NUTRITION AND CANCER, *42*(2), 206–210

Regulation of Male Sex Hormone Levels by Soy Isoflavones in Rats

Mi-Ae Yi, Hyun M. Son, Jung-Soon Lee, Chong-Suk Kwon, Jin Kyu Lim, Young Keun Yeo, Young Sik Park, and Jong-Sang Kim

Abstract: *Several studies have suggested that soybean intake is associated with a lower risk of prostate cancer. However, the mechanism of prostate cancer prevention by soybeans remains unclear. Because prostate cancer is reported to have an association with an increased level of dihydrotestosterone (DHT) and soybean isoflavones are known to inhibit 5α-reductase, which is involved in the conversion of testosterone to DHT, the effects of soybean extract and isoflavones on the plasma levels of male sex hormones were investigated using male rats. In Experiment I, Sprague-Dawley rats were fed diets with and without soy flour; in Experiment II, rats were fed diets containing 2% soy methanol extract or 0.2% semipurified isoflavones or a control diet. The study showed a reduction of plasma DHT along with an increase in total plasma androgen in rats fed soy flour or semipurified isoflavones for 1 wk. These results suggest that soy isoflavone intake may reduce plasma DHT level.*

Introduction

Epidemiological studies show a lower incidence of hormone-related cancer in Asians, including Koreans, Japanese, and Chinese, than in their Western counterparts (1,2). Most Asians eat a low-fat, high-fiber diet that is a rich source of phytoestrogens, and there is an extremely low incidence of prostate cancer. The soy-based foods included in this diet may play a role in decreasing the prevalence of prostate cancer in Asian communities (3,4). The isoflavone levels are much higher in the prostatic fluid from Hong Kong and Chinese men who consume soybeans than in Portuguese and British men (3). In animal models, most studies investigating the effects of soybeans show a reduced tumorigenesis (5). For instance, a soy-containing diet was reported to reduce the severity of prostatitis in rats (6) and prevent the development of dysplastic lesions of the prostate in neonatal diethylstilbestrol-treated mice (7). Recently, soy isoflavones have been shown to inhibit the growth of transplantable human prostate carcinoma and tumor angiogenesis in mice, thereby suggesting multiple interacting mechanisms in prostate cancer inhibition (8). In its early stages, prostate cancer is hormone dependent, with castration causing tumor regression (9). Meanwhile, isoflavones of soy origin may play a major role in the promotion and growth of prostate cancer by modulating the metabolism of sex hormones. Genistein and daidzein, the main isoflavones in soybeans, are known to inhibit 5α-reductase in vitro (9). This enzyme is involved in the conversion of testosterone to 5α-dihydrotestosterone (DHT), the main prostatic androgen responsible for the development of prostate cancer (1,10). Therefore, there is a strong possibility that soy isoflavones generate a significant effect on the DHT level, inasmuch as they are known to be strong inhibitors of 5α-reductase.

This communication reports that soy flour and isoflavones tended to lower the level of plasma DHT with a limited effect on the total androgen level in rats. In addition, a significant amount of isoflavones was retained in the plasma from rats fed an isoflavone-supplemented diet.

Materials and Methods

Animals and Diets

Male Sprague-Dawley rats (Korean Experimental Animal Center, Umsung, South Korea; 200–300 g body wt) were housed individually in stainless steel cages and divided into two and three experimental groups for two rounds of experiments with 8 or 10 rats per group. The animals were maintained at 22 ± 2°C on a 12:12-h light-dark cycle (lights on at 0700) with free access to food and water. The diet compositions used in Experiment I are presented in Table 1. Autoclaved soy flour was blended into the powdered diet at 442.7 g/kg diet. In Experiment II, the rats in the control group were fed ad libitum an AIN-76 diet containing 200 g of casein, 3 g of DL-methionine, 150 g of corn starch, 500 g of sucrose, 50 g of cellulose, 50 g of corn oil, 35 g of mineral mixture, 10 g of vitamin mixture, and 2 g of choline bitartrate per kilogram diet. The treatment groups were fed an AIN-76 diet in which the cornstarch was partially re-

J.-S. Lee, J. K. Lim, Y. K. Yeo, Y. S. Park, and J.-S. Kim are affiliated with the Department of Animal Science and Biotechnology, Kyungpook National University, Taegu 702-701, South Korea. M.-A. Yi is and H. M. Son was affiliated with the School of Food Science, Inje University, Kimhae 621-749, South Korea. C.-S. Kwon is affiliated with the Department of Food Science and Nutrition, Andong National University, Andong 760-380, South Korea.

Table 1. Composition of Diet Used in Experiment I

Ingredients[a]	Basal	Treatment[b]
Casein	200	0
DL-Methionine	3	5
Cornstarch	116.7	5.3
Sucrose	500	500
Cellulose	48.2	0
Corn oil	81.9	0
Mineral mix[c]	35	35
Vitamin mix[d]	10	10
Choline bitartrate	2	2
Soy flour[e]	0	442.7

a: Ingredients were obtained from ICN Biochemicals (Cleveland, OH).
b: Diet was normalized to the same approximate composition as basal diet after chemical analysis of soy flour. Approximate composition of soy flour was as follows (g/100 g): 6.5 water, 37.5 crude protein, 18.4 crude fat, 5.0 ash, 12.6 total digestible fiber, 20 sugars.
c: AIN vitamin mix provided (mg/kg diet) 19.8 retinyl acetate, 1.38 ergocalciferol, 110 DL-α-tocopheryl acetate, 495 ascorbic acid, 55 inositol, 2,227 choline, 24.7 menadione, 55 ρ-aminobenzoic acid, 46.7 niacin, 11 riboflavin, 11 pyridoxine HCl, 11 thiamin HCl, 33 D-calcium pantothenate, 0.2 biotin, 0.99 pteroylmonoglutamic acid, 0.015 cyanocobalamin.
d: AIN mineral mix provided (mg/kg) 15 calcium phosphate dibasic, 2.2 sodium chloride, 6.6 potassium citrate monohydrate, 1.56 potassium sulfate, 0.7 magnesium oxide, 0.105 manganous carbonate, 0.18 ferric citrate, 0.048 zinc carbonate, 0.009 cupric carbonate, 0.0003 potassium iodate, 0.0165 chromium potassium sulfate.
e: Total isoflavone content of soy flour was 1.92 mg/g.

placed with 20 g of soy methanol extract (isoflavone content 3.38 mg/g) or 2 g of semipurified isoflavone (Pulmuwon, Seoul, South Korea; isoflavone content 218 mg/g) per kilogram diet.

Preparation of Isoflavone-Rich Extract From Soy Flour

The soy flour was defatted twice for 12 h each using 1,000 ml of hexane and refluxed twice for 5 h each using 1,000 ml of 80% (vol/vol) methanol. The extract was centrifuged for 20 min at 1,000 g, and then the supernatant was removed, concentrated in a rotary evaporator (Eyela) at 45°C until the volume was reduced to approximately one-fifth of that of the original extract, and freeze-dried (Ilsin, Seoul, South Korea). The total concentrations of genistein and daidzein were measured as free isoflavones after hydrolysis in 1 M HCl (10 ml/g). The isoflavone extract was analyzed by high-performance liquid chromatography (HPLC) as described previously (11).

Experimental Design

The rats were randomly assigned to one of two or three treatment groups and fed the experimental diets for 1 wk. In Experiment I, the rats were fed a basal diet or a treatment diet containing soy flour (Table 1). In Experiment II, the rats were divided into three groups and fed a control diet (AIN-76) or a diet containing soy extract (20 g/kg diet) or soy isoflavone (2 g/kg diet). After 1 wk, the rats ($n = 10$ or 8 rats/group for Experiments I and II, respectively) were killed by CO_2 asphyxiation, and their blood was collected via cardiac puncture. The plasma was separated from the blood by centrifugation and stored at −70°C until further analysis for the presence of hormones and isoflavones.

Sample Analysis

Testosterone and DHT were extracted from the plasma twice using 3 vol of diethyl ether. The ether extracts were then evaporated to dryness under a gentle stream of nitrogen and redissolved in a 1.5-ml assay buffer. Testosterone and DHT concentrations were determined using a radioimmunoassay kit (BIOTRAK TRK600, Amersham International, Buckinghamshire, UK) as described in the supplier's manual. All assays were performed in duplicate. The radioactivity was determined using a liquid scintillation counter (model LS 7500, Beckman, Fullerton, CA).

The isoflavone content in the rat plasma was determined as described previously (12,13). Briefly, 1 ml of plasma was incubated at 37°C overnight in 0.17 M ammonium acetate, pH 5.0, containing 2×10^3 U β-glucuronidase (Sigma, St. Louis, MO) to hydrolyze the conjugates. Samples were extracted twice with 2 ml of diethyl ether, and the pooled extracts were dried at room temperature with a stream of nitrogen before reconstitution in 200 µl of HPLC mobile phase. The HPLC analysis was conducted using a Hewlett-Packard (Palo Alto, CA) model HP1100LC/MSD with a 25 × 0.46 cm ID C_{18} reverse-phase column (Waters, Milford, MA) using a mobile phase consisting of 60:40 (vol/vol) methanol-1 mM ammonium acetate at a flow rate of 1 ml/min. The injection volume was 20 µl. The isoflavones in the eluate were detected on the basis of their absorbance at 254 nm and then identified using a mass spectrometer.

Statistical analyses, including one-way analysis of variance and Duncan's multiple range test, were conducted using the Statistical Package for the Social Sciences (SPSS, Chicago, IL). The statistical tests were considered to be significant at $P < 0.05$.

Results

The daily food consumption and body weights of rats were not significantly different among the experimental diet groups in Experiment I. During the 1-wk experimental period, the mean food intakes for the control and treatment groups were 23.9 ± 1.9 and 22.4 ± 1.3 g/day, respectively, and the mean body weight gains were 8.9 ± 1.3 and 9.1 ± 1.6 g/day, respectively. Inasmuch as the soy flour diet contains 67.2 mg isoflavones/kg, rats in the soy flour group were estimated to have consumed 19.0 mg isoflavones/day.

In Experiment II, which included three treatment groups, the rats fed the isoflavone-supplemented diet (isoflavone group) showed a lower dietary intake and body weight gain than those fed the control and soy extract diets, possibly be-

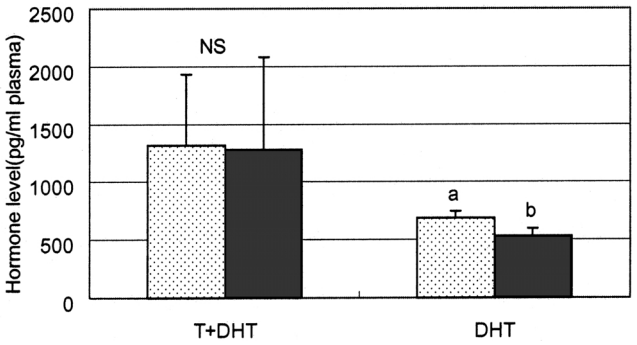

Figure 1. Decreased serum testosterone (T) and dihydrotestosterone (DHT) levels in male rats fed soy flour diet. Values are means for 10 animals; error bars indicate SD. Bars with different superscripts (a, b) are significantly different from each other ($P \leq 0.05$). NS, not statistically significant ($P > 0.05$).

cause of the bitter taste of the isoflavones. During the experimental period, the mean dietary intakes for the control, soy extract, and isoflavone diet groups were 17.1 ± 1.4, 16.2 ± 2.0, and 13.9 ± 1.3 g/day, respectively. The isoflavone intake level of each group was estimated to be 0.9 and 3.3 mg/day for the soy extract and isoflavone groups, respectively. Meanwhile, the mean body weight gains were 3.6 ± 0.7, 3.6 ± 0.5, and 1.5 ± 0.7 g/day, respectively. Again the isoflavone group showed less weight gain than the control or the soy extract group.

The total plasma androgen and DHT levels are presented in Fig. 1. The level of testosterone plus DHT in the plasma did not differ significantly between the control and soy flour groups; however, the plasma DHT level was significantly reduced in rats fed the soy flour diet.

On the basis of the results in Figs. 2 and 3, it would appear that the isoflavone-supplemented diet significantly increased the total plasma androgen levels and yet significantly lowered the DHT level ($P \leq 0.05$), on average, 60% less than the control diet. The total plasma androgen level in the rats fed the soy extract diet for 1 wk was not significantly different from that in the control group, yet it did tend to increase. In contrast, the plasma DHT level in the rats fed the soy extract diet was not significantly different from that in the control group.

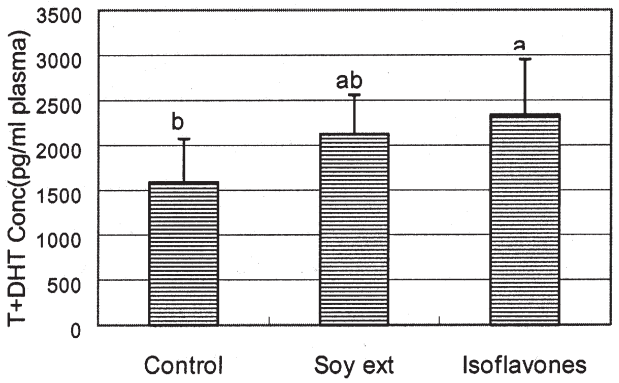

Figure 2. Regulation of plasma testosterone level by soy extract- or isoflavone-supplemented diet in male rats. Values are means for 8 animals; error bars indicate SD. Bars with different superscripts (a, b) are significantly different from each other ($P \leq 0.05$).

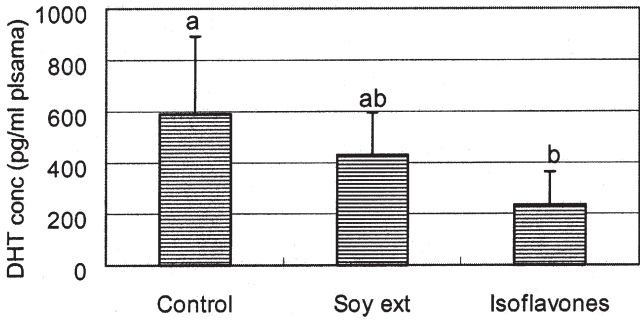

Figure 3. Regulation of plasma DHT level by soy extract- or isoflavone-supplemented diet in male rats ($n = 8$). Values are means for 8 animals; error bars indicate SD. Bars with different superscripts (a, b) are significantly different from each other ($P \leq 0.05$).

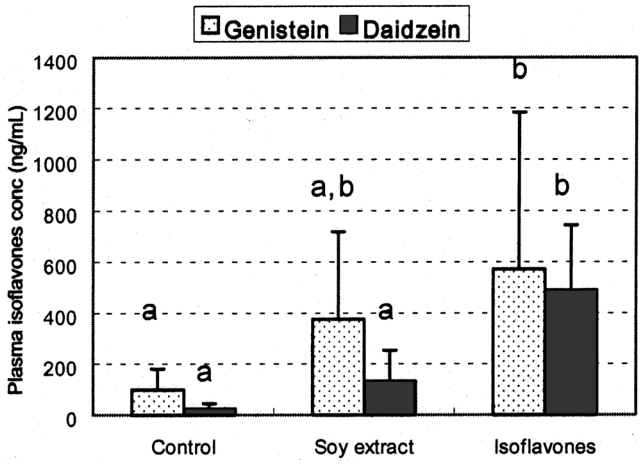

Figure 4. Plasma isoflavone concentrations in rats ($n = 8$) fed control and soy extract- and isoflavone-supplemented diets. Values are means for 8 animals; error bars indicate SD. Bars with different superscripts (a, b) are significantly different from each other ($P \leq 0.05$).

The plasma isoflavone concentrations in the rats fed a soy extract- or isoflavone-supplemented diet are shown in Fig. 4. Rats in the isoflavone diet group ($n = 8$) exhibited a significantly high level of isoflavones in plasma, whereas rats in the soy extract group ($n = 8$) did not retain significantly high isoflavones in plasma compared with the control group, which is consistent with data for plasma sex hormone levels.

Discussion

Although the exact mechanism of prostate cancer remains unknown, most accounts of the subject stress the importance of hormonal factors as well as genetic predisposition in pathogenesis (13). Among the various hormonal factors, DHT would appear to play a major role in the development of prostate cancer (10,14). 5α-Reductase is the key enzyme involved in the conversion of testosterone to DHT; accordingly, the inhibition of this enzyme along with a re-

duction of 5α-reductase may lower the plasma DHT level and thereby inhibit the development of prostate cancer (1).

The major objective of this study was to test the hypothesis that soy isoflavones can reduce the plasma DHT concentration in male rats. This study revealed that dietary soy flour and isoflavones significantly reduced the plasma DHT level, and dietary isoflavone-rich soy extract also tended to lower the DHT level in rat plasma, although this was not statistically significant. A diet supplemented with 0.2% semipurified isoflavone, but not 2% soy methanol extract, caused a significant increase in total plasma androgen level. This might be one of the homeostatic mechanisms to compensate for the lowered DHT level. Pollard and others (15) observed a significant reduction in serum testosterone level in Lobund-Wistar rats fed soy protein isolate plus isoflavones. These findings imply that isoflavones modulate the male sex hormone level in plasma. Therefore, the potential of isoflavones to prevent the development of prostate cancer might be associated with the regulatory activity of the compounds on the plasma androgen level. Furthermore, the concentration of isoflavones in the diet should be critical in affecting plasma sex hormone level, since the plasma androgen level was changed by the diet with the higher isoflavone content (0.24 and 0.85 mg isoflavones/g) but not by the soy extract diet with the relatively low content (0.07 mg isoflavones/g diet), which is consistent with the data of plasma isoflavone levels in each group (Fig. 4). It appears that sex hormone levels in the plasma are affected by plasma isoflavone concentration.

DHT is the main prostatic androgen, and the prostate cannot grow or function in its absence (9,16). It is interesting that lower levels of 3-α, 17-β-androstanediol glucuronide and androsterone glucuronide, which are recognized as indexes of 5α-reductase activity, are reported in young Japanese men than in young adult white and black men (17). It is possible that life-long dietary isoflavonoids have a significant influence on the development of hormone-dependent tumors via the regulation of specific sex hormone levels in plasma. Urinary excretion of soy isoflavones is also higher in young Japanese men (18) than in their Western counterparts, and plasma levels of soy isoflavones are higher. In addition, the prostate size does not increase with age as dramatically in Japanese men as in men from Western countries (19).

Isoflavones, including genistein and daidzein, have previously been shown to inhibits α-reductase and 17β-hydroxy steroid dehydrogenase activities in human tissue (8). The precursors of these compounds are present in relatively large amounts in soy and its products such as tofu and soy milk. These are metabolized into aglycones by gut bacterial enzymes and then absorbed to exert physiological activity in the human body.

Soy has also been found to protect against prostatic dysplasia in an estrogenized animal model. In particular, genistein, a soy isoflavone, was found to inhibit the growth of prostate cancer tissue from recently removed surgical specimens and tested in vitro (20). It would appear that genistein exerts antiproliferative activity by regulating the growth factors and receptors of tyrosine kinase (21–23) and inhibiting topoisomerase II by promotion, angiogenesis, or some other mechanism (24), such as affecting the cell growth or inducing apoptosis (25,26). These mechanisms can explain the significantly lower incidence of sex hormone-dependent as well as -independent cancers in the population with a high consumption of soy products. It has been reported that the development and growth of prostate cancer depend on the androgen receptor and its high-affinity binding of DHT (10). Accordingly, lowering of plasma DHT by dietary soy isoflavones may be a mechanism for preventing the development of prostate cancer development. Hormonal and nonhormonal mechanisms might operate to prevent prostate cancer with soy isoflavones. Clinical studies are required to confirm whether a similar trend occurs in human subjects.

Acknowledgments and Notes

This work was supported by Korean Science and Engineering Foundation Grant KOSEF 97-04-02-06-01-3. Present address of H. M. Son: Cellontech, South Korea. Address correspondence to J.-S. Kim, Dept. of Animal Science and Biotechnology, Kyungpook National University, Taegu, 702-701, South Korea. E-mail: vision@knu.ac.kr.

Submitted 11 July 2001; accepted in final form 6 December 2001.

References

1. Moyad MA: Soy, disease prevention, and prostate cancer. *Semin Urol Oncol* **17,** 97–102, 1999.
2. Lee C, Lee ES, Choi H, Koh SK, Lee JM, et al.: Incidence estimation of genitourinary cancer in Korea. *J Kor Med Sci* **7,** 154–161, 1992.
3. Morton MS, Chan PS, Cheng C, Blacklock N, Matos-Ferreira A, et al.: Lignans and isoflavonoids in plasma and prostatic fluid in men: samples from Portugal, Hong Kong, and the United Kingdom. *Prostate* **32,** 122–128, 1997.
4. Wakai K, Egami I, Kato K, Kawamura T, Tamakoshi A, et al.: Dietary intake and sources of isoflavones among Japanese. *Nutr Cancer* **33,** 139–145, 1999.
5. Bingham SA, Atkinson C, Liggins J, Bluck L, and Coward A: Phytoestrogens: where are we now? *Br J Nutr* **79,** 393–406, 1998.
6. Sharma OP, Adlercreutz H, Strandberg JD, Zirkin BR, Coffey DS, et al.: Soy of dietary source plays a preventive role against the pathogenesis of prostatitis in rats. *J Steroid Biochem Mol Biol* **43,** 557–564, 1992.
7. Pylkkanen L, Makela S, and Santti R: Animal models for the preneoplastic lesions of the prostate. *Eur Urol* **30,** 243–248, 1996.
8. Zhou JR, Gugger ET, Tanaka T, Guo Y, Blackburn GL, et al.: Soybean phytochemicals inhibit the growth of transplantable human prostate carcinoma and tumor angiogenesis in mice. *J Nutr* **129,** 1628–1635, 1999.
9. Evans BA, Griffiths K, and Morton MS: Inhibition of 5α-reductase in genital skin fibroblasts and tissue by dietary lignans and isoflavonoids. *J Endocrinol* **147,** 295–302, 1995.
10. Gregory CW, He B, Johnson RT, Ford OH, Mohler JL, et al.: A mechanism for androgen receptor-mediated prostate cancer recurrence after androgen deprivation therapy. *Cancer Res* **61,** 4315–4319, 2001.
11. Wang G, Kuan SS, Fransis OJ, Ware GM, and Carman: A simplified HPLC method for the determination of phytoestrogens in soybean and its processed products. *J Agric Food Chem* **38,** 185–190, 1990.
12. King RA, Broadbent JL, and Head RJ: Absorption and excretion of the soy isoflavone genistein in rats. *J Nutr* **126,** 176–182, 1996.
13. Record IR, Jannes M, Dreosti IE, and King RA: Induction of micronucleus formation in mouse splenocytes by the soy isoflavone genistein in vitro but not in vivo. *Food Chem Toxicol* **33,** 919–922, 1995.

14. Pollard M: Prevention of prostate-related cancers in Lobund-Wistar rats. *Prostate* **39,** 305–309, 1999.
15. Pollard M, Wolter W, and Sun L: Prevention of induced prostate-related cancer by soy protein isolate/isoflavone-supplemented diet in Lobund-Wistar rats. *In Vivo* **14,** 389–392, 2000.
16. Griffiths K, Eaton CL, Harper ME, Peeling B, and Davies P: Steroid hormones and the pathogenesis of benign prostatic hyperplasia. *Eur Urol* **20** Suppl 1, 68–77, 1991.
17. Ross RK, Bernstein L, Lobo RA, Shimizu H, Stanczyk FZ, et al.: 5α-Reductase activity and risk of prostate cancer among Japanese and US white and black males. *Lancet* **339,** 887–889, 1992.
18. Adlercreutz H, Honjo H, Higashi A, Fotsis T, Hamalainen E, et al.: Urinary excretion of lignans and isoflavonoid phytoestrogens in Japanese men and women consuming a traditional Japanese diet. *Am J Clin Nutr* **54,** 1093–1100, 1991.
19. Oesterling JE, Kumamoto Y, Tsukamoto T, Girman CJ, Guess HA, et al.: Serum prostate-specific antigen in a community-based population of healthy Japanese men: lower values than for similarly aged white men. *Br J Urol* **75,** 347–353, 1995.
20. Geller J, Sionit L, Partido C, Li L, Tan X, et al.: Genistein inhibits the growth of human-patient BPH and prostate cancer in histoculture. *Prostate* **34,** 75–79, 1998.
21. Linassier C, Pierre M, Le Pecq JB, and Pierre J: Mechanisms of action in NIH-3T3 cells of genistein, an inhibitor of EGF receptor tyrosine kinase activity. *Biochem Pharmacol* **39,** 187–193, 1990.
22. Nishimura J, Huang JS, and Deuel TF: Platelet-derived growth factor stimulates tyrosine-specific protein kinase activity in Swiss mouse 3T3 cell membranes. *Proc Natl Acad Sci USA* **79,** 4303–4307, 1982.
23. Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, et al.: Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J Biol Chem* **262,** 5592–5595, 1987.
24. Bergan R, Kyle E, Nguyen P, Trepel J, Ingui C, et al.: Genistein-stimulated adherence of prostate cancer cells is associated with the binding of focal adhesion kinase to β_1-integrin. *Clin Exp Metastasis* **14,** 389–398, 1996.
25. Jewell K, Kapron-Bras C, Jeevaratnam P, and Dedhar S: Stimulation of tyrosine phosphorylation of distinct proteins in response to antibody-mediated ligation and clustering of α_3- and α_6-integrins. *J Cell Sci* **108** Pt 3, 1165–1174, 1995.
26. Kyle E, Neckers L, Takimoto C, Curt G, and Bergan R: Genistein-induced apoptosis of prostate cancer cells is preceded by a specific decrease in focal adhesion kinase activity. *Mol Pharmacol* **51,** 193–200, 1997.