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Effects of environmental endocrine disruptors upon mouse spermatogenic cell death and their possible molecular mechanisms

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Key words: endocrine disruptor, germ cell death, Fas/Fas ligand, Bcl-2/Bax, steroid hormone receptors, mouse testis

Abstract:

For a better understanding of the effects of various environmental toxic compounds including endocrine disruptors (ED) on mammalian reproduction, the direct study on germ cell death in fetal, neonatal and adult animals would be required. In the project of this year, we focused on the effect of estradiol-3-benzoate (EB; 1 ng - 100 μg, 2 mg, 4 mg/kg BW), diethylstilbestrol (DES; 1, 5, 10, 20 and 50 mg/kg BW), bisphenol A (BPA; 4 μg - 4 mg, 20 mg, 200 mg/kg BW) and dichlorodiphenyl dichloroethene (DDE; 10 mg, 50 mg/kg BW) upon germ cell apoptosis in a short term protocol, where ICR male mice were injected subcutaneously with a compound dissolved in 5% ethanol/corn oil in every 5 days and killed on 20 days after the first injection. When germ cell apoptosis was examined by TUNEL, the number of TUNEL positive cells were increased in a dose-dependent fashion with EB. DES and DDE increased the number of TUNEL positive germ cells to a maximum at a dose of 20 mg/kg BW and 10 mg/kg BW, respectively, and thereafter declined to the control level, indicating a "low-dose effect". BPA also increased the number of TUNEL positive germ cells at a dose of higher than 400 μg/kg BW. Very interestingly, however, BPA significantly inhibited spontaneously occurring apoptosis at a dose of 4 - 40 μg/kg BW. These results indicated that ED has wide-ranging effects on germ cell apoptosis depending upon each chemical nature. To analyze the molecular mechanism underlying the induction of germ cell apoptosis, we investigated the involvement of Fas/Fas ligand and Bcl-2/Bax in EB and DES treated testes immunohistochemically. In both cases, the expression of Fas was increased in parallel with an increase of TUNEL positive spermatocytes and the expression of Fas ligand in Sertoli cells was kept nearly constant. Unlike EB, DES also increased markedly the expression of Bax at 20 mg/kg. Thus, the induction of spermatogenic cell death by DES can be mediated through both Fas and Bcl-2 systems. Since estrogenic compounds are supposed to affect cells expressing

estrogen receptor (ER) α and β, we examined the expression of ER α and β in EB- and DES-treated testes by immunohistochemistry. Our results revealed that ER α is expressed in only Leydig nuclei, while ER β is in the nuclei of spermatogonia and spermatocytes, suggesting the direct action of these comopounds upon germ cells through ER β. And the expression level of ER proteins was not changed so much by the treatments with EB and DES. Finally, to analyze the late effect of fetal exposure to ED, we performed histological examination of mouse testes on 2, 4 and 6 weeks after birth, which were exposed to EB or DES in fetal periods at various doses, and found that the onset of spermatogenesis might be occurred earlier. These accumulated results indicate that various environmental toxic compounds may affect the states of germ cell proliferation, differentiation and death in different ways. Further study to categorize these environmental toxic compounds, based on germ cell toxicity, would be helpful for prediction of reproductive risks of unanalyzed suspicious compounds.

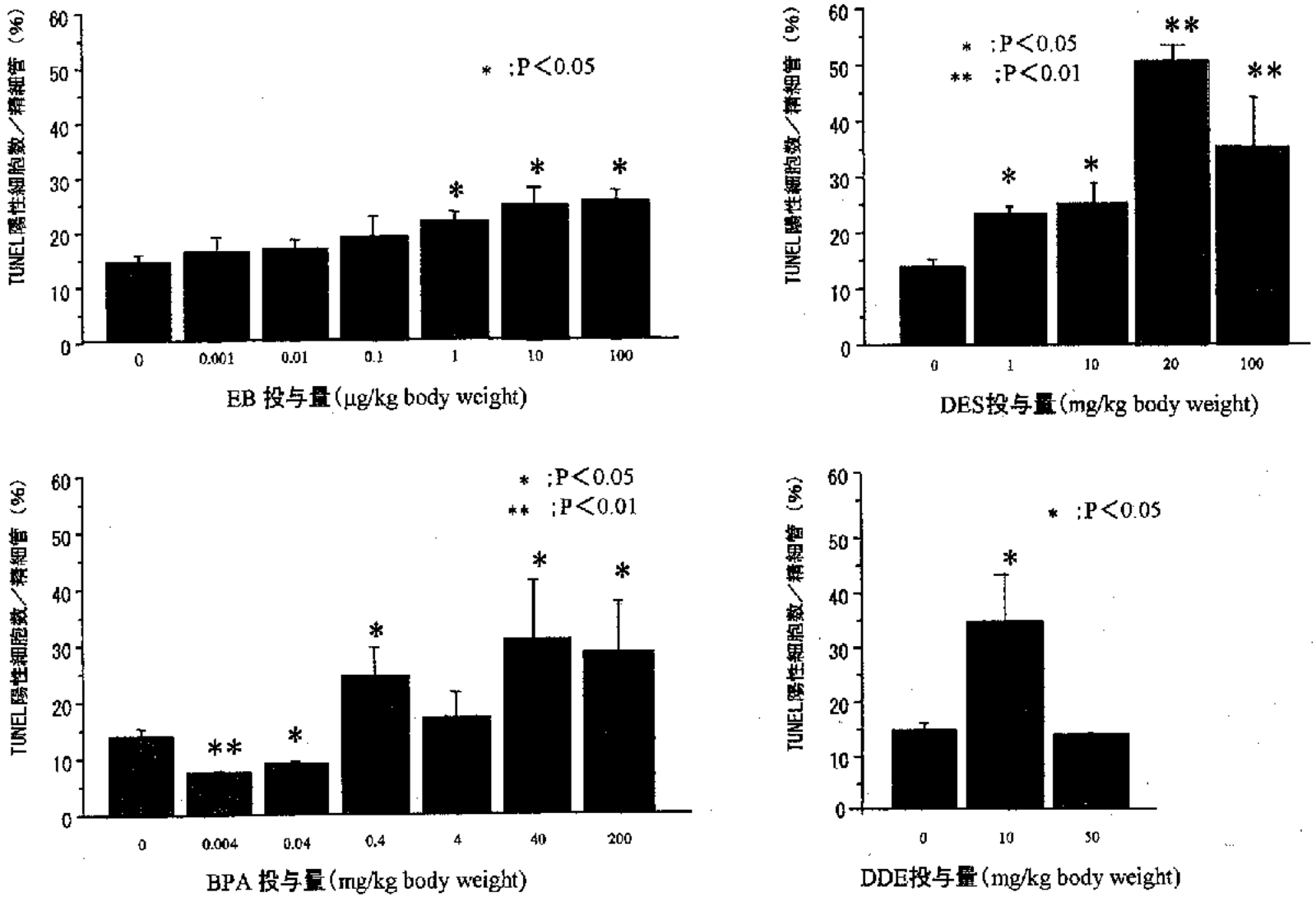

図１：精子形成細胞アポトーシスへの種々の内分泌攪乱物質の影響

成熟雄マウスに種々の濃度でEB、DES、BPA及びDDEを５日毎に皮下投与し、初回投与から２０日目に精巣を採取し、そのパラフィン切片を用いてDNA二本鎖切断部位を検出するTUNEL染色を行い、アポトーシス細胞を検出したもの。アポトーシス頻度は、精細管円形断面当たりのTUNEL陽性細胞数を%として示した。対照としては、5%エタノール/コーンオイルを投与した。