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Short communication

Xenobiotic phenols in early pregnancy amniotic fluid☆

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Abstract

We found detectable levels of three phytoestrogens (enterolactone, daidzein and genistein) and bisphenol A (BPA) in 21 residual amniotic fluid specimens that were collected before 20 weeks gestation. Samples were obtained by amniocentesis from women who were referred to the Mount Sinai Medical center because of advanced maternal age. Phytoestrogens were present in higher concentrations than BPA. Enterolactone was detected at the highest concentration (median 95.9 μg/L), followed by daidzein and genistein (9.5 and 1.4 μg/L, respectively). BPA was present at very low concentrations (10% > LOD of 0.5 μg/L). The relative concentration of the chemicals measured in amniotic fluid were identical to those in urine reported by other studies, i.e. enterolactone > daidzein > genistein ≫ BPA. Amniotic fluid is a source of fetal exposure to polar xenobiotics that come from the mother.

Keywords: Amniotic fluid; Bisphenol A; Phytoestrogens; In utero

1. Introduction

Hormonally active chemicals, such as bisphenol A (BPA) and phytoestrogens, have been detected in amniotic fluid [1–3]. Phytoestrogens and BPA are weak hormone agonists. Phytoestrogens also possess anti-estrogenic activity possibly by competitive binding to the estrogen receptors, and can reduce genotoxic damage to cells by antioxidant and other mechanisms [4,5]. They can also inhibit cellular growth and proliferation by inhibiting tyrosine kinase cell-signaling activity and by downregulating certain membrane receptors (e.g. erbB2, EGFR) [6,7]. In addition, phytoestrogens are powerful antioxidants [8]. Moreover, the joint effect of multiple hormonally active low-level compounds could be biologically relevant [9]. Consequently, concern has been mounting that prenatal exposure to hormonally active agents may result in reproductive or neurological effects [10–12].

We examined the concentration of three phytoestrogens (enterolactone, daidzein and genistein) and BPA in residual amniotic fluid samples that had been collected early in pregnancy from a population of women in the US.

2. Materials and methods

Twenty-one consecutive amniotic fluid specimens were collected by amniocentesis before 20 weeks gestation from women who were referred to the Mount Sinai Medical Center with the sole indication of advanced maternal age (AMA). AMA indications are generally reserved for women over the age of 35 and are associated with a relatively low risk for fetal abnormalities (chromosomal) when compared to the risks associated with Mendelian inheritance or an abnormal ultrasound scan. Women with AMA indications were selected because women who receive an amniocentesis at younger ages generally have high-risk indications that could

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Table 1
Range and median levels of xenobiotic phenols measured in amniotic fluid collected before 20 weeks gestation at the Mount Sinai Medical Center, New York in 2004

Metabolite	Range (μg/L)	Median (μg/L)	Percentage detectable
Enterolactone	11.8–112	95.9	100
Daidzein	3.84–17.4	9.52	100
Genistein	0.20–7.88	1.38	100
Bisphenol A	0.5[a]–1.96	0.5[a]	10

[a] Limit of detection (LOD).

potentially be associated with exposure. Residual amniotic fluid (mainly noncellular supernatant) that remained after clinical care tests were completed was utilized for this investigation. Samples were de-identified prior to analysis and stored in polypropylene Falcon™ tubes at −20 °C.

The analytical method, as previously reported for analysis of polyphenols in urine, was used without alteration for amniotic fluid to determine phytoestrogens daidzein, genistein, and enterolactone and BPA. The method involves deconjugation of the phenol metabolites in 2 mL of amniotic fluid with glucuronidase, C18 column clean up, and liquid chromatography with electrochemical detection. The limits of detection (LOD, defined as three times the standard deviation of multiple blank determinations) ranged from 0.2 μg/L (genistein) to 0.9 μg/L (enterolactone) [13].

3. Results

Phytoestrogens, as expected, were present in higher concentrations than BPA. Enterolactone was detected at the highest concentration, followed by daidzein and genistein. BPA was present at very low concentrations and 10% were above the LOD (0.5 μg/L) (Table 1).

4. Discussion

The relative concentrations of the chemicals measured in amniotic fluid were identical to those in urine reported by other studies, i.e. enterolactone > daidzein > genistein ≫ BPA. Median phenol levels in amniotic fluid were 1/3 or less the values reported by CDC in the NHANES data (CDC 2003). However, levels in amniotic fluid (medians) were only 1.4–2 times lower than in the New York City adults reported Liu et al. [13]. This suggests the need to study amniotic fluid and urinary levels side-by-side, as xenobiotic levels vary widely by age, race, sex, and geographic location (CDC 2003).

In a study of 53 second trimester amniotic fluid samples from women presenting for amniocentesis at a southern California clinic, Foster et al. found lower median daidzein and genistein concentrations than our population (median 0.50 and 0.60 μg/L, respectively), with 46 and 61% having detectable values [1]. These differences may be attributable to differences in the exposure patterns between study populations.

Exposure to isoflavones (including daidzein and genistein) is largely through the use of soya in processed foods [14]. Enterolactone is the highest phytoestrogen in the Western diet, as well as in urine samples measured in most US studies [13,15]. It has never before been measured in amniotic fluid; however, it was the highest chemical present overall in our sample. Enterolactone is derived from lignan precursors in whole grains, seeds, nuts, vegetables, berries, tea, and coffee [16], and has been found to have both pro- and anti-estrogenic effects depending on the concentration. Between 0.5 and 2 μM, enterolactone stimulated the proliferation of MCF-7 cells; however, inhibition occurred above 10 μM [17,18]. We observed intraamniotic concentrations of enterolactone in the range of 0.4–4 μM in amniotic fluid. Therefore, these concentrations may be biologically relevant.

BPA is weakly estrogenic. Two studies have examined BPA concentration in early pregnancy amniotic fluid samples in Japanese populations from different locations, although none have been reported in a US population, which may have substantially different exposure patterns. Yamada et al. examined the change in amniotic fluid BPA concentration over a 10-year period, and found that median values ranged from 0.00 to 0.68 μg/L, with the 10-year overall median of 0.26 μg/L [3]. These values are very similar to those we observed in this study. However, another study from Japan reported a substantially higher mean BPA concentration in early pregnancy amniotic fluid samples (8.3 ± 8.9 μg/L); in term amniotic fluid samples the levels of BPA were within the range reported by ours and Yamada et al. (1.1 ± 1.0 μg/L) [2]. In another study of a Japanese population, median levels of BPA in urine declined as much as 2.2-fold between 1992 and 1999, possibly relating to industrial changes in the interior coating of canned beverages and foods in 1997 [19]. Silva et al. observed a median effect concentration of 0.8 μM for BPA [9]. In a recent comparison of the estrogencity of a variety of chemicals, BPA induced the highest estrogenic response of all the environmental chemicals tested, albeit at a potency several thousand times lower than 17β-estradiol [20]. The intraamniotic concentration of BPA in our subjects was low, and ranged from 0.01 to 0.1 μM. It remains to be seen whether exposure at this level presents a threat to fetal health.

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