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High Yields of Granulosa Cell Tumors/Luteomas in F344 Rat Ovaries after Transplacental Administration of N-Nitrosobis(2-oxopropyl)amine

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Ovarian tumors were induced at very high incidence in the offspring of F344 rats receiving 3 subcutaneous injections of 10 mg/kg of N-nitrosobis(2-oxopropyl)amine on the 14th, 18th and 20th days of gestation. Histologically, all ovarian tumors were of the granulosa cell tumor and/or luteoma type. Many of them consisted of large, polygonal cells with abundant eosinophilic or vacuolated cytoplasm, arranged in sheets or in a pseudo-palisaded pattern separated by thin fibrovascular stroma, and they exhibited typical luteoma morphological character. The high yields, and the similarities in morphology as well as putative hormonal influence suggest that this experimental system may serve as a good animal model for granulosa cell tumor and/or luteoma development in women.

Key words: Granulosa cell tumor — Luteoma — F344 rat — N-Nitrosobis(2-oxopropyl)amine — Transplacental carcinogenesis

While a number of methods have been used with various degrees of success in attempts to induce ovarian tumors in rats, there are a few reports of successful induction, as reviewed by Carter and Ird.[1] It was found that granulosa cell tumors could be induced in rats by transplantation of the ovary to various ectopic sites such as the spleen.[2] Hormonal factors may be important for development of tumors in transplanted ovaries. There is, however, little direct evidence that hormone treatment is responsible for induction of ovarian tumors. Experimentally, rats appear resistant to ovarian tumor induction by irradiation, although this is the oldest and most effective method in mice.[3] Polycyclic hydrocarbon carcinogens such as 7,12-dimethylbenzanthracene and 3-methylcholanthrene have been mainly used for chemical induction of ovarian tumors, but while they result in good tumor yields in mice, again there are only a few reports of success in rats.[1] Histologically, almost all ovarian lesions induced in rats and/or mice by these methods have been diagnosed as granulosa/theca cell tumors. Recently, however, new methods for induction of ovarian tumors with N-nitroso compounds have been introduced, and new histological types of ovarian neoplasm have been identified. For example, N-ethyl-N-nitrosourea (ENU) administered intraperitoneally or transplacentally to Sprague-Dawley and BD-IV rats induced Sertoli cell tumors.[4] We also reported Sertoli cell/granulosa cell tumors in Donryu rats after oral administration of ENU or N-propyl-N-nitrosourea,[5,6] although ENU did not induce equivalent lesions in F344 rats (unpublished data). Pour[7] has described transplacental induction of mixed stromal cell-coelomic type ovarian tumors in Wistar-derived MRC rats by N-nitrosobis(2-oxopropyl)-amine (BOP). In the present comparative study, BOP was similarly given transplacentally to F344 rats, to assess strain-differences in susceptibility to this carcinogen.

Eight- to 10-week-old female F344/DuCrj rats purchased from Charles River Japan Inc. (Kanagawa) were housed individually in plastic cages in an air-conditioned animal room (24±1°C, 55±5% relative humidity), and maintained on basal diet (CRF-1, Oriental Yeast Ind. Co., Tokyo). BOP, purchased from Ash Stevens Co. (Detroit, MI) was dissolved in physiological saline shortly before use. The experiment was performed to the same design reported by Pour.[7] The animals were mated with healthy male rats. The day of mating was considered the first day of gestation, and was ascertained by examination of vaginal smears. Pregnant rats were given 3 subcutaneous injections of 10 mg/kg of BOP on the 14th, 18th and 20th days of gestation. Control animals received saline only in the same manner. All the sucklings were weaned at the 4th week and grouped according to sex (53 males and 57 females in the BOP-treated group and 28 males and 17 females in the control group). Animals were then maintained under the laboratory conditions mentioned above, and observed for 87–93 weeks, when all survivors were killed. All organs and/or tissues including the reproductive organs were fixed in buffered 10% formalin, and paraffin-embedded sections were stained with hematoxylin and eosin (H-E), and

Table I. Sites, Types and Incidences of Tumors Observed in Offspring Receiving BOP Transplacentally

Organ	Type of tumors	Incidence of tumors (%)			
		BOP		Control	
		Males	Females	Males	Females
Effective no. of rats		53	57	28	17
Mean survival time (wk)		88	85	86	87
Testis	Interstitial cell tumor	91	—	93	—
Ovary	Granulosa cell tumor/Luteoma	—	72*	—	0
Adrenal	Pheochromocytoma	15	4	7	6
Thyroid	C-Cell adenoma	8	4	0	0
Pancreas	Islet cell adenoma	6	0	4	0
Liver	Hepatocellular adenoma/carcinoma	4	7	0	0
Preputial/clitoral	Adenoma	9	0	0	0
Pituitary	Adenoma	4	9	4	12
Brain	Glioma	4	0	4	0
Mammary	Fibroadenoma	0	4	0	0
Uterus	Endometrial stromal polyp	—	0	—	12
Prostate	Adenoma	2	—	0	0
Others		6	4	11	0

* $P < 0.001$ (Fisher's test).

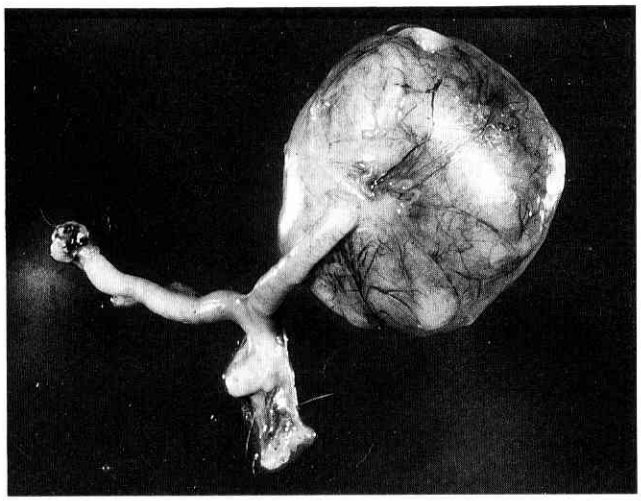

Fig. 1. Ovarian tumor of the left ovary observed in a 92-wk-old F344 rat. The animal had received 3 subcutaneous injections of 10 mg/kg of N-nitrosobis(2-oxopropyl)amine (BOP) on the 14th, 18th and 20th days of gestation. The right ovary is atrophic. Histologically, the tumor was typical luteoma.

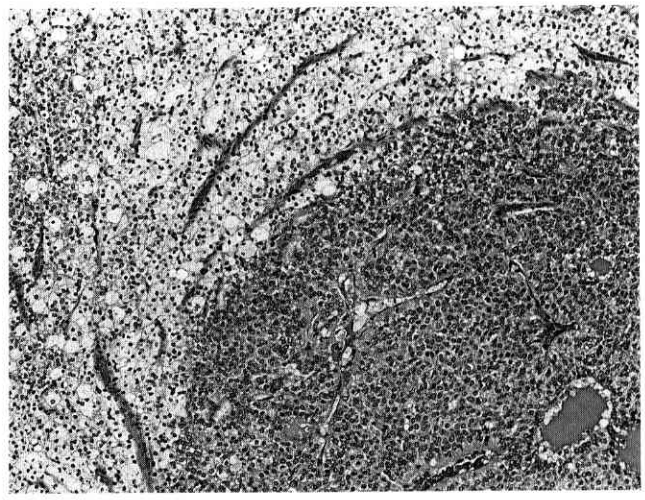

Fig. 2. Granulosa cell tumor mixed with luteoma from an 87-wk-old BOP-treated rat. A follicle-like area composed of uniformly rounded cells with eosinophilic cytoplasm is surrounded by large cells with vacuolated cytoplasm resembling luteal cells. Empty areas are visible within a follicle-like area. H-E. ×250.

examined microscopically. In a few animals, the plasma values of gonad steroids such as 17β-estradiol (E2) and progesterone (P) were assayed.

In the BOP-treated group, the average number of offspring and weaning rate were slightly lower than the control group values, although the differences were not significant. Seven males and eight females in the BOP-treated group died during the experimental period, although all of the control group except for one male survived to the end of the experiment. The mean body

weights in the treated group of both sexes at the end of the experiment were significantly ($P<0.01$) lower than those in the corresponding control.

Data for sites, types and incidences of tumors observed in the offspring receiving BOP transplacentally are summarized in Table I. Many tumors were observed in both treated and control groups, but ovarian tumors were found at high incidence (72%) only in the BOP-treated animals. Most of the induced ovarian tumors were observed unilaterally, although occasionally they occurred bilaterally. Macroscopically, they were round and/or oval in shape, and of various sizes (Fig. 1). Histologically, all of them were of the granulosa cell tumor and/or luteoma type. In the typical granulosa cell type tumors, solid masses of uniform round cells with scanty cytoplasm and ovoid nuclei, were arranged in sheets or in a pseudo-palisaded pattern separated by thin fibrovascular stroma. Empty areas or areas containing only few cells were visible within follicle-like structures consisting of diffusely arranged tumor cells. Many granulosa cell tumors also contained large, polygonal cells with abundant eosinophilic or vacuolated cytoplasm, resembling luteal cells (Fig. 2). When tumors consisted mainly of these cells, they were diagnosed as luteomas (Figs. 3 and 4), and most of the ovarian tumors observed in the present study were of the typical luteoma type. All tumors, except for the ovarian lesions, observed in both groups were histologically similar to those well known to occur spontaneously in this strain of rats,[8] although spontaneous ovarian tumors in F344 rats were very rare and the most frequently observed neoplasms were granulosa cell tumors.[9]

In the treated group, follicular cysts and atrophic changes such as absence of the follicle and/or corpus luteum were observed in all ovaries without tumors. As other lesions, squamous metaplasia, atypical change and/or adenomatous hyperplasia of the uterine endometrium were also more frequently observed in the treated group. Plasma values of gonad steroids, especially E2, were markedly decreased in the BOP-treated animals without ovarian tumors. The decrease (especially for E2), however, was much less pronounced in rats with ovarian tumors although values were still lower than those in the control animals. The E2/P ratio for rats with ovarian tumors was higher than in controls, although there was wide variation. There were no clear differences in the E2/P ratios between animals with typical granulosa cell tumors and luteomas.

In the report of Pour,[7] ovarian and testicular tumors were induced in the offspring of MRC rats after transplacental administration of BOP. Morphologically, the majority (80%) of the ovarian tumors were of a mixed stromal cell-coelomic type, and all testicular tumors demonstrated mixed Leydig cell-glandular character. In the present study, in contrast, all ovarian tumors were sex cord-stromal tumors and no coelomic type lesions were found. The reason for this strain difference is unclear.

Much of the work on the pathogenesis of ovarian granulosa cell tumors has been carried out in irradiated mice: it has been suggested that the destruction of the ovarian follicles, the disturbance of autoregulation in the pituitary-gonadal system and the exessive produc-

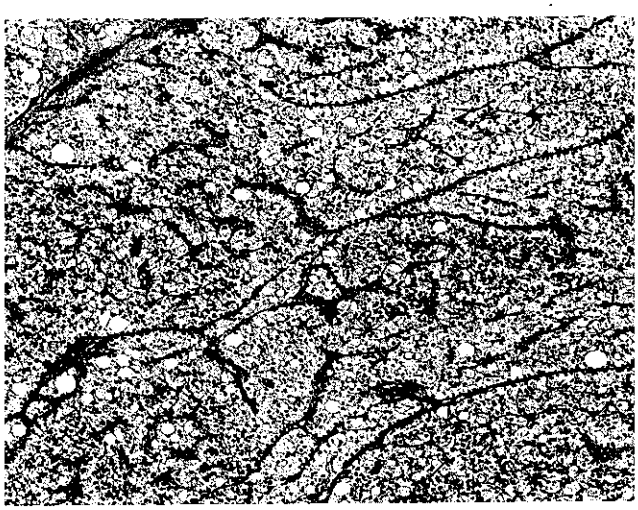

Fig. 3. Typical luteoma from an 89-wk-old BOP-treated rat. The tumor is composed of large polygonal cells with abundant vacuolated cytoplasm and small ovoid nuclei. The tumor cells are arranged in sheets and separated by thin fibro-vascular stroma. H-E. ×250.

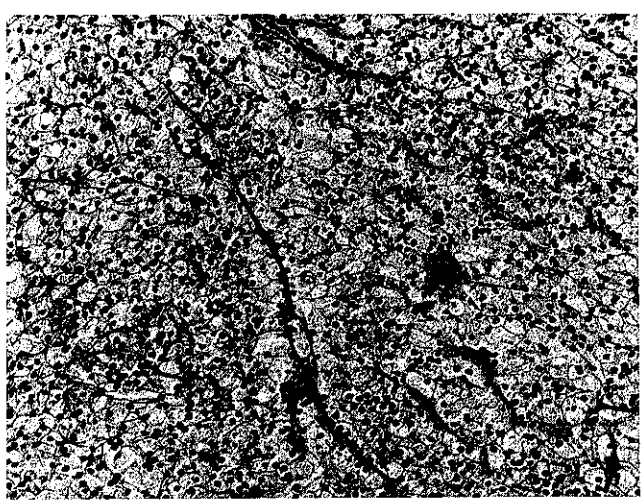

Fig. 4. Higher magnification of another luteoma from a 92-wk-old BOP-treated rat. H-E. ×400.

tion of gonadotrophic hormones may cause neoplastic growth.[10] Although the precise mechanism through which granulosa cell tumors/luteomas were induced by transplacental administration of BOP in the F344 rat cannot be deduced from the present study, the atrophic changes observed to occur in the ovaries of the treated group may be of key importance for the induction of the tumors. The influence of plasma FSH (follicle stimulating hormone) and LH (luteinizing hormone) levels and the possible hormonal function of ovarian tumors are still uncertain and are the subjects of ongoing research in our laboratory. In the present study, however, adenomatous hyperplasia of the uterine endometrium and ovarian atrophy were prominent in BOP-treated animals with ovarian tumors. The increased E2/P ratios found in these rats are also in good agreement with the histological finding of signs of estrogenization in the vaginal epithelium. In view of the similarities with human ovarian tumors, these results thus indicate that the described experimental system may serve as a good animal model for granulosa cell tumor and/or luteoma development in women.

In the present study, all testicular tumors observed in both groups were histologically diagnosed as interstitial cell (Leydig cell) neoplasms and no mixed Leydig cell-glandular type lesions were found. In the BOP-treated group, however, very small glandular structures were evident in 6 of a total of 48 rats with testicular tumors, while only 1 of 26 control rats with the tumors was similarly affected. Previously, we reported that the spontaneous interstitial cell tumor-associated glandular changes of the testis were found in F344 rats at relatively high incidence and the lesions consisted of Sertoli cell metaplastic changes.[11] These results indicate that transplacental administration of BOP did not induce Leydig cell-coelomic type tumors, but slightly increased spontaneous glandular changes of the testis in F344 rats.

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