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ScienceDirect
Int. J. Hyg. Environ.-Health 210 (2007) 429–432
International Journal of Hygiene and Environmental Health
www.elsevier.de/ijheh

Biomonitoring of environmental estrogens in human tissues

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Abstract

Two examples are presented for the application of the total effective xenoestrogen burden as biomarker of chemical exposure measured in tissue samples from patients recruited for two case–control studies. The first study focused on environmental chemicals with hormone mimicking activity, the so-called environmental estrogens, and their participation in the etiology of breast cancer. The second study investigated mother–child exposure to persistent organochlorine chemicals and assessed their combined effect on the risk of male urogenital malformations in the newborn.

Keywords: Biomarkers of effect; Human biomonitoring; Total effective xenoestrogen burden

Introduction

Endocrine disruptors (ED) are defined as exogenous substances or mixtures with the ability to disrupt normal endocrine homeostasis and alter endocrine system function, thereby producing adverse health effects in an intact organism, its progeny, or in (sub)-populations (EU, 2001). Some pesticides are ED, most of them characterized as acting in the living organism in an estrogen-like manner (Soto et al., 1995), e.g., DDT and endosulfan, although they can also induce anti-androgen activity, e.g., *p*,*p*′-DDE and vinclozolin, or more complex interactions affecting endocrine homeostasis. Because of their ubiquity and persistence in the environment, ED pesticides can be found in soil, water, wildlife, and in the adipose tissue of mothers, reaching children during pregnancy and lactation (Fernandez and Olea, 2006). If they play a role in the pathogenicity of human hormonal diseases, there is a need for biomonitoring of these chemicals as a key instrument to assess their impact on susceptible populations.

Over the past few years, the scientific community has begun to develop techniques that allow investigators to quantify exposure to ED and to discriminate between endogenous hormones and xenoestrogens. This methodology is critical for future application of biomarkers in epidemiological studies. To address this issue, we developed a method to assess the total effective xenoestrogen burden (TEXB) in human samples (Fernandez et al., 2004). High performance liquid chromatography (HPLC) is used to separate more lipophilic environmental estrogens (alpha-fraction) from endogenous hormones (beta-fraction). The combined estrogenic effect of both fractions is analyzed from their proliferative effect on MCF-7 human breast cancer cells and expressed as estradiol equivalent units (pM Eeq). The estrogenicity of the alpha-fraction, which contains no endogenous sex-hormones, can be considered a marker of the TEXB due to environmental organohalogenated estrogens.

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doi:10.1016/j.ijheh.2007.01.014

Two examples for this application on tissue samples are presented. The first study focused on environmental chemicals with hormone mimicking activity, the so-called environmental estrogens, and their participation in the etiology of breast cancer. The second study investigated mother–child exposure to persistent organochlorine chemicals and assessed their combined effect on the risk of male urogenital malformations in the newborn.

First study: biomonitoring of environmental estrogens in breast cancer

Spain has a low breast cancer incidence in comparison with other European countries, although it has shown an increase in the past few decades (Benach et al., 2003). Geographical differences in breast cancer incidence have been explained in terms of genetic, reproductive, and environmental factors, among others. Higher breast cancer risk has been associated with characteristics implying a greater lifetime accumulation of estrogens, e.g., age, early menarche, late menopause, nulliparity, late full-term pregnancy, and lack of breast feeding. Some sociodemographic and lifestyle factors, including alcohol and tobacco consumption, have also been widely considered. Although breast cancer onset cannot be directly attributed to any of the above risk factors, the length, extent, and timing of exposure to estrogens may play a key role in the vulnerability of the female breast (Gadducci et al., 2005).

Environmental chemicals with hormone mimicking activity, so-called environmental estrogens, have been found in mammary adipose tissue, and their participation in the etiology of breast cancer has been postulated. Over the past 20 years, numerous epidemiological studies have addressed the role of organochlorine compounds in breast cancer, but an association between exposure to chemicals and disease onset remains inconclusive (Lopez-Cervantes et al., 2004). Inconsistencies in results may have been caused by differences in the populations or ethnic groups studied, variability in the sensitivity of chemical analyses, or failure to adequately control for potential confounders. Chemicals may also interact with environmental, dietary, lifestyle, and reproductive factors that are not systematically measured across studies. More importantly, a hypothetical association between organochlorines and breast cancer risk cannot be tested on the basis of individual compound levels, and account must also be taken of possible interactions between the chemicals (Kortenkamp, 2006).

A hospital-based case control study was conducted from April 1996 to June 1998 in the three largest public hospitals serving Granada and Almeria provinces in southern Spain. Cases were recruited from women aged between 35 and 70 years undergoing surgery for newly diagnosed malignant breast carcinoma. Controls were matched for age (± 3 years) and hospital. Because adipose tissue was needed for the study, controls were recruited from women undergoing non-cancer-related surgery. Breast or abdominal adipose tissue from cases and controls were obtained from participants in the course of surgery and always before the initiation of chemotherapy or radiotherapy.

Structured face-to-face interviews before surgery were conducted at the hospitals by trained interviewers to gather data on sociodemographic characteristics, reproductive history and fertility, menopausal status, use of exogenous hormones, diet, tobacco and alcohol consumption, and family history of breast cancer. The questionnaire, chemical analysis, and estrogenicity assay were carried out in 198 (76%) cases and 260 (73%) controls.

Bioaccumulated compounds were extracted from samples (200 mg of adipose tissue) with hexane and eluted in a glass column filled with aluminum. The eluate obtained was concentrated and then injected into the preparative HPLC. The presence and identity of aldrin, dieldrin, endrin, lindane, methoxychlor, endosulfan I and II, mirex, *p,p′*-DDT, *o,p′*-DDT, *o,p′*-DDD, *p,p′*-DDE, endosulfan diol, sulfate, lactone, and ether was analyzed by gas chromatography with electron-capture detection, using *p,p′*-dichlorobenzophenone as internal standard and gas chromatography/mass spectrometry (GC/MS).

Leaner and postmenopausal women with the highest levels of estradiol equivalents in the alpha-fraction had a significantly greater risk of breast cancer versus other sub-groups (Ibarluzea et al., 2004). Among this leaner group, the women with highest levels of estradiol equivalents in the alpha fraction (>228.51 pM Eeq/g lipid; fourth quartile) had a 3.8-fold significantly greater risk of breast cancer versus those with the lowest levels (<0.47 pM Eeq/g lipid; first quartile) (95% CI 1.37–10.56; $p<0.01$). On the other hand, no association with breast cancer risk was found for TEXB-β or for TEXB-α and -β combined in either the whole study population or any subgroup. Indeed, separate analyses of the results for leaner women (BMI $<$median) provided the first demonstration of a significant relationship between breast cancer risk and estrogenicity of environmental chemicals bioaccumulated in adipose tissue.

In conclusion, this approach to biomonitoring exposure to environmental estrogens could be applied to human samples preserved in bio-repositories and would allow retrospective epidemiological studies to be performed on breast cancer and environmental factors.