Vitamin A Insufficiency Accelerates the Decrease in the Number of Sperm Induced by an Environmental Disruptor, Bisphenol A, in Neonatal Mice

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[SHORT COMMUNICATION]

Vitamin A Insufficiency Accelerates the Decrease in the Number of Sperm Induced by an Environmental Disruptor, Bisphenol A, in Neonatal Mice

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ABSTRACT—Exposure of neonatal mice to an estrogenic endocrine disruptor, bisphenol A, resulted in a malfunction of the testes when the animals became adults. The effect of bisphenol A was cancelled out by concurrent administration of retinol acetate, a naturally occurring metabolite of vitamin A. In contrast, the effect of endocrine disruption became more severe in mice neonatally exposed to bisphenol A and nursed by mothers fed a vitamin A-deficient diet only a few days before and after parturition. These results clearly show that maternal vitamin A is important for relieving in a baby the effect of endocrine disruption caused by environmental xenoestrogens, and suggest that the changes in the content of vitamin A and similar physiological factors in the habitat may be worth considering in studies on environmental disruptors.

INTRODUCTION

Recently, endocrine disruptors that cause a hormonal imbalance in animals have received a great deal of attention (Carson, 1962; Colborn *et al*., 1993, 1996; Cadbury, 1997), with reports of infertility in female sheep induced by a phytoestrogen (Obst *et al*., 1971), androgyny in conch caused by organic tin (Gibbs *et al*., 1987), and small penis in alligators exposed to polychlorinated biphenyls (Guillette *et al*., 1999), etc. Human health has also affected by endocrine disruptors; diethylstilbestrol which was used to prevent abortion in the 1960's induced abnormalities of the reproductive organs, such as vaginal cancer, prostatic hyperplasia, and testicular tumor (Herbst, 1981). And, it is claimed that the number of fertile sperm decreases in the young as a result of exposure to endocrine disruptors during fetal life (Sharpe and Skekkebaek, 1993), although there are conflicting reports (Safe, 1995). Most of the environmental endocrine disruptors that affect vertebrates are categorized as estrogenic substances interacting with estrogen receptors. This is the reason why most efforts have concentrated on determining the estrogenic potency of all sorts of chemicals (Soto *et al*., 1995). However, the action of estrogen may be modified by many factors. Here, we introduce bisphenol A (BPA) as an endocrine disruptor and vitamin A (VA) as an inhibitor of the effect of BPA on the mouse testis during the neonatal period.

Exogenous estrogen or estrogenic substances cause much more serious problems in the fetus and neonate than in adults. The genital tracts are particularly fragile during their development, the so-called critical period, and are often subjected to irreversible and fatal injuries even after short-term exposure to a low dose of estrogenic substances (Mori and Nagasawa, 1988). The critical period in the developing genital tracts corresponds to 3–4 months in the human fetus and to a few days pre- and postpartum in mice and rats. As the sensitivity differs between mother and child, mothers often keep taking estrogenic chemicals unaware of the effect to their children. BPA is one of the most widespread artificial estrogenic chemicals. For instance, it has been used in coating for the inside of cans, baby bottles, dental sealant, plastics for automobiles and many other products (Krishnan *et al*., 1993; Olea *et al*., 1996). Although BPA is easy to resolve and possesses rather weak estrogenic activity (10^2–10^4 times weaker than estradiol-17β) (Nagel *et al*., 1997), the concentration in certain environments is thought to be enough to evoke endocrine disruption in animals during the critical period.

VA is involved in regulating the function and differentiation of various epithelia, but is also a key factor inducing organogenesis in the embryo and fetus (Conlon, 1995). VA plays a role in spermatogenesis (van Pelt and Rooij, 1991) as well as oogenesis (Morita and Tilly, 1999). Estrogen, meanwhile, plays a crucial role in the reproductive activities of male as

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well as female animals (Robertoson *et al.*, 1999). Mahato *et al.* (2000) claimed that male germ cells do not require estrogen to develop or to function in fertilization, and suggested that estrogen acts within somatic cells of the male reproductive system, resulting in disruption of the excretion system of sperm. Previously, it was demonstrated that the concurrent injection of VA with estrogen into neonatal female mice inhibits the irreversible proliferation and cornification in the vaginal epithelium that is induced by neonatal treatment with estrogen alone (Mori, 1968). Therefore, we investigated the effects of BPA plus retinol acetate (RA) as VA on the reproductive function in neonatal male mice. Furthermore, the involvement of VA in the effect of BPA on the function was studied in neonatal mice nursed by mothers fed a VA-deficient diet before and after parturition.

MATERIALS AND METHODS

Male mice of the SHN strain were divided into 4 groups and given daily injections of 0.5 or 50 μg of BPA, 50 μg of BPA plus 100 IU of RA, or the vehicle only for 5 days from the day of birth. The vehicle consisted of 16 μl of sesame oil used for BPA and 4 μl of dimethylsulfoxide (DMSO) used for RA. The animals were weaned at 20 days of age and provided with a commercial diet (CE-7; Clea Japan Inc., Tokyo). They were killed at 14 weeks of age, and the sperm in the left caput epididymis were enumerated using a hemocytometer chamber under a microscope.

In addition, pregnant mice were fed a VA-deficient (VAD) diet (Low vitamin A diet; Clea Japan) from 3 days before gestation to postnatal day 5 of their pups. The pups nursed by the mothers taking the VAD diet were given 5 daily injections of BPA (0.5 μg) or the vehicle only from the day of birth (VAD/BPA or VAD mice). After 5 days, the mothers were fed a normal VA-containing diet (CE-7). After being weaned at 20 days of age, the pups were also given the normal diet until autopsy at 14 weeks of age. At autopsy, the number of sperm was determined as mentioned above.

RESULTS AND DISCUSSION

In the present study, mice treated neonatally with a high dose (50 μg for 5 days) of BPA showed a 35% reduction in the number of sperm in the caput epididymis as compared with unexposed controls when the animals became adults, while the treatment with a lower dose (0.5 μg for 5 days) affected little the number of sperm (Fig. 1).

Interestingly, neonatal treatment with high-dose BPA failed to induce a reduction in the number of sperm if RA was injected concurrently (Fig. 1). Neonatal treatment with RA alone had no effect on the sperm count (data not shown). Thus, RA could nullify the effect of BPA on the testis to some extent in neonatal mice.

The VAD mice nursed by mothers fed a VAD diet only 8 days before and after parturition, showed no decrease in the number of sperm when they became adults. Surprisingly, in the VAD mice, neonatal administration of a low dose of BPA (VAD/BPA mice) caused a marked decrease in the number of sperm, although the same dose hardly affected the sperm count at all in mice nursed by normal mothers (Fig.1).

Although the interaction of VA with BPA was not clarified,

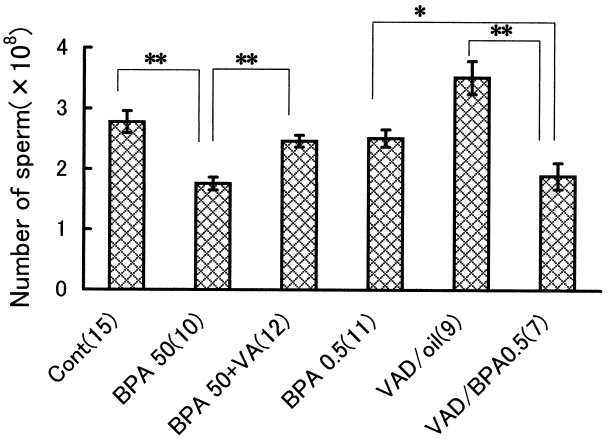

Fig. 1. Changes in the number of sperm in mice receiving neonataly BPA in combination with or without RA. Cont, mice were given the vehicle only for 5days from the day of birth; BPA 0.5 or BPA 50, mice were treated with 0.5 or 50 μg BPA for 5days from the day of birth; BPA 50+VA, mice received 50 μg BPA in combination with 100IU RA as VA for 5days from the day of birth; VAD/oil or VAD/BPA0.5, mice received vehicle or 0.5 μg BPA for 5days from the day of birth and were nursed by mothers taking a VAD diet for 8 days before and after parturition. The number in parentheses represents the number of mice examined.
*P<0.05, **P<0.01

there are two possible explanation for the decrease in the number of sperm in epididymis. First, BPA has an irreversible effect on the spermatogenesis and/or the excretion of sperm by disrupting the action of VA. Second, VA has the ability to make the developing testis and/or epididymis insensitive to BPA. However, a possible effect of BPA and VA on the hypothalamo-hypophyseal system could not be excluded because BPA could affect the secretion of gonadotropic hormones in adult mice (Tohei *et al.*, 2001). The preventive effect of VA on the action of BPA in the developing male reproductive organs is similar to the previous finding that VA inhibits the effect of estrogen on the developing vaginal epithelium (Mori, 1968). In all events, the development of the reproductive organs including gonads may be controlled by a fine balance between two biologically active substances, estrogen and VA. A few papers concerning the interactions between VA and endocrine disruptors have been published: poultry chicks receiving a low VA diet and exposed to 3, 4, 3', 4'-tetrachlorobiphenyl become hypothyroid in comparison with unexposed controls (Spear and Moon, 1986) and hydroxylated metabolites of polychlorinated biphenyls inhibit VA transport in mice and turtles (Kramer *et al.*, 1997).

In conclusion, depletion of VA in pregnant or lactating mothers may predispose the fetus and pups to the adverse effects of BPA as well as many other estrogenic chemicals in the environment. Alternatively, the presence of relatively large amounts of xenoestrogens in combination with a lack of vitamin A or the presence of chemicals disrupting the actions of VA would severely affect the development of gonads as well as genital tracts. The intake of plenty of VA by a mother who has been accidentally exposed to an excess amount of estro-

gen may be effective in protecting her fetus or pups from endocrine disruption. Therefore, assessment of the anti-VA activity of all kinds of artificial chemicals in the environment is important in the research of endocrine disruptors. It should be stressed that the *in vivo* data of the threshold for estrogenic action of endocrine disruptors were obtained under nutritionally favorable conditions.

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