Low-Dose Topical Delivery of All-*Trans* Retinoic Acid for Cervical Intraepithelial Neoplasia II and III

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Abstract

Objective: **The objective of this study was to determine an effective dose for *all-trans* retinoic acid (atRA) delivered with a cervical cap and sponge for 4 days to women with cervical intraepithelial neoplasia (CIN) II/III.**

Methods: **Study participants made up of 175 women with biopsy-proven CIN II/III were randomized to four consecutive days of atRA at one of three doses (0.16%, 0.28%, and 0.36%) or placebo. All subjects underwent a repeat colposcopy evaluation and biopsy of the cervix at 12 weeks.**

Results: **The study participants mean ages were 27.6 years. The racial distribution was 63% Caucasian, 27% African American, and 8% other. Among participants, 93% were human papillomavirus-positive at baseline with 68% positive for high-risk types. The disease response at 12 weeks to atRA or placebo was not significantly different (P = 0.49) among the four dose groups. Participants with CIN II at baseline were more likely to be free of disease at 12 weeks than participants with CIN III at baseline (P = 0.003). There were no reported systemic adverse events related to drug or placebo exposure and only mild local self-reported and clinician-detected toxicities.**

Conclusion: **Lower concentrations of atRA applied with a cervical cap for 4 days were no more effective than placebo. However, the rate of histologic regression in biopsied CIN II/III patients was high even over a short time interval, and emphasizes the importance of having a placebo arm and an adequate sample size.** (Cancer Epidemiol Biomarkers Prev 2004;13(12):2148–52)

Introduction

Retinoids have been studied as a chemoprevention strategy for various cancers including cervical neoplasia, one of the leading causes of cancer mortality for women worldwide (1). Retinoids are essential for cell growth, differentiation, and cell death. Various retinoids have been shown to inhibit cellular proliferation in cervical cancer cells in several studies, which suggests their potential as chemopreventive agents for cervical neoplasia (2-4). A series of studies (5-9) suggested that topically applied all-*trans* retinoic acid (atRA) is an effective chemopreventive agent for cervical neoplasia. The maximally tolerated dose has been defined as 0.37% (8) and shown to be significantly more effective in treating cervical intraepithelial neoplasia (CIN) II than a placebo agent (9).

The protocol used in those studies consisted of four consecutive days of treatment followed by repeat treatment for 2 days every 3 months for a year (9). This protocol is not acceptable for most women or physicians. The Phase III trial had cumulative losses of 40% of participants (9). Given the lack of keratinization of the cervical epithelium and delivery under occlusion, theoretically, lower concentrations should be effective. Earlier studies suggested that a concentration of 0.16% could represent the minimally effective dose (8). In addition, the range of clinically effective concentrations of atRA used on keratinized skin without occlusion is from 0.025% to 0.1%. At concentrations under 0.2%, atRA is a cream, whereas at higher concentrations, it is a liquid. Because the cream is easier to handle, lower concentrations would facilitate the development of self-application protocols. If lower concentrations of atRA were proven to be effective when given over a short period of time, then this chemopreventive strategy might be far more acceptable.

The goals of this study were 2-fold. First, to determine if doses < 0.37% of atRA delivered with a cervical cap and sponge to women with CIN II or III were equally effective as doses of 0.37%. The second goal was to determine if a one-time exposure of four consecutive days of treatment was adequate to eradicate evidence of CIN II or III.

Materials and Methods

Patient Eligibility Criteria. Patients from colposcopy units in Michigan and Ohio were recruited to

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participate in this study. Women ages 14 and older were deemed eligible if they had histologic confirmation of CIN II or III with a fully delineated ectocervical lesion and adequate colposcopic evaluation. The study participants were required to use an effective birth control method (excluding IUD's or natural family planning) or to be not capable of getting pregnant. No regular scheduled use of systemic steroids or anticoagulants were allowed. No nutritional supplements other than two multivitamins per day were allowed. Participants were excluded if they were pregnant or lactating, had pap smears suspicious for invasive carcinoma, or had positive endocervical curettage. Other reasons for exclusion were *in utero* exposure to diethylstilbestrol (DES), latex allergy, history of toxic shock, allergy to the drug or any form of immune suppression or immune disorder. Human subject institutional review boards at all participating sites approved the study.

Treatment Plan. The study intervention consisted of four consecutive days of atRA (gift of Ortho-McNeil Pharmaceuticals, Raritan, NJ) with a cervical cap and sponge at one of three doses (0.16%, 0.28%, and 0.36%) or a placebo agent. The sponge was placed in the cervical cap and then the liquid was poured onto the sponge from the syringe. After all of the liquid was absorbed, the drug or placebo, sponge, and cap were placed on the cervix each day by a trained nurse practitioner. The sponge was made of an inert synthetic material. The placebo agent was the same carrier base used to compound the active agent. The drug was compounded as previously described (6, 8, 9). The drug and drug delivery system are identical to the protocol previously published (9) with the exception that the previous studies used a collagen sponge, in contrast to the inert sponge used in this protocol. The participants, research staff, and all investigators were blinded to the drug concentration used. Questionnaires were completed at baseline visit, along with height and weight measurements in stocking feet, and collection of serum. The maximum time from cervical biopsy to delivery of the intervention was 12 weeks.

Toxicity. Toxicity was evaluated on days 1 through 5 of treatment and on follow-up at week 12. At each encounter, study participants provided ratings of vaginal burning, vaginal itching, and vaginal discharge on a scale of 0 to 10 (0 = no symptoms, 10 = unbearable). The adverse effects of vaginal discharge and vulvar, vaginal, and cervical erythema were evaluated at each visit by research staff using clinical and colposcopic assessments on a scale of 0 to 3 (0 = none, 1 = mild, 2 = moderate, and 3 = severe) refs. (9, 10). The local toxicity scores, both self-reported and clinician-assessed, were transformed to yes/no (yes for any report or assessment ≥ 1).

Assessment of Effect. The participants were examined 12 weeks after the last day of drug exposure. The outcome of interest was whether the subject still had disease requiring treatment, i.e., CIN II or worse. All subjects underwent a repeat colposcopic evaluation by the same provider who did the baseline evaluation. If the cervix seemed normal, then a cervical biopsy was taken from the same site that was previously determined at histology to be CIN II/III. If there was more than one site, all sites were biopsied again. All pathology samples at baseline and week 12 were reviewed by a single pathologist (Claire W,Michael CWM) blinded to the study arm assignment or disease status.

Human Papillomavirus Testing. Prior to application of acetic acid or iodine to the cervix, endocervical and ectocervical cells for human papillomavirus (HPV) testing were collected with a synthetic extend-tip spatula placed in the cervix and rotated 360 degrees. The spatula was washed with 2 mL 0.6% SDS, 0.01 mol/L EDTA. This sample was immediately placed on ice and transported to a −80°C freezer. After thawing and resuspension, a 0.5 mL aliquot was digested overnight with Proteinase K, extracted with phenol-chloroform, and ethanol-precipitated. The DNA was suspended in 80 μL distilled deionized water and stored at −20°C until tested. A 50-μL volume of extract was washed with 2 mL distilled deionized water in a Centricon column (Millipore Corp., Bedford, MA) and resuspended in 50 μL distilled deionized water; 10 μL was used in a 100 μL PCR. HPV detection and typing was done using the Roche line blot assay (reagents provided as a gift from Roche Molecular Systems, Alameda, CA). This assay uses HPV L1 consensus PCR with biotinylated PGMY09/11 primer sets and β-globin as an internal control for sample amplification (11, 12). Compared with the Food and Drug Administration–approved Digene hybrid capture method, this method avoids cross-hybridization between low-risk and high-risk types, provides type-specific information, and clearly identifies the presence of multiple HPV types. High-risk and low-risk HPV types were classified using the recent classification scheme (13).

Analysis. Statistical significance of cross-tabulation tables was determined by Fisher's exact test. Multivariate tests were derived from generalized linear models. Logistic regression was used with regression, no disease or CIN I at 12 weeks (yes/no) as the outcome and treatment, HPV status at baseline (high-risk or low-risk/no HPV) and baseline disease status as predictors. All calculations were done using SAS 9.0 (SAS Institute, Cary, NC).

Results

Patient Characteristics and Evaluability. We enrolled 176 women, of which 175 completed the study; the one study withdrawal was due to domestic violence. The study participants mean ages were 27.6 years, ranging from 14 to 52 years. The racial distribution was 63% Caucasian, 27% African American, 2% Asian, 2% Latina, and 4% others; 67% of the participants were married. The frequency of risk behaviors for cervical cancer was 42% for current smokers, 88% for those who have had more than three lifetime sexual partners, and 73% for those who have had sexual intercourse before the age of 18. Nearly all participants (93%) were HPV-positive at baseline, 68% had one or more high-risk types and 25% had only low-risk types. Women with CIN III at baseline were significantly more likely to have high-risk type HPV ($P = 0.004$) than women with CIN II at baseline (see Table 1).

Table 1. HPV status by disease at baseline

	High-risk HPV*	Low-risk HPV
	n (% disease group)	*n* (% disease group)
CIN II	71 (75%)	24 (25%)
CIN III	42 (95%)	2 (5%)

*P = 0.004.

Patient Response and Compliance. The distribution of CIN II/III at baseline and week 12 by treatment group are shown in Table 2. There were no significant differences between the treatment groups in terms of CIN stage, age, current smoking status, number of lifetime partners, age of first intercourse, HPV status, or race. The regression to no disease or CIN I at week 12 among women with CIN II at baseline was 70% on placebo, 83% on 0.16% atRA, 87% on 0.28% atRA, and 74% on 0.36% atRA. The regression to no disease or CIN I at week 12 among women with CIN III at baseline was 57% on placebo, 57% on 0.16% atRA, 58% on 0.28% atRA, and 35% on 0.36% atRA. Drug exposure had no effect even after collapsing the treatment categories into placebo and drug exposure.

Women with CIN II at baseline were significantly (P = 0.003) more likely to clear the disease than women with CIN III at baseline. Women with only low risk or no HPV at baseline were more likely to clear their disease at 12 weeks (P = 0.0003) than women with high-risk HPV (96% compared with 52%). Baseline characteristics of age, smoking status, number of lifetime partners, age of first intercourse, race, and body mass index were not related to disease response at week 12. The elimination of cervical disease at week 12 was not significantly different between the four treatment groups (P = 0.49). The adjusted odds ratios from the logistic regression for elimination of cervical disease at week 12 are shown in Table 3. There was no significant interaction in the logistic regression between HPV status or disease at baseline and treatment.

Patient Toxicity. There were no reported systemic adverse events related to drug or placebo exposure. There were some toxicities, self-reported and clinician-detected, but none were severe enough to cause participants to withdraw from the study. No participants withdrew or discontinued therapy due to toxicity.

Table 3. Adjusted odds ratios for disease elimination at 12 weeks by treatment exposure, baseline HPV status, and baseline disease status

	Odds ratio	95% Confidence limits	
0.16% atRA versus placebo	1.14	0.37	3.56
0.28% atRA versus placebo	1.34	0.42	4.34
0.37% atRA versus placebo	0.61	0.21	1.69
HPV status: high-risk versus low-risk or no HPV	0.08	0.005	0.46
Baseline disease status: CIN II versus CIN III	2.70	1.22	6.05

The mean and range of the self-reported scores for each of the local toxicities were vaginal burning (0.25, 0-9), vaginal itching (0.37, 0-8) and vaginal discharge (0.71, 0-7). The mean and range of the clinician-assessed local toxicities were cervical erythema (0.63, 0-3), vaginal erythema (0.07, 0-3), vulvar erythema (0.13, 0-2), and vaginal discharge (0.41, 0-3). The percentage of study participants with evidence of local toxicity were compared between the placebo and all participants receiving atRA as shown in Fig. 1. There was a significant increase in self-reported (P = 0.0005) and clinician-assessed (P = 0.0122) vaginal discharge over time in participants receiving atRA compared with placebo. Self-reported vaginal burning (P = 0.024) and vaginal itching (P = 0.0023) significantly increased over time in participants receiving atRA compared with placebo. Clinician-assessed cervical erythema (P = 0.0135) increased significantly over time in participants receiving atRA compared with placebo. There were no significant differences with respect to vaginal erythema.

Discussion

For nearly two decades, retinoids have been investigated as possible therapeutic agents for preinvasive cervical

Table 2. Disease status by drug exposure at baseline and week 12

Week 12 status	Baseline disease status							
	CIN II*				CIN III			
	Dose†				Dose†			
	N (%)				*N* (%)			
	Control	0.16%	0.28%	0.37%	Control	0.16%	0.28%	0.37%
Normal	15 (62)	18 (62)	16 (50)	17 (50)	3 (21)	7 (44)	6 (50)	2 (14)
CIN I	2 (8)	6 (21)	12 (37)	8 (24)	5 (36)	2 (13)	1 (8)	3 (21)
CIN II	6 (25)	5 (17)	3 (9)	4 (12)	4 (29)	5 (31)	0 (0)	7 (50)
CIN III	1 (4)	0 (0)	1 (3)	5 (15)	2 (14)	2 (13)	5 (42)	1 (14)
All	24	29	32	34	14	16	12	13

*P = 0.003 baseline CIN II more likely to clear the disease than CIN III at baseline.
†P = 0.78 response to placebo or atRA was not significantly different.

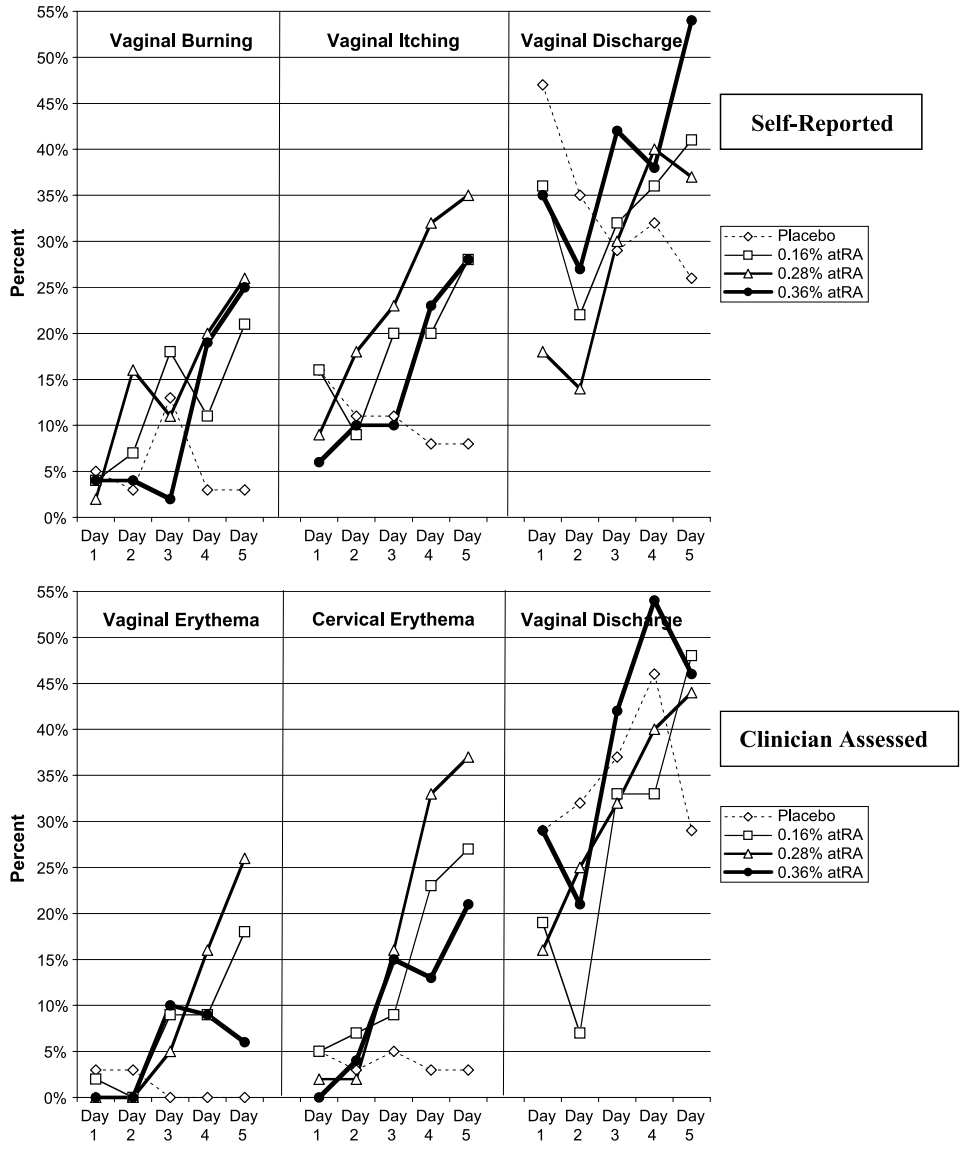

Figure 1. Self-reported and clinician-assessed local toxicities. Study participants in each dose group: placebo = 38; 0.16% atRA = 45; 0.28% atRA = 44; and 0.36% atRA = 48.

neoplasia. Although, the maximum tolerated dose of atRA has been shown to be effective in CIN II, the delivery method was not practical because of timing and mode of application. This study was designed to evaluate whether lower concentrations of atRA given as a single exposure of four consecutive days could eradicate CIN. This dosing scheme was chosen because, if effective, it could be self-administered with a cervical cap and a cream. This study, designed according to accepted criteria for CIN chemoprevention studies (14), fails to support the efficacy of the lowered concentration and shortened exposure.

After 4 days of treatment, the three concentrations of atRA were not significantly different in effectiveness at eradicating CIN II or CIN III 12 weeks postexposure. However, none of the doses were any more effective than placebo, including the maximum tolerated dose previously shown to be effective with multiple short-term exposures over a year (9). With the sample of 175 women, there was 80% power to detect a difference of regression to normal ranging from 15% to 30% with a significance of 5%. Therefore, the lack of detecting an effect of atRA compared with placebo was not a result of inadequate sample size.

The placebo response (47%) was significantly higher than previous studies using atRA (25-27%; ref. 9), but was in the range of other studies (14, 15). There are several possible etiologies for the higher placebo response. First, the synthetic sponge may have induced a more pronounced biological response than the collagen sponge used in previous studies (6, 7). Second, recent implementation of folate fortification increases the likelihood that the current study participants had adequate folate levels. However, we have no folate data on the study participants to verify this hypothesis. Folate alone has not been shown to be an effective therapy for CIN II or CIN III (16, 17), but interaction with other agents may have some impact. Third, the placebo may

have had biologically active ingredients. This is unlikely as the placebo agent was supplied by the same manufacturer as in previous studies. During the study, samples of the placebo had no evidence of atRA or any other agents than the compounding ingredients (Bio-Science Research Institute, Chino, CA; data not shown). Fourth, women on the active agent arms of the study may not have been exposed to the drug at specified concentrations. From each drug concentration batch compounded during the study, three samples were assayed for atRA. All drug samples were within ± 2% of specified atRA concentration (Bio-Science Research Institute, data not shown). Finally, the serial biopsies may have been an effective treatment for some women with small lesions or the biopsy may have missed the lesion at week 12. We were not able to require that all study participants undergo loop electrosurgical excision procedure, which would have provided a more definitive end-point assessment at week 12. We were also unable to follow participants beyond the completion of the study protocol.

These findings and a recent report on oral 9-*cis*-retinoic acid (15) suggest that retinoids, oral or topical, do not seem to be an effective chemopreventive strategy for women with CIN. Neither approach is more effective than placebo. The topical approach previously thought to be effective remains impractical (9) and does not provide any advantage over the current ablative strategies. It remains to be determined if longer acute exposure to topical atRA, such as 2 to 3 weeks, might be more effective than placebo. However, prophylactic and therapeutic HPV vaccines may prove to be more efficacious. As noted in Table 3, both baseline disease status and baseline HPV status are each significant predictors of elimination of disease at 12 weeks. Other measures linked to HPV status such as viral load, presence of HPV variants, and expression of E6 or E7 may further identify women likely to clear the disease.

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