Measurement of Estrone Sulfate in Postmenopausal Women: Comparison of Direct RIA and GC-MS/MS Methods for Monitoring Response to Endocrine Therapy in Women with Breast Cancer

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Abstract. Background: High concentrations of estrogen sulfate (E1S) are present in serum of pre- and postmenopausal women. Most assays for this estrogen conjugate involve enzyme hydrolysis and chromatographic purification prior to RIA. We have compared concentrations of serum E1S in postmenopausal women measured by direct RIA or GC-MS/MS methods. Patients and Methods: We analysed serum E1S concentrations using a direct ‘ultrasensitive’ RIA. Serum E1S concentrations were also measured by GC-MS/MS in which estrone conjugates are isolated using a solid-phase technique after which enzyme hydrolysis is employed to liberate estrone prior to GC-MS/MS analysis. Results: We analysed 32 serum samples collected from 8 postmenopausal women participating in a Phase I trial of the steroid sulfatase inhibitor 667 COUMATE. Concentrations of E1S were 998±86 pmol/l (mean±sem) and 912±114 pmol/l as measured by direct RIA and GC-MS/MS analysis respectively. There was a highly significant correlation (r=0.96, p<0.001) between concentrations of E1S measured by the different methods. Conclusion: We conclude that the direct ‘ultrasensitive’ RIA for the measurement of serum E1S provides a reliable method for assay of serum concentrations of this estrogen conjugate and should be useful in monitoring the response to endocrine therapy in postmenopausal women with hormone-dependent breast cancer.

Estrone sulfate (E1S) is the most abundant circulating estrogen in both pre- and postmenopausal women (1). It is formed by the actions of estrogen- and phenolsulfo-
and at a number of time points up to 12 days after starting treatment. After centrifugation (2800 g) for 15 min the serum obtained was aliquoted and stored at −20°C until assayed. We measured serum E1S concentrations using an ‘ultrasensitive’ direct RIA kit obtained from DSL (DSL-54200, Webster, TX, USA) according to the manufacturer’s instructions. The limit of quantification for this assay was 0.4 pmol/l. Concentrations of E1S were also measured using a GC-MS/MS method (SFBC Taylor, Princeton, NJ, USA). This method employs extraction of steroid conjugates using solid phase cartridges, enzyme hydrolysis of estrone conjugates and quantification of the liberated estrone by GC-MS/MS. The limit of quantification for this assay was 2.3 pmol/l. Coefficients of variation for these assays were <10%.

Results

We analysed 32 serum samples from these patients by both analytical procedures. Concentrations of E1S measured by the direct RIA were 998±86 pmol/l (mean±sem) and 912±114 pmol/l by GC-MS/MS. There is a highly significant correlation between the concentrations as measured by the two methods ($r=0.96$, $p<0.001$, Pearson’s correlation coefficient) (Figure 1). Pre-treatment serum concentrations of E1S were 823±187 pmol/l and 732±138 pmol/l as measured by the direct RIA and GC-MS/MS methods respectively. These concentrations of E1S in serum from postmenopausal women are in good agreement with those we previously obtained in our laboratory (841±496 pmol/l), using a method employing extraction of unconjugated estrogens, enzyme hydrolysis, thin layer chromatographic purification and RIA of estrone (1).

Discussion

Measurements of unconjugated and conjugated estrogens in postmenopausal women are difficult due to the low serum concentrations of these hormones (11). These difficulties are compounded when samples are obtained from women receiving aromatase inhibitor therapy. As it has been suggested that measurements of E1S concentrations may provide a better indicator of the level of aromatase inhibition there is a need for a reliable, robust, E1S RIA. The finding of a highly significant correlation between the two methods employed in this study suggests that the
ultrasensitive RIA method used could fulfil these requirements. While GC/MS/MS remains the 'gold standard' for the assay of steroids this method is not widely available. In conclusion, results obtained in this study have revealed that serum E1S concentrations, as measured using an 'ultrasensitive' direct RIA, show a good correlation with those obtained using a GC/MS/MS method. The use of a direct RIA assay should greatly facilitate investigations into the role of E1S in normal and pathological conditions. Such an assay should also be of value in monitoring the extent of inhibition in women receiving aromatase inhibitor therapy.

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References


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