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Effect of Retinoic Acid on HPV Titration and Colposcopic Changes in Korean Patients With Dysplasia of the Uterine Cervix

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Abstract Retinoids, a family of molecules capable of profound impact on many biological functions, have antiproliferative, differentiative, and immunomodulatory properties. The present study assessed the effect of 13-*cis*-retinoic acid (13-CRA) treatment in 13 chronic cervicitis and 52 cervical intraepithelial neoplasia patients. We examined low- and high-risk human papilloma virus titer (using the hybrid capture method) and made a colposcopic and cervicographic examination before and after treatment with 13-CRA at 1 mg/kg for 4 to 12 weeks. Patients were between 27 and 64 years, the average age being 36.6 years. Histology revealed chronic cervicitis in 13 cases, mild dysplasia in 18 cases, moderate dysplasia in 18 cases, and severe dysplasia in 16 cases, totaling 65 cases. The expression rate of high-risk human papilloma virus (HPV 16, 18) was 9 of 13 cases (69%) in chronic cervicitis, 7 of 18 cases (38%) in mild dysplasia, 9 of 18 cases (50%) in moderate dysplasia, and 12 of 16 cases (75%) in severe dysplasia, with the overall expression rate being 37 of 65 cases (57%). Following 13-CRA treatment, decreases in high-risk titer were observed in 6 of 9 cases (66%) of chronic cervicitis, 4 of 11 cases (36%) of mild dysplasia, 7 of 9 cases (77%) of moderate dysplasia, and 8 of 12 cases (75%) of severe dysplasia. Overall, HPV titer decreased in 25 of 41 cases (61%). Minimal changes were found in colposcopic and cervicographic observations during the study. In summary, high-risk HPV titer decreased after treatment with 13-CRA in the majority of patients with cervical intraepithelial neoplasia. This study supports the potential of retinoids to interrupt multi-step carcinogenesis, possibly by down-regulation of gene products (E6,E7) produced by HPV infection. J. Cell. Biochem. Suppls. 28/29:133–139.

Key words: retinoic acid (RA); cervical intraepithelial neoplasia (CIN); HPV; dysplasia; colposcopic examination

The use of retinoids in oncology clinical trials is a recent and exciting development, which has increased understanding of the role of vitamin A analogues in the normal development process [1–4]. Several nucleus receptors for retinoids, which function as transcription and modulatory factors regulating specific gene expression, have been discovered [5–7].

Retinoids have demonstrated potential in preventing and treating oral leukoplakia, head and neck cancer, stage I non-small cell lung cancer (NSCLC), acute promyelocytic leukemia (APL), T-cell lymphoma, and juvenile chronic myelogenous leukemia. Additionally, IFNα 2a and 13-*cis* retinoic acid (13-CRA) in combination have been used to treat renal cell carcinoma and squamous cell carcinoma of the skin and the cervix. Together, these studies demonstrate enormous potential for retinoids in oncology [8,9].

After human papilloma virus (HPV) infection in the cervix, viral oncoproteins E6 and E7 are continuously synthesized. Encoded by HPV 16 and HPV 18, the E6 and E7 oncoproteins exhibit immortalizing activity by binding the protein products of certain tumor suppressor genes and negating their normal function [10,11]. For example, E6 binds the tumor suppressor p53; the E6-p53 complex is then degraded by a ubiquitin-dependent proteolysis pathway [12,13]. If treating cervical intraepithelial neoplasia (CIN) with 13-CRA reduces the number of HPV-infected cells, cervical tumorigenesis mediated by viral oncoproteins may be interrupted. We performed clinical trials of 13-CRA in patients with CIN, with the objective of providing a

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quantitative measure of high-risk HPV in CIN after 13-CRA treatment.

MATERIALS AND METHODS

Sixty-five patients underwent cytologic examinations, cervicography, HPV DNA tests, colposcopy, and histologic examinations between January 1995 and September 1996 in the Department of Obstetrics and Gynecology, College of Medicine, The Catholic University of Korea. All patients were diagnosed histologically as having either chronic cervicitis (13), CIN I (18), CIN II (18), or CIN III (16). The retinoid used in this study was 13-CRA, at 1 mg/kg for 4–12 weeks. During treatment, patients were examined every 4 weeks; patients who tested positive for HPV DNA were checked at 4-week intervals for the duration of the 12-week study.

HPV DNA Test Procedure

Specimens for HPV DNA testing were stored at -20°C for analysis by the hybrid capture method. Each specimen was tested for a group of common cancer-associated high-risk HPV types (16, 18, 31, 33, 35, 45, 51, 52, and 56) and another group of low-risk types (6, 11, 42, 43, and 44) not associated with cancer. Specimens were denatured and the liberated single-strand DNA was hybridized in solution with a ribonucleic acid (RNA) probe mix consisting of low-risk or high-risk HPV types. Each reaction mixture, containing any RNA-DNA hybrids formed, was immobilized by transfer to a capture tube coated with RNA-DNA hybrid antibodies. Unreacted material was removed by washing and a dioxetane-based chemiluminescent substrate (Lumi-Phos 530, Lumigen, Inc., Detroit, MI), which binds with the alkaline phosphatase, was added. Light produced by the ensuing reaction was measured by a luminometer and expressed as relative light units. Solutions of HPV 11 or HPV 16 DNAs at 10 pg/ml served as positive controls for lower-risk HPV probes or cancer-associated HPV probes, respectively. All relative light unit measurements for specimens were divided by the relative light units of the appropriate controls to give a ratio. A specimen ratio of $\geq$1.0 was regarded as positive for HPV DNA and a ratio of $<$1.0 was regarded as negative. Because the amount of light produced by the hybrid capture assay is proportional to the amount of target DNA in each specimen, the results were quantitative: the higher the ratio, the greater the amount of target HPV DNA indicated in the specimen. All HPV DNA testing was strictly masked with regard to the clinical results.

TABLE I. Patient Characteristics

Histologic diagnosis	Number	Age (average)
1 Chronic cervicitis	13	33–46 (39.5)
2 CIN I	18	35–56 (35.8)
3 CIN II	18	23–48 (35.6)
4 CIN III	16	25–64 (35.5)
Total	65	23–64 (36.6)

TABLE II. HPV DNA Assay in Chronic Cervicitis and Cervical Intraepithelial Neoplasia*

Diagnosis	$1 > PC\bar{x}/NC\bar{x}$ ratio (%)	$1 \leq PC\bar{x}/NC\bar{x}$ ratio (%)
1 Chronic cervicitis	4 (31)	9 (69)
2 CIN I	11 (61)	7 (39)
3 CIN II	9 (50)	9 (50)
4 CIN III	4 (25)	12 (75)

*Overall positive expression rate 37/65 (57%). $PC\bar{x}$: positive control mean; $NC\bar{x}$: negative control mean.

RESULTS

Characteristics of patients enrolled in the study were as follows: chronic cervicitis (13), CIN I (18), CIN II (18), and CIN III (16). The age distribution was 23–64; average age was 36.6 years (Table I). Nine of 13 chronic cervicitis patients, seven of 18 CIN I, nine of 18 CIN II, and twelve of 16 CIN III patients showed positive high-risk HPV ($1 \leq PC\bar{x}/NC\bar{x}$ ratio) (Table II). In six of nine HPV DNA-positive cases in the chronic cervicitis group, the HPV titer decreased after 13-CRA treatment; three of nine showed an increase. Five of six patients who experienced a decrease in titer also showed a change from positive to negative. Four of nine HPV-negative patients showed a negative change during treatment (Fig. 1). In three of seven HPV DNA-positive CIN I cases, the titer decreased after 13-CRA treatment. Four of seven showed an increase or no change. Two of three patients who experienced a decrease in titer also showed a change from positive to negative. One of three decreased within 4 weeks after treatment. Eleven HPV-negative patients showed a negative change during treatment

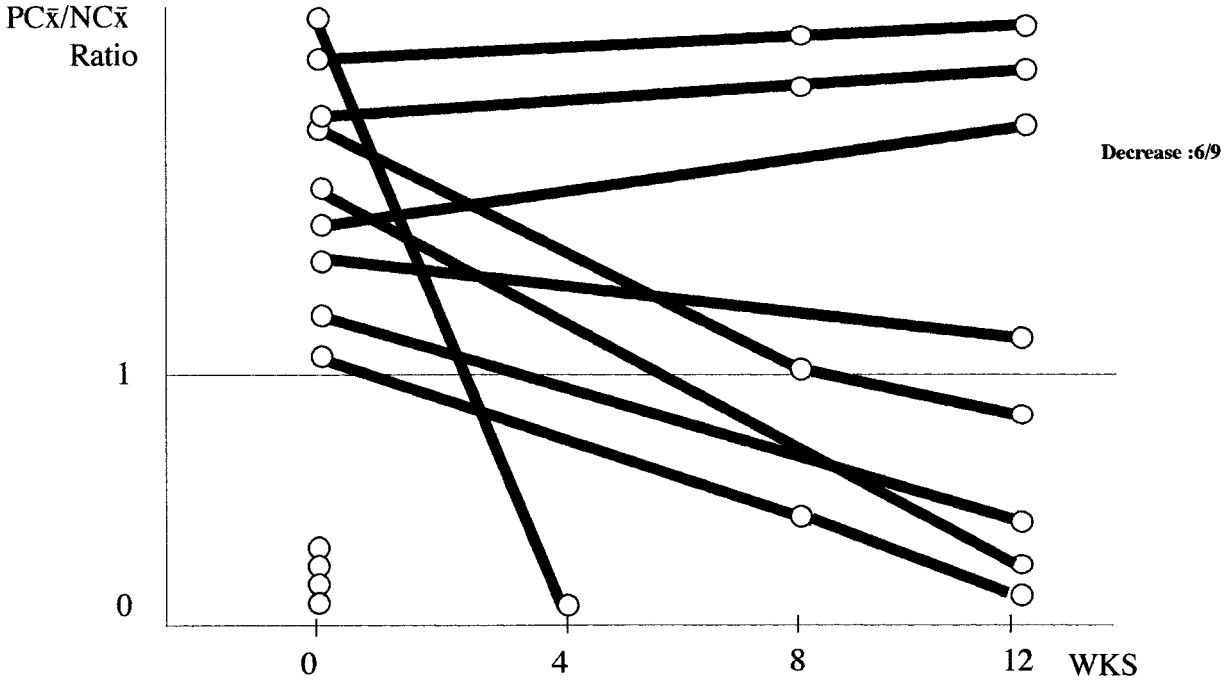

Fig. 1. Changes of high-risk human papilloma virus (HPV 16,18) titers after treatment of chronic cervicitis patients treated with 13-*cis* retinoic acid.

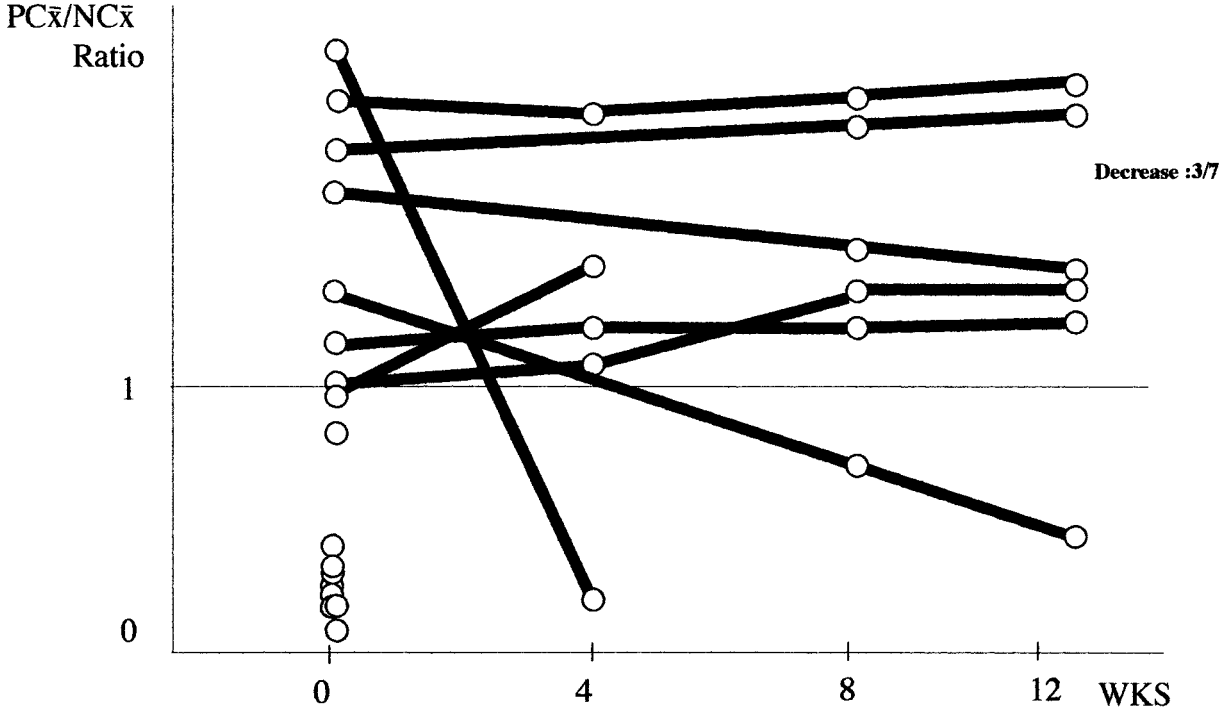

Fig. 2. Changes of high-risk human papilloma virus (HPV 16,18) titers after treatment of CIN I patients treated with 13-*cis* retinoic acid.

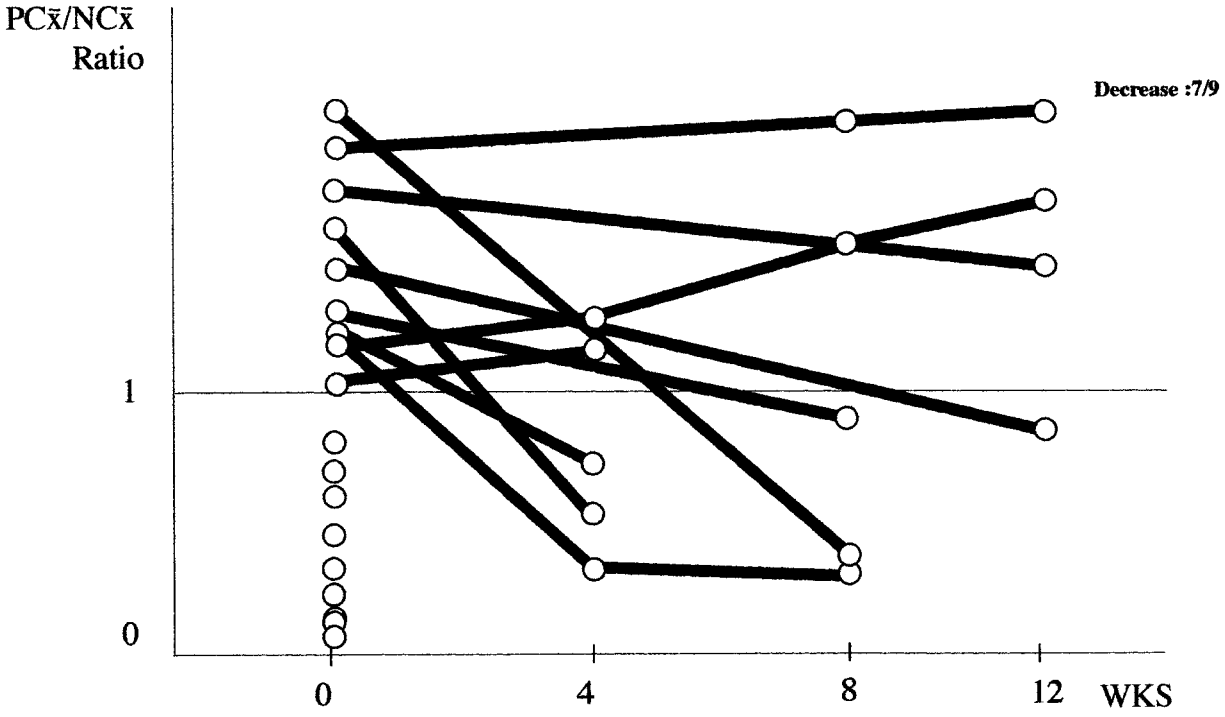

Fig. 3. Changes of high-risk human papilloma virus (HPV 16,18) titers after treatment of CIN II patients treated with 13-*cis* retinoic acid.

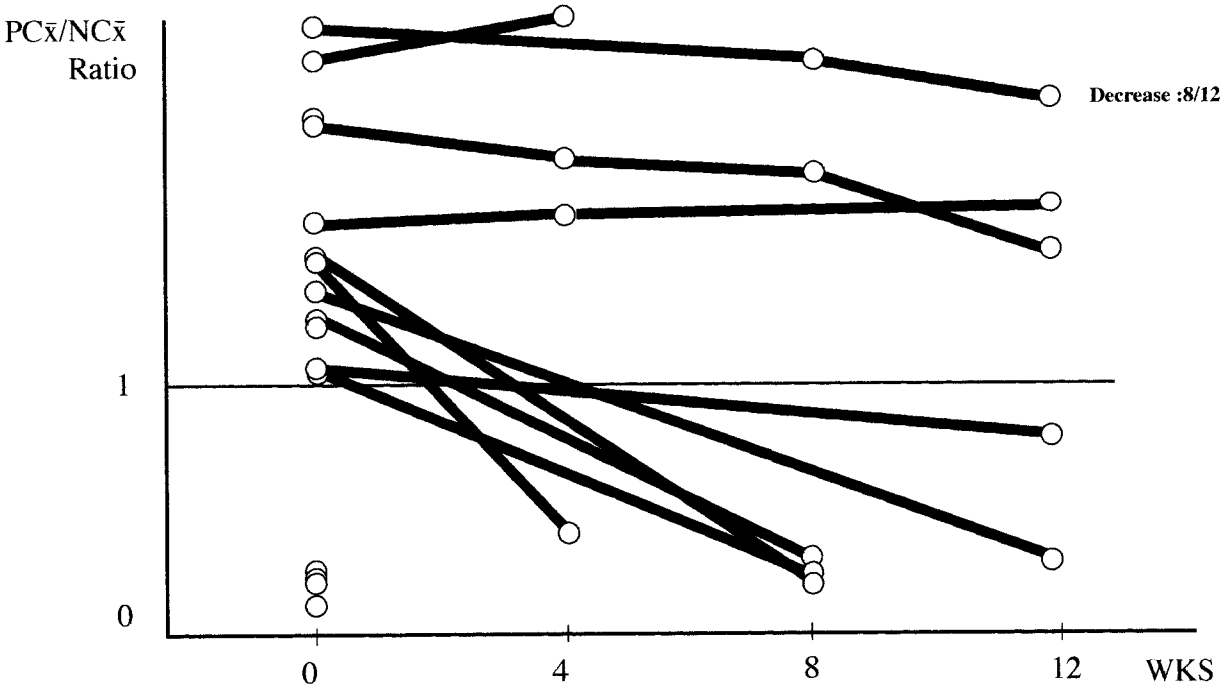

Fig. 4. Changes of high-risk human papilloma virus (HPV 16,18) titers after treatment of CIN III patients treated with 13-*cis* retinoic acid.

TABLE III. Toxic Effects*

Effect	Toxicity grade 0	1	2	3	4
Cheilitis	0	41	21	3	0
Conjunctivitis	31	27	7	0	0
Hypertriglyceridemia	23	39	3	0	0
Fatigue	27	31	7	0	0
Anorexia	33	25	7	0	0
Aspartate transferase	21	31	11	2	0
Alkaline phosphatase	8	46	11	0	0

*Toxic effects were graded according to the common toxicity criteria from the Division of Cancer Treatment, National Cancer Institute. 0 = least toxic, 4 = most toxic.

(Fig. 2). In seven of nine HPV DNA-positive CIN II cases, the titer decreased after 13-CRA treatment. Two of nine patients showed an increase. Six of seven patients who experienced a decrease in titer also showed a change from positive to negative. One of six decreased within 4 weeks after treatment. Nine HPV-negative patients showed a negative change during treatment (Fig. 3). In eight of 12 HPV DNA-positive CIN III cases, the titer decreased after 13-CRA treatment. Four of 12 patients showed an increase. Five of six patients who experienced a decrease in titer also showed a change from positive to negative. Four HPV negative patients showed a negative change during treatment (Fig. 4).

Toxicity

Adherence to treatment was 100%. This outpatient regimen was well tolerated (Table III). All patients developed grade 1 or 2 cheilitis and also dry skin. Thirty-four patients developed mild conjunctivitis, and grade 1 or 2 fatigue occurred in 38 patients. Grade 1 or 2 hyperglyceridemia developed in 42 patients. Only three patients developed grade 3 toxicity (cheilitis), which required a dose reduction. Grade 4 clinical toxicity was not observed.

DISCUSSION

Cervical cancer is the second most common malignancy in women worldwide and a significant health problem. Prevention of cervical cancer and its precursors are important objectives [14].

HPV DNA is present in more than 85% of squamous cell cervical cancers [15]. Although no conclusive evidence exists that HPV by itself is capable of causing cervical dysplasia or cancer, it is an important factor in the pathogenesis of cervical cancer [16–18]. HPV E6 protein plays a central role in the development of cervical cancer via its interactions with the p53 gene product [19]. Also, HPV may activate the *myc* oncogene through chromosomal translocation, insertional mutagenesis, and amplification.

Dietary vitamin A (retinol) and other retinoids maintain normal cervical cell function and inhibit the growth of cervical tumors [20–22]. HPV 16 immortalization enhances cervical cell sensitivity to retinoids, therefore cytokeratin expression may be useful as a marker for predicting the success of retinoid therapy in vivo [23–27]. Retinoids do not necessarily directly inhibit proliferation of HPV-immortalized cervical cells. Instead, retinoids may have an effect on HPV E6 and E7 RNA levels and may inhibit cervical cell proliferation by suppressing the activity of the EGF and TGF signaling pathway [28].

HPV DNA titer correlates with progression of the CIN lesion from CIN I to CIN III [29,30]. In this study, chronic cervicitis patients showed 69% HPV DNA-positive titer, because we selected patients from the HPV-positive cytology and cervicography findings. Cervicographically atypical lesions are sometimes obvious in mild cases, even though HPV-infected lesions revealed chronic cervicitis histologically. Six of nine chronic cervicitis patients with a positive HPV-DNA test showed a decrease in HPV DNA titer after treatment with retinoids. One or two of these cases showed a sudden drop of HPV titer after exposure to retinoids. We observed this same phenomenon of a rapid response in CIN I-CIN III patients. Though definitive changes after treatment are not easily seen [31,32], considerable evidence of a change in HPV DNA titer was found. During the short time that the study was conducted, morphological changes in cytology and cervicography were not observed. Using nucleomorphometry, we could explore whether nuclear differences or other trends are associated with changes in titer.

The pattern of decreasing and increasing ratio of titer varies according to the CIN stage; patients with an increased ratio will likely have progressed; a decreased ratio suggests they will have regressed. In CIN III, HPV titer decreased in 8 of 12 patients after treatment. These re-

sults suggest that CIN is a good model for chemoprevention clinical trials of 13-CRA. When a double-blind placebo-controlled clinical trial is developed, retinoids may show positive results in treating cervical intraepithelial neoplasia.

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