A methanol extract of banana peel (BPEx, 200 mg/kg, p.o.) significantly suppressed the regrowth of ventral prostates and seminal vesicles induced by testosterone in castrated mice. Further studies in the androgen-responsive LNCaP human prostate cancer cell line showed that BPEx inhibited dose-dependently testosterone-induced cell growth, while the inhibitory activities of BPEx did not appear against dehydrotestosterone-induced cell growth. These results indicate that methanol extract of banana peel can inhibit 5α-reductase and might be useful in the treatment of benign prostate hyperplasia.

Key words: suppressive effect on benign prostate hyperplasia; inhibitor of 5α-reductase; banana peel extract; anti-androgen effect; mice

Enlargement of the prostate, which affects 50% of men aged 60 and 90% of men by age 80, is referred to as benign prostate hyperplasia (BPH).1) BPH is a slow, progressive enlargement of the fibromuscular and epithelial structures of the prostate gland.2) Substantial evidence indicates that the androgens testosterone (T) and dihydrotestosterone (DHT) contribute to the production of BPH.3) The principal serum androgen T is converted by 5α-reductase (5αR) to DHT, which is about 5 times more potent than T itself. DHT binds to androgenic receptors (AR) in the prostate, where it indicates that BPEx inhibits growth of the prostate and that BPEx has anti-androgenic activity through inhibition of 5αR.

Materials and Methods

Extract preparation. Fresh peel (2.4 kg) of organically-cultivated bananas was cut and extracted with methanol at room temperature for 2 d. The extracts were filtered, concentrated under a vacuum, and freeze-dried. BPEx (56 g) was stored in a refrigerator before assay.

Chemicals. The chemicals used and their sources were as follows: testosterone and dihydrotestosterone from Wako Pure Chemical Industries (Osaka, Japan); flutamide from Sigma–Aldrich Japan (Tokyo); finasteride from LKT Labs. (St. Paul, MN); and pentobarbital from Dainippon Sumitomo Pharma (Osaka, Japan).

Animals. Std: ddY mice were obtained from SLC (Hamamatsu, Japan). They were maintained at 24 ± 1 °C at 50 ± 2% humidity under a 12 h light-dark cycle (lights on from 08:00 to 20:00). Animal studies were performed according to the 2006 guidelines entitled Notification No. 88 of the Ministry of the Environment of Japan.

Evaluation of growth-suppressive effects of mice prostates and seminal vesicles. Assay of growth suppression in castrated mouse prostates and seminal vesicles was performed based on the OECD protocol.5) The testes of ddY mice were removed at 7 weeks of age under anesthesia by intraperitoneal injection of pentobarbital (50 mg/ml/kg). After 1 d, testosterone propionate at (TP) 2 mg/kg or DHT 6 mg/kg was
injected intraperitoneally (i.p.) into the mice once daily for 10 d. Enlargement of the reproductive organs in the mice was demonstrated dose-dependently due to testosterone propionate in our preliminary studies. To evaluate the effects on reproductive organs, each of various doses of BPEx, finasteride (Fl, 2 mg/kg) and flutamide (FL, 10 mg/kg) was suspended in 1% ethanol and orally administered to the BPH model mice once daily. After 10 d, the mice were weighed, and sacrificed by cervical dislocation. The lengths of the short and long axes of the prostates were then measured with vernier calipers, and the seminal vesicles were removed and weighed. In particular, the weights of the seminal vesicles were sensitive to androgenic effects in our mouse model.

Cell culture and growth studies of human prostate cancer cells. The LNCaP human prostate cancer cell line is a well-established androgen-dependent cell line. AR-positive human prostate cancer LNCaP cells were obtained from the Riken BRC Cell Bank (Tsukuba, Japan). The cells were plated onto a 96-well plate at a density of $2 \times 10^5$/well and supplemented with 10% charcoal stripped fetal bovine serum (CSFBS) obtained from Invitrogen Japan (Tokyo). Twenty-four h later, the cells were treated with either vehicle control or androgens (T or DHT) in the presence and the absence of each concentration of BPEx for another 3 d. BPEx was dissolved in ethanol and added to the cells after further dilution so that the final volume of ethanol was 1% or less. After culture, cell proliferation was determined to measure cell viability by 3-[4,5-dimethyl thiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay. The cells were treated with 1 mg/ml of MTT for 2 h, and precipitated dye was dissolved into dimethylsulfoxide. The absorbance of each well was measured at 570 nm.

Statistical analysis. Data were analyzed statistically by Student’s $t$-test to determine significant differences in the data among the groups. The $p$ values less than 0.05 were considered significant. The values were expressed as mean $\pm$ S.E.

Results

Effects of BPEx on growth of mouse prostates and seminal vesicles

The effects of BPEx on the growth of mouse prostates and seminal vesicles were studied as compared with those of flutamide and finasteride in the BPH model mice for 10 d. As shown in Fig. 1, the short (A) and long (B) axes of the ventral prostates and the weights of the seminal vesicles (C) were severely reduced, and when TP (2 mg/kg i.p.) was injected, significant growth of prostates and seminal vesicles was induced. BPEx (200 mg/kg) produced a reduction in prostate weight. Finasteride (2 mg/kg) and flutamide (10 mg/kg), well-known anti-androgens, showed larger reductions in prostate weight. Similar results were observed with regard to seminal vesicle weights.

When DHT (6 mg/kg, i.p.) was injected in place of T, significant growth of the prostate and seminal vesicles was induced. BPEx did not inhibit these effects of DHT (Fig. 2).

Dose-dependency of effects of BPEx on mouse prostates and seminal vesicles

The inhibitory effects of BPEx on the growth of mouse prostates and seminal vesicles were estimated after 10 d of administration (Fig. 3). BPEx dose-dependently reduced the prostate size and the weights of seminal vesicles, and showed almost maximal effect at 200 mg/kg.

Inhibitory effects of BPEx on prostate cancer cells

The effects of BPEx on the proliferation of prostate cancer cells (LNCaP cells) were investigated. LNCaP cells show most of the characteristics of human prostatic carcinoma, such as dependence on androgens, the presence of ARs, and the production of acid phosphatase and prostate-specific antigen. The LNCaP cell line is used as an attractive model for in vitro studies of the biology of human prostate cancer. LNCaP cells were incubated with different concentrations of BPEx (3.125–100 μg/ml) with and without T or DHT for 3 d. In the absence of BPEx, T alone stimulated LNCaP cell number about 20.5 $\pm$ 0.5 $\times 10^5$/well (Fig. 4A), and DHT alone stimulated LNCaP cell numbers to about 30.4 $\pm$ 2.1 $\times 10^5$/well (Fig. 4B). Treatment of LNCaP cells with BPEx in the presence of T resulted in dose-dependent inhibition of cell growth. In the presence of T, both finasteride and flutamide inhibited cell proliferation. However, in the presence of DHT, while flutamide inhibited cell proliferation, finasteride did not.

Discussion

We studied the in vivo potency of BPEx in immature castrated mice. First, when we evaluated dose-depend-
The growth of these organs is androgen-dependent. BPEx inhibited TP-induced growth of prostates and seminal vesicles in castrated mice, although it was less potent than finasteride at the same doses. While BPEx inhibited the action of TP, it did not inhibit DHT-induced organ growth. Similar results were found with finasteride, while flutamide reduced the sizes of these organs. In addition, BPEx inhibited cell proliferation in the presence of T, but not in the presence of DHT. These results suggest that BPEx suppressed the growth of prostates and seminal vesicles by inhibiting the conversion of T to DHT, rather than by blocking the binding of androgen and its receptor. BPEx may have reduced prostate size and seminal vesicle weight by inhibiting 5αR.

In further study, we found that treatment of LNCaP cells with BPEx inhibited T-induced cell proliferation. The inhibition of this effect of T might have been due, at least in part, to inhibition of 5αR or to antagonism of androgen binding to the AR. Since 25 μg/ml of BPEx inhibited 5αR activity by about 30%, we expected to find that this dose would inhibit T action. Similar results have been found with finasteride, a well-known inhibitor of 5αR, at doses above 25 μg/ml. Similar results were also found with flutamide, an antagonist of androgen binding to the AR. On the other hand, treatment of LNCaP cells with BPEx in the presence of DHT did not result in a dose-dependent inhibition of cell growth. These results suggest that inhibition of cell growth in the presence of BPEx was not the result of a cell cytotoxic effect, but rather was due to an anti-androgen effect, such as inhibition of 5αR.

According to a previous report describing discrimination between cytotoxic and cytostatic effects of integrants on LNCaP cells, cytotoxic effects are detectable as a decrease in MTT conversion to a level below that of the starting cells. In our experiment, growth of androgen-induced LNCaP cells was suppressed without decreasing from the starting level in the presence of BPEx during the experimental period. Therefore, a cytostatic effect was estimated for suppression of BPEx on LNCaP cell growth. However, since no index markers related to apoptotic signaling were measured in our experiments, the possibility of a non-cytostatic effect should be considered and confirmed in further detailed investigation.

There are generally two ways to suppress prostate regrowth in animal experiments: by inhibiting 5αR activity, and through the use of an androgen receptor antagonist. An androgen antagonist can suppress DHT-induced prostate and seminal vesicle regrowth. Therefore, blocking DHT from binding to androgen receptors in the prostate and seminal vesicle is considered to be a possible mechanism of action (other than 5αR inhibition). To examine this possibility, the effects of BPEx on prostate growth induced by DHT were investigated. If the suppression of prostate growth is caused only by inhibition of 5αR, DHT-induced prostatic regrowth should not be suppressed. Ten days after castration, the weights of mouse prostates were markedly reduced, and prostate size was restored by i.p.
injections of TP or DHT. BPEx had no effect on the sizes of the prostate and seminal vesicle of castrated mice that received DHT, whereas flutamide, an androgen receptor antagonist, significantly reduced prostate weight (Fig. 3). In our study, blood levels of testosterone and samples were not measured, but the anti-androgenic activity of BPEx is of scientific interest. Recently, there are research reports on components of the banana peel: dietary fiber and pectin, and phytosterol from unripe banana.

For several years, SPEx has been used as a popular phytotherapeutic agent for the treatment of BPH. SPEx cells were incubated with various concentrations of BPEx (shaded column), finasteride (an inhibitor of 5αR, slanted column) or flutamide (an inhibitor of androgen receptor, closed column) in the presence of testosterone (T, 10 mg/ml) (A) and of dihydrotestosterone (DHT, 10 mg/ml) (B) for 24 h. Then cell proliferation was determined to measure cell viability by the 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay as described in "Materials and Methods." Each value represents the mean ± S.E., n = 3. *, p < 0.05; **, p < 0.01; ***, p < 0.005 vs. control (T- or DHT-treated without samples).

**Fig. 4.** Effects of BPEx on LNCaP Cell Growth in the Presence of Testosterone and of Dihydrotestosterone. LNCaP cells were incubated with various concentrations of BPEx (shaded column), finasteride (an inhibitor of 5αR, slanted column) or flutamide (an inhibitor of androgen receptor, closed column) in the presence of testosterone (T, 10 mg/ml) (A) and of dihydrotestosterone (DHT, 10 mg/ml) (B) for 24 h. Then cell proliferation was determined to measure cell viability by the 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay as described in "Materials and Methods." Each value represents the mean ± S.E., n = 3. *, p < 0.05; **, p < 0.01; ***, p < 0.005 vs. control (T- or DHT-treated without samples).

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**References**