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 estrone 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 methods 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 assaying 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 phenolsulfotransferases in peripheral tissues (2). Estrogen sulfates have a much longer half-life (10-12 h) compared with that of unconjugated estrogens (20-30 min) (3). This enzyme has an important role in regulating the synthesis of estrogens in breast tumors and a number of potent STS inhibitors have now been developed (5). One such inhibitor, 667 COUMATE, has just completed the first Phase I trial of this class of drug in postmenopausal women with breast cancer (6).

Although blood concentrations of E1S are much higher than those of estradiol or estrone its measurement has proved to be difficult and there is a need for a robust direct RIA. Initial attempts to develop antisera for its direct measurement proved unsuccessful due to a lack of specificity, although some success with direct assays has recently been achieved (7, 8). However, most assays for E1S involve solvent extraction of unconjugated estrogen, enzyme hydrolysis to cleave the sulfate moiety remaining in the aqueous phase, a chromatographic purification step, followed by RIA of the isolated estrone (1, 9).

Measurement of E1S concentrations are proving to be important in monitoring the efficacy of aromatase inhibitor therapy. The decrease in plasma E1S concentrations gives a better indication of the extent of aromatase inhibition than measurements of estrone or estradiol (10). As part of the first phase I trial of a STS inhibitor two different assays were used to measure serum E1S concentrations. The first was a direct 'ultrasensitive' RIA developed by DSL. The second was a method involving enzyme hydrolysis and quantification of the liberated estrone using a gas chromatographic – tandem mass spectrometric (GC-MS/MS) procedure.

Patients and Methods

We collected blood from 8 patients participating in a phase I trial of the STS inhibitor 667 COUMATE after having obtained their informed consent to the study. The investigation was approved by the Ethics Committee of the participating hospitals. Whole blood (20 ml) was collected into plain glass tubes before (pre-treatment)

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Key Words: Estrone sulfate, breast cancer, aromatase, steroid sulfatase.

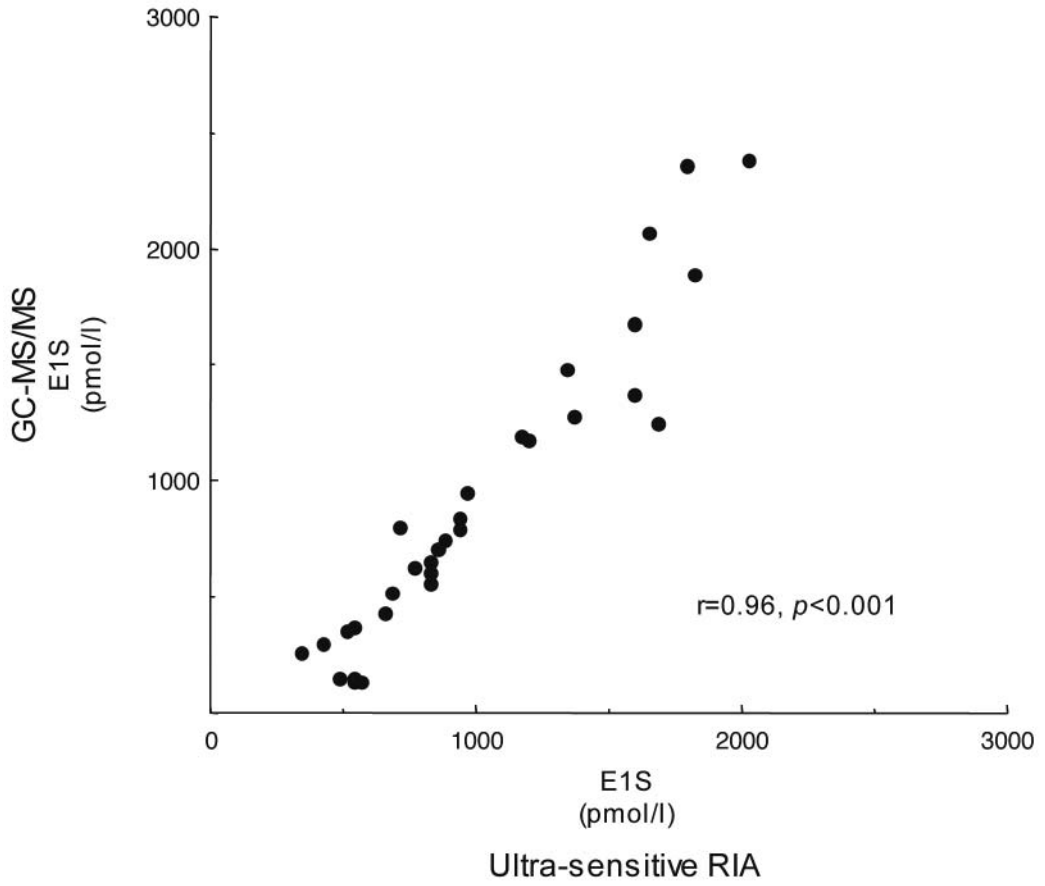

Figure 1. *Correlation between serum concentrations of estrone sulfate (E1S) measured by gas chromatographic-tandem mass spectrometric (GC-MS/MS) method and direct RIA.*

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.

Acknowledgements

This research was supported by Sterix Ltd, a member of the Ipsen Group.

References

1 Noel CT, Reed MJ, Jacobs HS and James VHT: The plasma concentration of oestrone sulphate in postmenopausal women: lack of diurnal variation effect of ovariectomy, age and weight. J Steroid Biochem *14*: 1101-1105, 1981.
2 Hobkirk R: Steroid sulfation. Trends Endcrinol Metab *4*: 69-74, 1993.
3 Ruder HJ, Loriaux DL and Lipsett MB: Estrone sulfate: production rate and metabolism in man. J Clin Invest *51*: 1020-1033, 1972.
4 Reed MJ, Purohit A, Woo LWL and Potter BVL: The development of steroid sulphatase inhibitors. Endocr Rel Cancer *3*: 9-23, 1996.
5 Reed MJ, Purohit A, Woo LWL, Newman SP and Potter BVL: Steroid sulfatase: molecular biology, regulation and inhibition. Endocr Rev *26*: 171-202, 2005.
6 Stanway SJ, Purohit A, Woo LWL, Sufis S *et al*: Phase I study of STX64 (667 COUMATE) in breast cancer patients: the first study of a steroid sulfatase inhibitor. Clin Cancer Res *12*: 1585-1592, 2006.
7 Ranadive GN, Mistry JS, Damodaran K, Khosraul MJ *et al*: Rapid, convenient radioimmunoassay of estrone sulfate. Clin Chem *44*: 244-249, 1998.
8 Giton F, Valleix A, Boudou P, Villelte JM *et al*: Specific radioimmunoassay of estrone sulfate: application to measurement in male plasma. J Steroid Biochem. Mol Biol *81*: 85-94, 2002.
9 Lonning PE and Ekse D: A sensitive assay for measurement of plasma estrone sulphate in patients on treatment with aromatase inhibitors. J Steroid Biochem Mol Biol *55*: 409-412, 1995.
10 Geisler J, Haynes B, Anker G, Dowsett M and Lonning PE: Influence of letrozole and anastrozole on plasma estrogen levels in postmenopausal breast cancer patients evaluated in a randomised, cross-over study. J Clin Oncol *20*: 751-757, 2002.
11 Dowsett M and Folkerd E: Deficits in plasma oestradiol measurements in studies and management of breast cancer. Breast Cancer Res *7*: 1-4, 2005.

Received May 22, 2007
Accepted May 29, 2007