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REGULAR ARTICLE

INFLUENCE OF PETROCHEMICAL INDUSTRY ENVIRONMENTAL CONTAMINANTS ON ANIMAL OVARIAN CELLS

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ABSTRACT

The aim of our studies was to examine (1) the effect of environmental contaminants (benzene, toluene and xylen) on basic ovarian cell functions (proliferation, apoptosis, secretory activity) in different animal species (rabbit, pig, cow), and (2) whether gonadotropic hormone (FSH) and plant molecules (quercetin, resveratrol or extract of yucca) can affect these functions and modify effect of environmental contaminants.

It was observed, that the culture of either porcine or bovine ovarian cells with benzene, toluene or xylen promote apoptosis (accumulation of apoptosis markers bax and p53) and proliferation (accumulation of PCNA). Furthermore, additions of these contaminants were able either up- or down-regulate the release of progesterone, oxytocin, insulin-like growth factor I (IGF-I) and prostaglandin F by cultured porcine, rabbit and bovine ovarian cells and their response to addition of FSH. FSH additions promoted proliferation, apoptosis and

release of molecules listed above by porcine granulosa cells. Moreover, FSH was able to modify and to prevent. Some effects of BTEX on these cells. The effects of either quercetin or resveratrol on basic porcine ovarian cell functions were observed, but these plant molecules were not able to prevent BTEX effect. Feeding of rabbits with yucca extract caused changes in release of progesterone, IGF-I and prostaglandin F by their ovarian cells, as well as to modify and prevent the influence of benzene on ovarian hormone release.

The obtained data suggest that (1) the negative effect of BTEX on reproduction can be due to their influence on ovarian cell apoptosis, proliferation, turnover and release of peptide and steroid hormones and growth factors, and that (2) FSH and plant molecules can regulate ovarian cell functions and prevent some effects of BTEX on these cells.

Keywords: benzene, toluene, xylen, ovarian, proliferation, apoptosis, hormone

INTRODUCTION

One of the most dangerous and stable environmental contaminants are products of petrochemical industry – hydrocarbons benzene, toluene, ethylbenzene and xylen (BTEX).

The most dangerous effects of BTEX could be their influence on the most biological important process – reproduction, especially female reproduction. Female organism accumulate 3.7-6.8 times more xylene than males, whilst ovaries are the important accumulation site of this hydrocarbon (**Suter-Eichenbeger et al., 1998**). Contacts of female workers to benzene was associated with ovarian hypo- and hyperplasia, ovarian and uterine retardation (**Maronpot, 1987**) reduction in duration of luteal phase of the menstrual cycle (**Chen et al., 2000, 2001**) and with retardation in fetal growth during pregnancy (**Aguilera et al., 2010**). Exposure of cows to benzene increased incidence of odd calves (**Waldner, 2008**). Toluene in rats suppressed development of growing but not primordial ovarian follicles (**Tap et al., 1996**). Inhalation of toluene increased incidence of maternal and fetal morbidity and embryonal malformations in women (**Kuczkowski. 2007; Hannigan and Bowen, 2010**), cows (**Waldner, 2008**) and rats (**Bowen et al., 2006; Jarosz et al., 2008**), although other studies did not detect any adverse effects on implantation, number and viability of rat fetuse. Inhalation of **ethylbenzene** did not affect rat ovarian structure, function and fertility (**Faber et al., 2006**). The accessible databases do not contain publications concerning reproductive effects of **xylens,** although decreased embryobnal growth (**Ungváry et al., 1981**) and

increased prenatal mortality (**Hood and Ottley,1985**) in rats exposed to xylen has been reported.

Although extra- and intracellular mechanisms of BTEX action could help to explain and prevent their negative effects on reproduction, they have been very poorly studied yet. There are some evidence that BTEX can affect reproduction and fertility via hypothalamic, pituitary and peripheral hormones. Inhalation of **toluene** by rats reduced hypothalamic level of GnRH and plasma level of gonadotropins, changed FSH:LH rate and increased plasma level of anti-gonadotropin prolactin. These changes were associated with reduced blood progesterone and estradiol level, but not with their response to gonadotropin administration (**Stepanov et al., 1990**). Inhalation of para-**xylene** decreased plasma progesterone and estradiol level, but not the release of these hormones by ovaries in rats (**Ungváry et al., 1981**). All these observations suggest BTEX can reduce output of ovarian steroid hormones via inhibition of upstream hypothalamic GnRH/pituitary gonadotropin production. Direct effect of BTEX on ovarian hormone release has not been studied yet.

The ability of BTEX to inhibit growth and to induce cell death suggest that they can suppress cell proliferation and promote cell apoptosis. Nevertheless, there is only one paper demonstrating inhibitory effect of BTEX on markers/promoters of ovarian cell cycle yet. In the experiments of **Lin et al. (2011)** the analogue of **xylene** (L^1 = α,α'-diamino-p-xylene, L^2 = 4,4'-methylenedianiline) arrested cycle of cultured human cancer cells (COC1) at G2 or M phase, probably via dose-dependent influence on accumulation and phosphorylation of proliferation-related protein kinases CHK1/2, ERK1/2, and p38 MAPK.

The analysis of the available literature shows, that the current available related knowledge are poor and superficial. Not all BTEX are studied in relation to the main reproductive processes. The majority of results were obtained on *in-vivo* model, therefore their physiological/cellular interpretation and identification the primary BTEX target is difficult. The endocrine and intracellular mechanisms of BTEX action, whose discovering could help to understand, to characterize, to predict and to modify biological effects of BTEX, are very poor studied. Finally, practically nothing is known about the ways, how to prevent or neutralize the negative effects of BTEX on reproduction at physiological level. It is known, that some effects of stress on ovarian functions could be prevented or neutralized by some plants containing antioxydants and other adaptogenes (**Huang and Chen, 2008; Liang and Yin, 2010**), hormones, growth factors (**Sirotkin, 2010, 2011**). Nevertheless, character of effect of BTEX on various ovarian functions, extra- and intracellular mechanisms of their

action and the molecules whose could be practically used for prevention and neutralization of negative effect of BTEX remain to be studied yet.

The aim of our studies was to examine (1) the effect of environmental contaminants (benzene, toluene and xylen) on basic ovarian cell functions (proliferation, apoptosis, secretory activity) in different animal species (rabbit, pig, cow), and (2) whether gonadotropic hormone (FSH.) and plant molecules (quercetin, resveratrol or extract of yucca) can affect these functions and modify effect of environmental contaminants.

MATERIAL AND METHODS

Granulosa cells isolated from bovine and porcine ovaries, as well as rabbit ovarian fragments were processed and cultured with- and without BTEX, FSH, plant molecules quercetin or resveratrol (at different doses) and their combinations as it was described previously (**Sirotkin, 2010, 2011; Pavlova et al., 2011**). Rabbit ovaries were isolated from animas fed with yukka powder at doses 0, 200 and 500 mg/kg food/day during 2 months. Accumulation of markers of apoptosis (bax and p53) and proliferation (PCNA) was evaluated by SDS PAGE-Western immunoblotting, the release of progesterone, oxytocin, insulin-like growth factor I (IGF-I) and prostaglandin F by cultured porcine, rabbit and bovine ovarian cells was measured by RIA as it was published previously (**Pavlova et al., 2011**).

RESULTS AND DISCUSSION

It was observed, that the culture of either porcine or bovine ovarian cells with benzene, toluene or xylen promote apoptosis (accumulation of apoptosis markers bax and p53) and proliferation (accumulation of PCNA). Furthermore, additions of these contaminants were able either up- or down-regulate the release of progesterone, oxytocin, insulin-like growth factor I (IGF-I) and prostaglandin F by cultured porcine, rabbit and bovine ovarian cells and their response to addition of FSH. FSH additions promoted proliferation, apoptosis and release of molecules listed above by porcine granulosa cells. Moreover, FSH was able to modify and to prevent. Some effects of BTEX on these cells. The effect of FSH, benzene and their combination on the release of progesterone by cultured porcine granulose cells is illustrated by Table 1.

Table 1 Progesterone release (ng/10^6 cells/day) by porcine ovarian granulose cells cultured with and without FSH and benzene.

FSH dose added (IU/ml media)	No benzene addition	Addition of benzene (1%)
0	7.64±0.40	16.38±0.72 [b]
0.001	10.03±0.34[a]	21.81±1.19 [ab]
0.01	14.14±0.76 [a]	23.81±0.33 [ab]
0.1	9.99±0.33 [b]	25.36±1.89 [ab]

Values are means ± SED.

[a] – effect of FSH (significant, $P<0.05$ differences between cell cultured with and without (0 IU/ml media) FSH

[b] - effect of benzene (significant, $P<0.05$ differences between corresponding cell cultured with and without benzene

Our data are in line with previous reports concerning influence of BTEX on hormonal regulators of reproduction (**Ungváry et al., 1986; Stepanov et al., 1990; Tap et al., 1996; Burmistrov et al., 2001**), although these publications demonstrated that BTEX affect gonads indirectly, via hypothalamo-hypophysial system. Our observations are the first evidence for direct action of BTEX on ovarian functions (release of ovarian hormones, proliferation and apoptosis) and ovarian response to physiological upstream stimulator (FSH). Moreover, it is the first demonstration that FSH can modify effect of BTEX on ovarian cells.

The effects of either quercetin or resveratrol on basic porcine ovarian cell functions were observed, but these plant molecules were not able to prevent BTEX effect. Feeding of rabbits with yucca extract caused changes in release of progesterone, IGF-I and prostaglandin F by their ovarian cells, as well as to modify and prevent the influence of benzene on ovarian hormone release. Table 2 illustrates, that feeding of rabbits with yucca did not affect the ability of their ovaries to produce prostaglandin F, but prevented and even inverted stimulatory effect of benzene on this parameter.

Table 2 Prostaglandin F release (ng/10^6 cells/day) by cultured fragments of ovaries isolated from rabbits fed and not fed with yucca.

	No yucca feeding	Feeding with yucca
No benzene addition	12.54±1.56	12.67±0.50
Benzene addition (1%)	15.31±0.57[a]	7.27±1.06 [ab]

Values are means ± SED.

[a] – effect of benzene (significant, P<0.05 differences between cell cultured with and without benzene

[b] - effect of yucca (significant, P<0.05 differences between corresponding cell originated from animals fed and not fed with yucca

This is the first demonstration of the interrelationships between BTEX and plant molecules in control of ovarian functions. Our observation suggest that yucca, but probably not quercetin or resveratrol is able to modify and even to invert the response of ovarian cells to BTEX. Therefore, some plants or their molecules could be potentially useful for natural prevention of negative effect of BTEX on reproduction.

CONCLUSION

The obtained data suggest that (1) the negative effect of BTEX on reproduction can be due to their influence on ovarian cell apoptosis, proliferation, turnover and release of peptide and steroid hormones and growth factors, and that (2) FSH and plant molecules can regulate ovarian cell functions and prevent some effects of BTEX on these cells.

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