PLENARY LECTURE

THE COMBINED EFFECT OF ENVIRONMENTAL HORMONES AND THE RISK OF HORMONE DEPENDENT DISEASES

Nicolas Olea, M.D.

Lab. Medica Investigations. Hospital Clinico. University of Granada. Granada. Spain

Confirmation that humans are exposed to chemicals that interfere with the hormonal system (endocrine disrupters) has prompted many epidemiological studies, but an association between chemical residues and disease remains elusive. Exposure to these chemicals occurs mainly through diet, water, environment, indoor air, dust, soil, and also in an occupational setting. For example, organochlorine pesticides and polychlorinated biphenyls (PCB) enter the human organism via food and water but they also may reach humans by inhalation and contact, especially among those professionally exposed in industrial and agricultural settings. Many of these organohalogenated derivatives accumulate in adipose tissues because of their solubility in lipids and their inefficient metabolism.

Since 1970, the use of some organochlorines has been banned, leading to a reduction in their accumulation in the environment and a decrease, albeit slower, in the human body burden. Other organohalogenated chemicals are still used and frequently found in the environment, human tissues, and fluids. Over the past 16 years, many studies have reported a wide range of pesticide content in the adipose tissue of breast cancer patients, not only dichlorodiphenyltrichloroethane (DDT) and its metabolites, but also hexachloronbenzene, mirex, chlordane, and b-hexachlorocyclohexane. Experimental data confirmed the estrogenicity of many of these fat-soluble chemicals, which are now classified as xenoestrogens (Sonnenschein and Soto, 1998). However, there has been no demonstration to date of a clear connection between exposure to these chemicals and hormone-dependent diseases, or of the role of these xenobiotics in the modulation of cancer growth.

It was stated (Woodruff *et al.* 1994) that the design of retrospective cancer studies based on the analysis of human samples should consider the hypothesis to be tested, the chemicals to be measured, and the biological activity to be analysed. However, these considerations are frequently disregarded. For example: i) many studies have included chemicals that are not hormonally active, taking no account of the role of estrogens in the pathogenesis of the disease, ii) other studies have only measured a single residue because of the time, resources and the large sample sizes required to determine more chemicals, and iii) most of the studies have disregarded cumulative effects and interactions between chemicals.

There is a need to develop markers of hormonal exposure that go further than the quantification of isolated environmental hormones. For instance, it has been proposed (Soto *et al.* 1997) that rather than measuring the levels of identified xenoestrogens, it might be more meaningful to assess the risk of endocrine disruption by measuring the biological activity resulting from the xenobiotics.

In order to facilitate the rigorous testing of a putative link between exposure and disease, we adopted this approach and developed and standardized a method to assess the total effective xenoestrogen burden (TEXB) in human adipose tissue and serum (Sonnenschein et al., 1995; Rivas et al., 1997; Pazos et al., 1998; Rivas et al., 2001). High performance liquid chromatography (HPLC) is used to extract and separate organohalogenated xenoestrogens from sex-steroids, and the combined estrogenic biological effect of the extracts is then determined in the E-Screen biosassay from their proliferative effect on MCF-7 cells (Soto et al., 1995). Two fractions of interest are eluted by the HPLC

process, denominated the alpha and beta fractions. The alpha-fraction contains organohalogenated compounds, whereas the beta-fraction contains endogenous sex-steroids and more polar xenoestrogens, distinct from those eluted in the alpha-fraction. Extensive testing (Rivas et al., 1997; Pazos, et al., 1998;; Rivas et al, 2001) demonstrated that the pesticides DDT and metabolites, dieldrin, aldrin and lindane, among other organochlorines, as well as other chlorinated and/or brominated organohalogenated chemicals all elute in the alpha-fraction. Thus, the estrogenicity of the alpha fraction, which contains no endogenous sex-hormones, can be considered a good marker of the total effective xenoestrogen burden from organohalogenated chemicals (Rivas et al., 2001).

Endogenous sex-hormones such as estradiol, estrone and their sulfates, and androstenedione and androstenediol elute in the beta-fraction, as well as certain synthetic estrogens like bisphenols, alkylphenols and polyphenols (Rivas et al., 2001). In this context, the presence of endogenous sex-steroids in adipose tissue has been documented, and nonylphenol, octylphenol and bisphenol-A were stored in adipose tissue of treated animals and humans.

Our method was applied to adipose tissue samples collected in a hospital-based, case-control study on breast cancer. The combined effect of chemical residues was assessed in a biological assay for estrogenicity (E-Screen) , and patients were classified according to their total effective xenoestrogen burden (TEXB) and adipose tissue content of sixteen environmental estrogens.

Our results suggested that the combined effect of xenoestrogens in adipose tissue may increase the risk of breast cancer. When different groups (body weight and menopausal status) of the women were considered, relationships of interest emerged: Estrogenicity due to organohalogenated chemicals (alpha fraction) could be distinguished from that due to endogenous estrogens and the most polar xenoestrogens (beta fraction), and the estrogenicity due to organohalogenated chemicals is a risk factor for breast cancer.

In addition, we found that the organochlorines pesticides aldrin and lindane may increase the risk of breast cancer. In the whole series, breast cancer risk was significantly associated only with aldrin levels, when comparing the highest percentile with the lowest. Aldrin can degrade to dieldrin, so that the measurement of either chemical indicates a similar exposure, and a significant relationship between blood levels of dieldrin and the risk of breast cancer has previously been described. Interestingly, when our postmenopausal women were considered separately, breast cancer risk was associated with both aldrin and lindane. Both aldrin and lindane may act as estrogen disrupters, suggesting the mechanism by which breast cancer risk is increased by these pesticides, and supporting a biological relationship between exposure and effect in hormone-dependent cancer.

We found no differences in adipose tissue DDE levels between the breast cancer patients and their paired controls. DDE levels were lower in our series compared with other case-control studies, which may be because the pesticide has not been used since 1980. It has been recommended (Snedeker, 2001) that the association between DDE and breast cancer be explored in populations with much more recent exposure, and studies in Colombia and Mexico City, where the pesticide was in use until recently, found a moderately high risk of breast cancer in women with higher blood levels of DDE. However, negative results were observed in previous studies carried out in Mexico, Brazil and North Vietnam.

In summary, concerns about the role of environmental and dietary estrogens as contributors to the increasing incidence of hormone-related diseases have prompted epidemiologists and clinicians to design patient-based studies in which to test their hypotheses (Fernández et al., 1998). However, the study of etiologic factors in diseases is not a simple task when the period of exposure is far removed from the clinical presentation of the disease. This can be observed in studies that have attempted to link breast cancer risk to organochlorines content in adipose tissue. It is possible that a relationship between long-latency diseases and organochlorine exposure cannot be demonstrated from the levels of a single xenoestrogen or of only a few of them. Most studies have estimated the circulating levels or adipose

content of one or a small number of chemicals, and have ignored the impact of other chemicals and also the combined effects of chemical mixtures in the environment, which cannot be assessed by the testing of isolated chemicals. The approach we proposed may help to establish a relationship between the content of environmental estrogens and the risk of hormonal diseases.

References

Sonnenschein C., Soto A. (1998) An update review of environmental estrogen and androgen mimics and antagonist. *J. Steroid Biochem. Molec. Biol.,* 65, 143-150.

Woodruff T., Wolff M.S., Davis D.L. *et al.* (1994) Organochlorine exposure estimation in the study of cancer etiology. *Environ. Res.*, 65, 132-144.

Soto A, Fernández MF, Luizzi M, Oles Karasko AS, Sonnenschein C (1997). Developing a marker of exposure to xenoestrogen mixtures in human serum. *Environ Health Pespect:* 105:647-63.

Sonnenschein C., Soto A., Fernández MF. M.F., Olea N., Olea-Serrano MF., Ruiz-López MD. (1995) Development of a marker of estrogenic exposure in human serum. *Clin Chem.*, 41, 1888-1895.

Pazos P, Pérez P, Rivas A, Nieto R, Botella B, Crespo J. (1998) Development of a marker of estrogenic exposure in breast cancer patients. *Adv Exp Med Biol* 444: 29-40.

Rivas A., Olea N., Olea-Serrano F. (1997) Human exposure to endocrine-disrupting chemicals: assessing the total estrogenic xenobiotic burden. *TRAC,* 16, 613-619.

Rivas A, Fernández MF, Cerrillo I, Ibarluzea J, Olea-Serrano MF, Pedraza V, Olea N. (2001). Human exposure to endocrine disrupters: Standardisation of a marker of estrogenic exposure in adipose tissue. APMIS 109:185-97.

Soto A., Sonnenschein C., Chung K. Fernández, M.F., Olea, N., Olea-Serrano, M.F (1995) The E-SCREEN Assay as a Tool to Identify Estrogens: An update on Estrogenic Environmental Pollutants *Environ. Health. Perspect.*, 103, 113-122.

Snedeker S. Pesticides and Breast cancer risk: a review of DDT, DDE, and Dieldrin. (2001) *Environ Health Perspect* 109: 35-47.

Fernández M.F., Pedraza V., Olea N. (1998) Estrogens in the environment: is there a breast cancer connection?. *Cancer J.*, 11, 11-17.