Chemoprevention of MNU and Testosterone induced prostate carcinogenesis by Calcitriol (vitamin D₃) in adult male albino Wistar rats

Senthilkumar K., Arunkumar A., Sridevi N., Vijayababu M. R.,
Kanagaraj P., Venkataraman P., Aruldas M. M., Srinivasan N. and Arunakaran J.

Department of Endocrinology, Dr.ALM PG Institute of Basic Medical Sciences,
University of Madras, Taramani, Chennai-600 113. INDIA

Abstract

Background Calcitriol is a steroid hormone, inhibits the proliferation and promotes the differentiation of human prostate cancer cells. Calcitriol markedly inhibits the invasiveness of human prostate cancer cells *in vitro*. These properties support the use of calcitriol as differentiation therapy in prostate cancer. Chemopreventive role of calcitriol on prostate cancer remains unknown. Prostatic intraepithelial neoplasia (PIN) is the most common precancerous state and represents the major target for chemoprevention of prostate cancer.

Methods Prostate cancer was induced in Wistar rats using MNU+T (N-Methyl nitroso urea + Testosterone). Calcitriol (0.5 µg/kg body weight) was administered weekly thrice as *i.p.* injection simultaneously to MNU+T treated rats. The control group received vehicle alone. After 16 weeks of experimental period ventral and dorsolateral lobes were removed for histopathological evaluation and serum prostatic acid phosphatase (PAP) was determined.

Results MNU+T treated rats showed hyperplasia, dysplasia and PIN (70%, 60% and 30%) changes in dorsolateral prostate and ventral prostate 60% 50% 30%, respectively. Where as MNU+T along with calcitriol treated rats, the incidence of hyperplasia, dysplasia and PIN in the ventral was 10% each and in dorsolateral it was 20%, 10% and 10%, respectively. Hyperplasia, dysplasia and PIN were less common in these rats. Serum PAP significantly increases in MNU+T treated rats, whereas decreased in the calcitriol treated rats. The results of this study suggests that calcitriol may have chemopreventive activity in rat prostate carcinogenesis.

Interpretation During the treatment with calcitriol on MNU+T induced prostate carcinogenesis, calcitriol might be capable of inhibiting the initiation of prostate cancer. Hence, calcitriol may have useful for the prevention of prostate cancer.

Key Words: Calcitriol, Chemoprevention, Methyl nitroso urea, Prostate cancer, Prostatic intraepithelial neoplasia

(Received February 20, 2006; Accepted April 20, 2006)

Introduction

Prostate cancer is the second most common cancer among men World wide. In the year 2003, there were 220,900 new cases with prostate cancer diagnosed among American men¹. In India, prostate cancer ranks in its fifth incidence and 4th in mortality rate². The mechanisms leading to the initiation and progression of prostate cancer are largely unknown. One of the reasons that the progress of the work has been slow which is due to the lack of suitable animal models. Although there are a number of animal carcinogenesis models, they are based on single sex hormone, testosterone, or a combination of testosterone and estrogen^{3,4}. The combination of carcinogen (N-methyl-N-nitrosourea) and testosterone

one has the advantage of inducing higher incidence of prostate carcinogenesis in Wistar-Unilever rat⁵. Our laboratory report also suggested that MNU model was used in higher incidence of prostate cancer induction in Sprague-Dawley rat⁶. This particular model has several advantages including the development of hyperplasia to dysplasia and prostatic intra epithelial neoplasia in short term treatments (4 months) in Wistar rats.

Prostate intraepithelial neoplasia is the precursor for prostate cancer⁷. Histopathologically, PIN is divided into two grades: low grade PIN (LGPIN) and high grade PIN (HGPIN). HGPIN is characterized by cell proliferation within presenting ducts and alveoli with cytologic changes mimicking cancer, including hyper chromatic nuclei and mono nucleoli, but HGPIN retains an intact or fragmented basal layer^{8,9}. Prostate cancer mortality increases with decreased exposure to sunlight^{10,11} and vitamin D metabolites are also decreased with increasing age in circulation¹². Presently there are no effective

Correspondence to: Dr. J. Arunakaran, Department of Endocrinology, Dr.ALM PG Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai-600 113. INDIA.

treatments are available for prostate cancer. Calcitriol ($1,\alpha$ -25 dihydroxy vitamin D_3), is the active form of vitamin D metabolite, which is involved in the classical role in bone metabolism and causes growth inhibition and differentiation in a variety of cell types including breast and prostate normal and cancer cells^{13,15}. Vitamin D and vitamin D analogs are significant potency to inhibit prostate cancer cells and also involved in cell cycle arrest and regulation of growth factor signalling¹⁶. However, there is no report available on the role of calcitriol on prostate cancer initiation in *in vivo* model. Therefore, the chemoprevention study was conducted to evaluate the effect of calcitriol in prostate carcinogenesis in albino Wistar rats.

Materials and Methods

Chemicals

Cyproterone acetate and Testosterone propionate were purchased from Sigma Chemical Co., USA. Calcitriol was purchased from ICN Chemicals, USA. Other chemicals were obtained from Sisco Research Laboratories (SRL), India. All the chemicals used were extra pure and of analytical grade.

Animals

Healthy adult male albino rats of Wistar strain *Rattus norvegicus* weighing 180 - 200g were used in the present study. The animals were housed in clean polypropylene cages and maintained in an air-conditioned animal house with constant 12 hours light and 12 hours dark schedule. At all times during the studies, rats were permitted free access to drinking water. The animals were fed with standard rat pellet diet (Lipton India Ltd., Mumbai, India). Experiment was approved by the Institute animal ethical committee (IAEC-No 03/014/04).

Induction of prostatic carcinogenesis using carcinogen and hormone

Carcinogen and hormone exposure followed protocols published previously⁵. First, each rat received daily gavage administration of cyproterone acetate (50 mg/kg body weight) (Sigma Chemicals, USA) for 21 consecutive days. One day after the last dose of cyproterone acetate, rats received daily *i.p.* injection of 100 mg testosterone propionate/kg body weight (Sigma Chemicals, USA) in 0.3 ml propylene glycol for 3 days. One day after the testosterone propionate injection, all the rats received a single *i.v.* dose (50 mg/kg body weight) of Methyl Nitroso Urea (MNU) (dissolved in saline at 50 mg/ml), through the tail vein. One week after MNU administration, rats received daily *i.p.* injection of 2 mg/kg body weight testosterone propionate/kg body weight for 90 days.

Experimental design

A total of 40 rats were divided into four groups. Each group consists of 10 animals.

Group I

Rats received vehicle (propylene glycol) alone by intra peritoneal (*i.p.*) injection as control.

Group II

Rats received calcitriol 0.5 μ g/kg body weight as intra peritoneal (*i.p.*) injection as drug control.

Group III

Rats were induced prostate cancer by using carcinogen and hormone.

Group IV

Rats were induced prostate cancer and simultaneously treated with calcitriol 0.5 μ g/kg body weight given weekly thrice as *i.p.* (intra peritoneal) injection started one week before administration of cyproterone acetate and throughout the studies. The dose were selected based upon the previous studies by Carlos *et al.*¹⁷. We have taken at low dose level of 0.5 μ g/kg body weight calcitriol for the present investigation.

Blood and tissue collection

After the treatment period rats were killed, blood sample were collected and prostate was removed from the adhering connective tissue, washed several times with physiological saline, weighed accurately, separated the ventral and dorsolateral lobes and were fixed with 10% neutral buffered formalin. After fixation, prostatic lobes were embedded in paraffin stained with H&E for histopathological evaluation and examined histologically for tumor types, using histological criteria⁴.

Serum prostatic acid phosphatase

The serum PAcP activity was measured by Tenniswood *et al.*¹⁸. The substrate reaction buffer (0.5 ml of 0.1 M sodium citrate (pH 4.85) + 0.5 ml of 0.4% para nitrophenyl phosphate substrate) was added to 0.02 ml of serum. The reaction was arrested by the addition of 3.8 ml of 0.1 N NaOH. The amount of *p*-nitrophenol released was measured spectrophotometrically at 410 nm.

Statistical analyses

The data obtained were evaluated by One way Analysis of Variance. $P < 0.05$ was accepted as the level of significance.

Results

Body weight and prostatic weight

Fig.1a represents the effect of calcitriol on body

Fig. 1a Average body weight of rats treated with Methyl Nitroso Urea (MNU) + testosterone (T), Calcitriol and vehicle. The number of animals in each group was 10. Group I: Control, II: Calcitriol control, III: MNU+T treated, IV: MNU+T plus calcitriol treatment. There was no significant difference in body weights among the calcitriol treated rats. The MNU+T rats showed significant decrease ($p < 0.05$) in body weight compared with other groups. a: Control Vs MNU+T, b: MNU+T Vs calcitriol plus MNU+T treated rats at $p < 0.05$ level.

Fig. 1b Organ weight of ventral and dorsolateral prostates of rats treated with Methyl Nitroso Urea (MNU) + testosterone (T), calcitriol and vehicle. Group I: Control, II: Calcitriol control, III: MNU+T treated, IV: MNU+T plus calcitriol treatment. There was no significant difference in organ weights among the calcitriol treated rats. The MNU+T rats showed significant increase ($p < 0.05$) in ventral and dorsolateral prostate weight compared with other groups. a: Control Vs MNU+T, b: MNU+T Vs calcitriol plus MNU+T treated rats at $p < 0.05$ level.

weight of Wistar rats during MNU+T induced prostate carcinogenesis. Body weight was significantly decreased in MNU+T treated rats compared than control animals. Simultaneous treatment with calcitriol treated rats didn't show weight loss compared than MNU+T treated.

Fig.1b depicts the effect of calcitriol on ventral and dorsolateral prostate weight of albino rats during MNU and testosterone induced prostate carcinogenesis. Weight of ventral and dorsolateral lobes was significantly increased in MNU+T treated rats. Simultaneous treatment with calcitriol showed decreased ventral and dorsolateral prostate weight.

Serum prostatic acid phosphatase

Fig.2 represents the effect of calcitriol on serum prostatic acid phosphatase activity in MNU+T induced prostate carcinogenesis of male Wistar albino rats. MNU+T treated rats showed the increased serum PACP activity, whereas on simultaneous calcitriol treatment rats showed decreased prostatic acid phosphatase.

Pathology

The results are summarized in table 1. Survival was limited to 16 weeks from the start of the treatments. Prostatic Intraepithelial neoplasia is a pattern of epithelial cell proliferation, preneoplastic lesion containing epithelial cells protruding into the lumen. The tubules of varying sizes contained regions, which were composed of more than one layer of epithelial cells. Numerous papillary projections were present in the MNU+T treated rats. Prostatic intraepithelial neoplasia (PIN) was found

in 3 (30%) animals out of 10 in the dorsolateral prostate lobe as well as ventral lobe of the prostate. The 2 -6 PIN regions were found in the T+ MNU treated rats in each rat. About 10% (1 out of 10) of animals were developed PIN in the ventral and dorsolateral lobes of calcitriol treated rats. MNU+T treated rats showed hyperplasia and dysplasia about 60% (6) and 50% (5), respectively. As well as in the dorsolateral region its 7 (70%), 6 (60%) of hyperplasia and dysplasia. In the calcitriol treated group, occurrence of hyperplasia, dysplasia and PIN lesion were less in ventral (10% hyperplasia, 10% dysplasia and 10% PIN) and dorsolateral (20% hyperplasia, 10% dysplasia and 10% PIN) lobes. The hyperplastic, dysplastic and PIN lesion was reduced in the ventral and dorsolateral lobes of calcitriol treated group significantly, from that of control animals (Fig. 3 and Fig. 4).

Discussion

In this *in vivo* study we have demonstrated that calcitriol (Vitamin D₃), an steroid hormone has significant potency as an inhibitor of cancer induction in albino Wistar rats. Epidemiological data showed that vitamin D maintains the differentiated phenotype of prostatic cells and that vitamin D deficiency may be a risk factor for prostate cancer mortality^{10,11}. Calcitriol causes growth inhibition in a variety of cell types including breast, colon and skin^{19,21}. MNU+Testosterone model is relevant to human disease²². PIN lesion is now widely accepted as a premalignant condition of prostate cancer. In MNU+T treated animal show significant reduction in the body weight; whereas simultaneous calcitriol treated rats did

Fig. 2 Assay of serum prostatic acid phosphatase in rats treated with Methyl Nitroso Urea (MNU) + testosterone (T), calcitriol and vehicle. Group I: Control, II: Calcitriol control, III: MNU+T treated, IV: MNU+T plus calcitriol treatment. Decreased prostatic acid phosphatase was observed in the calcitriol treated rats. The MNU+T rats showed significant increase ($p < 0.05$) in prostatic acid phosphatase compared with other groups. a: Control Vs MNU+T, b: MNU+T Vs calcitriol plus MNU+T treated rats at $p < 0.05$ level.

not show weight loss. Weight of ventral and dorsolateral prostate in MNU+T treated rats are significantly increased. MNU is a cancer inducing agent, after a single dose; continuous stimulation by testosterone induces fibro muscular tissues, induces multiplication of squamous epithelium of the prostate. This could explain calcitriol inhibits the initiation of prostate cancer growth.

In order to find out the status of malignant growth, serum prostatic acid phosphatase levels were measured in the control and experimental groups. The prostate is a major organ, which secretes acid phosphatase and the level of serum acid phosphatase of prostatic origin increase markedly in human with extensive or metabolic carcinoma of the prostate²³. This enzyme is excellent marker of androgen dependent function of rat prostatic tissue^{18,24}. There was an increased activity of serum PACp, observed in the MNU+T treated animals. Simultaneous administration of calcitriol treated rats shows decreased in serum PACp activity. In malignant state, elevated serum levels of PACp in prostate cancer could be due to an increase in cell number of the tumor mass, increased leakage of the PACp from plasma membranes of carcinoma cells²⁵. Thus, decreased activity of PACp could explain involvement of calcitriol in the inhibition of prostate cancer initiation.

The MNU+T induced pathological changes closely mimic the histological features found in human prostatic dysplasia, also termed PIN, which is considered to be a precursor of prostate cancer. In an animal model, a single dose of MNU (carcinogen) was used to induce prostate carcinogenesis²⁶. In MNU+T treated animals, more

Table 1 Effect of calcitriol on hormone and carcinogen induced prostate carcinogenesis in albino Wistar rats. Number of animals in each group – 10; Hyp – Hyperplasia, Dys – Dysplasia, PIN – Prostatic Intraepithelial Neoplasia, DLP – Dorsolateral prostate, VP – Ventral prostate.

Treatment	DLP			VP		
	Hyp	Dysplasia	PIN	Hyp	Dysplasia	PIN
Control	-	-	-	-	-	-
Calcitriol	-	-	-	-	-	-
MNU+T	70% (7)	60% (6)	30% (3)	60% (6)	50% (5)	30% (3)
MNU+T and Calcitriol (0.5 µg/kg b.wt.)	20% (2)	10% (1)	10% (1)	10% (1)	10% (1)	10% (1)

number of hyperplastic, dysplastic and PIN lesion were observed, which showed the induction of prostate tumor, PIN is primarily accepted as a morphologically identifiable early stages in prostate cancer²⁷. Calcitriol treated animals showed less PIN morphology than MNU+T treated animals. Tumor occurs preferentially at DLP than VP. Although, Christov *et al.*²⁸ indicated that PIN could be used for assessing the efficacy of chemopreventive agents on prostate carcinogenesis.

In our present investigation, vitamin D₃ treatment significantly reduced hyperplastic, dysplastic and PIN changes. In this current model, MNU+T induced prostate carcinogenesis clearly showed the presence of hyperplasia, dysplasia and prostatic intraepithelial neoplasia. Here testosterone appears to be as promoter role and MNU was found to be a tumor initiator^{29,30}. Our results clearly demonstrated that calcitriol inhibits the initiation of prostate cancer.

Vitamin D₃ has an important role in regulating the prostatic cell growth. Vitamin D₃ action is mediated through vitamin D receptor (VDR) and was demonstrated in the human prostate cancer cell line LNCaP³¹. Vitamin D₃ inhibits tumor growth by induction of cyclin dependent kinase inhibitor p21 and G1-G0 cell cycle arrest³². Vitamin D₃ has been shown to exert significant antiangiogenic effects in rat mammary tumors and murine retinoblastomas^{33,34}. Vitamin D₃ arrests cell cycle. It induces apoptosis and also inhibits the metastasis of cancer cells. This may be the mechanism contributing the regression of cancer cell growth of vitamin D₃ treated rats.

A) Control

B) MNU + T treated

C) MNU + T treated

D) Calcitriol treated

Fig. 3. A) Histopathological appearance of normal dorsolateral prostate (DLP). The tubules are lined by a single layer of cuboidal cells lines the tubules with secretion in the lumen. Epithelial tubules, surrounded by a thin layer of smooth muscle cells, are distributed within the loosely organized stroma. B) MNU+T treated rats. Hyperplastic changes showing thickening of the epithelium and the formation of papillary processes. C) MNU+T treated rats. Prostatic intraepithelial neoplasia in DLP. D) Calcitriol treated rats. Note that absence of dysplastic and hyperplastic nodules with normal stromal compartment. It also shows cell death in the epithelial cells. Sections are H & E - stained ($\times 200$). E – Epithelium, L – Lumen, S – Stroma.

A) Control

B) MNU + T treated

C) MNU + T treated

D) Calcitriol treated

Fig. 4. A) Normal ventral prostate (VP). The acini are mainly lined by a single layer of cuboidal cells composed of columnar epithelial cells, intact and enclosing a lumen containing secretions. B) MNU+T treated rats. Hyperplastic sites were seen within the same glandular epithelium. It shows the papillary formation within the lumen & C) MNU+T treated rats. Pattern of prostatic intra epithelial neoplasia in VP. D) Calcitriol treated rats. Note that absence of dysplastic and hyperplastic nodules, stroma is very loosely organized. Sections are H & E - stained ($\times 200$). E – Epithelium, L – Lumen, S – Stroma.

In conclusion, our result of present study indicates chemoprevention of MNU +T induced prostate carcinogenesis at low dose level (0.5 µg/kg body weight) of calcitriol have significant potency to inhibits prostatic hyperplasia, dysplasia and PIN, and also decreased serum PACp activity. Calcitriol may be an effective therapy for the treatment of early prostate cancer.

Acknowledgements

This work was funded by grants from UGC, New Delhi as UGC SAP DRS Phase II and DST FIST programme to the department.

References

- 1) American Cancer society (2003) Cancer Facts and Figures 2003.
- 2) Quinn, M. and Babb, P. (2002) Patterns and trends in prostate cancer incidence, survival, prevalence and mortality. Part I: international comparisons, BJU International 90: 162-164.
- 3) Leav, I., Ho, S.M., Ofner, P., Merk, F.B., Kwan, P.W. and Damassa D. (1988) Biochemical alterations in sex hormone-induced hyperplasia and dysplasia of the dorsolateral prostates of Noble rats. J. Natl. Cancer Inst. 80: 1045-1053.
- 4) Wong, Y.C., Wang, Y.Z. and Tam, N.N.C. (1998) The prostate gland and carcinogenesis It. J. Anat. Embryol. 103: 237-252.
- 5) McCormick, D.L. and Rao, K.V.N. (1999) Chemoprevention of hormone-dependent prostate cancer in the Wistar-Unilever Rat. Eur. Urol. 35: 464-467.
- 6) Arunkumar, A., Vijayababu, M.R., Venkataraman, P., Senthilkumar, K. and Arunakaran, J. (2006) Chemoprevention of rat prostate carcinogenesis by diallyl disulfide, an organosulfur compound of garlic. Biological and Pharmaceutical Bulletin. (29: 375-379).
- 7) Bostwick, D.G., Pacelli, A. and Antonio, L.B. (1996) Molecular biology of prostatic intraepithelial neoplasia. Prostate 29: 117-134.
- 8) Haggman, M.J., Wojno, K.J., Pearsall, C.P. and Macoska, J.A. (1997) Allelic loss of 8p sequences in prostatic intraepithelial neoplasia and carcinoma. Urology 50: 643-647.
- 9) Bostwick, D.G. (1995) High-grade prostatic intraepithelial neoplasia: the most likely precursor of prostate cancer. Cancer 75: 1823-1836.
- 10) Schwartz, G.G. and Hulka, B.S. (1990) Is vitamin D deficiency a risk factor for prostate carcinoma? (hypothesis). Anticancer Res. 10: 1307-1311.
- 11) Hanchette C.L., Schwartz G.G. (1992) Geographic patterns of prostate cancer mortality evidence for a protective effect of ultra-violet radiation. Cancer 70: 2861-2869.
- 12) MacLaughlin, J. and Holick, M. (1985) Aging decreases the capacity of human skin to produce vitamin D₃. J. Clin. Invest. 76: 1536-1538.
- 13) Fife, R.S., Sledge, G.W. and Proctor, C. (1997) Effects of vitamin D₃ on proliferation of cancer cells in vitro. Cancer Lett 120: 65-69.
- 14) Van den Bemd, G.J., Pols, H.A. and Van Leewen, J.P. (2000) Antitumor effects of 1,25-dihydroxyvitamin D₃ and vitamin D analogs. Curr. Pharm. Des. 6: 717-732.
- 15) Colston, K.W., Chander, S.K., Mackay, A.G. and Coombes, R.C. (1992) Effects of synthetic vitamin D analogues on breast cancer cell proliferation in vivo and in vitro. Biochem. Pharmacol. 44: 693-702.
- 16) Stewart, L.V. and Weigel, N.L. (2004) Minireview: Vitamin D and prostate cancer. Exp. Biol. Med. 229: 277-284.
- 17) Carlos, M., Stable, P., Schwartz, G.G., Farinas, A., Finegold, M., Binderup, L., Howard, G.A. and Roos, B.A. (2002) The Gγ/T15 Transgenic mouse model of androgen independent prostate cancer: Target cells of carcinogenesis and the effect of the vitamin D analogue EB1089. Cancer Epid. Biol. Prev. 11: 555-563.
- 18) Tenniswood, M., Bird, C.E. and Clark, A.F. (1976) Acid phosphatases: Androgen dependent markers of rat prostate. Can. J. Biochem. 54: 350-357.
- 19) Verlinden, L., Verstuyf, A., Convents, R., Marcelis, S., Van Camp, M. and Bouillon, R. (1998) Action of 1,25(OH) 2D₃ on the cell cycle genes, cyclin D1, p21 and p27 in MCF-7 cells. Mol. Cell. Endocrinol. 142: 57-65.
- 20) Ootshi, T., Iwata, H., Kitano, M., Nishizawa, Y., Morii, H., Yano, Y., Otoni, S. and Fukushima, S. (1995) Inhibition of intestinal tumor development in rat multiorgan carcinogenesis and aberrant crypt foci in rat colon carcinogenesis by 22-oxa-calcitriol, a synthetic analogue of 1α,25-dihydroxyvitamin D₃. Carcinogenesis 16: 2091-2097.
- 21) Kensler, T.W., Dolan, P.M., Gange, S.J., Lee, J.K., Wang, Q. and Posner, G.H. (2000) Conceptually new deltenoids (vitamin D analogs) inhibit multistage skin tumorigenesis. Carcinogenesis 21: 1341-1345.
- 22) Liao, Z., Boileau, T.W.M., Erdman, J.W. and Clinton, S.K. (2002) Interrelationships among angiogenesis, proliferation, and apoptosis in the tumor microenvironment during N-methyl-N-nitrosourea androgen-induced prostate carcinogenesis in rats. Carcinogenesis 23: 1701-1712.
- 23) Zuke, M.C. and Coffey, D.S. (1994) The male accessory tissue: structure, Androgen action and physiology. In: Knobil E. and Neul J.D., editors, The physiology of Reproduction. New York: Raven Press Ltd.; p. 1436-1479.
- 24) Shao, T.C., Kong, A.Y. and Cunningham, G.R. (1987) Acid phosphatase activity a marker of androgen status action in prostate explant cultures. Prostate 10: 69-77.
- 25) Reif, A.F., Schilesinger, R.M., Fish, C.A. and Robinson, C.M. (1973) Acid phosphatase isoenzyme in cancer of the prostate. Cancer 31: 689-699.
- 26) Bosland, M.C. (1996) Chemical and hormonal induction of prostate cancer in animal models. Urol. Oncol. 2: 103-110.
- 27) Foster, C.S., Bostwick, D.G., Bonkhoff, H., Damber, J.E., van der Kwast, T., Montironi, R. and Sakr, W.A. (2000) Cellular and molecular pathology of prostate cancer precursors. Scand. J. Urol. Nephrol. Suppl. 205: 19-43.
- 28) Christov, K.T., Moon, R.C., Lantvit, D.D., Boone, C.W., Steele V.E., Lubet, R.A., Kelloff, G.J. and Pezzuto, J.M. (2002) 9-cis-Retinoic Acid but Not 4-(Hydroxyphenyl) retinamide Inhibits Prostate Intraepithelial Neoplasia in Noble Rats. Cancer Res. 62: 5178-5182.
- 29) Bosland, M.C., Prinsen, M.K., Dirksen, T.J.M. and Spit, B.J. (1990) Characterization of adenocarcinomas of the dorsolateral prostate induced wistar rats by N-methyl-N-nitrosourea, 7,12-di-methyl-4-aminobenz(a)anthracene and 3,2'-dimethyl-4-aminobiphenyl following sequential treatment with cyproterone acetate and testosterone propionate. Cancer Res. 50: 7000-7009.
- 30) Rao, K.V.N., Johnson, W.D., Bosland, M.C., Lubet, R.A., Steele, V.E., Kelloff, G.A. and McCormick, D.L. (1999) Chemoprevention of rat carcinogenesis by early and delayed administration of dehydroepiandrosterone. Cancer Res. 59: 3084-3089.
- 31) Miller, G.J., Stapleton, G.E., Ferrara, J.A., Lucia, M.S., Pfister, S., Hedlund, T.E. and Upadhyay, P. (1992) The human prostatic carcinoma cell line LNCaP expresses biologically active, specific receptors for 1 alpha,25 dihydroxyvitamin D₃. Cancer Res. 52: 515-520.
- 32) Miller, G. (1999) Vitamin D and prostate cancer: biologic interactions and clinical potentials. Cancer metastasis Rev. 17: 353-360.
- 33) Oikawa, T., Yoshida, Y., Shimamura, M., Ashino-Fuse, H., Iwaguchi, T. and Tominaga, T. (1991) Antitumor effect of 22-oxa-1α,25dihydroxyvitamin D₃, a potent angiogenesis inhibitor, on rat mammary tumors induced by 7,12-dimethylben-z(a)anthracene. Anticancer Drugs 2: 475-480.
- 34) Shokravi, M.T., Marcus, D.M., Alroy, J., Egan, K., Saornil, M.A. and Albert, D.M. (1995) Vitamin D inhibits angiogenesis in transgenic murine retinoblastoma stomas. Invest. Ophthalmol. 36: 83-87.