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Uterine Adenocarcinoma in *N*-Ethyl-*N′*-nitro-*N*-nitrosoguanidine-treated Rats with High-dose Exposure to *p-tert*-Octylphenol during Adulthood

Shin-ichi Katsuda,[1] Midori Yoshida,[1] Hiroyuki Kuroda,[1] Jin Ando,[1] Masakazu Takahashi,[1] Yuji Kurokawa,[1] Gen Watanabe,[2] Kazuyoshi Taya[2] and Akihiko Maekawa[1, 3]

[1]*Department of Pathology, Sasaki Institute, 2-2 Kanda Surugadai, Chiyoda-ku, Tokyo 101-0062 and* [2]*Laboratory of Veterinary Physiology, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu, Tokyo 183-8509*

Since many risk factors are associated with the development of uterine adenocarcinomas in humans, the etiology is unclear in most cases, although it has been pointed out that estrogen may play essential roles. To clarify the effects of exposure to *p-tert*-octylphenol (OP), an environmental xenoestrogen, on uterine carcinogenesis, adult Donryu rats were initiated with a single intrauterine treatment of *N*-ethyl-*N′*-nitro-*N*-nitrosoguanidine (ENNG) at 11 weeks of age and exposed thereafter to 100 mg/kg OP by s.c. injection until 15 months of age. Adult ovariectomized (OVX) rats were also treated in a similar way. OP had no effect on occurrence of persistent estrus in middle age, although uterotrophic effects were obvious in OVX rats. At the termination, development of uterine adenocarcinomas was significantly increased in animals exposed to OP during adulthood. No tumors, but a few focal hyperplasias, developed in OVX rats. These findings suggest that OP has tumor-promoting effects on ENNG-treated endometrium of rats, possibly due to direct action on the uterus, as indicated by the uterotrophic effect when a high dose of OP was given. The results provide clues to the mechanisms of influence of hormonal disrupters on uterine carcinogenesis.

Key words: Octylphenol — Rat — Uterine adenocarcinoma — Adulthood

Recently, the possible adverse consequences arising from the release of man-made substances with estrogenic, anti-estrogenic or androgenic properties, so-called endocrine disrupting chemicals (EDCs), into the environment have become an important social issue. *p-tert*-Octylphenol [OP; *p*-(1,1,3,3-tetramethylbutyl)phenol, Fig. 1], one of the alkylphenols (APs), is listed as an EDC with estrogenic activity *in vitro*[1, 2] and *in vivo*.[3, 4] Environmental OP is thought to be derived from biodegradation of non-ionic surfactants, alkylphenol polyethoxylates (APEOs),[5] and is found in the sludge of sewage-treatment plants as well as in the river and sea sediments.[1, 6] It has been pointed out that human exposure to OP may occur not only through drinking water extracted from polluted rivers and foods from fields contaminated with sewage sludge, but also by contact with manufactured and/or breakdown products, such as absorption through skin from shampoos and cosmetics, or inhalation and ingestion from pesticide sprays.[7] While APs, including OP, exist at only very low concentrations in the environment (below 1 μg/liter in water in Europe)[8] and are markedly less estrogenic than estradiol-17β (E2), this does not rule out potential toxicity of chronic exposure to animals and human beings, taking into account the evidence of bioaccumulation in fish.[9]

Carcinogenicity is the most important possible adverse consequence of chemicals including EDCs. In fact, it has been hypothesized that environmental estrogens, including APs, may be causative agents for breast cancer in humans.[10] The uterine adenocarcinoma is one of the most common malignant tumors in women.[11] While its etiology remains largely unclear, it has been pointed out that hormones such as estrogen may play essential roles.[12–15] Menoxenia, polycystic ovary syndrome, chronic anovulation, estrogen replacement therapy, obesity, hypertension, diabetes, and the nulliparous state have been listed as risk factors.[16–20] Recently, epidemiological evidence has accumulated with regard to endometrial cancers as second primaries after the use of tamoxifen, an anti-estrogen, for the treatment of breast cancer.[21] In experimental studies, however, there is only limited evidence that environmental chemicals/hormones induce uterine adenocarcinomas in rodents, as reviewed recently.[22] We have documented that the Donryu rat is a high incidence strain for spontaneous development of uterine adenocarcinomas, associated with a hormonal imbalance characterized by an increased estrogen-progesterone ratio.[23–25] The incidence of spontaneous uterine adenocarcinomas in this rat strain showed a tendency to decrease in animals having reproductive experience, compared to the nulliparous case, suppression being associated with changes in the hormonal milieu.[26] These results indicate that the Donryu rat may be a good animal model for uterine adenocarcinoma linked to endogenous estrogens in humans. An elevated incidence of such tumors develops in this rat strain with a single intra-uterine administration of *N*-ethyl-*N′*-nitro-*N*-nitrosoguanidine

[3] To whom requests for reprints should be addressed.
E-mail: amaekawa@sasaki.or.jp

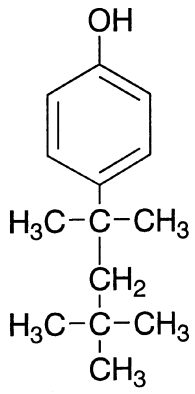

Fig. 1. Chemical structure of *p-tert*-octylphenol.

(ENNG) and a two-stage rat uterine carcinogenesis model has already been established.[27] This animal system has advantages for clarification of the tumor-promotive effects of long-term exposure to estrogens and/or estrogenic compounds during adulthood, with acceleration of cell proliferative activity in the uterus and/or indirect effects such as perturbation of endocrine regulation.[22]

We performed a series of comprehensive experiments to study the influence of OP on uterine carcinogenesis using Donryu rats. In the rats, the first ovulation, termed puberty, usually begins at 5 or 6 weeks of age. Thereafter, a 4- or 5-day sexual cycle is repeated and the rat after 11 or 12 weeks of age has fully grown to be an adult. In the current study, to clarify the effects of long-term OP exposure during adulthood on uterine carcinogenesis, both ovary-intact and ovariectomized (OVX) adult female animals, initiated with a single intra-uterine administration of ENNG at 11 weeks of age, were given a high dose of OP s.c. until 15 months of age. In the adult OVX rat, 2 consecutive injections of 100 mg/kg OP caused marked estrogenic effects.[4] We selected this dose to confirm the maximum effect of OP on the uterine carcinogenesis. As suggested by Certa *et al.*,[8] to minimize the metabolism of OP during first passage through the liver, animals were treated with OP by s.c. administration.

MATERIALS AND METHODS

Animals and housing conditions Female Crj:Donryu rats were obtained from Charles River Japan, Inc. (Kanagawa). They were housed in plastic cages and kept in an air-conditioned animal room under constant conditions of 24±2°C and 55±10% humidity with a 12 h light/dark cycle, and maintained on basal diet, CRF-1 (Oriental Yeast, Inc., Tokyo) and tap water *ad libitum*. Animal care and use followed the NIH Guide for the Care and Use of Laboratory Animals.

Experimental design Four experimental groups were prepared, as shown in Fig. 2. Groups 1 and 2 were ovary-intact, and groups 3 and 4 were OVX adult rats with or without long-term OP treatment from 11 weeks of age until 15 months of age.

Groups 1 and 2: ovary-intact rats with or without OP treatment When rats were 11 weeks of age, at which time they are more sensitive to chemical carcinogens than when older,[22] a single dose of 20 mg/kg ENNG (Nacalai Tesque, Inc., Kyoto) dissolved in polyethylene glycol was given into a unilateral uterine cavity using a stainless catheter via the vagina, as reported previously.[27] Subsequently, rats were given s.c. injections with dimethylsulfoxide (DMSO) (group 1) or OP (Wako Pure Chemical Ind., Ltd., Osaka). The dose of OP was 100 mg/kg body weight, and this was applied 5 times/week for the first 2 weeks, 3 times/week for the next 11 weeks, and 2 times/week thereafter. In our previous study, the dose was ascertained to exert strong uterotrophic effects on OVX rats with treatment of OP for 2 weeks,[4] and after that, dosing times of the treatment were reduced because irritative responses such as induration and erythema were evident at the injection sites. For sequential histological observations and hormone assays, 6–8 animals in each group were sacrificed at 9 and 12 months of age (6 and 9 months after the beginning of dosing, respectively). All surviving rats (23–26 animals in each groups) were killed at 15 months of age (12 experimental months).

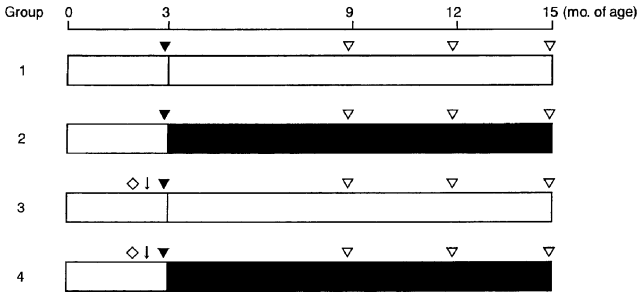

Fig. 2. Experimental design for examination of the effects of *p-tert*-octylphenol (OP) on uterine carcinogenesis. Intact (groups 1 and 2) and ovariectomized (groups 3 and 4) Donryu rats were initiated with *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine (ENNG) and then received s.c. injections of 100 mg/kg OP or vehicle alone (dimethylsulfoxide) until the end of the experiment (15 months of age). Dosings were made 5 times/week for the first 2 weeks, 3 times/week for the next 11 weeks, and 2 times/week thereafter. ▼ a single intra-uterine application of 20 mg/kg ENNG via the vagina, ▽ sacrifice, ◇ ovariectomy, ↓ priming with s.c. injections of 15 ng of estradiol-17β three times at 13, 14, and 15 days after ovariectomy, ■ s.c. injection of 100 mg/kg *p-tert*-octylphenol (OP). 1, control—ovary-intact animals; 2, OP-exposed—ovary-intact animals; 3, control—ovariectomized animals; 4, OP-exposed—ovariectomized animals.

Groups 3 and 4: OVX rats with or without OP treatment To assess the role of the ovary in uterine carcinogenesis, adult OVX rats initiated with chemical carcinogen were chronically exposed to OP. At 8 weeks of age, ovariectomy was performed by the dorsal route under general ether anesthesia. The success of the operation was confirmed by demonstration of castration vaginal smears characterized by predominant leukocytes with few epithelial cells over at least 4 days. The rats were then primed with s.c. injections of 15 ng of E2 (Wako Pure Chemical Ind., Ltd., Osaka) 3 times at 13, 14, and 15 days after ovariectomy, to increase the sensitivity to estrogens.[28] After 3 weeks (11 weeks of age), 20 mg/kg ENNG was administered into a uterine horn under laparotomy, and thereafter animals were s.c. injected with vehicle alone (group 3) or OP (group 4) in the manner described above and sacrificed following the same schedule (3–6 rats in 9 and 12 months of age, and 15–29 rats in 15 months of age).

Histological examination and hormonal assays All animals were checked for general condition every day and body weights were measured every 2 weeks. Vaginal smears were checked at 4 and 6 months of age and every 3 months thereafter to confirm the estrous cycle stage in groups 1 and 2, and estrous conversion in groups 3 and 4. At necropsy, animals were weighed and then sacrificed by decapitation. Serum samples were collected at necropsy and frozen at −70°C until analysis. The reproductive tract tissues and other representative organs such as the pituitary, lungs, liver, kidneys, and adrenals were quickly removed, weighed and fixed in 10% neutral buffered formalin, and then routinely processed for histopathological examination. Animals found dead or sacrificed when moribund were also autopsied and sampled for histopathology. Each uterus was dissected into about 12 slices in cross-section and proliferative endometrial lesions were classified into three degrees of hyperplasias (slight, +; moderate, ++; severe, +++) and adenocarcinoma using our categories for rat uterine proliferative lesions reported previously.[25] In addition, adenocarcinomas were subdivided into well–moderately (G1–2) and poorly differentiated (G3) types, and also classified as to the degree of invasion; I–II, tumors limited in the uterus; III–IV, tumors invaded into the serosa and/or surrounding adnexae, including cases with distant metastases, in accordance to the simplified FIGO histopathological grades of human uterine cancers.[29] Serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were measured with NIDDK-rat-FSH and -LH radioimmunoassay (RIA) kits (NIAMDD, NIH, Bethesda, MD).[30] Concentrations of prolactin (PRL), E2 and progesterone were also measured by RIA.[31] Immunoreactive inhibin in the serum was analyzed by double-antibody RIA using a rabbit anti-serum, TNDH-1.[32]

Statistical analysis Values for incidences were statistically analyzed using the Fisher's exact probability test. Other data were analyzed using ANOVA, and post hoc comparisons between OP-treated and control groups were made with Student's *t* test. A *P* value less than 0.05 was considered to be statistically significant.

RESULTS

Effects of OP treatment on vaginal smears and uterine weights of ovary-intact and OVX rats At 4 months of age (about 1 month after the beginning of the dosing), the estrous cycle stage could be easily identified from vaginal smears, a precise 4-day cycle being evident in group 1, as shown in Table I. In OP-treated animals (group 2), vaginal cytology of metestrous and/or diestrous stages was significantly disturbed with large amounts of contaminating epithelial cells, although the duration of the estrous cycle was not affected. Table II summarizes data for sequential changes in the incidence of persistent estrus in control (group 1) and OP-treated animals (group 2). Persistent estrus, characterized by vaginal smears exhibiting nucleated epithelial cells and/or cornified cells, began to appear after 6 months of age in both groups, with no difference in incidence between groups 1 and 2 throughout. On the other hand, in the OVX rats, OP injections caused marked uterotrophic effects (group 4; Fig. 3), although relative uterine weights were still only 1/5th to 1/6th of those of

able I. Effects of *p-tert*-Octylphenol (OP) on Estrous Cyclicity t 4 Months of Age (before the Beginning of Persistent Estrus)

Group	Abnormal estrous cycle/stage	
	Abnormal duration of estrous cycle[a)]	Disturbed estrous cycle stage[b)]
1: Control	0/35 (0)	0/35 (0)
2: OP-treated	3/35 (9)	19/35 (54)**

** Significantly different from group 1 ($P<0.01$).
Numbers in parentheses are percentages.
a) Term shortened or prolonged with estrous cycle less or more than 4 days.
b) Large amounts of contaminating epithelial cells in vaginal smears at metestrous and/or diestrous stages.

Table II. Sequential Changes in Incidences of Persistent Estrus in Ovary-intact Animals

Group	6	9	12	15 (months of age)
1: Control	11/35 (31)	29/35 (83)	27/28 (96)	21/21 (100)
2: OP-treated	16/33 (48)	27/31 (87)	26/28 (93)	26/26 (100)

Numbers in parentheses are percentages.
Vaginal cytology was examined.
OP, *p-tert*-octylphenol.

Table III. Incidences of Uterine Proliferative Lesions

Age	Group	*n*	–	Hyperplasia				Adenocarcinoma
				+	++	+++	Total	
9 months	1: Control	6	1	5	0	0	5	0
	2: OP-treated	6	2	4	0	0	4	0
	3: Control (OVX)	3	3	0	0	0	0	0
	4: OP-treated (OVX)	6	6	0	0	0	0	0
12 months	1: Control	6	1	2	3	0	5	0
	2: OP-treated	8	0	4	1	0	5	3
	3: Control (OVX)	3	3	0	0	0	0	0
	4: OP-treated (OVX)	7	6	1	0	0	0	0
15 months	1: Control	23	2	2	8	7	17	4
	2: OP-treated	26	0	1	8	5	14	12[a)]
	3: Control (OVX)	15	15	0	0	0	0	0
	4: OP-treated (OVX)	29	25	3	1	0	0	0

a) Incidence of adenocarcinomas is significantly different from the controls ($P<0.05$).
OVX, ovariectomy; OP, *p-tert*-octylphenol.

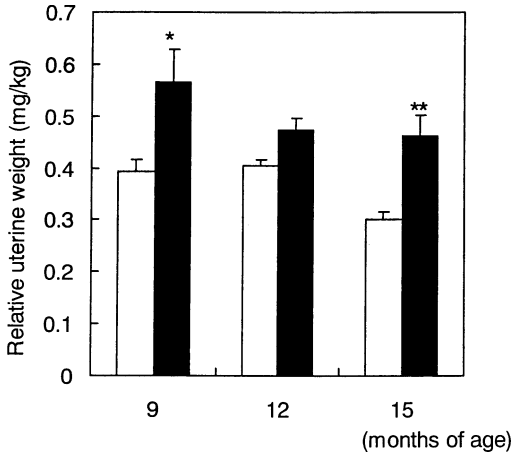

Fig. 3. Uterotrophic effects in adult ovariectomized rats treated with *p-tert*-octylphenol (■ group 4), and the controls (□ group 3). Data are expressed as mean±SE values. * $P<0.05$, ** $P<0.01$.

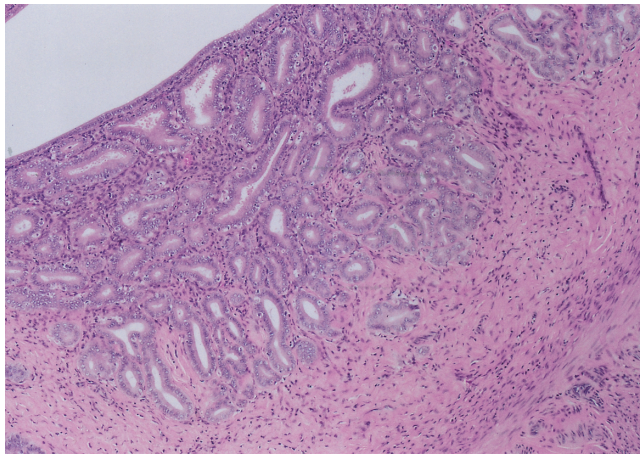

Fig. 4. Endometrial hyperplasia (moderate, ++) with focal proliferation of uterine glands found in a rat of group 2. ×90.

ovary-intact groups with or without OP (groups 1 and 2) (data not shown). Estrus conversion in vaginal smears appeared in 83% of OP-treated animals (group 4) at 4 months of age, but thereafter almost all animals showed no estrus.

Uterine proliferative lesions and other histopathological findings A comparison of sequential development of uterine proliferative lesions in control and OP-exposed rats (groups 1 and 2) is given in Table III. In both controls and rats treated with OP (groups 1 and 2), endometrial hyperplasias increased in number and severity with age (Table III), most being focal proliferations of uterine glands with apparent duct structures in the stroma of the endometrium (Fig. 4).

Uterine adenocarcinomas were significantly increased in ovary-intact rats treated with OP (group 2), as compared with group 1 ($P<0.05$) (see Table III). The results of subclassification of adenocarcinomas at 15 months of age, regarding degree of differentiation and invasion, are shown in Table IV. All adenocarcinomas in group 1 and almost adenocarcinomas in group 2 were of well-differentiated type (Fig. 5), but 3 cases in group 2 were of poorly

Table IV. Degrees of Differentiation and Invasion of Uterine Adenocarcinomas Found at 15 Months of Age

Age	Group	Total	Differentiation[a)]		Invasion[b)]	
			G1–2	G3	I–II	III–IV
15 months	1: Control	4	4 (100)	0 (0)	4 (100)	0 (0)
	2: OP-treated	12	9 (75)	3 (25)	10 (83)	2 (17)

Numbers in parentheses are percentages.
Adenocarcinomas were classified in accordance with the simplified FIGO histopathologic grades of human uterine cancers.
a) Histopathological grades of uterine adenocarcinomas by tumor differentiation. G1–2, well to moderately differentiated; G3, poorly differentiated.
b) Degree of invasion of uterine adenocarcinomas. I–II, tumors limited to the uterus; III–IV, tumors invading into the serosa and/or surrounding adnexae, including intra-abdominal and distant metastases.
OP, *p-tert*-octylphenol.

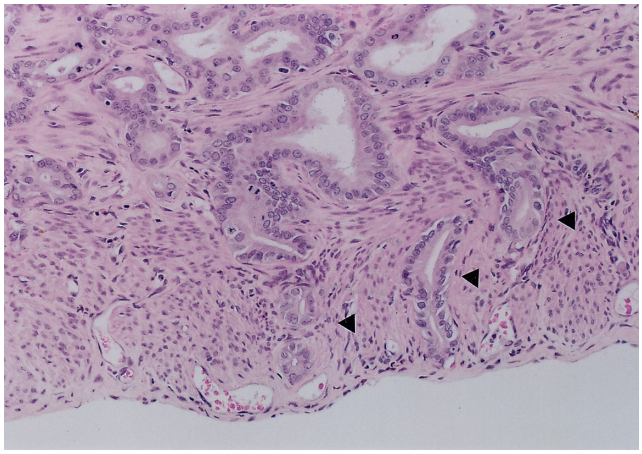

Fig. 5. Well-differentiated uterine adenocarcinoma found in a rat of group 2. Arrowheads show tumor cells invading the myometrium. ×180.

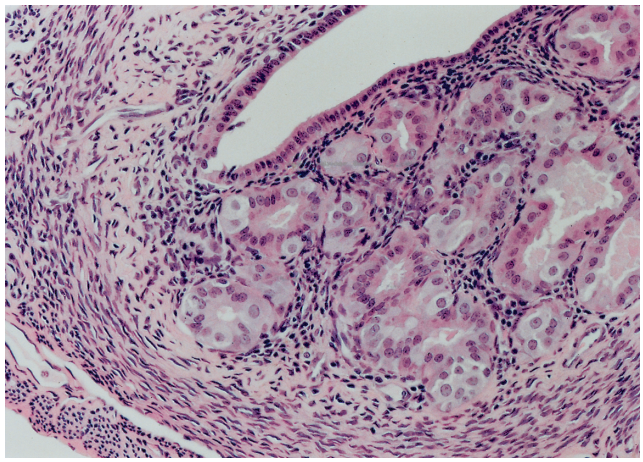

Fig. 6. Endometrial hyperplasia (weak, +) with focal proliferation of uterine glands found in a rat of group 4. ×210.

differentiated type, invading into the serosa of the corpus uteri and/or adnexae. No tumors were observed in OVX rats with or without OP treatment (groups 3 and 4) at any age examined. Although all uteri were thin and atrophic in these animals, five OP-exposed uteri (group 4) at 15 months of age had focal hyperplasias, ranging from slight to moderate (Fig. 6).

At terminal necropsy, ovarian atrophy/cyst formation and lack of corpora lutea were observed in almost all animals of groups 1 and 2.

Endocrine environment Hormonal profiles of serum FSH, LH, PRL, E2, progesterone, and inhibin in groups 1 and 2 are shown in Fig. 7. Although FSH levels were not different between the two groups, LH levels in OP-treated rats were significantly lower than the control values. As compared to the controls, the serum E2 level in OP-treated rats was lower at 9 months of age. Although serum progesterone levels did not significantly differ between the groups, serum inhibin levels in OP-treated rats (group 2) were higher at 12 months of age and thereafter.

DISCUSSION

Estrogens are well established to be important etiological agents for uterine carcinogenesis in humans.[12–15)] Although the exact roles still remain to be detailed, the tumor-promotive effects of up-regulation of cell proliferation have long been suggested. Natural and synthetic estrogenic hormones express their effects mainly by binding to estrogen receptors. OP is a typical putative endocrine disrupter, binding to ER and exerting estrogenic effects.[1, 33)] Recently we determined that OP increased the proliferation of endometrial cell components in adult OVX rats, in a dose- and period-dependent manner.[4)] Until now,

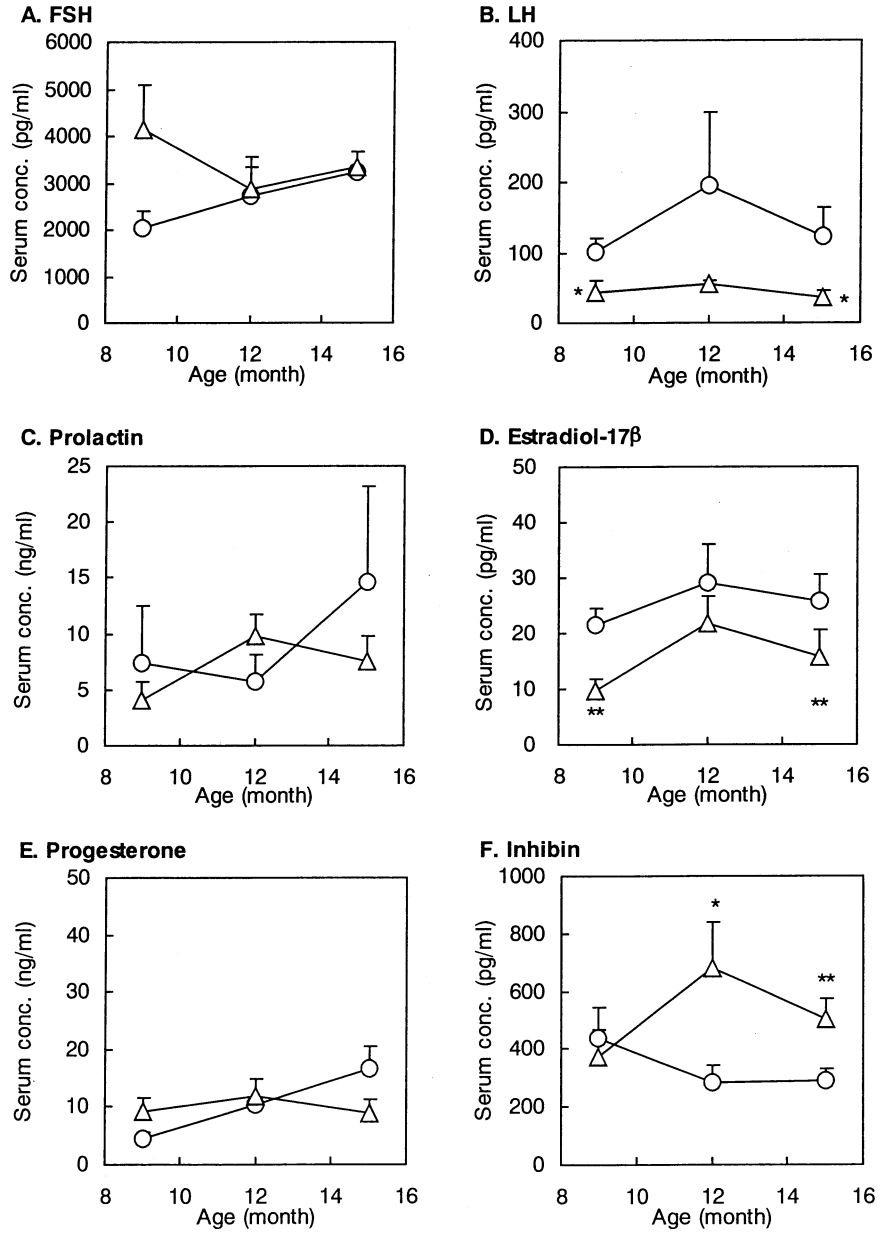

Fig. 7. Serum hormone levels in rats of the control (group 1, ○) and OP-treated (group 2, △). Animals were sacrificed at 9, 12, and 15 months of age and serum samples were collected. Data are expressed as mean±SE values. * $P<0.05$, ** $P<0.01$. OP, *p-tert*-octylphenol.

however, there has been no report concerning carcinogenic effects of OP on the female genital tract, including the uterus, in rodents. Thus, we performed the present study, in which a high dose was administered during adulthood. The present results provide support for the hypothesis that exposure to high doses of OP may result in promotive effects on uterine carcinogenesis in rats.

In considering the carcinogenic effects of hormones and/or hormone-like chemicals such as OP, it is very important to clarify whether the mechanisms involve a direct action on the target organs or an indirect influence via perturbation of hormonal regulation. In the present study, long-term OP exposure caused apparent uterotrophic effects in OVX rats. On the other hand, in ovary-intact rats, age-related persistent estrus was not perturbed by OP treatment at 6 months of age and thereafter, although abnormal estrous cycling was significantly increased at 4 months of age. Age-related ovarian atrophy/cyst formation was evident in ovary-intact groups (groups 1 and 2), but there were no differences in incidence or morphology. These findings suggest that dosing with OP during adulthood in this experiment had clear uterotrophic

effects, but little influence on hypothalamic function. There is some experimental evidence of the development of tumors following treatment with xenoestrogens, irrespective of endogenous estrogen levels, e.g., mammary tumor by estradiol benzoate in the rat,[34] and renal tumor by diethylstilbestrol (DES) in the hamster.[35] Accordingly, OP may impact directly upon the uterus as a xenoestrogen, the tumor-promotive effect depending on its estrogenic activity additional to that of endogenous hormones. The fact that adenocarcinomas were not induced in OVX rats exposed to OP indicates that endogenous ovarian hormones are needed for uterine tumor development. After 9 months of age, the hormonal environment was affected by OP exposure. Low levels of E2 in rats treated with OP during adulthood might be induced by negative feedback due to OP exposure, as LH levels were consistently lower in those animals. In the present study, the relative estrogen level (E2/progesterone ratio), known to be very important for uterine carcinogenesis in humans and rodents,[22] was low at 15 months of age. Therefore, it should be clarified whether an additional hormonal effect of OP might play a key role in uterine carcinogenesis or not, although ovarian hormones are essential to tumor development as mentioned above. The persistent-estrous state in aging rats, characterized by a lack of ovulation and absence of estrous cycles, was associated with enhanced inhibin subunit mRNA expression in large and anovulatory follicles of the ovaries.[36] High levels of inhibin in rats treated with OP during adulthood may indicate that residual granulosa cells in the atrophic ovaries possess the ability to secrete inhibin, and/or that small follicles which lack estrogen-secreting ability might exist.

Previous observations concerning spontaneous uterine adenocarcinoma development in Donryu rats indicated that uterine adenocarcinomas can arise from both lining epithelium and uterine glands, but especially the latter.[25] In the present case of rats treated with OP, endometrial hyperplasias due to focal proliferation of uterine glands were frequently seen. The fact that many tumors were well to moderately differentiated with definite duct-structures closely resembling uterine gland may also have histogenetic significance. On the contrary, neonatal exposure to OP caused marked depression of the number of uterine glands.[37] It was reported that DES-treated mice had decreased numbers of uterine glands, when exposed during neonatal period.[38] Neonatal treatment of rats with tamoxifen induced uterine adenocarcinoma without hyperplasia, and it was concluded that the suppression of uterine-gland genesis by exposure to tamoxifen may account for the low incidence of hyperplasias.[39] Based on this evidence, we hypothesized that OP might affect the uterine gland differently depending upon the age at exposure, and induce different types and incidences of tumors from the present case.

In conclusion, a high-dose OP exposure during adulthood can enhance the development and/or growth of uterine adenocarcinomas in rats. The present evidence shows that a xenoestrogenic compound can act as a tumor promoter through its estrogenic activity, additional to that of endogenous hormones. Since APs including OP exist at only very low concentrations in the environment, the relevance to the human situation of the present findings is not clear. To clarify this point, additional studies are now under way, using lower doses of OP by the oral route.

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REFERENCES

1) White, R., Jobling, S., Hoare, A., Sumpter, J. P. and Parker, M. G. Environmental persistent alkylphenolic compounds are estrogenic. *Endocrinology*, **135**, 175–182 (1994).
2) Abraham, E. J. and Frawley, L. S. Octylphenol (OP), an environmental estrogen, stimulates prolactin (PRL) gene expression. *Life Sci.*, **60**, 1457–1465 (1997).
3) Blake, C. A. and Ashiru, O. A. Disruption of rat estrous cyclicity by the environmental estrogen 4-*tert*-octylphenol. *Proc. Soc. Exp. Biol. Med.*, **216**, 446–451 (1997).
4) Katsuda, S.-I., Yoshida, M., Isagawa, M., Asagawa, Y., Kuroda, H., Watanabe, T., Ando, J., Takahashi, M. and Maekawa, A. Dose- and treatment duration-related effects of *p-tert*-octylphenol on female rats. *Reprod. Toxicol.*, **14**, 119–126 (2000).
5) Giger, W., Brunner, P. H. and Schaffner, C. 4-Nonylphenol in sewage sludge: accumulation of toxic metabolites from nonionic surfactants. *Science*, **225**, 623–625 (1984).
6) Purdom, C. E., Hardiman, P. A., Bye, V. J., Eno, N. C., Tyler, C. R. and Sumpter, J. P. Estrogenic effects of effluents from sewage treatment works. *Chem. Ecol.*, **8**, 275–285 (1994).
7) Blake, C. A. and Boockfor, F. R. Chronic administration of the environmental pollutant 4-*tert*-octylphenol to adult male rats interferes with the secretion of luteinizing hormone, follicle-stimulating hormone, prolactin, and testosterone. *Biol. Reprod.*, **57**, 255–266 (1997).
8) Certa, H., Fedtke, N., Wiegand, H. J., Müller, A. M. F. and Bolt, H. M. Toxicokinetics of *p-tert*-octylphenol in male

Wistar rats. *Arch. Toxicol.*, **71**, 112–122 (1996).
9) Shiraishi, H., Carter, D. S. and Hites, R. A. Identification and determination of *tert*-alkylphenols in carp from the Trenton Channel of the Detroit River, Michigan, USA. *Biomed. Environ. Mass Spectrom.*, **18**, 478–483 (1989).
10) Davis, D. L. and Bradlow, H. L. Can environmental estrogens cause breast cancer? *Sci. Am.*, **273**, 167–172 (1995).
11) Masubuchi, K., Nemoto, H., Masubuchi, S., Fujimoto, I. and Uchino, S. Increasing incidence of endometrial carcinoma in Japan. *Gynecol. Oncol.*, **3**, 335–346 (1975).
12) Lingeman, C. H. Hormones and hormonomimetic compounds in the etiology of cancer. *Recent Results Cancer Res.*, **66**, 1–48 (1979).
13) Hilliard, G. D. and Norris, H. J. Pathologic effects of oral contraceptives. *Recent Results Cancer Res.*, **66**, 49–71 (1979).
14) Zeil, H. K. Estrogen's role in endometrial cancer. *Obstet. Gynecol.*, **60**, 509–515 (1982).
15) Fox, H. Endometrial carcinogenesis and its relation to oestrogen. *Pathol. Res. Pract.*, **179**, 13–19 (1984).
16) Baker, V. V. Potential criteria for cohort selection in chemoprevention trials of uterine adenocarcinoma. *J. Cell. Biochem. Suppl.*, **23**, 184–188 (1995).
17) Noumoff, J. S. and Faruqi, S. Endometrial adenocarcinoma. *Microsc. Res. Tech.*, **25**, 246–254 (1993).
18) Wood, G. P. and Boronow, R. C. Endometrial adenocarcinoma and the polycystic ovary syndrome. *Am. J. Obstet. Gynecol.*, **124**, 140–142 (1976).
19) The British Gynaecological Cancer Group. Oestrogen replacement and endometrial cancer. *Lancet*, **i**, 1359–1360 (1981).
20) Kvale, G., Heuch, I. and Ursin, G. Reproductive factors and risk of cancer of the uterine corpus: a prospective study. *Cancer Res.*, **48**, 6217–6221 (1988).
21) Cohen, I., Altaras, S., Lew, S., Tepper, R., Beyth, Y. and Ben-Baruch, G. Ovarian endometrioid carcinoma and endometriosis developing in a postmenopausal breast cancer patient during tamoxifen therapy: a case report and review of the literature. *Gynecol. Oncol.*, **55**, 443–447 (1994).
22) Maekawa, A., Takahashi, M., Ando, J. and Yoshida, M. Uterine carcinogenesis by chemicals/hormones in rodents. *J. Toxicol. Pathol.*, **12**, 1–11 (1999).
23) Maekawa, A., Onodera, H., Tanigawa, H., Furuta, K., Matsuoka, C., Kanno, J., Ogiu, T. and Hayashi, Y. Spontaneous neoplastic and non-neoplastic lesions in aging Donryu rats. *Jpn. J. Cancer Res.*, **77**, 882–890 (1986).
24) Nagaoka, T., Onodera, H., Matsushima, Y., Todate, A., Shibutani, M., Ogasawara, H. and Maekawa, A. Spontaneous uterine adenocarcinomas in aged rats and their relation to endocrine imbalance. *J. Cancer Res. Clin. Oncol.*, **116**, 623–628 (1990).
25) Nagaoka, T., Takeuchi, M., Onodera, H., Matsushima, Y., Ando-Lu, J. and Maekawa, A. Sequential observation of spontaneous endometrial adenocarcinoma development in Donryu rats. *Toxicol. Pathol.*, **22**, 261–269 (1994).
26) Nagaoka, T., Takegawa, K., Takeuchi, M. and Maekawa, A. Effects of reproduction on spontaneous development of endometrial adenocarcinomas and mammary tumors in Donryu rats. *Jpn. J. Cancer Res.*, **91**, 375–382 (2000).
27) Ando-Lu, J., Takahashi, M., Imai, S., Ishihara, R., Kitamura, T., Iijima, T., Takano, S., Nishiyama, K., Suzuki, K. and Maekawa, A. High-yield induction of uterine endometrial adenocarcinomas in Donryu rats by a single intrauterine administration of *N*-ethyl-*N*′-nitro-*N*-nitrosoguanidine via the vagina. *Jpn. J. Cancer Res.*, **85**, 789–793 (1994).
28) Schatz, R., Soto, A. M. and Sonnenschein, C. Estrogen-induced cell multiplication: direct or indirect effect on rat uterine cell? *Endocrinology*, **115**, 501–506 (1984).
29) Pecorelli, S., Benedet, J. L., Creasman, W. T. and Shepherd, J. H. FIGO staging of gynecologic cancer. *Int. J. Gynecol. Obstet.*, **64**, 5–10 (1999).
30) Taya, K. and Sasamoto, S. Age-related changes in the secretory pattern of FSH and LH in response to LH-RH in prepubertal female rats. *Endocrinol. Jpn.*, **35**, 349–355 (1988).
31) Watanabe, G., Taya, K. and Sasamoto, S. Dynamics of ovarian inhibin secretion during the oestrous cycle of the rat. *J. Endocrinol.*, **126**, 151–157 (1990).
32) Noguchi, J., Hikono, H., Sato, S., Watanabe, G., Taya, K. and Sasamoto, S. Ontogeny of inhibin secretion in the rat testis: secretion of inhibin-related proteins from fetal Leydig cells and of bioactive inhibin from Sertoli cells. *J. Endocrinol.*, **155**, 27–34 (1997).
33) Mueller, G. C. and Kim, U.-H. Displacement of estradiol from estrogen receptors by simple alkyl phenols. *Endocrinology*, **102**, 1429–1435 (1978).
34) Inao, H., Yamanouchi, H., Suzuki, K., Onoda, M. and Wakabayashi, K. Estradiol-17β as an initiation modifier for irradiation-induced mammary tumorigenesis of rats ovariectomized before puberty. *Carcinogenesis*, **16**, 1871–1877 (1995).
35) Li, J. J., Li, S. A., Oberley, T. D. and Parsons, J. A. Carcinogenic activities of various steroidal estrogens in the hamster kidney: relation to hormonal activity and cell proliferation. *Cancer Res.*, **55**, 4347–4351 (1995).
36) Jih, M. H., Lu, J. K., Wan, Y. J. and Wu, T. C. Enhanced ovarian inhibin subunit gene expression in aging rats is due to chronic anovulation. *Biol. Reprod.*, **49**, 1208–1214 (1993).
37) Katsuda, S.-I., Yoshida, M., Watanabe, G., Taya, K. and Maekawa, A. Irreversible effects of neonatal exposure to *p-tert*-octylphenol on the reproductive tract in female rats. *Toxicol. Appl. Pharmacol.*, **165**, 217–226 (2000).
38) Yoshida, A., Newbold, R. R. and Dixon, D. Effects of neonatal diethylstilbestrol (DES) exposure on morphology and growth patterns of endometrial epithelial cells in CD-1 mice. *Toxicol. Pathol.*, **27**, 325–333 (1999).
39) Carthew, P., Edwards, R. E., Nolan, B. M., Martin, E. A., Heydon, R. T., White, I. N. and Tucker, M. J. Tamoxifen induces endometrial and vaginal cancer in rats in the absence of endometrial hyperplasia. *Carcinogenesis*, **21**, 793–797 (2000).