—Full Paper—

**Long-Term Treatment with Bromocriptine Inhibits Endometrial Adenocarcinoma Development in Rats**

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**Abstract.** The effects of long-term blockade of prolactin (PRL) action by bromocriptine (BRC) treatment on uterine carcinogenesis and on related ovarian physiology were investigated using a rat uterine cancer model. Ten-week-old cycling female Donryu rats, a high yield strain for uterine corpus tumors (endometrial adenocarcinomas), were treated with N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG), as a tumor initiator, and injected with 1 mg/kg body weight BRC subcutaneously 4 times per week until 14.5 months of age to block the proestrus PRL surge. The study was terminated at 15 months of age, and the results showed that long-term BRC treatment significantly inhibited endometrial adenocarcinoma development in terms of both incidence (34.6% to 13.0% with significant difference at 5%) and multiplicity (0.35 to 0.18 with significant difference at 5%), which indicates the number of adenocarcinomas per animal. While BRC did not affect estrous cyclicity in the treated animals, a significant decline was evident in the serum 17β-estradiol (E2) to progesterone (P) ratio (E: P ratio), and the serum E2 level showed a decreased tendency at 15 months of age. While the precise pathway to the inhibitory effect could not be determined, the pathway by which ovarian hormonal imbalance decreases the serum E: P ratio most likely plays a crucial role.

**Key words:** Bromocriptine, Long-term treatment, Prolactin blockage, Rat, Uterine carcinogenesis


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dominant steroidhormone imbalance contributes to uterine car-
icogenesis in human beings [1]. Maekawa et al. [2–4] have provided evidence that a similar imbalance, especially elevated 17β-estradiol (E2) levels relative to progesterone (P) levels (the E: P ratio), plays a crucial role in promoting endometrial adenocarcino-
cina development in rodents. While it is well-established that E2 and estrogenic chemicals play supportive or sometimes initia-
tive roles in uterine carcinogenesis in rats as well as women, P may exert an inhibitory influence on human uterine cancer development [5–7]. However, the effects of other hormones related to the pitu-
ity or ovaries on uterine carcinogenesis are not well known.

Prolactin (PRL) is one of the important regulators of the corpus luteum as well as luteinizing hormone (LH), prostaglandin and vas-
cular endothelial growth factors and platelet derived growth factors in rats [8–10]. The function of PRL as a luteotrophic and luteolytic hormone, however, is very complex [11–17]. Repeated injection of PRL has been shown to stimulate rapid regression of the persistent corpus luteum, with a concomitant decline in total steroidogenic capacity [12, 18]. These studies indicate the possibility that the modulation of P production induced by PRL or its inhibitor affect uterine carcinogenesis through ovarian hormonal imbalance. On the other hand, PRL receptors, especially long-form ones (PRLR-L) [19], are present in the uteri of rats. PRL is known to play important roles in adenomyosis formation in Swiss mice [20–22]. Bromocriptine (BRC), a dopamine agonist, inhibits inverted growth of the uterine epithelium to the muscle layer in the uterine cervix in ovariectomized mice treated with E2 and PRL [23]. These reports indicate that direct action of PRL on the uterus should be considered as one factor related to uterine carcinogenicity in rats. Blockade of the proestrus PRL surge by treatment with BRC increases the weight of the ovary and the number of corpora lutea in rats without affecting estrous cyclicity [16, 24]. However the long-term effects of BRC treatment on the ovary or uterus have not yet been fully investigated.

In the present study, we therefore focused on the long-term effects of BRC on the ovary and uterine carcinogenesis using the Donryu rat, a high yield strain for endometrial adenocarcinomas.

**Materials and Methods**

**Animals and housing conditions**

A total of eighty female Crj:Donryu rats were purchased from Charles River Japan (Yokohama, Japan) at 7–8 weeks of age. The animals were maintained under conditions of controlled temperature (24 ± 2°C), humidity (55 ± 10%), and lighting (12-h light/dark cycle). They were housed in plastic cages (3 or 4 animals/cage). Commercial powder diet (CRF-1, Oriental Yeast, Tokyo, Japan) and drinking water were available ad libitum throughout the study. Animal care and use were handled in accordance with the guidelines for the care and use of laboratory animals established by the Ethics Committee for Animal Experiments of Sasaki Institute and followed the NIH Guide for the Care and Use of Laboratory Animals.
Animals.

Chemicals and selection of the dosing for bromocriptine

BRC (α-ergocryptine; Sigma-Aldrich, St. Louis, MO, USA) was dissolved in DMSO (Wako Pure Chemicals, Osaka, Japan) to produce a dose of 1 mg/kg body weight for subcutaneous administration in 1 ml DMSO/kg body weight. This dose level was selected since it is known to block the proestrus PRL surge [18]. Control animals were administered DMSO only in the same manner.

Impact on uterine carcinogenesis of long-term treatment with BRC

Assessment of the long-term effects of BRC on uterine carcinogenicity was performed using the Donryu rat initiation-promotion assay model for uterine corpus cancer [25]. Briefly, fifty rats at 10 weeks of age were initiated with a single injection of 20 mg/kg N-ethyl-N’-nitro-N-nitrosoguanidine (ENNG; Nacalai Tesque, Kyoto, Japan) into one uterine horn via the vagina using a stainless steel catheter. This initiation is known to not be carcinogenic in organs other than the uterus, and to not affect estrous cyclicity [26]. The animals were allocated into 2 groups, one receiving subcutaneous treatment with BRC five times per week up to 14.5 months of age and the other given DMSO only as the controls. Half a month before termination of the experiment, the treatment was ceased to avoid direct effects of the treatment on the serum hormone profiles. The Donryu strain rat has very regular 4-day estrous cyclicity, and the other given DMSO only as the controls. Half a month before termination of the experiment, the treatment was ceased to avoid direct effects of the treatment on the serum hormone profiles. The Donryu strain rat has very regular 4-day estrous cyclicity, and about 60 to 70% of the PRL surge was estimated to be blocked in the treated animals. Clinical signs, body weight changes and estrous cyclicity were checked throughout the study. The animals were allocated into 2 groups, one receiving subcutaneous treatment with BRC five times per week up to 14.5 months of age and the other given DMSO only as the controls. Half a month before termination of the experiment, the treatment was ceased to avoid direct effects of the treatment on the serum hormone profiles. The Donryu strain rat has very regular 4-day estrous cyclicity, and about 60 to 70% of the PRL surge was estimated to be blocked in the treated animals. Clinical signs, body weight changes and estrous cyclicity were checked throughout the study. At 15 months of age, all surviving animals were euthanized by decapitation and necropsied for histopathological assessment and hormone assays as described below. Animals euthanized when moribund or found dead were also examined for histopathology.

Pathology

After complete necropsy of all rats, the ovaries, uteri, adrenals and livers were weighed. These organs and related tissues, including the pituitary, thymus, mammary gland, brain, vagina and sites with macroscopic abnormalities, were fixed in 10% neutral buffered formaldehyde solution and routinely processed for histopathological examination. Both the ovaries were dissected at the maximum transverse sections. In the present study, the upper, middle and lower parts of each uterine horn and the uterine cervix were cut into 3 pieces each in cross-section to detect uterine neoplastic lesions, and the lesions were classified into three degrees of atypical hyperplasia (slight, moderate or severe) and adenocarcinomas, according to the criteria described previously [27]. Atypical hyperplasia was considered to be a precancerous lesion of endometrial adenocarcinoma [27]. Uterine neoplastic lesions were evaluated using the following 2 indicators: the number of animals bearing the most serious neoplastic lesions and the frequency of each neoplastic lesion per animal were expressed as the incidence and multiplicity, respectively.

Radioimmunoassays

Serum samples obtained after decapitation were stored at –80°C until assay. The serum concentrations of follicle-stimulating hormone (FSH), lutenizing hormone (LH), inhibin, E2, P and PRL were determined using double-antibody radioimmunoassays and 125I-labelled radio-ligands. National Digestive and Kidney Disease (NIDDK) radioimmunoassay kits were employed for rat FSH and LH (NIAMDD, NIH, Bethesda, MD, USA) as described by Taya et al. [28] and Watanabe et al. [29], respectively. Immunoreactive inhibin in the serum was analyzed using a rabbit anti-serum, TNDH-1[31]. The serum concentrations of E2 and P were also measured as described by Taya et al. [29].

Statistical analysis

Incidences values were statistically analyzed using the Fisher’s exact probability test. Other data were assessed using t-test (2 groups), and post hoc comparisons between the treated and control groups were made with the Dunnett’s t-test.

Results

Long-term BRC-treatment did not affect the growth curve or general condition (data not shown). With regard to estrous cyclicity, the vaginal cytology of the BRC-treated group was not different from that of the control group throughout the study. The results were similar to in regard to the occurrences of persistent estrus (PE), abnormal cyclicity including irregular estrous cycle and alterations of cellular components of vaginal smears between the control and BRC-treated groups (Fig. 1). At the end of the study, most of the ovaries were markedly atrophic in both the control and BRC-treated animals (Fig. 2), and no significant differences were detected in the absolute and relative weights of the ovary, uterus, pituitary, liver and spleen between the two groups (Table 1).

Histopathologically, the long-term BRC treatment caused significant reduction in the incidence of endometrial adenocarcinoma and the multiplicity of uterine neoplastic lesions, including both endometrial atypical hyperplasias and adenocarcinomas (Table 2). All adenocarcinomas observed in the control and treated-groups were well-differentiated; poorly- or moderately-differentiated ones were not observed in the BRC-treated animals. The incidence of adenocarcinomas was decreased in BRC-treated animals, whereas the incidence of severe atypical endometrial hyperplasia was...
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increased in this group. The atrophic ovaries of both the BRC-treatment and control groups exhibited similar degrees of cystic or atretic follicles associated with few or no corpora lutea. The occurrences of adenomas and/or adenocarcinomas in the anterior pituitaries or mammary fibroadenomas in the BRC-treated group were comparable to those in the controls. The incidences and intensities of other neoplasms and precancerous lesions were also comparable in the BRC-treated animals and control animals.

At the end of study, the levels of ovarian-derived hormones, such as E2, P and inhibin, at 15 months of age were not significantly different between the BRC-treated and control animals. The E2 levels showed a tendency of decrease in the BRC-treated group (Fig. 3). The ratio of E2 to P was significantly depressed in the BRC-treatment group. The values of pituitary derived hormones, such as PRL, FSH and LH, varied and showed no particular trends.

**Discussion**

In the present study, long-term BRC treatment significantly inhibited uterine endometrial adenocarcinoma development with regard to both incidence and multiplicity. The incidence of severe atypical endometrial hyperplasia accepted as a precancerous lesion in multi-step tumorigenesis was paradoxically increased, indicating that BRC treatment might inhibit some process in the development of precancerous lesions and step up cancer formation, although the precise mechanism of this was not determined.

Considering the mechanisms underlying the inhibitory effects, decrease of the serum E:P ratio via through ovarian hormonal imbalance most likely plays a crucial role, since the hormonal changes, including the tendency of the serum E2 level to decrease and the significant decrease in the serum E:P ratio, were observed.

**Table 1.** Relative organ weights after long-term of treatment with BRC

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>BRC-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats at termination</td>
<td>25</td>
<td>23</td>
</tr>
<tr>
<td>Pituitary</td>
<td>3.18 ± 2.11</td>
<td>2.14 ± 0.31</td>
</tr>
<tr>
<td>Ovaries</td>
<td>12.9 ± 0.72</td>
<td>14.0 ± 0.38</td>
</tr>
<tr>
<td>Uterus</td>
<td>325.4 ± 40.1</td>
<td>308.5 ± 16.4</td>
</tr>
<tr>
<td>Spleen</td>
<td>173.0 ± 25.1</td>
<td>186.1 ± 14.9</td>
</tr>
<tr>
<td>Liver</td>
<td>348.0 ± 48.9</td>
<td>332.1 ± 43.2</td>
</tr>
</tbody>
</table>

*a) Organ weights (mg)/body weight (g) × 100.  
b) Mean ± SD.

**Table 2.** Incidence and multiplicity of uterine neoplastic lesions

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>None</th>
<th>Hyperplasia</th>
<th>Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Slight</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
<tr>
<td>Incidence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>26</td>
<td>2</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>BRC-treated</td>
<td>23</td>
<td>0</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Multiplicity</th>
<th>Slight</th>
<th>Moderate</th>
<th>Severe</th>
<th>Adenocarcinoma</th>
<th>Average number of neoplastic lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.23 ± 0.51</td>
<td>0.64 ± 0.64</td>
<td>0.38 ± 0.64</td>
<td>0.35 ± 0.49</td>
<td>1.58 ± 0.81</td>
</tr>
<tr>
<td>BRC-treated</td>
<td>0.23 ± 0.51</td>
<td>0.17 ± 0.49</td>
<td>0.52 ± 0.59</td>
<td>0.18 ± 0.49*</td>
<td>1.35 ± 0.49</td>
</tr>
</tbody>
</table>

* Significantly different from the control groups (P<0.05).
occurrence of atrophic ovaries with cystic atretic follicles and lack of consistent elevation of the serum E2: P ratio manifested by early modulate regular estrous cyclicity in the present study. The sensitivity related to the inhibitory effect on uterine cancer development. Subsequently, a number of studies showing that PRL, E2 and/or their receptors are important for promoting effects on uterine carcinogenesis. There is not only a high yield strain, but also has the following 3 similarities to humans [1, 4]: 1) multi-step development from atypical hyperplasia of the glandular epithelium to adenocarcinoma; 2) change in morphological and gene expression profiles; and 3) consistent elevation of the serum E2: P ratio manifested by early occurrence of atrophic ovaries with cystic atretic follicles and lack of a corpus luteum, resulting in persistent estrus (PE) on vaginal cytology [2, 3]. These features indicated that ovarian hormonal imbalance is crucial for uterine carcinogenesis in rats as well as humans [2–4]. Many previous studies have provided evidence that delayed onset of PE and/or depression of the serum E2 level can prevent adenocarcinoma development in rats [31–33]. The present study supports our hypothesis that an increased E:P ratio is very important for promoting effects on uterine carcinogenesis. There are a number of studies showing that PRL, E2 and/or their receptors control each other through endocrine and autocrine/paracrine mechanisms [34–36]. While the BRC treatment did not affect estrous cyclicity in the present study, the decrease in the serum E2: P ratio and tendency of the serum E2 levels to decrease might be different from that in adults with normal estrous cyclicity.

BRC might act directly on the uterus in related to uterine carcinogenesis, because PRLR-L is predominantly located in the rat uterus [19]. The effects of PRL and BRC on the uterine proliferating lesions in the uteri of mice are controversial [23]. Mori et al. reported that an increase in the plasma level of PRL induces adenomysis in mice through increased expression of PRL receptor mRNA in the uterus [20–22]. On the other hand, BRC-treatment for 30 days induces proliferation of endometrial epithelial cells in mice [37]. The present study did not provide any clear evidence showing long-term BRC treatment has any direct action on uterine carcinogenesis in rats.

When rodent model data are extrapolated for human carcinogenicity predictions, it is very important to pay attention to the differences in modes of action between rodents and humans. The functional effect of PRL on the rat ovary is quite different from that in humans. Whereas PRL directly leads to atrophic corpora lutea or luteolysis in rodents, this does not occur in women [38, 39]. Although BRC has been used clinically for therapy in patients with prolactinomas for long-term, there is little information available showing that therapeutic BRC affects ovarian and uterine function, and this suggests that the influence observed in the present study may be restricted to the rat. However, there remain unclear points in regard to the effects of BRC on uterine carcinogenesis in the rat model. Therefore, further investigation is necessary to clarify the differences between the data collected from animals and women.

In conclusion, the present results indicate that long-term BRC treatment inhibits uterine cancer development. The major pathway to the inhibitory effect could not be determined; however, there is a very plausible link to ovarian hormonal alterations resulting in a decrease in the serum E: P ratio.

Acknowledgements

This study was supported by a Grant-in-Aid for Research on Risk Assessment of Chemicals (2004-Chemical-001) from the Ministry of Health, Labor and Welfare of Japan.

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