (n = 15) at the standard adult daily dose of 600 mg had plasma concentrations of 17β-estradiol (mean ± SD, 1233 ± 364 ng/L) up to 26-fold higher than the upper limit of normal (75 ng/L), as determined by ELISA (AIA 21; Tosoh). In the same series, all recipients (n = 62) of currently marketed antiretroviral drugs other than efavirenz (NRTIs, NNRTIs, and PIs) had normal or slightly increased 17β-estradiol concentrations (97 ± 26 ng/L), well below the concentrations recorded in the subjects under treatment with efavirenz (P <0.001; Fig. 1). There were no significant differences in terms of distribution of the other components of HAART between efavirenz recipients and the rest of the group, and pituitary hormones were consistently within the appropriate reference intervals in both efavirenz recipients and the rest of the series. Concentrations of 17β-estradiol were within the reference interval in all efavirenz recipients before introduction of the drug, as established by the analysis of stored serum samples. Prospectively collected sera made it possible to see how the effect of efavirenz on 17β-estradiol concentrations became evident as early as 1 day after the first administration, with an apparent trend toward rising concentrations with time during the first 10–14 days, followed by a mean decrease of 20% after 4 weeks of treatment, a pattern closely resembling the pharmacokinetics of efavirenz itself. (As a P450 inducer, efavirenz increases the rate of its own metabolism, thus leading to a terminal half-life much shorter than otherwise expected.)

In spite of these high circulating 17β-estradiol concentrations, urinary estradiol metabolites, as measured by gas chromatography, were within the appropriate reference intervals in the subjects treated with efavirenz. To investigate this discrepancy, we repeated the measurement of 17β-estradiol (on aliquots of the same serum samples tested by ELISA) with a competitive chemiluminescent immunoassay (Estradiol-6; Chiron Diagnostic) and with a RIA-based method (SPECTRIA Estradiol; Orion Diagnostics). With both such methods, the 17β-estradiol concentrations measured were within the reference interval (Fig. 1), thus indicating a difference between the values obtained with the first test (ELISA) and those obtained with the two subsequently used immunoassays that is too large to be explained by ordinary interassay variability (4, 5). A plausible explanation for the exceedingly high 17β-estradiol concentrations measured by ELISA is that of false findings, likely to result from the binding of efavirenz to some component(s) of the assay. The remarkable similarity between the 17β-estradiol decay over time in efavirenz recipients (here measured erroneously by ELISA) and the pharmacokinetics of efavirenz (as described by the manufacturer) (3) seem to support this view.

Because the pharmacological interactions and untoward effects engendered by antiretrovirals are well known, metabolic and endocrinologic serum measurements are increasingly performed in the clinical setting; these data thus may help avoid an incorrect interpretation of ELISA-determined 17β-estradiol concentrations in patients under treatment with efavirenz.

Although there should not be any relationship between the artifact described here and the established metabolic interaction between efavirenz and coadministered estrogens (as de-

Fig. 1. Blood 17β-estradiol concentrations measured by three different immunoassays (ELISA, RIA, and chemiluminescent immunoassay) in patients with HIV infection under treatment with HAART.

Recipients of efavirenz are indicated by black ovals; all other patients are represented by gray symbols.
Thiobarbiturates Interfere with the Dade Behring aca Ammonia Test

To the Editor:

A 13-month-old boy with myoclonic epilepsy gave abnormal results for ammonia concentration in EDTA plasma and urine on an automatic clinical analyzer (aca; Dade Behring), which printed out negative concentration values with an error message. The assay reaction mixture in the aca pack showed no turbidity or precipitates, and there was no clue for the explanation of these results at that time. Approximately 6 weeks later, a 21-month-old boy with heart disease also gave negative ammonia concentrations. Both had received injections of sodium thiopental solution for prevention of convulsions, but no other drug had been given to both boys in common. The concentration of thiopental, a thiobarbiturate, in the plasma of the first boy was 135 mg/L by an HPLC-ultraviolet detection method.

This assay interference could be reproduced with thiopental added to control plasma and also with tiamylal (another thiobarbiturate) added to control plasma. Two barbiturates, pentobarbital and phenobarbital, gave no interference when added to control plasma.

Various amounts of thiopental were added to plasma specimens for the dose–response study. As the result for one specimen shows (Table 1), the ammonia concentration measured by the aca test was inversely proportional to the thiopental concentration. The clinically effective concentration of thiopental in plasma for anesthesia is 25–80 mg/L according to a manufacturer of this drug (Tanabe Pharmaceutical Company). This concentration range would produce a decrease in the ammonia concentration of 20–64 μmol/L. Because the reference interval for ammonia is 11–35 μmol/L, plasma samples from patients receiving thiopental would give anomalously decreased or even negative ammonia values as seen in the boys described above.

The ammonia test is based on the following reaction of glutamate dehydrogenase: \( \text{NH}_4^+ + \alpha\text{-ketoglutarate} + \text{NADPH} \rightarrow \text{l-glutamate} + \text{NADP}^+ + \text{H}_2\text{O} \). The analyzer measures the decreasing absorbance of NADPH at 340 nm. Another ammonia test kit, Deteminer \(^\circledR\) NH\(_3\) (Kyuwa Medix), based on the same enzyme reaction, was implemented on a Hitachi 717 automatic analyzer (Hitachi). To our surprise, no interference was observed with this test (Table 1). Following are the differences between the aca and Deteminer methods: (a) source of glutamate dehydrogenase, bovine liver vs yeast; (b) pH of assay buffer, pH 7.4 vs pH 8.25; (c) additives, e.g., surfactant, pH buffer salt, and chelating reagents, although detailed descriptions are not given for these ingredients; (d) absorbance measurement mode, single wavelength of 340 nm vs double wavelength of 340 nm (primary) and 405 nm (secondary); (e) reaction monitoring method, rate assay vs end–point assay.

According to Dade Behring Japan Ltd., there had been no reports on ammonia assay interference by thiobarbiturates. The mechanism of this interference is not yet clear. No reports have been found on the interaction of thiobarbiturates with this enzyme. Another possibility is that unknown extra reactions occur and counteract the decrease in NADPH absorbance or even produce NADPH from traces of NADP\(^+\) in the reaction vessel.

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**Table 1. Apparent ammonia concentrations in plasma with added thiopental.**

<table>
<thead>
<tr>
<th>Sodium thiopental in plasma, mg/L</th>
<th>Ammonia concentration in plasma, μmol/L</th>
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<tr>
<td></td>
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<td>200</td>
<td>-65.3</td>
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