Lack of Modifying Effect of Arctiin on ENU-Induced Uterine Carcinogenesis in ICR Mice

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Abstract: The present study was performed to investigate the modifying effects of arctiin, a plant lignan isolated from Arctium lappa (burdock) seeds, on uterine carcinogenesis induced by N-ethyl-N-nitrosourea (ENU) initiation in mice. Female ICR mice aged 5 weeks were administered a single intra-uterine injection of ENU at a dose of 50 mg/kg via the vagina. After 1 week, the animals were fed a soybean-free diet containing 0 (control), 0.004, 0.02 and 0.1% of arctiin for 26 weeks. Histopathological examinations revealed the development of uterine proliferative lesions such as adenocarcinoma, atypical hyperplasia, adenomyosis, and endometrial hyperplasia. However, there were no significant differences in the incidences and severities of these lesions among the groups. These results indicate that arctiin showed no definite modifying effects on uterine carcinogenesis under the present experimental conditions.

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Key words: arctiin, lignan, phytoestrogen, uterine carcinogenesis, ICR mice

Materials and Methods

Animals

Female specific pathogen-free ICR mice aged 4 weeks were purchased from Japan SLC, Inc (Shizuoka, Japan). Throughout the acclimation and experimental periods, the animals were housed individually in wire-bottom stainless-steel cages in an air-conditioned animal room (room temperature, 23 ± 2°C; relative humidity, 51 ± 13%; and lighting cycle, 12 hours light/12 hours dark). All the animals were transferred to clean cages every 2 weeks. They were quarantined for 1 week in the animal room assigned for the study, and only those without any abnormal findings at the end of the acclimation period were selected for experimentation. A pulverized basal diet (NIH-07 soybean free; Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water were available ad libitum throughout the acclimation and experimental periods. This study was performed in accordance with the Guidelines for Animal Care and
Experimentation of Kissei Pharmaceutical Co., Ltd.

**Chemicals**

Arctiin was provided by the National Institute of Health Sciences (Tokyo, Japan). Test diets with admixtures of arctiin were prepared at Oriental Yeast Co., Ltd., and were stored at 4°C until use. ENU was purchased from Nacalai Tesque, Inc (Kyoto, Japan).

**Experimental design**

After the end of the acclimation period, 80 females were divided into 4 groups, comprised of 20 females each. Each mouse received a single intra-uterine injection of ENU in physiological saline via the vagina at a dose of 50 mg/kg body weight. After 1 week, the animals were administered a diet containing 0 (control), 0.004, 0.02 or 0.1% arctiin for 24 weeks. The dietary dose levels were selected based on the results of previous studies that had reported the modifying effects of arctiin on carcinogenesis in the liver, and mammary glands10,12. During the experimental period, all the animals were observed daily, and any clinical abnormality was recorded. Body weight and food consumption were measured once a week. At the end of the experimental period, the animals were subjected to an autopsy, and their uterus and ovaries were weighed. Most of the organs and tissues were fixed in 10% phosphate-buffered formalin, embedded in paraffin, sectioned at 3 µm, and stained with hematoxylin and eosin (HE) for histopathological analysis.

**Statistics**

Data for incidence of uterine proliferative lesions were analyzed by Fisher’s exact probability test. Data for body weight, food consumption, and organ weights were analyzed by Bartlett’s test for homogeneity of variance between the control and each arctiin-treated group. If the variances were homogeneous or heterogeneous, Dunnett’s tests for parametric or non-parametric multiple comparisons, respectively, were performed.

**Results**

During the experimental period, 1 animal in each of the 0.004% and 0.1% groups died, and 1 animal in each of the control and 0.004% groups was sacrificed in a moribund condition, mainly due to the development of malignant lymphoma. These animals were not included in the analyses. Changes in body weight and food consumption were not statistically significant for any group (Figs. 1 and 2).

At necropsy, morphological abnormalities such as discoloration and nodule formation were observed in the uterus of almost all the animals. No statistical differences were found in uterus and ovary weights among the groups (Table 1). The uterine proliferative lesions were diagnosed according to the criteria of the World Health Organization13, and were classified as adenocarcinoma, atypical hyperplasia, adenomyosis, and endometrial hyperplasia. In particular, endometrial hyperplasia was classified into 3 grades of severity, slight (+), moderate (++), and severe (+++), on the basis of cellular atypia and size according to the criteria described by Nagaoka et al.14. The histopathological results of the uterine proliferative lesions are summarized in Table 2. Adenocarcinoma (Fig. 3) was observed in the uterus of 1 animal in each of the 0.1% and 0.004% groups. The incidences of atypical hyperplasia in the control, 0.004, 0.02 and 0.1% groups were 26.3, 17.6, 10.5 and 26.3%, respectively; those of adenomyosis were 42.1, 29.4, 42.1 and 15.8%, respectively. The incidences of these uterine proliferative lesions were not statistically significant between the control and arctiin-treated groups. Deciduoma or decidual reaction, endometrial stromal polyp and leiomyoma were also found in this study. However, their incidences in the arctiin-treated groups were not significantly different from that in the control group.

**Discussion**

As a possible mechanism in the development of uterine cancer, it is well known that endogenous estrogens or
chemicals with estrogenic activity typically bind the estrogen response elements (ERE) and that transcription factors are recruited to the estrogen receptor-ligand/ERE complex, resulting in enhanced mRNA synthesis and gene expression of polypeptide growth factors such as epidermal growth factor and transforming growth factor (TGF)-alpha, both of which can stimulate cell proliferation\textsuperscript{15-17}. In the present study, uterine proliferative lesions such as endometrial hyperplasia, atypical hyperplasia, adenocarcinoma, and adenomyosis were observed in ICR mice with ENU initiation. However, arctiin treatment did not increase the incidences and severities of these lesions in any treated group. These results indicate that arctiin has no potential to promote uterine carcinogenesis through estrogenic activity.

The dried fruits of \textit{Arctium lappa} contain about 0.04\% by weight of arctiin\textsuperscript{18}, and the recommended daily dosage when

<table>
<thead>
<tr>
<th>Dose (%)</th>
<th>No. of animals</th>
<th>Uterus Absolute (g)</th>
<th>Uterus Relative (%)</th>
<th>Ovaries Absolute (g)</th>
<th>Ovaries Relative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>19</td>
<td>0.361 ± 0.176</td>
<td>0.902 ± 0.443</td>
<td>0.031 ± 0.010</td>
<td>0.075 ± 0.023</td>
</tr>
<tr>
<td>0.004</td>
<td>17</td>
<td>0.352 ± 0.143</td>
<td>0.906 ± 0.373</td>
<td>0.026 ± 0.007</td>
<td>0.067 ± 0.020</td>
</tr>
<tr>
<td>0.02</td>
<td>19</td>
<td>0.387 ± 0.222</td>
<td>0.973 ± 0.539</td>
<td>0.026 ± 0.007</td>
<td>0.066 ± 0.020</td>
</tr>
<tr>
<td>0.1</td>
<td>19</td>
<td>0.481 ± 0.360</td>
<td>1.256 ± 1.008</td>
<td>0.031 ± 0.020</td>
<td>0.082 ± 0.059</td>
</tr>
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</table>

Values are mean ± SD.

Table 2. Incidences of Uterine Proliferative Lesions in Female Mice Treated Orally with Arctiin for 26 Weeks after ENU Initiation

<table>
<thead>
<tr>
<th>Incidence (%)</th>
<th>Finding</th>
<th>Dose (%)</th>
<th>No. of animals</th>
<th>0</th>
<th>0.004</th>
<th>0.02</th>
<th>0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Endometrial hyperplasia</td>
<td>+</td>
<td>19</td>
<td>5.3</td>
<td>5.9</td>
<td>5.3</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>36.8</td>
<td>17</td>
<td>41.2</td>
<td>36.8</td>
<td>47.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+++</td>
<td>47.4</td>
<td>19</td>
<td>41.2</td>
<td>47.4</td>
<td>42.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>89.5</td>
<td>19</td>
<td>88.2</td>
<td>89.5</td>
<td>94.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Atypical hyperplasia</td>
<td></td>
<td>19</td>
<td>26.3</td>
<td>17.6</td>
<td>10.5</td>
<td>26.3</td>
</tr>
<tr>
<td></td>
<td>Adenocarcinoma</td>
<td></td>
<td>19</td>
<td>0</td>
<td>5.9</td>
<td>0</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>Adenomyosis</td>
<td></td>
<td>19</td>
<td>42.1</td>
<td>29.4</td>
<td>42.1</td>
<td>15.8</td>
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<tr>
<td></td>
<td>Deciduoma/decidual reaction</td>
<td>21.1</td>
<td>17</td>
<td>17.6</td>
<td>10.5</td>
<td>26.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Endometrial stromal polyp</td>
<td>42.1</td>
<td>17</td>
<td>47.1</td>
<td>42.1</td>
<td>52.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leiomyoma</td>
<td></td>
<td>19</td>
<td>5.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 3. Uterine adenocarcinoma in a mouse of the 0.04\% arctiin group. All sections shown were stained with HE. A: The tumor shows irregular proliferation of atypical glands, ×20. B: Higher magnification of A showing invasion of tumor cells into the muscle layer, ×50.
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the dried fruits are used as Chinese herbal medicines is 8 g\textsuperscript{19}. Considering these results, the arctiin intake in this study appears to be significantly higher than that usually recommended for humans, and therefore, it is unlikely that arctiin has a promoting effect on uterine cancer in women.

Epidemiological and experimental studies have also provided evidence that some phytoestrogenic compounds are associated with a reduced risk of endometrial cancer\textsuperscript{8,20,21}. Furthermore, Goodman \textit{et al.}\textsuperscript{22} and Horn-Ross \textit{et al.}\textsuperscript{23} reported an inverse association between phytoestrogen consumption and the risk of endometrial cancer. Despite phytoestrogens having similar relative affinity to estrogen receptor (ER) as estradiol, their estrogenic activities are 1000-fold less potent than that of estradiol\textsuperscript{24,25}. Therefore, phytoestrogens that essentially act as partial antagonists of endogenous estrogens would exhibit anti-carcinogenic effects on endometrial cancer. Arctiin has been reported to inhibit heterocyclic amine-induced mammary and hepatic carcinogenesis in rats\textsuperscript{10,12}. It has also been reported that arctigenin, an aglycone of arctiin, has an inhibitory effect on mouse skin tumor induced by 7,12-dimethylbenz(a)anthracene (DMBA) and 12-O-tetradecanoyl phorbol-13-acetate (TPA)\textsuperscript{26,27}. Considering these results, it is likely that arctiin has a chemopreventive effect on uterine carcinogenesis. However, our present study showed neither inhibitory nor promoting effects of arctiin on uterine carcinogenesis. Although the precise cause remains unknown, the dose levels of arctiin selected in this study might not have been sufficient to inhibit uterine carcinogenesis under the present experimental conditions, as in the case of a study by Zeng \textit{et al.}\textsuperscript{28} in which arctiin showed no significant chemopreventive effect on prostate carcinogenesis in probasin/SV40 T antigen transgenic rats. Further studies are necessary to conclude whether arctiin has the potential to inhibit uterine carcinogenesis.

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References


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Fig. 4. Uterine endometrial hyperplasia in control mice. All sections shown were stained with HE and magnified at \( \times20 \). A: Slight hyperplasia (+); focal proliferation of uterine glands is found in the endometrium. B: Moderate hyperplasia (++); increased uterine glands are found focally to diffusely in the endometrium. C: Severe hyperplasia (+++); marked proliferation of uterine glands is found in the endometrium without any invasion into the muscle layer.


