Reproductive Toxicology 32 (2011) 418–424

Contents lists available at SciVerse ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Xenoestrogenic chemicals effectively alter sperm functional behavior in mice

Yoo-Jin Park, El-Sayed A. Mohamed, Woo-Sung Kwon, Young-Ah You, Buom-Yong Ryu, Myung-Geol Pang*

Department of Animal Science and Technology, School of Bioresource and Bioscience, Chung-Ang University, Ansung, Gyeonggi-Do 456-756, Republic of Korea

ARTICLE INFO

Article history:
Received 9 May 2011
Received in revised form 30 August 2011
Accepted 28 September 2011
Available online 20 October 2011

Keywords:
Eendocrine disruptors
Sperm function
Capacitation
Acrosome reaction
Estrogen
Progesterone
Heparin

ABSTRACT

Xenoestrogenic compounds (XCs) can disrupt endogenous hormone function and affect sperm function by binding to receptors on sperm membrane. Albeit spermatozoa are potentially a useful model for screening estrogenic activities of endocrine disruptors, high-quality *in vitro* test system that examination of the XCs effects on sperm function is required. The objective of this study was to compare the effects of XCs (genistein and 4-tert-octylphenol) to those of steroids (estrogen and progesterone) and heparin on *in vitro* capacitation and acrosome reaction (AR) in mouse spermatozoa. Mouse spermatozoa were incubated with various concentrations (0.001–100 μM) of each chemical for 15 or 30 min, and then capacitation and AR were assessed using chlortetracycline. All chemicals studied effectively alter capacitation and/or AR in mouse spermatozoa with different manner. Therefore, we believed that our system will provide a good *in vitro* model system to characterize the physiological effect of XCs especially when compared with steroids.

1. Introduction

Many chemicals released into the environment can disrupt endocrine function in animals and human, including chemicals that occur naturally in plants, such as phytoestrogens, and man-made chemicals like xenoestrogens [1]. Genistein (Gen), a phytoestrogen, is a naturally occurring isoflavone that interacts with estrogen receptors (ERs) and multiple other molecular targets [2] and that may trigger many of the biological responses that are evoked by physiological estrogen (E2) [3]. Human exposure to Gen is predominantly through consumption of soy products, including soy-based infant formula and dietary supplements [2]. Gen is also present in virtually all natural-ingredient rodent diets that use soy as a source of protein [4].

Dietary Gen has been shown to produce effects in multiple estrogen-sensitive tissues in males and females rats that are generally consistent with estrogenic activity. These effects occurred within exposure ranges achievable in humans [2]. However, several contradictory results have been published about the endocrine disrupting effect of Gen in animal models [1,5,6] with a general finding that these effects occurred at the high doses [4] and that females are more sensitive than are males [6,7]. Most *in vivo* studies have focused on the reproductive developmental toxicity of Gen, with little attention given to its effect on sperm function.

It cannot be ignored that Gen exposure in males around and after puberty may have an impact on sexual maturation and sperm function. To our knowledge, only a handful of reports have shown that very low dosages of Gen have direct effects on the function of spermatozoa, significantly accelerating capacitation and the acrosomal reaction (AR) in human [8], mice [9] and porcine [10] spermatozoa. The sensitivities of animal species and strains have been a matter of concern when selecting an assay type for testing chemical estrogenicity [11]. In conjunction with previous contradictory *in vivo* results, this led us to believe that additional *in vitro* studies are needed using different species, strains and experimental designs.

The alkylphenol 4-tert-octylphenol (OP) is commonly used in industrial manufacturing and is present at significant concentrations in the environment [12]. OP bind to the estrogen receptor and responses to a similar extent as E2 [13]. Extensive results were published about the toxicity and endocrine disruptor effect of OP [12,14–16]. However, only one study is available about its effect on sperm capacitation and the AR [10].

For first time, we evaluated the effects of two xenoestrogenic compounds (Gen and OP) on mouse sperm capacitation and the AR in comparison to those of two dominant endogenous hormones in the female genital tract (E2 and P4) and the heparin used in *in vitro* capacitation/AR protocols. In this experimental design, we also considered the effect of treatment duration and concentration on sperm function. Furthermore, since the sensitivity differences

* Corresponding author. Tel.: +82 31 675 9001.
E-mail addresses: legend0508@naver.com (Y.-J. Park), eamohamed79@yahoo.com (E.A. Mohamed), returnmaster@naver.com (W.-S. Kwon), yerang02@naver.com (Y.-A. You), byryu@cau.ac.kr (B.-Y. Ryu), mgpang@cau.ac.kr (M.-G. Pang).

0890-6238/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.reprotox.2011.09.011

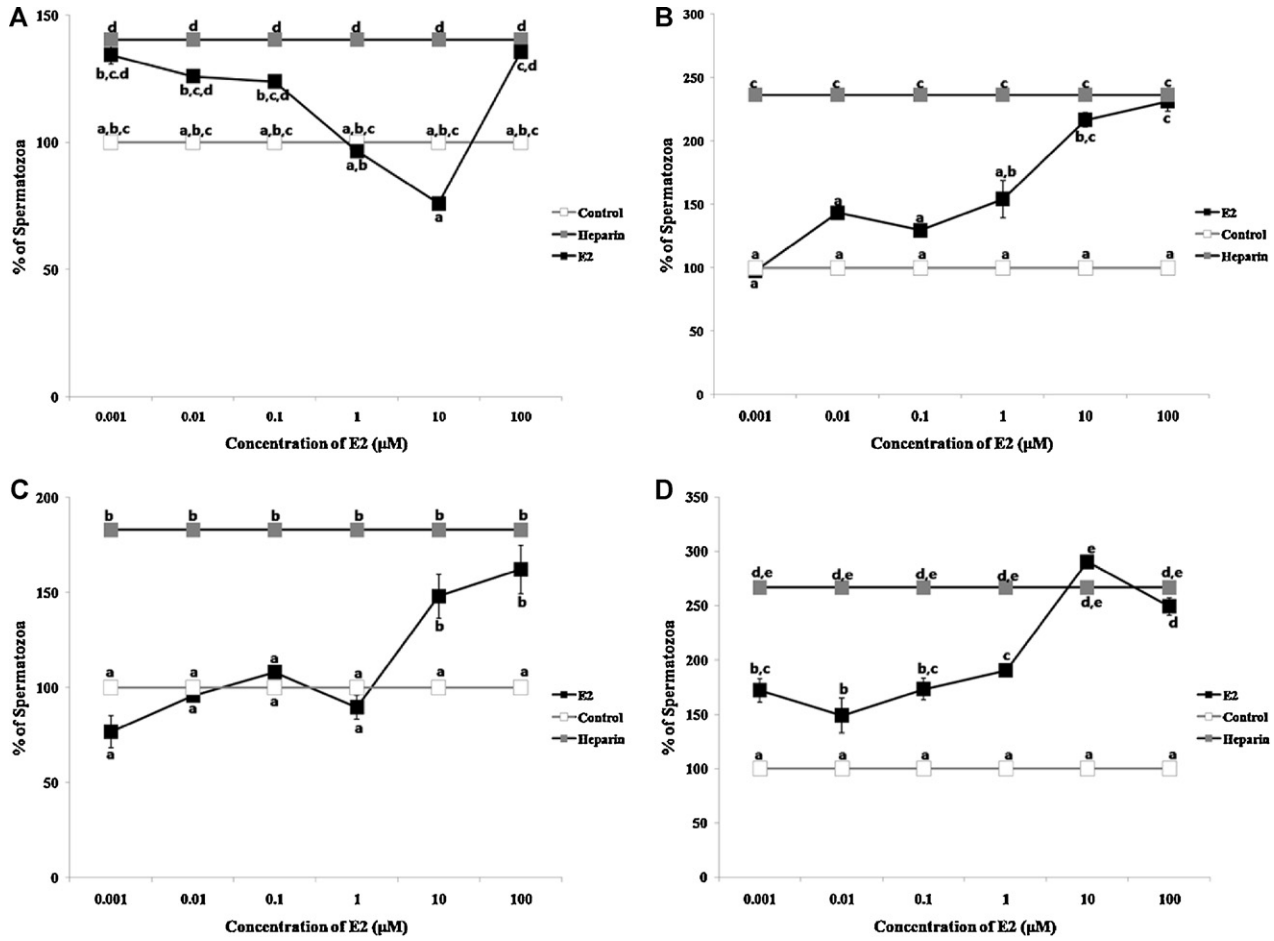

Fig. 1. Effects of 15 min of incubation with E2 on capacitation (a), the AR (b), and effects of 30 min of incubation with E2 on capacitation (c) and the AR (d). Data are expressed as the mean ± SEM. A *P*-value of <0.05 was considered to be significant. [a–e]Within E2, means with the same superscripts were not significantly different ($P < 0.05$).

among the different species and strains to the endocrine disruptors has been reported, it is useful to investigate the effect of these chemicals using different strains of mice [17,11].

2. Materials and methods

All procedures were performed according to the approved guidelines for the ethical treatment of animals as established in the regulations of Chung-Ang University (AEC-20080428-2), in compliance with standard international regulations.

2.1. Medium and reagents

Mouse spermatozoa were treated in basic medium [modified tissue culture media 199 comprised of TCM 199 with Earle's salts and 10% fetal calf serum]. Stock solutions of 1000 μM E2 (17 β-estradiol) or P4 (Sigma, St. Louis, MO, USA) were prepared in DMSO and stored at −20 °C. The other stock solutions (100 μM) of GEN (Sigma) or OP (Sigma) were prepared in absolute ethanol and stored at −20 °C. Working stock solutions were prepared daily using basic medium as a diluent.

2.2. Preparation of spermatozoa

Epididymal mouse spermatozoa were collected from nine-week-old male ICR mice (Central Laboratory Animal, Seoul, Korea). After animals were sacrificed, the caudal epididymides from three animals were separated from the surrounding tissues. For sperm sampling, both caudal epididymides were minced in 1 ml basic medium. The sperm suspensions were then allowed to disperse for 5 min on a warming tray at 37 °C and were used to determine a motile sperm count. The final sperm concentration was adjusted to 2×10^6 cells/ml. To evaluate effects on capacitation and the AR, these spermatozoa were incubated for 15 min or 30 min with or without (negative control) different concentrations (0.001, 0.01, 0.1, 1, 10, and 100 μM) of chemicals in an atmosphere of 5% CO_2 at 37 °C. Motile spermatozoa were also incubated in capacitation medium (containing 20 μg/ml of heparin) for 20 min for use as a positive control. The analysis of each condition was replicated three times.

2.3. Combined Hoechst 33258/chlortetracycline (H33258/CTC) fluorescence assessment of spermatozoa

The CTC staining assay was performed as described previously [18]. Briefly, 135 μl of spermatozoa (2×10^6 cells/ml) were added to 15 μl of H33258 solution (10 μg H33258/ml D-PBS) for 10 min at 37 °C in water bath. Excess dye was removed by layering the mixture over 250 μl 2% polyvinylpyrolidone in D-PBS and centrifuging at 400 × *g* for 10 min. The sperm pellets were resuspended with 500 μl of D-PBS and added to 500 μl of CTC solution (750 μM CTC, 5 mM cysteine, 130 mM NaCl, 20 mM Tris, pH 7.8) and then fixed with 10 μl of 12.5% (v/v) paraformaldehyde in 1 M Tris–HCl (pH 7.4). Samples were observed with a Nikon microphot-FXA under epifluorescence illumination using UV BP 340-380/LP 425 and BP 450-490/LP 515 excitation/emission filters for H33258 and CTC, respectively. Four kinds of CTC staining patterns were identified: an F pattern (uncapacitated spermatozoa), with uniform fluorescence over the whole head; a B pattern (capacitated spermatozoa), with a green fluorescence over the acrosomal region and a dark postacrosome; an AR pattern (acrosome-reacted spermatozoa), with almost no fluorescence in the head; and the D pattern (dead spermatozoa) showed bright blue fluorescence over the sperm head. At least 400 spermatozoa were evaluated per sample.

2.4. Statistics

Differences among groups were detected with one-way ANOVA (SPSS, Version 12.0; Chicago, IL, USA). Each single experiment was carried out in triplicate, and significant differences between means were determined using Tukey's test. A *P*-value of <0.05 was considered significant. Data are expressed as the percentage of each corresponding negative control (100%) ± the standard error (SE).

3. Results

3.1. Effects of E2

Heparin reliably induces capacitation and the AR and therefore was used as a positive control (Fig. 1). After 15 min of exposure to E2,