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Review

Analysis of estrogens and androgens in postmenopausal serum and plasma by liquid chromatography–mass spectrometry

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ABSTRACT

Liquid chromatography-selected reaction monitoring/mass spectrometry-based methodology has evolved to the point where accurate analyses of trace levels of estrogens and androgens in postmenopausal serum and plasma can be accomplished with high precision and accuracy. A suite of derivatization procedures has been developed, which together with modern mass spectrometry instrumentation provide investigators with robust and sensitive methodology. Pre-ionized derivatives are proving to be useful as they are not subject to suppression of the electrospray signal. Postmenopausal women with elevated plasma or serum estrogens are thought to be at increased risk for breast and endometrial cancer. Therefore, significant advances in risk assessment should be possible now that reliable methodology is available. It is also possible to conduct analyses of multiple estrogens in plasma or serum. Laboratories that are currently employing liquid chromatography/mass spectrometry methodology can now readily implement this strategy. This will help conserve important plasma and serum samples available in Biobanks, as it will be possible to conduct high sensitivity analyses using low initial sample volumes. Reported levels of both conjugated and non-conjugated estrogen metabolites are close to the limits of sensitivity of many assays to date, urging caution in the interpretation of these low values. The analysis of serum androgen precursors in postmenopausal women has not been conducted routinely in the past using liquid chromatography/mass spectrometry methodology. Integration of serum androgen levels into the panel of metabolites analyzed could provide additional information for assessing cancer risk and should be included in the future.

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Abbreviations: 2-hydroxy-estradiol, 2,3-dihydroxy-17β-estradiol; 2-methoxy-estradiol, 2-methoxy-3-hydroxy-17β-estradiol; 4-methoxy-estradiol, 3-hydroxy-4-methoxy-17β-estradiol; 4-hydroxy-estradiol, 3,4-dihydroxy-17β-estradiol; 16α-hydroxy-estradiol, 3,16α-dihydroxy-17β-estradiol; estradiol, 17β-estradiol; APCI, atmospheric pressure chemical ionization; BMI, body mass index; CYP, cytochrome P-450; D, dansyl; DHEA, dehydroepiandrosterone; ECAPCI, electron capture atmospheric pressure chemical ionization; ESI, electrospray ionization; GP, Girard P; GT, Girard T; G/S, β-glucuronidase/arylsulfatase; LC, liquid chromatography; HSD, hydroxysteroid dehydrogenase; MPPZ, 1-(2,4-dinitro-5-fluorphenyl)-4,4,-dimethylpiperazinyl; MS, mass spectrometry; NMN, N-methyl-nicotinyl; NMP, N-methyl-2-pyridyl; NMPS, N-methyl-pyridynium-3-sulfonyl; P, picolinoyl; PFB, pentafluorobenzyl; PS, pyridyl-3-sulfonyl; SRM, selected reaction monitoring; SULT, sulfotransferase.

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1. Introduction

There is a compelling need for reliable methodology capable of quantifying estrogens in the serum of postmenopausal women because increased levels appear to be associated with increased breast cancer risk [1,2]. Estrogen carcinogenesis arises through a dual mechanism in which estradiol can act either as a hormone to stimulate aberrant cell proliferation or as the precursor to the formation of genotoxic catechol metabolites [3]. Estrogen levels in the breast tissues of postmenopausal women are dependent upon the availability of circulating C-19 androgen precursors, which are converted to estrogens in the tissue (Fig. 1). Estrogens can then be released into the circulation, providing biomarkers of tissue estrogen biosynthesis if it is assumed that the circulating levels are reflective of tissue concentrations. This assumption has been questioned because tissue levels of estrogens are significantly higher than the corresponding circulating levels and breast tissue-specific metabolism is known to occur. A pharmacokinetic model has been proposed in which there is rapid equilibrium between tissue and plasma estrogens that may might explain this conundrum [4].

The analysis of circulating androgens concentrations can provide insight into availability of relevant androgen precursors, such as androstenedione and testosterone, which can be taken up into tissue (Fig. 1). In postmenopausal women, such an analysis could provide useful additional biomarkers of breast cancer risk. Circulating sulfate conjugates have the potential to provide a source of estrogens in breast tissue through the action of sulfatases, which would release the corresponding non-conjugated steroids [5]. This is particularly relevant to circulating estrone-3-sulfate (a precursor to estrone) and dehydroepiandrosterone (DHEA) sulfate, a precursor to DHEA, which is a substrate for 3β-hydroxysteroid dehydrogenase (HSD)-mediated conversion to androstenedione. The androstenedione can in turn be converted to estrone by aromatase (Fig. 1). However, there is little evidence that the conversion of circulating sulfate conjugates to tissue androgens and estrogens actually takes place [4]. Furthermore, the polar nature of the sulfate conjugates suggests that they are not good substrates for passive diffusion from the plasma into breast tissue. However, the ability of multiple drug transporter (MRP)-1 (ABCC1) to transport estrone-3-sulfate [6] and MRP-1 and MRP-4 (ABCC4) to transport DHEA sulfate [7] does provide an alternative mechanism for the conjugated steroids to be taken up by breast tissue. Therefore, the analysis of circulating estrone-3-sulfate and DHEA sulfate in postmenopausal women could also be informative.

Aromatase inhibitors have significantly improved the recurrence-free and overall survival rates in breast cancer patients [8]. Unfortunately, only incremental progress has been made over the last decade in preventing breast cancer among postmenopausal women. There is a compelling need to improve this situation in view of the aging world population and the role of aging as an important determinant of breast cancer risk [9,10]. It is clear that implementation of breast cancer prevention programs will require selection of women with high breast cancer risk in order to maximize the benefit/risk ratio [11,12]. It is anticipated that significant advances in risk assessment will be possible if reliable methodology is available to quantify estrogens and androgens in the plasma or serum of postmenopausal women [9]. These measurements can be coupled with other risk factors such as mammographic density [13], bone density [14], body mass index (BMI) [15], and single-nucleotide polymorphisms associated with breast cancer [16] to provide an improved model of breast cancer risk [11]. The present review will focus on the analysis of non-conjugated and conjugated estrogens and

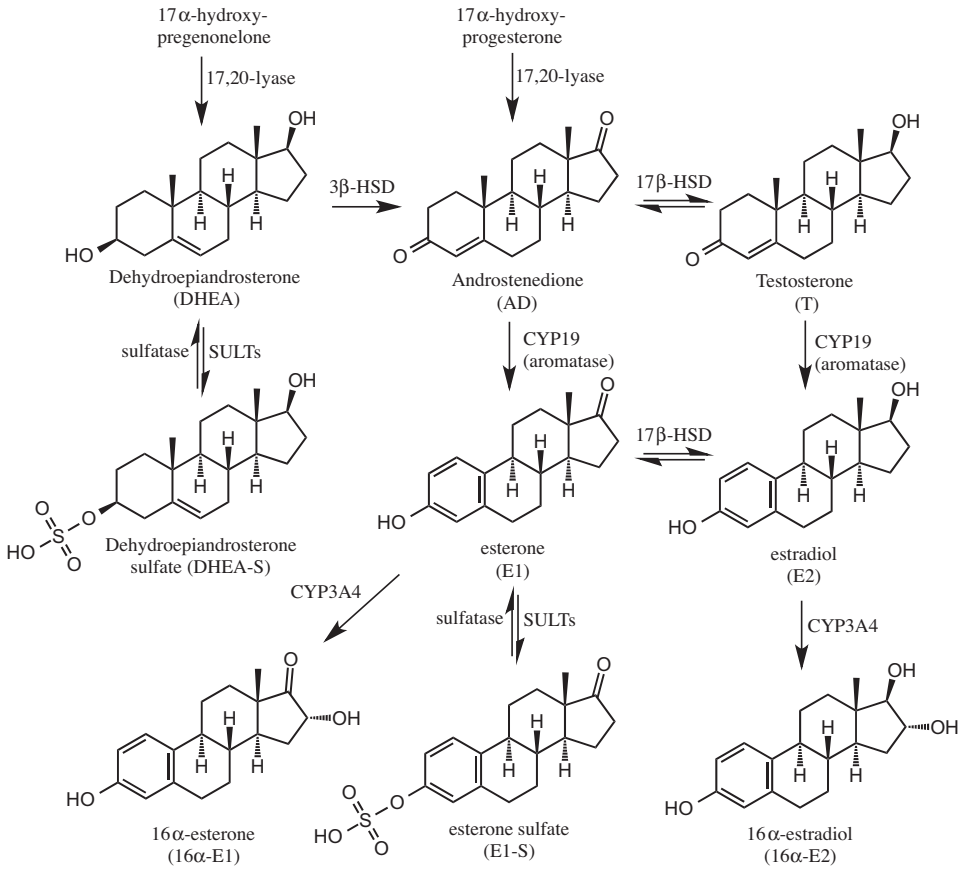

Fig. 1. The formation of estrogens in the tissue postmenopausal women from circulating C-19 androgens and sulfate precursors.

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