Female Reproductive Tract and Mammary Disorders Caused by Endocrine Disruptors

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Abstract: Several possible endocrine disruptors, including bisphenol A, chlorobenzenes, benzo (A) pyrene, phthalate, PCB, and chlordane, were assayed in cord blood, maternal venous blood, breast milk and ascitic fluid to investigate the mechanisms of endocrine disrupting action in human subjects. The data indicate that, once reliable techniques for quantitation are established, it may be possible to elucidate endocrine disrupting action through comprehensive studies of the real levels of endocrine disruptors in human samples and the in vivo presence of the receptors and their metabolisms.

Key words: Endocrine disruptors; Female reproductive tracts; Cord bloods; Ascitic fluids

Introduction

It has been some time now since endocrine-disrupting chemical substances caught the attention of society, and as reports on environmental exposure and biogenesis come out sporadically, it is only recent that the directions we should be taking to study this field are becoming clarified. The initial social confusion over endocrine-disrupting chemical substances had started in reports on abnormal biogenetic phenomena and their fleeting detection in such natural environments as water, soil and fishes, where they were treated as “somewhat scary substances.”

The comprehensive and basic research into these substances that followed—in health sciences, for example—suggested how to proceed with research and operations. Additionally, the basic facts concerning the connection between endocrine-disrupting chemical substances and the reproductive functions have gradually been established.\(^1,2\)
Establishment of Endocrine-Disrupting Chemical Substance Assays

The first fundamental of endocrine-disrupting chemical substance research is the establishment of endocrine-disrupting chemical substance assays that are satisfactory in sensitivity and specificity. This is because, without the establishment of an assay, there can be no discussion of the effect of endocrine-disrupting chemical substances on reproductive functions.

The handling of samples must not be overlooked among the elements of an assay. All the substances are present in vivo only in minuscule amounts. It is especially important to establish the methodology for each step of the process, from taking biological samples to preventing background interference and admixture of contaminants during the separation and storage processes and through obtaining reliable absolute values.

We have already studied this field in cooperation with the Health Sciences Research report “Establishment of analysis of endocrine-disrupting chemical substances derived from consumer goods comprised of polymer materials” (Lead researcher: Prof. Hiroyuki Nakazawa, Hoshi University).

Biological Exposure to Endocrine-Disrupting Chemical Substances

Over 70 kinds of so-called endocrine-disrupting chemical substances are present in natural environments, but in a discussion of their effect on human health and reproductive functions, the second important issue is the exact level of biological exposure, i.e. analysis of in vivo concentrations of these substances.

In the Health Sciences Research report “Development of Biological Sample (Cord Blood, etc.) Analytical Methods Relating to Endocrine-Disrupting Chemical Substances and Research into Their Effects on Human Health Based on the Results of Sample Analysis” (Lead researcher: Tsunehisa Makino), working in concert with the Nakazawa team mentioned above, assays were established that enabled routine obtaining of stable results, and the report identified the following substances as candidate endocrine-disrupting chemical substances that cannot be neglected in the past and current volume of Japanese industrial production.

1) Bisphenol A
2) Chlorobenzenes
3) Parabens
4) Phthalate
5) Benzo (A) pyrene
6) PCB
7) Chlordane
8) Butyl tin compounds

The human biological samples subjected to assay were primarily reproductive system samples, (a) cord blood, (b) maternal venous blood, (c) breast milk, and (d) ascites. To the extent possible, samples (a) through (d) were taken simultaneously from each case subject, and we also examined the concentration gradients among in vivo internal organs of individual subjects.

Our results, as previously reported in detail at several opportunities,1,2) were that bisphenol A, still produced in volumes of 300,000 tons annually as a raw material of plastics, was detected in all such samples as (a) through (d) and was found to have in vivo concentrations in the range 0.21–0.79 ppb.

Among chlorobenzenes, we assayed hexachlorobenzene and detected it in 100% of general peripheral blood and maternal peripheral blood samples and in 88% of cord blood samples, and found it to have concentrations in range of 0.03–0.10 ppb. Hexachlorobenzene concentrations in samples taken from individual subjects showed a significant positive correlation (coefficient of rank correlation $\rho = 0.722, n = 12, p = 0.017$) between human peripheral bloods and ascites.

Among parabens, we detected methyl paraben in cord blood and maternal milk, and
inferred that parabens to which the pregnant women were exposed migrated via their blood to maternal milk and cord blood.

**Phthalate**, which is used as a plasticizer in plastics and the like, was detected in peripheral blood and ascites in concentrations averaging 1–5 ng/ml in the forms MBP (monobutyl phthalate), MBzP (monobenzyl phthalate), and MEHP (mono-2-ethylhexyl phthalate).

**Benzo (A) pyrene**, released into the atmosphere through the incomplete combustion of fossil fuels, was detected in male urines in the form OH-BaP, and we plan to go on to study the status of exposure to it in maternal milks, cord bloods, maternal peripheral bloods, and ascites.

The production of **PCB** (polychlorinated biphenyl), used as an incombustible and insulator, has been suspended since 1972, but it was detected as 35 different isomers in maternal milk, maternal peripheral blood, and cord blood in concentrations in the range, on a fat basis, of 60–99 ng/g.

The production of **chlordane**, used in the extermination of termites and other pests, has been suspended since 1986, but trans-nonachlor was detected in 63% (0.06–0.17 ppb) and cis-nonachlor in 17% (0.03–0.05 ppb) of samples, whereas none of heptachlor epoxide, oxychlordane, trans-chlordane or cis-chlordane was detected in any of the samples at all.

For **butyl tins**, used as ship’s bottom paints and fishing-net anti-contaminants and use of the open systems of which has now been partially suspended, results varied in different assays performed. They were detected (5–45 ppb) in 33% to 77% of hair samples. We reported in 1999 cases of high concentrations (41–45 ppb) detected within a single family.

Such volatile organic compounds as **toluene**, **benzene**, **xylene**, and **styrene** were detected in peripheral blood and ascites in concentrations of 0.6–4.0 ppb. 80% of samples and they were positive for toluene, 49% for P-dichloro benzene, 29% for O-xylene, and 26% for styrene. Naphthalene was not detected at all.

**In Vivo Action and Expression**

The third important task in the study of the effects of endocrine-disrupting chemical compounds on human health and reproductive functions is the investigation of their mechanisms of action **in vivo** in human beings. Specifically, this entails the investigation of (1) whether there are **receptors** for these substances in the human body, (2) whether they display **action** and **expression** as hormones, and (3) what the mechanisms of **metabolism** and **detoxification** of these substances are in the human body.

**Receptors** for endocrine-disrupting chemical substances have been identified in the human body similar to such **in vivo** estrogens as human adrenocortical-derived cells (H295R) and human mammary cells (T47D). In detailed studies of receptors with human endometrial cells (HHUA) and human mammary-derived cells (MCF-7), it has been confirmed that they bound with estrogen alpha and beta receptors. In addition to known receptors, we have also decided to investigate the existence of so-called “orphan receptors” hitherto unknown.

With respect to the **in vivo action** and **expression** of endocrine-disrupting chemical substances, we established that they regulated the cortisol production of human adrenocortical cells. We also confirmed that they stimulated the multiplication of mammary cells and endometrial cells. We found that in mice butyl tins were active in the immune system and affect the induction of oral tolerances, and that in rats benzo (A) pyrene affected the process of differentiation of trophoblast stem (TS) cell lines.

Work that remains to be done in the study of the action and expression of these substances is a study to find out what actions, if at all, are expressed within the range of exposure in which these substances are actually present **in vivo**.

Much scope remains for further research into the **metabolism** and **detoxification** of
endocrine-disrupting chemical substances. To take bisphenol A as an example, we found that in rats the bulk of the substance was glucuronidated in the gastrointestinal tract and the liver. On the other hand, it was surmised that it was not metabolized in the kidneys, but only filtered and excreted. We identified the presence of an enzyme (beta-glucuronidase) that broke down glucuronate conjugates into the original endocrine-disrupting chemical substance, and we are planning to study it in the human body in the future.

Conclusion

We have thus assayed exact levels of biological exposure on the basis of the establishment of assays for several substances derived from polymers. Through investigation of in vivo receptors, action and expression, and metabolism and detoxification of endocrine-disrupting chemical substances, we continue further research towards our primary objective of working towards a conclusion on their effects on human health and reproductive functions.

REFERENCES

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