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A Phase I Trial of β-*all-trans*-Retinoic Acid Delivered via a Collagen Sponge and a Cervical Cap for Mild or Moderate Intraepithelial Cervical Neoplasia[1,2,3]

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ABSTRACT—A phase I trial was conducted of the vitamin A derivative β-*all-trans*-retinoic acid (vitamin A acid; TRA), delivered via a collagen sponge and cervical cap for mild or moderate intraepithelial cervical neoplasia. On the basis of known skin and mucosal membrane toxicity, a concentration of 0.05% TRA in a cream-based vehicle was selected as the starting dose and was escalated later with the use of a modified Fibonacchi scale. The delivery device and the TRA were changed daily for 4 days, and side effects were assessed on days 1, 2, 3, 4, 8, and 30 by clinical and colposcopic examination. Vaginal, cervical, and systemic toxicity were evaluated in 35 patients. No dose-related systemic effects were found; mild cervical inflammation increased in many patients at higher doses. Unacceptably high vaginal toxicity was reached at a TRA concentration of 0.484%. A concentration of 0.372% TRA is recommended for use in phase II trials in mild and moderate cervical intraepithelial neoplasia.—JNCI 1983; 71:921–925.

Whether they have been initiated by physical, chemical, or viral carcinogens, vitamin A and its natural and synthetic derivatives (retinoids) can block the phenotypic progression of cells to cancer (*1, 2*). Numerous investigators have shown that retinoids inhibit cellular proliferation of many transformed cells (*3*), an event accompanied by evidence of cellular differentiation or maturation in many systems including murine (*4*) and human melanoma (*5*), human neuroblastoma (*6*), and promyelocyte (*7*) cells. Clinical responses of preneoplastic (*8, 9*) and neoplastic (*10, 11*) skin lesions to TRA have been reported, and we have recently detected activity of 13-*cis*-RA against neoplastic lesions of squamous cell histology (*12*).

Chemoprevention for certain human cancers should be possible on the basis of data on the retinoids (*1, 2*) and on many other compounds (*13*) in experimental animals. A major problem with pharmacological intervention for human precancerous conditions is the inevitable toxicity that is expected to occur with the ingestion of almost any chemopreventive agent (*14, 15*). Although a tremendous effort was committed to develop nontoxic chemopreventive agents for human use, particularly with regard to retinoids, it is unlikely that the design of a completely nontoxic, tissue-selective chemopreventive compound will be achieved. As we have discussed elsewhere, an alternative approach is to develop devices capable of selective local or regional delivery of the chemopreventive compound (*14*).

We have previously tested a collagen sponge and diaphragm insert to deliver TRA to the cervix, but we found an undesirable leakage of the retinoid preparation onto the vagina and vulva (*16*). Other side effects were mild, and we noted the development of early changes in the intraepithelial neoplasias. These results were sufficiently encouraging, so we developed an alternative and effective delivery device, which consists of an inert collagen sponge and cervical cap and eliminates the problem of leaking. We report here a phase I trial of TRA, delivered via a collagen sponge serving as a drug reservoir fitted into a cervical cap, for mild or moderate intraepithelial cervical neoplasia in 35 patients.

MATERIALS AND METHODS

Patient characteristics.—All patients entered into this trial had colposcopically directed biopsies which showed mild or moderate dysplasia. The lesion and the entire transformation zone (squamocolumnar junction) were completely visualized. Endocervical currettage had negative results in all patients, and the pretreatment Papanicolaou smear result was consistent with that of the ectocervical biopsy. In all patients the extent of disease was documented with colpophotographs; all had negative vaginal cultures for *Staphylococcus aureus.*

TRA administration.—TRA was dissolved in a cream-based vehicle, which contained polyethylene glycol 400, butylated hydroxytoluene, and 55% alcohol. TRA was incorporated into the cream under special minimal UV lighting (Sylvania Red F96T12-R tubes) to prevent photoisomerization and decomposition. Uniformity and content of TRA was confirmed by reverse-phase high-pressure liquid chromatography with the use of a previously published assay method (*17*). The prepared creams were also negative for endotoxin pyrogens according to the

ABBREVIATIONS USED: RA=retinoic acid; TRA=β-*all-trans*-retinoic acid.

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[3] Research procedures were in accord with the ethical standards of the University of Arizona Committee on Human Subjects.

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Limulus amebocyte lysate method (Pyrogent; Mallinckrodt Inc., St. Louis, Mo.). On the basis of our prior studies (*16*), and skin and mucosal side effects to TRA previously reported by us (*11, 12*) and by others (*10*), we selected 1 ml 0.05% TRA as the initial dose. Escalation of dosage followed a modified Fibonacchi scale (*see* table 3).

Delivery system.—The delivery system consisted of a cervical cap within which a collagen sponge was inserted (fig. 1). The cervical cap was made of the hydrogel Hypan (Sky Biopolymers, Princeton, N.J.) which, when in contact with wet tissue surfaces, adheres to them by a force of differential osmotic pressures. The characteristics and properties of the collagen sponge are extensively described elsewhere (*18*). The sponges were made from pure collagen isolated from bovine skin, swollen at pH 3.0, and stabilized into the physical form of a sponge layer. Glutaraldehyde was used as a cross-linking agent to provide high resilience and fluid-binding capacity. The average pore size was 400 Å (range 80–1,400 Å). The sponges were cut into thin, round wafers approximately 3–4 mm thick and 7 mm in diameter.

Schedule of TRA administration.—One milliliter of fresh retinoid preparation was applied to the sponge, and the sponge–cervical cap device was carefully inserted by the investigator into the vaginal vault. The position of the cap around and against the cervix was documented by clinical examination. Patients returned daily for removal of the sponge–cap insert. One milliliter of fresh drug and vehicle was applied to a new sponge, and the delivery device was reapplied. This procedure was repeated daily for a total of 4 days.

Evaluation of side effects.—We evaluated toxicity by clinical examination, colposcopy, and colpophotographs at the time of the second, third, and fourth collagen sponge applications and 1 week and 1 month after the initial application. Liver function tests were performed prior to entry into study and on the 4th day of therapy. Our grading system for cervical and vaginal toxicity is outlined in tables 1 and 2, respectively.

TABLE 1.—*Clinical and colposcopic grading for cervical effects*

Grade of cervical effect	Description of symptoms	Optional action (at each 24 hr)
0	None	Continue treatment
1^+ (mild)	Erythema	Continue treatment another 24 hr at same concentration
2^+ (moderate)	Erythema, vascularity	Continue treatment another 24 hr at same concentration
3^+ (severe)	Ulceration	Stop trial in patient. Do not escalate dose in next patient and treat 3 more patients at this dose before further escalation.

RESULTS

Side effects.—The systemic, cervical, and vaginal toxicity in these patients as related to the dose of RA is given in table 3.

Systemic side effects were mild and not clearly related to the RA dose. Patients demonstrated acceptable cervical side effects (1^+–2^+) to all dose levels tested. We did not note cervical changes by colposcopy until after 2 days of application in most cases. Mild inflammation was the most frequent side effect in 22 patients followed by a moderate increase in vascularity in 19 cases. Only 1 of 7 patients treated with less than 0.067% RA experienced cervical inflammation. Most patients experienced cervical inflammation with increasing dose and increased duration of treatment. Inflammations among the 35 patients were recorded 2, 3, 4, and 8 days after the start of the trial in 10, 11, 21, and 7 patients, respectively. Subsequent to the inflammatory changes, an increase in cervical vascularity was noted in 8, 12, 16, and 3 patients 2, 3, 4, and 8 days, respectively, after initiation of the trial.

Vaginal side effects occurred at all dose levels and were mild in 23 patients at concentrations of 0.05–0.372% TRA. Moderate vaginal toxicity was noted in 5 of 21 patients at TRA concentrations of 0.1167–0.372%. At an RA concentration of 0.484%, all 3 patients treated developed a moderate amount of discharge and 1 of 3 patients developed both bleeding after the last application, lasting for 1 day, and severe and prolonged vaginal burning, lasting 12 days after the last application.

Follow-up results.—Table 4 shows a summary of the colposcopic, Papanicolaou cytology, and tissue biopsy changes in patients whose pretreatment and 6-month follow-up results were available. Twenty-nine patients were evaluated by colposcopic mapping. The extent and/or severity of the lesion were decreased more than 50% in 23 patients. The lesion did not worsen in any of the patients. Among the 25 patients in whom Papanicolaou's cytology test was available, fewer dysplastic cells

TABLE 2.—*Grading of vaginal toxicity*

Grade of toxicity	Description of toxicity	Optional action (at each 24 hr)
0	None	Continue treatment.
1^+ (mild)	Mild burning, irritation, itching, or discharge not bothersome to patient (elicited from the patient)	Continue another 24 hr at same concentration.
	Moderate burning, irritation, itching, or discharge not bothersome to patient (described by the patient)	Continue another 24 hr at same concentration.
2^+ (moderate)	Severe (bothersome) burning, itching, or irritation; discharge requiring a pad; erythema and/or increasing vascularity of vaginal mucosa	Stop trial in that patient. Do not escalate dose in next patient and treat 3 more patients at this dose before further escalation.
3^+ (severe)	Burning, itching, irritation, or discharge leading to discontinuation of treatment; ulceration and/or bleeding	Stop trial in that patient. Do not escalate dose in next patient and treat 3 more patients at this dose before further escalation.

TABLE 3.—*Side effects and toxicity of TRA applied to cervical dysplasia: Phase I trial*[a,b]

Patient	Concentration of TRA	Grades of signs and symptoms on days: Cervical		Vaginal				Systemic						
		Inflammation	Vascularity	Discharge	Itching	Irritation	Burning	Appetite loss	Fatigue	Headache	Nausea	Dizziness	Change in moods	Lower abdominal discomfort
#1	0.05		2^+, $D_{3,4}$	1^+, $D_{3,8}$										
#2	0.05		2^+, $D_{3,4,5}$	1^+, D_5				1^+, D_4						
#3	0.05		2^+, $D_{2\,3,4}$	1^+, $D_{3,4,5}$	1^+, $D_{6,7}$		1^+, D_4	1^+, D_5	1^+, D_5	1^+, D_5				
#4	0.05		2^+, $D_{4,8}$	1^+, $D_{2,3,4}$	1^+, $D_{5,6,7}$									
#5	0.0667		2^+, $D_{2,4}$											
#6	0.0667	1^+, $D_{2-4,8}$	2^+, $D_{2,3,8}$											
#7	0.0667						1^+, $D_{4,5}$							
#8	0.0833			1^+, $D_{3,4,5}$		1^+, $D_{2,4,5}$								
#9	0.0833	1^+, D_4	2^+, D_4	1^+, D_{4-8}	1^+, D_3				1^+, D_{5-9}					
#10	0.0833	1^+, $D_{3,4}$	2^+, $D_{3,4}$	1^+, $D_{4,5}$	1^+, D_6		1^+, $D_{4,6}$		1^+, D_{4-9}	2^+, $D_{2,5-7}$			2^+, D_2	
#11	0.0833	1^+, $D_{3,4}$	2^+, $D_{1,3,4}$			1^+, $D_{3,4}$	1^+, $D_{2,4}$							
#12	0.1167			1^+, $D_{2,3,4}$										
#13	0.1167			1^+, D_4					1^+, D_4		1^+, D_4	2^+, D_4		
#14	0.1167	1^+, $D_{3,4}$	2^+, D_3											
#15	0.1167	1^+, $D_{3,4,5}$	2^+, D_3	1^+, $D_{2,3}$			1^+, $D_{7,8}$	1^+, D_3						Mild D_2
#16	0.1167	1^+, $D_{2,4}$	2^+, $D_{2,4}$	1^+, D_{5-8}								2^+, $D_{2,3}$		
#17	0.1583	1^+, $D_{3,4}$		1^+, D_{2-7}		2^+, $D_{4,5}$								
#18	0.1583	1^+, $D_{2-4,8}$	2^+, $D_{3,4}$	1^+, D_4					1^+, D_3	1^+, D_4				Mild $D_{3,4}$
#19	0.1583	1^+, $D_{2-4,9}$	2^+, $D_{1-4,9}$	2^+, D_{3-8}	1^+, D_{4-8}	1^+, D_{4-8}								Moderate–severe
#20	0.1583			1^+, $D_{2,4,5}$										
#21	0.21			1^+, $D_{3,4}$			1^+, D_4			1^+, D_2				
#22	0.21	1^+, $D_{2-4,8}$		1^+, $D_{2,3}$										Mild $D_{1,2,3}$
#23	0.21	1^+, $D_{2,3,4}$		2^+, $D_{3,4}$				1^+, D_2						
#24	0.28	1^+, D_2		1^+, $D_{2,3,4}$						1^+, D_3				
#25	0.28	1^+, D_4		1^+, $D_{2,3,4}$	2^+, D_8	2^+, D_8	2^+, D_4						2^+, D_{3-8}	Mild D_2
#26	0.28	1^+, $D_{2,3,4}$	2^+, $D_{2,3,4}$	1^+, $D_{2,3}$	1^+, D_8	1^+, D_2							2^+, D_{2-8}	Mild D_4
#27	0.28	1^+, $D_{2,3,4}$	2^+, $D_{3,4}$	2^+, D_{2-5}	2^+, D_{4-8}	2^+, D_{4-8}	2^+, D_{2-3}			1^+, D_4			2^+, D_{4-8}	
#28	0.372	1^+, $D_{3,4}$		1^+, $D_{3,4,5}$							1^+, $D_{4,5}$			Mild D_4
#29	0.372	1^+, D_4		1^+, D_{2-5}	2^+, $D_{4,5}$	2^+, $D_{4,5}$	2^+, D_{4-7}							
#30	0.372			1^+, D_{3-6}	1^+, D_6		1^+, D_3							
#31	0.372	1^+, $D_{2-4,8}$	2^+, $D_{2,3,4}$	1^+, D_{2-5}										
#32	0.372	1^+, $D_{2,3,4}$		1^+, $D_{2,3,4}$			2^+, D_{5-8}							
#33	0.484			2^+, D_{2-5}			3^+, D_{5-16}							
#34	0.484	1^+, $D_{2,3,4}$	2^+, $D_{2,3,4}$	2^+, D_{2-7}	1^+, $D_{2,3,4}$		2^+, D_{4-8}			1^+, D_2				Mild D_2
#35	0.484	1^+, D_4	2^+, $D_{3,4}$	2^+, D_{2-9}	2^+, D_{4-7}	2^+, $D_{2,3}$	2^+, $D_{5,6,7}$							

[a] *See* tables 1 and 2 for key to grades of signs and symptoms.
[b] D = day of trial at which side effect occurred; No. of days are indicated by subscripts.

TABLE 4.—*Changes in intraepithelial lesions 6 months after retinoid treatment*

Extent and severity of lesions	No. of cases
Colposcopy	
Increased	0
No change	4
Decreased 25%	2
Decreased 50%	6
Decreased 75–100%	17
Papanicolaou's cytology test	
Worsened	4
No change	12
Improved	9
Biopsy	
Worsened	3
No change	12
Improved	7

developed in 9 patients, whereas in 4 cases more dysplastic cells were identified. Biopsy results of involved tissue or of an area adjacent to the worst initial lesion (identified by colposcopy) were available from 22 patients. In the majority, (12 patients), the lesion was unchanged. However, in 7 patients the lesion became more benign, whereas in 3 the histology worsened.

DISCUSSION

Chemotherapy of cancers in humans by the reversal of established preneoplasia is a new concept that evolved from encouraging results in an extensive number of laboratory and clinical studies (*1–13*). The present chemoprevention trial provides data on the side effects and toxicity of a collagen sponge–cervical cap delivery system for topically applied TRA to intraepithelial neoplasia of the cervix.

Systemic side effects were mild and probably related to the close monitoring and frequent pelvic examinations, rather than to TRA, inasmuch as no dose-response relationship was evident. In our previous study no evidence of systemic absorption of retinoids could be documented by high-pressure liquid chromatographic measurements (*16*). Cervical alterations by colposcopy were noted in 19 of 37 patients on day 8. None of the TRA concentrations used in this study produced unacceptable cervical toxicity. The effects of TRA on the cervix were mild and similar to those we have reported with the use of a collagen sponge–diaphragm delivery system (*16*), and they represent clear evidence of the effect of the retinoid. In addition, we used tritium labeled TRA to document uptake into the cervical tissue (Meyskens FL, Alberts DS, Survit EA: Unpublished data).

Vaginal side effects were predominantly mild from a dose of 0.05–0.372% TRA and consisted of increased discharge, itching, irritation, and burning during the 4-day application period. With a concentration of 0.372% of TRA, moderate (2^+) itching, irritation, and burning occurred after completion of the 4-day application in 2–5 patients. With a concentration of 0.484% TRA, all 3 patients experienced unpleasant vaginal side effects. Since the worst vaginal toxicity occurred after the cervical delivery device was removed, we presume that these side effects represented continued leaching of TRA from a saturated cervix.

The evaluation of cervical intraepithelial neoplasia is complex. Most studies in animals indicate that the retinoid must be available continuously for maximal preventive benefit. Preliminary assessment consists of cytologic analysis and colposcopic evaluation to provide information on the extent of the lesion. Complete normalization of cytologic and colposcopic findings and a negative tissue biopsy specimen indicate the resolution of intraepithelial neoplasia. In this study, we did not see complete resolution of preneoplastic conditions. Nevertheless, our pretreatment evaluations suggest that colposcopic and histologic improvement can be achieved in some patients. However, a definitive statement will require extensive follow-up, and in particular, a randomized trial as discussed below.

Data from this study suggest that TRA delivered to the cervix via a collagen sponge–cervical cap insert is both feasible and well tolerated. The absence of significant systemic side effects after 4 days of treatment suggests that the development of a local "maintenance" retreatment protocol is reasonable. A concentration of 0.484% TRA appears to be dose limiting because it causes delayed vaginal toxicity. On the basis of these conclusions, we plan to treat patients with moderate intraepithelial neoplasia with a 4-day application of 0.372% TRA, then retreat for 2 days every 3 months. These patients will be carefully monitored for systemic effects in this long-term trial. The results from such a trial will have important implications for the long-term use of retinoids for preneoplasia of the cervix and for other precancerous conditions.

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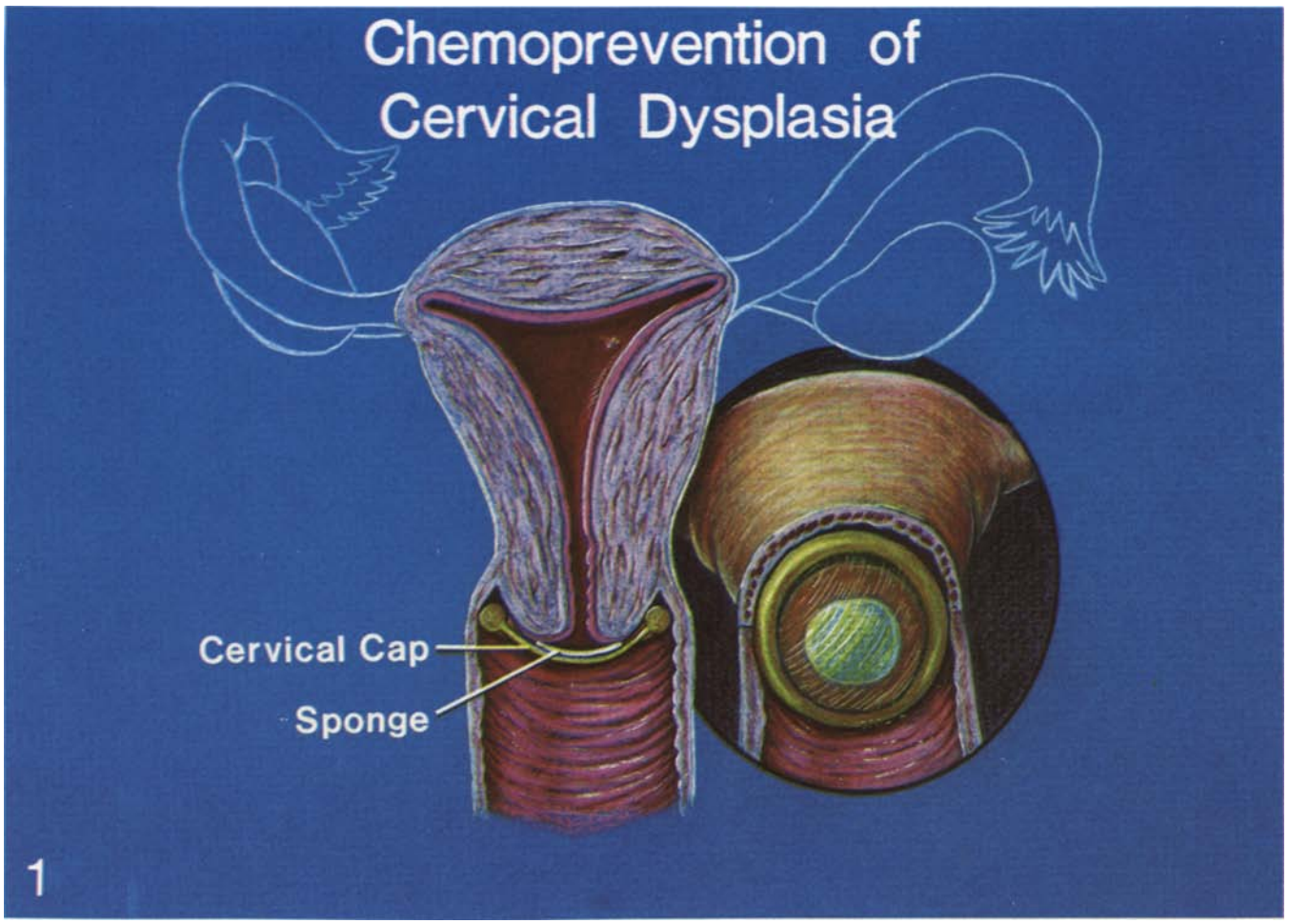

FIGURE 1.—Collagen sponge and cervical cap delivery device. The inert collagen sponge was impregnated with 1 ml TRA and placed in the cervical cap. The entire delivery device was placed over the cervix and remained attached by differential osmotic pressure, which created a leak-proof seal. The complete setup was changed daily for a total of 4 days, and systemic, vaginal, and cervical toxicities were evaluated.