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Note

Effect of Royal Jelly on Bisphenol A-Induced Proliferation of Human Breast Cancer Cells

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Royal jelly is known as a functional food containing many useful minerals. In this study, we found an anti-environmental estrogen activity of royal jelly. Bisphenol A (BPA) is an environmental estrogen that stimulates proliferation of human breast cancer MCF-7 cells. Royal jelly inhibited the growth-promoting effect of BPA on MCF-7 cells, even though it did not affect the proliferation of cells in the absence of BPA. In addition, this inhibiting effect of royal jelly was heat-stable.

Key words: royal jelly; breast cancer cell; bisphenol A

Bisphenol A (BPA) is a well-known environmental estrogen that is used to manufacture polycarbonate plastics and synthetic resins applied in the linings of beverage cans and wrapping paper for foods, and in dental sealants. Recent epidemiologic evidence suggests that BPA is related to diseases of women.[1)] A relationship between blood levels of BPA and body fat in women has been reported.[2)] Moreover, Takeuchi *et al.* reported that BPA is related to ovarian disease in women.[2)] Disruption of cellular function by BPA occurred at doses as low as 1 pM.[3)] This suggests that BPA is a potent risk factor for women's health even at low dose. Hence it is important to find substances having anti-BPA activity, because women are confronted with the risk of exposure to significant amounts of BPA *via* multiple sources.

Previous reports indicate that a major royal jelly protein 3 (MRJP3) in royal jelly modulates an immune response both *in vitro* and *in vivo*.[4,5)] In this study, we identified an effect of royal jelly on the proliferation of MCF-7 cells stimulated by BPA.

Royal jelly was supplied by the Yamada Apiculture Center (Okayama, Japan). Royal jelly (0.1 g) was dissolved with PBS (10 ml), and then the solution was centrifuged at 15,000 *g* for 15 min, and the top clear layer was collected. The number of adhesive cells was counted with a hematometer after trypsin treatment (0.2% trypsin with 0.025% EDTA in PBS). MCF-7 cells suspended in RPMI 1640 medium were inoculated in a 24-well culture plate at 4×10^4 cells/well suspended in RPMI 1640 medium (Nissui Pharmaceutical, Tokyo) containing 5% FCS (PAA Laboratories, Pasching, Austria), and cultured for 24 h at 37 °C in a humidified atmosphere containing 5% CO_2. The medium was then changed to phenol red-free RPMI 1640 (Sigma, St. Louis, MO) containing BPA (Sigma) and royal jelly with 5% charcoal-treated FCS, and cultured for 72 h. The royal jelly solution was added to the medium at 5 v/v %.

A previous report suggests that estrogen and estrogenic compounds enhance proliferation of breast cancer cells.[6)] BPA also has estrogenic activity and binds to estrogen receptors.[7,8)] BPA might expose female health to the risk of human breast cancer.[9)] First, to examine the effects of BPA on proliferation of MCF-7 cells, the cells were cultured in BPA-supplemented medium. As shown in Fig. 1, BPA stimulated the proliferation of MCF-7 cells dose-dependently. The effect of royal jelly on the cell proliferation activity of MCF-7 was investigated. Figure 2A shows the effect of royal jelly on the cell

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Abbreviations: BPA, bisphenol A; ER, estrogen receptor; FCS, fetal calf serum; PBS, phosphate-buffered saline

proliferation of MCF-7 cells inoculated in the medium without BPA. Royal jelly solution slightly inhibited the proliferation of MCF-7 cells. A recent report indicates that royal jelly has estrogenic activities through interaction with estrogen receptors (ER),[10] but the royal jelly obtained from the Yamada Apiculture Center did not enhance the proliferation of MCF-7 cells. It is possible that there are differences in components between the royal jelly used here and other samples. It has been reported that a food component, for example, conjugated linoleic acid (CLA), inhibits E2-induced proliferation of MCF-7 cells.[11] Hence the effect of royal jelly on the growth-promotion activity of BPA was investigated. As indicated in Fig. 2B, the growth-promotion effect of BPA on MCF-7 cells was obviously inhibited by royal jelly. This is the first repot concerning the anti-environmental hormone activity of royal jelly.

To characterize the inhibition activity of royal jelly against BPA, royal jelly was heat-treated at 100 °C for 30 min and assayed for activity against BPA. As shown in Fig. 3, heat-treated royal jelly inhibited the BPA-induced growth-promotion effect on MCF-7 cells, as well as intact royal jelly. This suggests that the active substance in royal jelly is heat-stable.

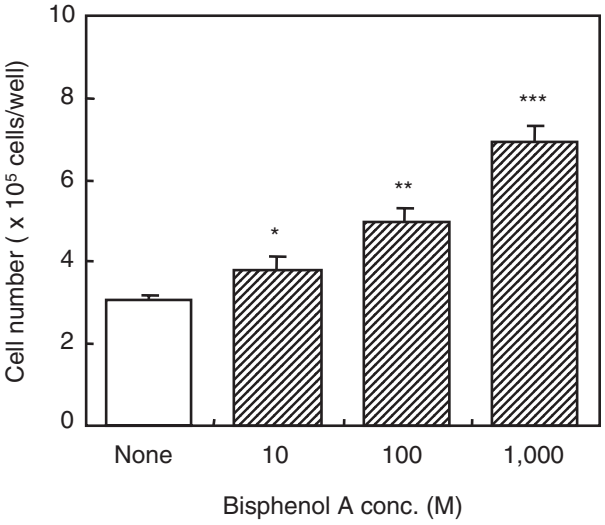

Fig. 1. Effect of Bisphenol A on the Proliferation of MCF-7 Cells.
MCF-7 cells were treated with BPA (10–1,000 nM) for 72 h, and then the cell number was counted. Data are represented as means ± SD (n = 3). Data with asterisk marks are significantly different from the values in the control group at $p < 0.05^*$, 0.01^{**} or 0.01^{***}.

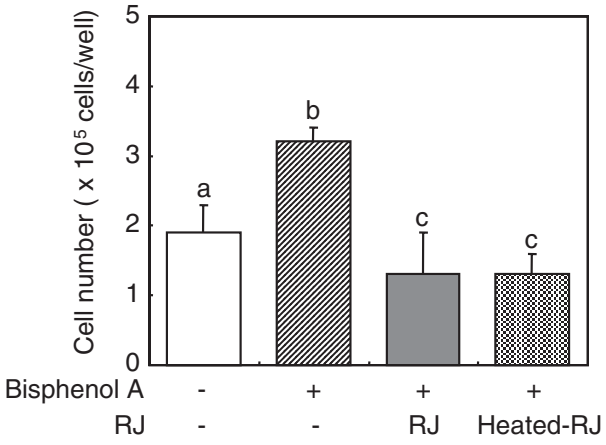

Fig. 3. Effect of Heat-Treated Royal Jelly on BPA-Induced Proliferation of MCF-7 Cells.
MCF-7 cells were treated with royal jelly or heat-treated royal jelly in the presence of BPA (1,000 nM) for 72 h, and then the cell number was counted. Data are means ± SD (n = 3). Letters that are different from each other denote significant difference at $p < 0.05$.

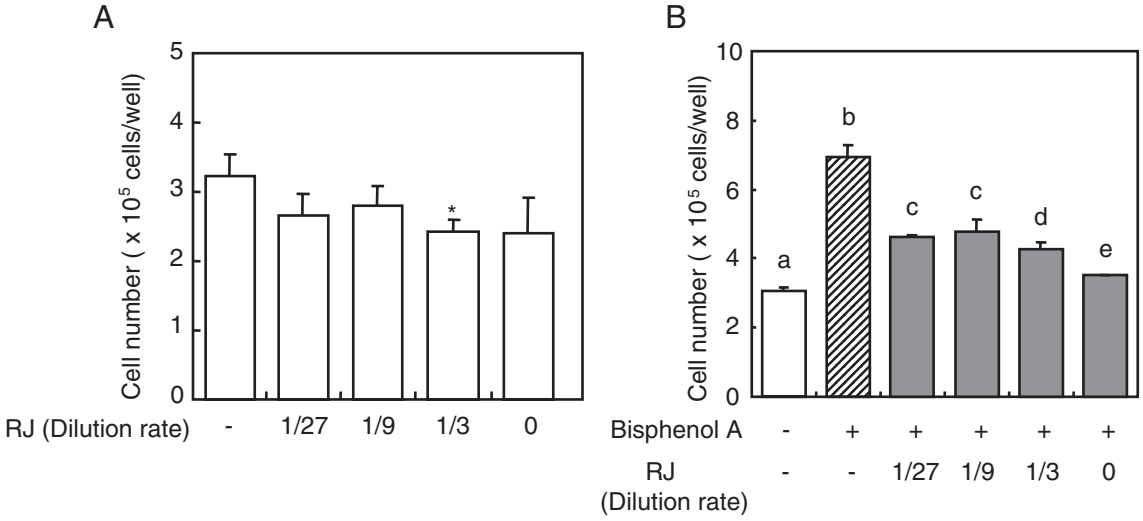

Fig. 2. Effect of Royal Jelly on the Proliferation of MCF-7 Cells.
A, MCF-7 cells were treated with royal jelly for 72 h, and then the cell number was counted. Data are represented as means ± SD (n = 3). Data with asterisk marks are significantly different from the values in the control group at $p < 0.05^*$. B, MCF-7 cells were treated with royal jelly in the presence of BPA (1,000 nM) for 72 h, and then the cell number was counted. Data are represented as means ± SD (n = 3). Letters that are different from each other denote significant difference at $p < 0.05$.

Our preliminary experiments indicated that pre-treatment with E2 for 1 h enhanced the proliferation of MCF-7 cells cultured in a medium without E2. Royal jelly also inhibited E2-induced proliferation of MCF-7 cells in coexistence with E2. Moreover, royal jelly inhibited the growth-promotion of cells pre-treated with E2 prior to assay. These findings suggest that royal jelly contributes to breaking E2-induced signaling in cell proliferation, not to suppressing E2 binding to ER (data not shown).

10-hydroxy-2-decenoic acid has been isolated as a royal jelly component possessing anti-tumor activity *in vitro*,[12–14] but 10-hydroxy-2-decenoic acid was ineffective at inhibiting BPA-induced proliferation of MCF-7 cells (data not shown). Identification of the active substance in royal jelly is now in progress. In addition, it is important to uncover its physiological properties. We are routinely exposed to BPA derived from plastic containers of foods and beverages, dental composites, and many products in houses and work places. Recent data indicate that BPA might be capable of altering important events during critical periods of brain development,[15] and that it causes DNA damage in MCF-7 cells.[16] However, anti-BPA substances in food have not been identified. The anti-environmental hormone activity found here is a novel function of royal jelly. This result may contribute to a decrease in the risk of BPA-induced breast cancer and improvements in health.

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