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REVIEW ARTICLE

Neoplasia as development gone awry: the role of endocrine disruptors

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Summary

The hypothesis that prenatal exposure to endocrine disruptors might cause cancer arose from challenging two well-accepted notions: (i) mammalian development is merely the unfolding of a genetic programme and (ii) only mutagenic agents can cause cancer. This hypothesis required challenging genetic determinism. The ecological developmental biology (eco-devo) movement revitalized the concept of developmental plasticity through the occurrence of polyphenisms (a single genotype produces diverse phenotypes which are determined by environmental cues). Based on the principles of eco-devo and the *tissue organization field theory of carcinogenesis and neoplasia*, we tested the hypothesis that exposure to xenoestrogens during foetal development in rats increased the propensity to develop mammary cancer during adulthood. We chose exposure to bisphenol A (BPA) as a model for environmental oestrogen exposure. This endocrine disruptor induced the development of ductal hyperplasias and carcinoma in situ. These highly proliferative lesions contained an increased number of oestrogen receptor α-positive cells. Thus, foetal BPA exposure was sufficient to induce the development of oestrogen-sensitive pre-neoplastic and neoplastic lesions in the mammary gland in the absence of any additional treatment aimed at increasing tumour incidence.

Introduction

At the beginning of the 21st century, we are experiencing a paradigm change in biology. The realization that reductionism has failed to bring about an understanding of complex phenomena has resulted in reappraisals of old research concepts in embryology and cancer research in the context of the scientific and technological advances acquired since their inception. The dominant view during the last 50 years has been that development is the unfolding of a genetic programme where the environment plays virtually no relevant role, despite studies from the end of the 19th century illustrating the phenomenon of environmentally triggered polyphenisms. Two factors among the many that contributed to the dominance of the genetic programme were the choice of model organisms that reproduce in laboratory facilities where the environment is practically invariable and the dominance of a genocentric view originating from the molecular biology revolution. These models and views converged into the construction of developmental genetics, 'a discipline that explicitly treated phenotype as a direct ''readout'' of the nuclear genome' (Gilbert, 2005).

This rigid view of development is now rapidly changing, as epidemiological studies reveal the developmental plasticity of the human foetus (Barker & Hanson, 2004). Several paths have been identified that could mediate environmental cues into the building of a phenotype, namely, (i) the neuroendocrine route, whereby the nervous system monitors the environment and transfers signals to the endocrine system; (ii) the epigenetic route, whereby environmental agents change the methylation pattern of genes, thereby altering their transcriptional capabilities; and (iii) direct modulation of gene expression, particularly by hormonally active agents (Gilbert, 2005).

The new emphasis on ecological development biology has prompted scientists to hypothesize that foetal exposure to xenoestrogens may be the underlying cause of the increased incidence of uterine leiomyoma, testicular cancer and breast cancer observed in European and US populations over the last 50 years (Skakkebaek *et al.*, 1998;

Markey *et al.*, 2003). This hypothesis implies comparable paradigm changes in cancer research.

According to the current dominant view, the *somatic mutation theory*, cancer is a cell-based disease caused by mutations in the DNA of a single cell (Hahn & Weinberg, 2002). The research programme emanating from this theory has failed to explain the cancer pathogenesis and to provide successful therapies. Probably because of these shortcomings, an older research tradition focussed on the tissue level of organization has been updated as the *tissue organization field theory of carcinogenesis and neoplasia* and is gaining momentum (Soto & Sonnenschein, 2004). This tradition originated in the late 19th century when pathologists began describing the histological pattern of tumours and suggested that altered tissue organization is at the core of neoplasia, thus linking carcinogenesis to embryonic development (Soto & Sonnenschein, 2004). A central motif in this theory is the persistence of morphogenic fields throughout adult life; these fields orchestrate histogenesis and organogenesis before birth as well as tissue maintenance and regeneration throughout postnatal life. The *tissue organization field theory* posits that neoplasms result from a flawed interaction among cells and tissues and that carcinogenesis is potentially reversible (Maffini *et al.*, 2005).

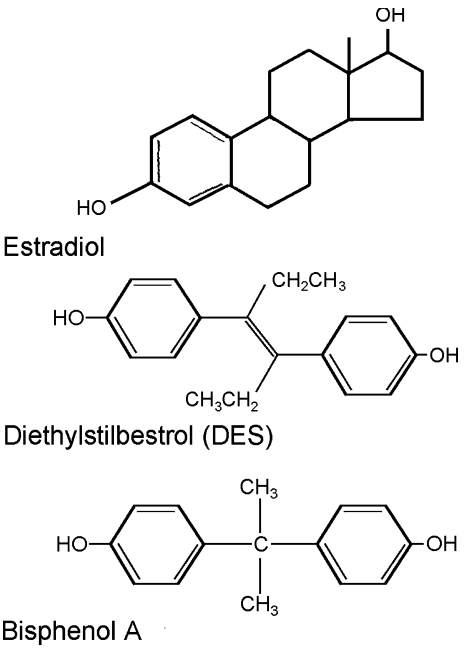

Figure 1 Chemical structures of oestradiol, diethylstilbestrol (DES) and bisphenol A (BPA). The structures of BPA and DES are more similar to one another than they are to the endogenous oestradiol, indicating that chemicals with variable structures are capable of binding to the oestrogen receptor.

Developmental origins of breast cancer

Epidemiological studies suggest that fluctuating oestrogen levels in the foetal environment have long-term consequences regarding the risk of developing breast cancer (Trichopoulos, 1990). Given the long latency period between exposure and effect, epidemiological studies designed to explore this hypothesis have used prenatal markers of foetal oestrogen exposure (i.e. twin pregnancy as an indicator of high-oestrogen exposure and pre-eclampsia for low-oestrogen exposure). Increased risk of breast cancer correlated with twin dizygotic birth and pre-eclampsia was associated with lowered breast cancer risk (Potischman & Troisi, 1999).

Direct evidence of prenatal oestrogen exposure and associated breast cancer risk is now being collected from the cohort of women born to mothers treated with diethylstilbestrol (DES) during pregnancy (Fig. 1). These women are now reaching the age at which the incidence of breast cancer becomes more prevalent. In the group of women aged 40 years and older exposed during gestation to DES, there is a 2.5-fold increase in the incidence of breast cancer compared with unexposed women of similar age (Palmer *et al.*, 2006). Likewise, experiments in rats showed that prenatal exposure to DES resulted in increased mammary cancer incidence during adulthood (Boylan & Calhoon, 1983). These data are consistent with the hypothesis that excessive foetal oestrogen exposure may increase the risk of breast cancer. However, in the above-mentioned studies, DES was administered at pharmacological doses, while twinning and pre-eclampsia represent a physiological range of endogenous hormone levels to which foetuses are exposed. Additionally, there is a third type of exposure that needs to be addressed (i.e. the inadvertent and continuous exposure of foetuses to xenoestrogens).

Xenoestrogens alter mammary gland development

During postnatal life, the mammary gland undergoes massive architectural changes similar to those observed during organogenesis. These changes occur in response to endogenous hormones associated with puberty and pregnancy. Exogenous synthetic hormones can alter normal patterns of tissue organization (Markey *et al.*, 2003; Munoz de Toro *et al.*, 2005). Among these compounds, bisphenol A (BPA) is receiving increased attention because of its ubiquitous presence in the environment and chronic human exposure (Fig. 1). BPA is used in the manufacture of polycarbonate plastics and epoxy resins; it leaches from food containers (Brotons *et al.*, 1994), beverage containers (Biles *et al.*, 1997) and dental sealants and composites (Olea *et al.*, 1996) under normal conditions of use (Markey *et al.*, 2003). BPA has been measured in

maternal and foetal plasma and placental tissue in humans (Ikezuki *et al.*, 2002; Schonfelder *et al.*, 2002). A recent study reported that BPA was found in 95% of urine samples from a reference US adult population (Calafat *et al.*, 2005).

Prenatal exposure of mice to environmentally relevant levels of BPA resulted in alterations in the stroma and epithelium of the mammary gland such as an advanced maturation of the mammary fat pad as well as ductal alterations including decreased cell size, delayed lumen formation and increased ductal area at embryonic day 18 (Vandenberg *et al.*, 2007). At puberty, there was an increased number of terminal end buds (TEBs) relative to the ductal area, decreased apoptosis in the TEBs and an increased number of epithelial cells expressing progesterone receptor (Munoz de Toro *et al.*, 2005). At 4 months of age, there was a significant increase in lateral branching (Munoz de Toro *et al.*, 2005). By 6 months, there was an overall increase in epithelial structures including terminal ends and a premature appearance of alveolar buds, normally associated with pregnancy in the mouse (Markey *et al.*, 2001). The mammary glands of BPA-exposed mice that were ovariectomized before puberty showed an enhanced sensitivity to oestradiol demonstrated by an increased number of TEBs, TEB area, TEB density and ductal extension (Wadia *et al.*, 2007). If these effects were observed in humans, it would have suggested an increase in the risk of developing breast cancer.

Does prenatal exposure to BPA induce mammary neoplasia?

To explore this hypothesis, we used a rat model because it mimics the human breast disease regarding oestrogen dependence and histopathology (Singh *et al.*, 2000) more closely than the available mouse models (Nandi *et al.*, 1995). BPA was used at doses ranging from 2.5 to 1000 μg/kg body weight (bw)/day (the estimated tolerable daily intake was set at 10 μg/kg bw/day by the European Commission and 50 μg/kg bw/day by the US-EPA). Foetal exposure to BPA resulted in the development of carcinomas in situ in the mammary glands of 33% of the rats exposed to 250 and 1000 μg BPA, while none of the unexposed animals developed carcinomas in situ (Murray *et al.*, 2006). Neoplasms were observed at 50 and 95 days of age. Foetal exposure to BPA also increased the number of pre-neoplastic lesions (intraductal hyperplasias) by three- to fourfold (Fig. 2). The number of intraductal hyperplasias observed at 50 days of age was quantitatively similar at all the BPA doses tested, but they persisted longer in the animals exposed to the lowest dose. Moreover, prenatal exposure to 25 μg BPA/kg bw/day, followed by the treatment of these animals at puberty with a 'subcarcinogenic' single dose of the chemical carcinogen N-nitrosomethylurea resulted in the development of tumours only in the animals exposed in utero to BPA (Durando *et al.*, 2007).

The lesions observed in the BPA-exposed animals were highly proliferative and contained abundant oestrogen receptor positive cells (Murray *et al.*, 2006), suggesting that the proliferative activity in these lesions may be oestrogen mediated. As mentioned above, mammary carcinomas in rats as well as in humans are predominantly oestrogen dependent, a feature that strengthens the relevance of these findings.

These results buttress the link between foetal exposure to BPA and the development of neoplasms in the adult mammary gland. These neoplasms may have their origin

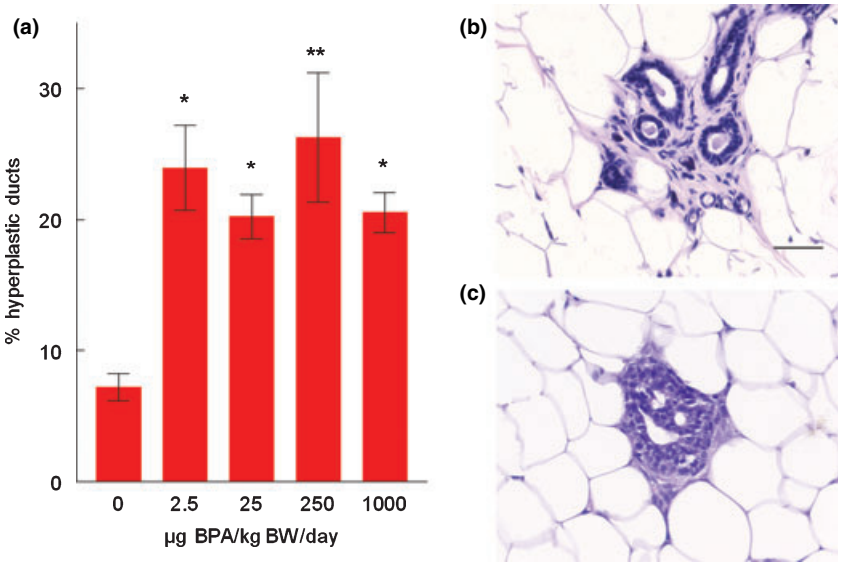

Figure 2 Preneoplastic and neoplastic lesions in animals exposed prenatally to bisphenol A (BPA). (A) The percentage of intraductal hyperplasias is significantly increased in BPA-exposed animals at postnatal day 50. (B, C) Histological sections of mammary glands stained with haematoxylin and eosin. (B) normal ducts; (C) carcinoma in situ at postnatal day 95 after prenatal exposure to 250 μg BPA/kg body weight/day. Scale bar: 200 μm.

in the altered morphogenesis that occurs in the foetus during the period of BPA exposure (Vandenberg *et al.*, 2007). Additionally, these data support the hypothesis that exposure to xenoestrogens during foetal life contributes to the increased incidence of breast cancer observed over the past five decades.

Conclusions

The findings reviewed above have both practical and theoretical implications. From a practical perspective, it is evident that wildlife and humans are affected by environmental exposure to hormonally active chemicals at levels previously considered to be irrelevant. Extrapolating data from animal studies to humans should be carried out cautiously, yet rodents have been shown to be an excellent surrogate model for the understanding of the DES syndrome. Thus, one cannot ignore the increasing evidence coming from controlled experiments in the laboratory and from exposed wildlife, alongside the increasing incidence of comparable anomalies in human populations exposed to the same chemicals. All of this evidence encourages the application of the precautionary principle by the banning or substitution of chemicals that are harmful to normal development.

From a theoretical perspective, research on endocrine disruptors is providing evidence that mammalian development is far more malleable than previously thought, as oestrogen exposure during development results in morphological and functional effects that persist into adulthood. Research on endocrine disruption is poised to contribute to the understanding of the mechanisms that underlie the development of hormone-target organs. This quest will require the use of both bottom-up approaches (from genes to organisms) and top-down approaches (from organisms to genes), as well as a new conceptual framework that would take into account the existence of emergent properties (Soto & Sonnenschein, 2005) (i.e. properties that cannot be explained from those of their isolated components). The properties at one level of complexity (e.g. tissues) cannot be ascribed directly to their individual parts (cells and extracellular matrix) but arise only because of the interactions among these parts. Developmental and cancer biology, guided by this integrative thinking, now have the tools to successfully revisit the old tradition of ecological regulation of development (Gilbert & Sarkar, 2000; Soto & Sonnenschein, 2005).

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Panel discussion

J. McLachlan

A difficulty in all cases of hormonal carcinogenesis is how to tell the difference between cells which show phenotypic abnormalities and those which are truly neoplastically transformed.

A. Soto

If we assume that mutations are readily seen we must rely on tissue architecture in the same way as pathologists. It is known that cells which are certified as being malignant cancer cells can be normalized if they are dispersed and introduced into the appropriate stroma. The latest such experiment has shown that very malignant melanoma cells which kill animals if injected subcutaneously can differentiate into normal neural crest cells if injected into early embryos. Cancer can therefore be considered as being an epigenetic phenomenon, and implicit in this definition it is a reversible process depending on the organization of the tissues. This is true for hormone carcinogenesis and all types of cancer.

H. Leffers

We have performed similar studies using phytoestrogens rather than bisphenol A (BPA) and found the same effect as oestradiol (E2) in the developing breast. Do you see the same effect with BPA as with E2 and other oestrogens?

A. Soto

We have not experimented with E2 or phytoestrogens. Mice which have been exposed *in utero* to BPA show intra duct hyperplasia in the breast at the age of 9 months which is identical to the pre-cancerous lesion seen in rats at 50 days. Studies by Dr R Newbold indicate that phytoestrogen has the same effect as diethylstilboestrol (DES) indicating that the type of oestrogen is not important, all producing the same phenotype. BPA appears to be acting as an oestrogen in this regard with similar effects as E2 and DES.

E. Rajpert De-Meyts

Do you think that we are about to see an increasing rate of breast cancer in women?

A. Soto

We have already seen a threefold increased risk of breast cancer in the last 50 years and perhaps a further rise will occur. In the USA there has recently been a slight reduction in breast cancer which may be related to fewer women taking hormone replacement therapy (HRT). If women continue to be exposed to oestrogens they must accept the consequence of possible increased risk of breast cancer.

M. Schlumpf

Is there any indication of an epigenetic effect resulting from exposure to BPA?

A. Soto

"Epigenetic" is a loose term implying that there is no alteration to the linear sequence of DNA. There are indications that BPA causes epigenetic effects similar to those induced by DES, such as extemporaneous gene expression, changes in DNA methylation and chromatin reorganisation. DES has also resulted in histone modifications. J McLachlan has indicated that similar changes occur in the uterus following exposure to DES, including changes of genes in the WNT pathway which may be due to alterations in the pattern of methylation.

E. Rajpert De-Meyts

In general terms, how do endocrine disrupters work? Their actions may be: (a) an epigenetic phenomenon; (b) direct effect on genes; (c) disruption of development; (d) changing morphogenesis; (e) activation of stem cells; or all of these.

A. Soto

There are several experimental examples of most of these activities when studying the effect of DES on tissues. There is extemporaneous gene induction as seen when the uterus is exposed to DES: there is alterations of methylation: and there are unpublished data on histone acetylation. There are also effects pertaining to the human situation. In my experiments, I exposed the mammary gland to oestrogens at a time when the oestrogen receptor (ER) is only present in the stroma but the effect is proliferation in the epithelial cells. I have no direct evidence of stem cell activation.

E. Rajpert De-Meyts

Perhaps the definition of endocrine disrupters should be enlarged because we are seeing disruption in more than just the endocrine function in the body.