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Cigarette Smoke Carcinogen Benzo(a)pyrene (BP) Affects Mouse Oogenesis

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The toxic effect of cigarette smoke carcinogen benzo(a)pyrene (BP) on morphology and function of mouse ovary was studied. ICR sexually mature female mice were treated with 50, 100 or 300 mg/kg BP for 10, 20 or 30 days. The ovarian tissue was proceeded for routine histological, autoradiographic and electron microscopic investigation. The obtained results showed that BP affects morphology and function of mouse ovary by inducing dose-dependent apoptotic degeneration of all ovarian cell types. The most sensitive to BP action were small follicles. In medium and large sized follicles the disturbances were registered in the oocyte as well as in the granulosa cells. The treatment with 300 mg/kg BP depresses irreversibly mouse ovulation. It is suggested that BP may induce apoptosis via intrinsic and/or extrinsic death pathway or may induce transitory disturbance of pituitary/hypothalamus interactions which in turn affects ovarian function.

Key words: benzo(a)pyrene, mouse ovary, apoptosis.

Introduction

Benzo(a)pyrene (BP), a major carcinogen in cigarette smoke is a polycyclic aromatic hydrocarbon (PAH) identified also in the air, water and in char-broiled foods. BP is absorbed following inhalation, oral and dermal routes of administration and rapidly distributed to several tissues in experimental animals. The metabolism of BP is complex and induces formation of a carcinogen benzo(a)pyrene 7,8 dial-9,10-epoxide. It is known that cytochcrome P450 superfamily of enzimes (P450) contribute to the detoxication of the variety of xenobiotic compounds and to the activation of many compounds to toxic, mutagenic or carcinogenic derivatives. Several P450 are inducible and their complex regulation can take place at transcriptional, post-transcriptional and post-translational level [10]. The constituitive expression of cytochrome P450 - 1A1 (CYP1A1) is highly inducible in liver and other tissues by a variety of compounds including PAHs. Regulation of the CYP1A1 gene in response to PAH occurs at transcriptional level and is mediated via ligand-dependent activation of the aryl hydrocarbon receptor (AhR). In the inactive form AhR has been shown to be a part of a cytosolic complex consisting of heat shock proteins (HSP) 70 and 90 and other proteins [3]. It is suggested that after administration of BP, HSPs 70 and 90 may be involved in modulation of apoptotic process [9].

Due to its long duration, the process of oogenesis is highly sensitive to different environmental toxic agents, including BP. It is shown that administration of 10 mg/kg BP

during gestation caused reduced fertility and reproductive capacity in offspring in mice. In addition single intraperitoneal administration of 20 mg/kg BP causes degeneration of mice primordial ovarian follicles up to 25% [5]. Since smoking is still widely spread between female human population, it is of a particular interest to follow up the effect of BP on the mammalian ovary.

The aim of the present work was to study the effect of BP on mouse ovarian morphology and function.

Material and Methods

Female ICR sexually mature mice, 30 g body weight were distributed in four groups: A/ control group (30 mice); B/ treated with 50 mg/kg BP (21 mice), C/ treated with 100 mg/kg (21 mice) and D/ treated with 300 mg/kg (7 mice). BP was dissolved in sterile sunflower oil and injected intraperitoneally (i.p). in a total volume 1 ml per animal. At 10, 20 and 30* day from the beginning of the experiment the animals were sacrificed under ether anesthesia. One hour before the end of the experiment, the animals were injected with 3H-thymidine (spec. act. 24 Ci/mMol) in a dose 1.5μCi/g body weight. The ovaries were fixed in Serra's fixative and embedded in paraffin wax. Paraffin sections 5 μm thick were stained with Mayer's haematoxylin. Part of the sections were covered with liquid nuclear emulsion (K2, Ilford, England) and exposed for three weeks at 4°C. After appropriate development the autoradiographs were stained with Mayer's haematoxylin. The observations were made on Zetopan Reichert microscope. The percentage of labelled follicular cells in large sized follicles as well as the mean number of follicles in the ovary after treatment with 50 and 100 mg/kg BP was estimated by using Student's t-test.

Pieces of ovaries were fixed in 2,5% glutaraldehyde, postfixed in OsO_4 and embedded in durcupan resin. Ultrathin sections were stained with uranyl acetate and lead nitrate and examined on an Opton 109 (Germany) Electron Microscope.

*Mice treated with 300 mg/kg B(a)P were sacrificed at day 30 only.

Results

The ovary of sexually mature female mice contains a large pool of nonproliferating primary (small) follicles each consisting of an oocyte arrested in meiotic prophase surrounded by a single or several layers of granulosa (follicular) cells. In addition medium and large sized follicles with increased layer thickness of proliferating granulosa cells were observed (Fig. 1a) as well as corpus luteum.

Ten days after administration of 50 and 100mg/kg BP majority of follicles showed normal appearance. Small and large sized follicle number was reduced (Fig. 3) and the percentage of labelled granulosa cells of large sized follicles was decreased (Fig. 4). In few follicles the contacts between granulosa cells and oocyte were destroyed and some follicular cells showed apoptotic appearance, stronger expressed after 100 mg/kg BP.

Twenty days after administration of 50 and 100 mg/kg BP the more profound changes in the morphology of the follicles were registered (Fig. 1b). Failure in the corona radiata integrity and partial or total loss of contact with oocyte was visible (Fig. 1b). The number of degenerating granulosa cells was increased, percentage of labelled granulosa cells as well as the number of small and medium sized follicles were decreased (Figs. 3 and 4). Corpora luthea number was obviously decresed. At the ultrastructural level in all oocytes the perinuclear space was expanded. In the oocyte cytoplasm of small follicles, vacuolization of endoplasmic reticulum and mitochondria with dilatated or disrupted cristae was observed (Fig. 2a).

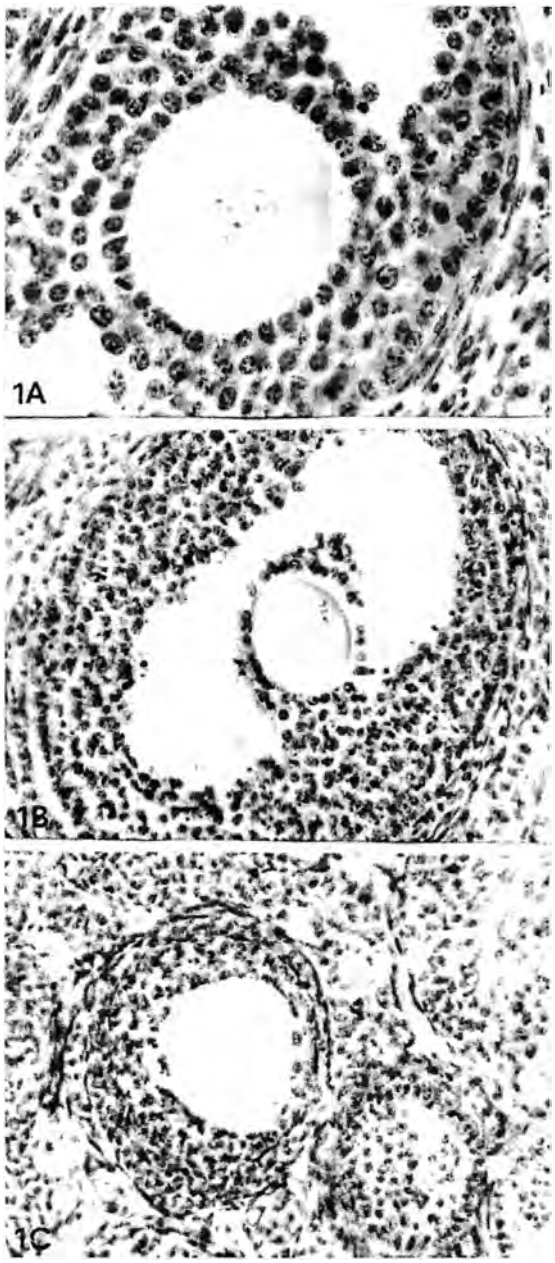

Fig. 1. Light microscopy of mouse ovary in control and after BP treatment
A — large sized follicle of control mice. Serra's fixative, Mayer's haematoxylin, ×400; B — large sized follicle from mice 20 days after treatment with 50 mg/kg BP. Disintegration of corona radiata cells and pyknosis of oocyte nucleus. Same methods, ×400; C — large sized follicle from mouse ovary in the process of degeneration 30 days after treatment with 100 mg/kg BP. Same methods, ×400

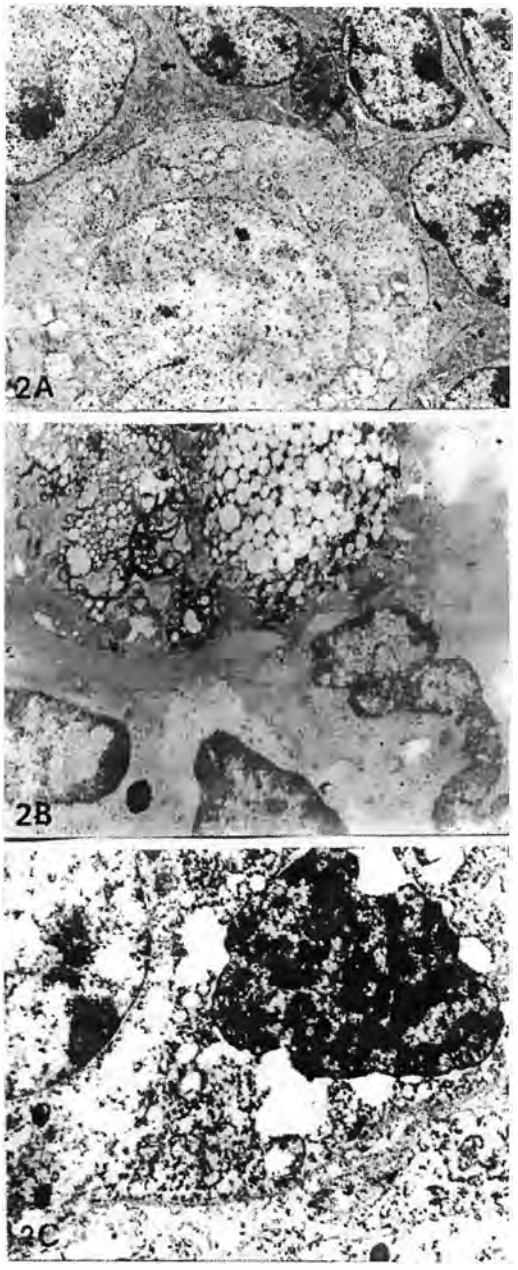

Fig. 2. Transmission electron micrographs of mouse ovary after administration of benzo(a)pyrene
A — small sized follicle 20 days after treatment with 50 mg/kg BP. Vacuolization of endoplasmic reticulum cysternae and mitochondrial martix is visible. ×3000; B — complete degeneration of oocyte from small follicle 30 days after treatment with 100mg/kg BP. ×7000; C — apoptotic appearance of follicular cell 30 days after treatment with 300 mg/kg BP. ×7000

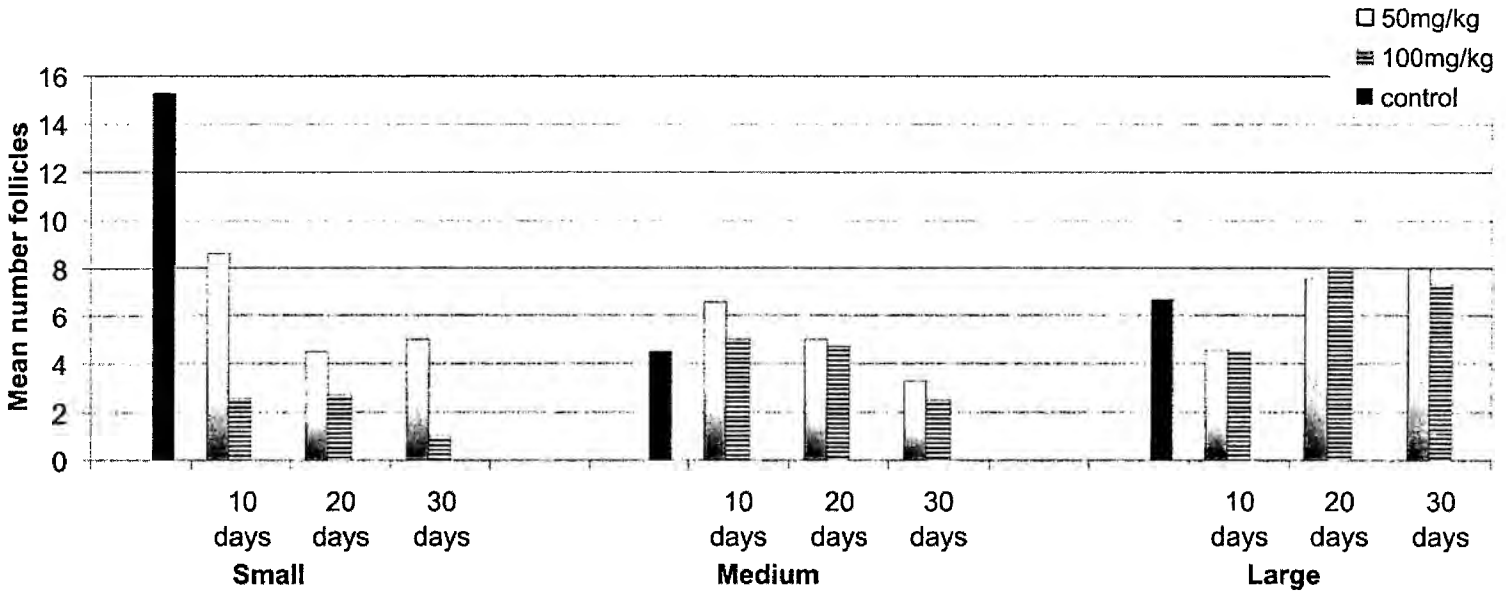

Fig. 3. Mean number of small, medium and large sized follicles in mouse ovary in control and after treatment with 50 and 100 mg/kg BP

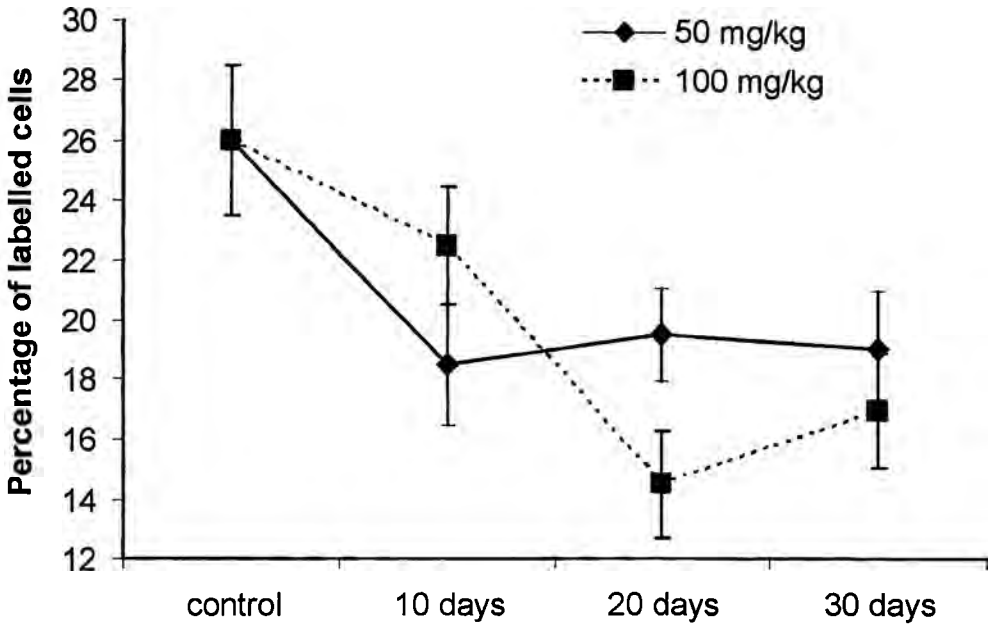

Fig. 4. Mean percentage of labelled follicular cells in large sized follicles of mouse ovary after treatment with 50 and 100 mg/kg BP

Thirty days after administration of 50, 100 and 300 mg/kg BP, sharp decrease in small follicle number was registered (Fig. 3) and majority of large sized follicles were in the process of degeneration (Fig. 1c). Some oocytes showed complete apoptotic (pyknotic) appearance (Fig. 2b). Corona radiata was disintegrated and vacuolization of granulosa cell cytoplasm was registered. The most prominent changes in morphological and ultrastructural characteristics in all kinds of follicles were visible after application of 300mg/kg BP. In more advanced stages of apoptosis the cytoplasm of some follicular cells was totally disintegrated, the perinuclear space was highly expanded and the nuclear chromatin was condensed (Fig. 2c). Corpora lutea were almost not detected in the ovary.

Discussion

The obtained results showed that intraperitoneal administration of BP induces dose-response changes in morphological and functional status of mouse ovary. The most sensitive were small follicles even after application of low dose of 50mg/kg. Ultrastructural analysis showed that apoptotic degeneration involved basic ovarian cell types. Doses of 100 and especially 300 mg/kg BP resulted in extreme depression of ovulation, demonstrated with sharp decrease in the number of corpora lutea. The molecular mechanisms which mediate the clearance of apoptotic cells are numerous and not yet well characterized [8]. It is possible that BP induces apoptosis via extrinsic or receptor-mediated cell death pathway [1]. As it was described before, in response to PAH a cascade of signal transduction is induced which results in binding of the ligand to the AhR and consequent release of HSP 90(70) [2]. As a result from BP treatment, HSP accumulates in the cytosol and associates with a number of signalling proteins thus regulating cell death machinery [6]. This suggestion needs to be proved by immunocytochemical localization of mAbs against HSP90 (70) in the future studies. On the other hand, vacuolization of endoplasmic reticulum cisternae

and mitochondrial matrix may trigger intrinsic or mitochondrial death pathway of apoptosis through cytochrome c and procaspase-9 [1]. In addition to above - mentioned direct apoptotic mechanisms, as a consequence of BP treatment, the described degenerative changes in mouse ovary may be induced by gonadotropins or growth factors withdrawal [4] since pituitary gonadotropins play crucial role in the regulation of follicle growth and differentiation [7].

In conclusion administration of benzo(a)pyrene, the basic carcinogen in cigarette smoke affects morphology and function of mouse ovary by inducing dose-dependent apoptotic degeneration of all ovarian cell types. It is suggested that BP may induce apoptosis via intrinsic and/or extrinsic death pathway or may induce transitory disturbances of pituitary/hypothalamus interactions which in turn affects ovarian function.

References

1. Arya, R., M. Mallik, S. C. Lakhota. Heat shock genes — integrating cell survival and death. — J. Biosci., **32**, 2007, 595-610.
2. Backlund, M., I. Johanson, S. Mkrtchian, M. Ingelman-Sundberg. Signal transduction-mediated activation of the aryl hydrocarbon receptor in rat hepatoma H4IIE cells. — J. Biol. Chem., **272**, 1997, 31755-31763.
3. Carver, L. A., C. A. Bradfield. Ligand-dependent interaction of the alyl hydrocarbon receptor with a novel immunophylin *in vivo*. — J. Biol. Chem., **272**, 1997, 11452-11456.
4. Francavilla, S., P. D'Abrizo, G. Cordeschi, F. Pelliccione, S. Necozione, S. Ulisse, G. Properzi, F. Francavilla. Fas expression correlates with human germ cell degeneration in meiotic and post-meiotic arrest of spermatogenesis. — Mol. Hum. Reprod., **8**, 2002, 213-220.
5. MacKenzie, K. M., D. M. Angevine. Infertility in mice exposed in utero to benzo(a)pyrene. — Biol. Reprod., **24**, 1981, 183-191.
6. Parcellier, A., S. Gurbuxani, E. Schmitt, E. Solary, C. Garrido. Heat shock proteins, cellular chaperones that modulate mitochondrial cell death pathways. — Biochem. Biophys. Res. Comm., **304**, 2003, 505-512.
7. Roche J. F. Control and regulation of folliculogenesis. A symposium of perspective. – Rev Reprod., **1**, 1996, 19-27.
8. Savill, J., V. Fadok. Corpse clearance defines the meaning of cell death. — Nature, **407**, 2000, 784-788.
9. Sreedhar, A. S., P. Csermely. Heat shock proteins in the regulation of apoptosis: new strategies in tumor therapy: a comprehensive review. – Pharmacol. Ther., **101**, 2004, 227-257.
10. Verlinger, V., M. Backlund, A. Zhukov, M. Ingelman-Sundberg. Transcriptional and post-transcriptional regulation of CYP1A1 by primaquine. — Pharmacol. Exper. Ther., **297**, 2001, 206-214.