Effects of Bisphenol A and Diethylstilbestrol on Estrogen Receptor Expression and Male Fertility

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Résumé :

(traduction)

On définit l'infécondité comme l'incapacité d'un couple à concevoir un enfant après un an de rapports sexuels sans protection. Les pathologies masculines de reproduction sont la principale cause d'au moins 20% des cas d'infécondité, si bien que l'infécondité masculine est un problème important pour la santé globale de la population. Les agents perturbateurs endocriniens (APE) peuvent provoquer une dégradation de la spermatogenèse en provoquant des déséquilibres hormonaux et des changements morphologiques. Le DES et le BPA sont tous les deux des œstrogènes exogènes, également appelés xéno-œstrogènes. L'œstrogène joue un rôle important au niveau de la fertilité des hommes, ainsi que des femmes, car il influe sur l'équilibre endocrinien global requis pour permettre la spermatogenèse. On peut surveiller ses effets sur l'axe hypothalamique-pituitaire-gonadal en évaluant la sur-régulation des récepteurs des œstrogènes α et -B (ERα et ERβ), ainsi que le récepteur 30 couplé à une protéine G (GPR30), qui est un nouveau récepteur des œstrogènes. Il y a une controverse à propos des mécanismes de perturbation endocrinienne associés à ces produits chimiques, pour savoir si leurs effets négatifs sur la fertilité apparaissent à faible dose, ou seulement à forte dose, ce qui est systématiquement toxique. Une analyse de la documentation associée à cette recherche nous permettra de mieux comprendre les bases moléculaires de la dégradation de la spermatogenèse et de la fertilité masculine, associée à l'exposition aux APE, et en particulier au DES et au BPA. Les résultats de cette étude viendront améliorer les connaissances dans les domaines de la santé de la population, de la salubrité de l'environnement et de la génétique moléculaire. Nous espérons que, grâce à une connaissance plus approfondie de l'impact des APE sur la fertilité, les gouvernements pourront empêcher que les gens y soient exposés, en réglementant mieux l'emploi de produits comme le DES ou le BPA.

Mots-clés :

Bisphénol A, diéthylstilbestrol, Agents Perturbateurs Endocriniens (APE), xéno-oestrogènes, infécondité masculine, spermatogenèse, Récepteur des Œstrogènes Alpha (ERα), Récepteur des Œstrogènes Beta (ERβ), Récepteur couplé à une Protéine G (GPR30)

Abstract:

Infertility is defined as a couple's inability to conceive after one year of unprotected intercourse. Male reproductive pathologies are the predominate cause of at least 20% of cases of infertility; making male infertility is an important issue in overall population health. Endocrine disrupting chemicals (EDCs) can impair spermatogenesis by creating hormonal imbalances and morphological changes. DES and BPA are both exogenous estrogens, also known as xenoestrogens. Estrogen has an important role in fertility of males as well as females: it has a role in the overall endocrine balance required to allow spermatogenesis. Effects on the hypothalamic-pituitary-gonadal axis may be monitored by assessing for upregulation of estrogen receptors α and -B (ERα and ERβ), as well as the novel estrogen receptor g-protein coupled receptor 30 (GPR30). Controversy surrounds the mechanisms endocrine disruption associated with these chemicals, and whether the negative effects on fertility are relevant at low doses, or only at high doses which are systemically toxic. Analysis of literature associated with this ongoing research will enrich our understanding of the molecular bases for impairment of spermatogenesis and male fertility associated with exposure to EDCs, specifically DES and BPA. Once completed, this study will contribute to knowledge in the fields of population health, environmental health, and molecular genetics. With a more thorough understanding of the impact of EDCs on fertility, it is hoped that governments will further prevent exposure by better regulating the use of compounds such as DES and BPA.

Keywords:

Bisphenol A, diethylstilbestrol, Endocrine Disrupting Chemicals (EDCs), xenoestrogens, male infertility, spermatogenesis, Estrogen Receptor Alpha (ERα), Estrogen Receptor Beta (ERβ), G-protein Coupled Receptor (GPR30)

Introduction

One of the underlying causes of the decreasing fertility rates among the developed world in a decrease in fecundity, or the ability to conceive. While a number of factors may take part in this trend, endocrine disruptors have been shown to play a significant role. Male infertility is an important component of the overall issue; in fact it is the sole cause of 20% of cases of human infertility, and a contributor in 30-40% of cases (Phillips & Tanphaichitr, 2008). Factors affecting fertility in males must be studied distinctly from the female causes. While the role of estrogen in females is commonly well understood, estrogen is also an important hormone in the hypothalamic-pituitary- gonadal axis of males, which is the endocrine basis of reproductive capacity. Interest in this field has evolved since male mice with knock-out status for estrogen receptor α (ERα) were demonstrated to have impaired fertility (O'Donnell, Robertson, Jones, & Simpson, 2001).

BPA is a known endocrine disruptor. It acts as an exogenous estrogen, or a xenoestrogen. Exposure in humans may occur through occupational exposure, or environmental exposure (Li et al., 2009). BPA is used in industry for the manufacturing of epoxy resins and polycarbonates for food packaging (Merck Sharp & Dohme Corp, 2006a). BPA has been associated with impaired spermatogenesis and steroidogenesis. Public awareness of adverse health effects of exposure to BPA is rising, and governmental legislation is increasing aimed to reduce exposure.

DES is a synthetic, nonsteroidal estrogen which also falls into the category of endocrine disruptor and more specifically xenoestrogens. It is a known human carcinogen, and is used in some cases as an androgen suppressor in combined antineoplastic therapy (Merck Sharp & Dohme Corp, 2006b). DES was administered to pregnant women in the 1940's—1970's to prevent miscarriages and spontaneous abortions, as well as pre- mature deliveries (Adamsson, Brokken, Paranko, & Toppari, 2008; Hong et al., 2010). Attention to the negative effects on the human endocrine system arose when women exposed to DES neonatally, several years after it was administered to their mothers during pregnancy, developed rare vaginal and uterine cancers at a significantly elevated rate (Hong et al., 2010). Effects on the male reproductive system have more recently come to light. Exposure to DES has been shown to disrupt steroidogenesis and spermatogenesis in rodents (Cederroth, Schaad, Descombes, Chambon, Vassalli, & Nef, 2007; Hendry, Weaver, Naccarato, & Khan, 2006). Most of this research has been done based on in-utero and neonatal exposure (Hong et al., 2010). While few studies have examined the effects of exposure in adults, such as in the case of antineoplastic uses, it has been shown to drastically increase apoptosis in spermatocytes and spermatids (Ma, Yang, Wang, Shi, & Chen, 2010).

ERα was the first of the estrogen receptors to be implicated in male fertility. Expression of ERα has been demonstrated to be reduced by up to 50% in human embryonic testicular cells with exposure to high doses of BPA, and up to 75% with high doses of DES (Benachour, Moslemi, Sipahutar, & Seralini, 2007). ERβ has a similar functionality as ERα, but through a different mechanism. This is an example of redundancy in genetics, which may help compensate when one system is working inadequately.

GPR30 has only very recently been implicated as an estrogen receptor of the plasma membrane cells in the brain (Funakoshi, Yanai, Shinoda, Kawano, & Mizukami, 2006). Research by Otto *et al.* (2009) however, has indicated that GPR30 doesn't seem to be required for male reproductive function in mice. The novel nature of this ER necessitates further research before sufficient evidence is available to accept or reject a role for GPR30 in male fertility.

Materials and Methods

Inclusion Criteria

Only original journal articles, such as original cohort studies, case-control studies and review articles were included. Human, rodent and in vitro studies were all used. Only articles from the past 5 years (published in 2005 or later) were included. Articles were eliminated if they did not focus on 1) the male reproductive system, 2) BPA and/or DES specifically, and 3) increase in estrogen receptor expression as a potential means of impairment of fertility. Studies focusing mainly on brain function (ex: the role up dopamine uptake), and reproductive cancers were eliminated as they are beyond the scope of this review.

PubMed Search

Between the two searches on PubMed, 20 articles meeting the inclusion criteria were obtained. The search terms were as follows:

- ("bisphenol A "[Substance Name] OR "Diethylstilbestrol" [Mesh]) AND ("Estrogen Receptor

alpha" [Mesh] OR "Estrogen Receptor beta" [Mesh] OR "GPR30 protein, mouse "[Sub- stance Name] OR "GPER protein, human "[Sub- stance Name])

A total of 280 results were returned, 157 of these were from the past 5 years

- ("bisphenol A "[Substance Name] OR "Diethylstilbestrol" [Mesh]) AND ("Testis" [Mesh] OR "Vas Deferens" [Mesh] OR "Seminiferous Tubules" [Mesh])

A total of 428 results were returned, 62 of these were from the past 5 years

SCOPUS Search

Only 1 of the articles met the inclusion criteria, but it was an editorial commentary. The primary article on the qualitative study was accessed through PubMed and included in the systematic review. The search terms used to retrieve articles from this database were as follows:

- (Bisphenol A OR Diethylstilbestrol) AND ((Estrogen receptor alpha OR Estrogen receptor beta OR G-protein coupled receptor 30) OR (Testes OR Vas deferens OR Seminiferous tubules))

A total of 5 results were returned, all of which from the last 5 years

Ovid Search

The vast majority of these were eliminated because they were excerpts from textbooks rather than scientific journal articles. Each of the results meeting the inclusion criteria was already included in the PubMed collection. This indicates a high degree of data saturation, meaning that the likelihood of the existence of articles not included in the review collection which met the inclusion criteria is quite low. The search terms used to retrieve articles from this database were as follows:

- (Bisphenol A OR Diethylstilbestrol) AND ((Estrogen receptor alpha OR Estrogen receptor beta OR G-protein coupled receptor 30) OR (Testes OR Vas deferens OR Seminiferous tubules))

A total of 2069 Results were obtained, which was decreased to 1484 by deduplication. 665 of these results were published within the last 5 years.

Results

Of the 20 studies included in the systematic review, only 1 was inconclusive. Hong *et al.* (2010) demonstrated effects of DES on gene expression, but did not provide insight into the injurious effects, or lack thereof, on male fertility. Three other studies revealed a statistically insignificant relationship, or a complete lack of correlation. Kato *et al.* (2006) found no statistically relevant difference in semen analysis, fertility, copulatory rate or preputial separation in rats exposed to as much as 95mg/kg/day of BPA. Likewise, Tyl *et al.* (2008) found no change in sperm motility of percent of normal sperm in mice exposed to as much as 600mg/kg/day of BPA. Finally, Adamsson *et al.* (2008) were unable to prove impairment of androgen receptor (AR) protein synthesis resulting from exposure to DES. They concluded that perhaps the difference seen in previous studies were due to high doses that were cytotoxic (Anahara et al., 2006). In the case of endocrine disrupting chemicals, such findings can help prevent unnecessary public hysteria.

Conversely, from a public health perspective it is often preferable to err on the side of caution. Significant impairments to male fertility were described in 17 of the 20 studies included in this review. During a qualitative study of men with occupational exposure to BPA, Li *et al.* (2009) demonstrated that exposed factory workers reported a 4-fold decrease in sexual desire, a 4-fold increase in erection difficulty, and a 7-fold increased risk of ejaculation difficulty. With such results coming from interviews with people, it is important to use animal studies to determine possible mechanisms that would cause these effects. One such suggested mechanism is the disruption of steroidogenesis. Hendry, Weaver, Naccarato, and Khan (2006) describe an earlier, longer lasting pubertal testosterone surge. According to Cederroth *et al.* (2007) this occurs due to repression of ERα. Findings from Mikkilä, Toppari, and Paranko (2006) and Nakamura *et al.* (2010) demonstrate decreased intratesticular steroidogenesis, which they believe to be due to decreased testicular mass associated with exposure to both BPA and DES.

Besides testosterone, decreased production of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) was also observed (Shin et al., 2009; Warita et al., 2006). Kobayashi et al. (2009) conceded to the negative impact of steroidogenesis, but suggested that there was an increase

in androgen receptor (AR) expression to compensate. Salian, Doshi, and Vanage (2009a) associated BPA exposure with impaired intra-testicular expression of steroid receptor co-regulators; steroid receptor coactivator-1 (SRC-1) and nuclear receptor corepressor (NCoR). Likewise, steroidogenic acute regulatory protein (StAR) is inhibited by exposure to DES, allowing for an increase in ARs (Kobayashi et al., 2009; Warita et al., 2006). Mikkilä et al. (2006) also observed a decrease in stAR levels, which they determined was due to a 41-44% increase in anti-stAT antibodies. Similarly, Volle et al. (2009) demonstrated an increase in orphan nuclear receptor small heterodimer partner (NrOb- 2) which is a transcriptional repressor of ERs.

Another observed means of impairment of male fertility is disruption of spermatogenesis (Hendry et al., 2006; Salian, Doshi, & Vanage, 2009b). Two of the studies demonstrated a "sloughing" off of germ cells in the seminiferous tubules, following neonatal exposure to BPA or DES (Koh et al., 2006; Salian et al., 2009c, Warita et al., 2006). Hypoplasia of Leydig cells was also observed (Warita et al., 2006). Salian *et al*. (2009c) proposed that this effect is caused by disturbance to the blood-testes barrier. Reduced sperm count was also observed in an additional, separate study by Salian *et al*. (2009a). A significant increase in apoptosis of spermatocytes and spermatids was recorded by Ma, Yang, Wang, Shi, and Chen (2008) in DES exposed adult hamsters was extremely increased compared to control samples. Anahara *et al*. (2006) demonstrated a decrease in actin binding necessary for sperm motility, via a decrease in cortactin expression in the testes.

Overall, there seems to be sufficient evidence of impairment of male fertility by exposure to DES and BPA to warrant avoiding these EDCs. In terms of understanding the breadth of influence of these chemicals, it is important to note that synergistic and transgenerational effects have been observed. Human exposure to continuous low-doses of EDCs seldom occurs with one chemical in isolation. It is important to study the confounding effects EDCs present when one is exposed to more than one at a time (Benachour, Moslemi, Sipahutar, & Seralini, 2007).

Discussion

Once the issues are well understood, the presence of these chemicals in our environmental may be controlled and the problems arising from exposure may be treated. In this study, estrogen receptor expression was used as a parameter for measurement of fertility. Increased estrogen receptor expression in male reproductive tissues has been associated with negative impacts on fertility. The reproductive outcome parameters affected by upregulation of estrogen receptors in males include sperm count (Ma et al., 2008), reduced sperm quality (altered morphology (Kato et al, 2006)), reduced sexual desire/copulatory rate (Li et al., 2009; Kato et al., 2006), erectile dysfunction and ejaculatory difficulty in humans(Li et al., 2009), preputial separation (Kato et al., 2006), and serum testosterone levels (Cederroth et al., 2007; Kato et al., 2006; Kobayashi et al., 2009; Mikkilä et al., 2006; Nakamura et al., 2010).

A multitude of options for future research exist in this growing area of environmental and population health. First of all, downstream gene products may be studied with PCR, to determine if these receptors are being activated and not just expressed. This will give indication of the effect on the cell as a whole, by including the internal signalling pathway.

Important knowledge gaps include the role of GPR30 which has only recently been implicated in the effects of EDC exposure on male fertility. Further, many studies have assessed the effects of EDCs on gestation; however few have examined their pathophysiological effects on female fertility. For example, ER expression could be studied in female mice neonatally exposed to BPA and DES. The impacts on their ovulatory capacity, hormone levels, litter sizes, frequency of resorption of fetuses, and number of pregnancies could be examined. Other interesting avenues of research include methods of correcting the effects of BPA and DES exposure. Since the effects of these EDCs appear to permeate through several generations following exposure, the future impact on human reproductive health cannot be reduced solely by eliminating exposure to these chemicals. Preliminary research has been done on therapies for those affected by EDC exposure. For example, Vitamin A has been linked with re-establishing sperm motility and decreasing the number of malformed sperm following neonatal exposure to BPA (Hendry et al., 2006). Likewise, the use of ginkgo biloba as a possible therapeutic agent for testicular injury caused by DES exposure is currently being studied (Hong et al., 2010).

Conclusion

The role of EDCs on male fertility is not well under- stood. Molecular mechanisms have not been well- examined in-

cluding possible epigenetic changes following EDC exposure. With the characterization of novel estrogen receptor subtypes and isoforms more study is required into the role and mechanisms of EDCs on male fertility.

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