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Cervical tissue uptake of all-trans-retinoic acid delivered via a collagen sponge-cervical cap delivery device in patients with cervical dysplasia

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Key words: cervical dysplasia, all-trans-retinoic acid, tissue uptake of anticancer drug, collagen sponge-cervical cap

Abstract

The present study was undertaken to evaluate the systemic absorption and cervical tissue uptake of all-trans-retinoic acid (TRA), delivered via a collagen spongecervical cap delivery device in patients with intraepithelial cervical dysplasia. Ten patients with histologically proven mild or moderate cervical dysplasia were included in this pharmacologic study. The two TRA concentrations (0.05% and 0.372%) selected for study represent the starting and maximally tolerated doses used in phase I clinical trial. All-trans-retinoic-11-^{3}H acid (^{3}H-TRA, 500 μCi) was used to facilitate cervical tissue uptake studies. Cervical biopsies and post-treatment blood samples were obtained from each patient after TRA exposure. The uptake of TRA into cervical tissues four hours after drug administration was significantly increased at the maximally tolerated TRA dose. There was a rapid decrease in cervical tissue concentration of TRA at the 0.372% dose between 4 and 24 h after drug exposure, suggesting a relatively short elimination half-life of TRA in cervical tissues. HPLC analysis of post-treatment blood samples indicate that there was no systemic absorption of TRA after local cervical administration.

Introduction

The naturally occurring and synthetic retinoids have attracted interest in recent years as potential chemoprevention agents. These compounds have been shown to prevent several chemically induced animal tumors (1–5). In humans, the retinoids have also caused regressions of premalignant lesions such as actinic keratosis (6) and leukoplakia (7). On these bases, clinical trials have been organized to evaluate the activity of the retinoids against a variety of other precancerous lesions, including cervical dysplasia.

For the retinoids to prove useful for the treatment of precancerous lesions, the administration route should be associated with little or no systemic toxicity. An alternative approach to systemic delivery of the retinoids involves local or regional administration, which should minimize systemic exposure and obviate systemic toxicity. With this approach in mind, we designed and have reported a phase I clinical trial of all-trans-retinoic acid (TRA), delivered via a collagen sponge fitted into a cervical cap for mild or moderate intraepithelial cervical dysplasia (8). An important feature of this trial was an evaluation of systemic absorption and quantitation of cervical tissue uptake of TRA.

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Materials and methods

Patients

Ten patients with histologically proven mild or moderate cervical dysplasia, who were participating in our phase I study, were included in this pharmacologic study. All patients were administered TRA locally to the cervix using a cervical cap with a collagen sponge insert. All patients signed informed consent documents (approved by University of Arizona Institutional Review Board).

Materials

All-trans-retinoic acid (0.05% and 0.372%) and placebo cream (which also contained polyethylene glycol 400, butlyated hydroxytoluene, and 55% alcohol) was generously supplied by G. Thorne of Ortho Pharmaceuticals (Raritan, NJ). Uniformity and content of TRA in this vehicle was confirmed by high performance liquid chromatography (HPLC) analysis (9). All-trans-retinoic-11-^{3}H acid (^{3}H-TRA, specific activity 5.12 mCi/mg) was purchased from the National Cancer Institute (Bethesda, MD) to facilitate uptake studies. The purity of ^{3}H-labeled TRA was greater than 97%. Retinoid reference standards for HPLC analysis were purchased from Sigma Chemical Company (St. Louis, MO) or provided as a gift from Hoffman-La Roche (Nutley, NJ). All reagents were of highest chemical purity available (A.C.S. certified grade). All organic solvents were glass-distilled (Burdick and Jackson, Muskegon, MI).

Special precautions

All procedures involving the manipulation of retinoids or biological samples containing retinoids were performed in the dark to prevent ultraviolet light degradation.

TRA delivery system

The TRA delivery system consisted of a cervical cap within which a collagen sponge was inserted. The characteristics and properties of the cervical cap and collagen sponge have been described extensively elsewhere (8, 10). The cervical cap was made of hydrogel Hypan (Sky Biopolymers, Princeton, NJ), which when in contact with wet tissue surfaces, adheres to them by the force of differential osmotic pressures. 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-building capacity. The average pore size was 400°A. The sponges were cut into thin, round wafers approximately 3–4 mm thick and 7 mm in diameter.

TRA administration and tissue sampling procedures

To study the uptake of TRA into cervical tissues in patients with cervical dysplasia, 500 μCi of ^{3}H-TRA (0.0917 mg) plus 1 ml of cold TRA (0.05% and 0.372%) were applied to the collagen sponge. The sponge-cervical cap device was carefully inserted into the vaginal vault. The position of the cap around and against the cervic was documented by clinical examination. The sponge-cervical cap remained in position for 24 h.

The two TRA concentrations selected for study (i.e. 0.05% and 0.372%) represent the starting and maximally tolerated doses used in the phase I clinical trial. Because the 0.372% dose was well tolerated with minimal vaginal irritation and tolerable cervical inflammation, it was selected for future phase II clinical trials (11).

Cervical biopsies (endocervical curettage, deep stroma, and epithelial cervix) were obtained from each patient at 4 and 24 h after drug exposure. Biopsy samples were rinsed thoroughly with physiological saline and the wet weights were measured. Post-treatment blood samples were obtained at 0, 1, 2, 4, 8, 12 and 24 h after TRA ex-

posure in selected patients. Blood samples were centrifuged immediately and plasma was separated. All samples were foil-wrapped and stored at −80°C freezer for subsequent analysis.

Measurement of TRA uptake

To measure ^{3}H-TRA concentrations in cervical biopsy materials, samples were oxidized completely to tritiated water and CO_2 by a Packard Tri-Carb Sample Oxidizer (BO 306). The tritiated water was trapped using 12 ml monophase in glass vials. The radioactivity was counted for 10 minutes using a Beckmann Liquid Scintillation System (LS 100 C).

HPLC analysis

HPLC analysis of retinoids in plasma was performed as previously described (9). Briefly, 50 μl of 5% perchloric acid were added to a 500-μl aliquot of plasma in a microcentrifuge tube and vortexed for 30 s. A 500-μl aliquot of ethyl-acetate was added and the samples were vortexed for 60 s and centrifuged at 13,000 xg for 1 min using a microcentrifuge (Fisher Scientific Model 235). Fifty microliters of the resulting organic layer were analyzed by HPLC.

HPLC analysis was performed with the use of two Waters Associates (Milford, MA) Series M45 solvent delivery systems, a Model 710B WISP autoinjector, a Model 730 data module, a Model 720 system controller, and a Model 440 dual-wavelength UV detector. Two Bio-sil ODS-10 columns (150 × 4 mm, Bio-rad Laboratories, Richmond, CA) connected in series were used for all analyses. The mobile phase consisted of 75% acetonitrile and 25% of a 1% aqueous ammonium acetate solution delivered at a flow rate of 2.5 ml/min. Retinoids were detected at 340 and 365 nm.

Table 1. Uptake of TRA into cervix tissues in patients with mild and moderate cervical dysplasia.

Patient no.	Dose TRA[a] (%)	Dose TRA[a] (mg)		Tissue uptake of total TRA (ng/g wet wt)					
		Cold	^{3}H	At 4 hr			At 24 h		
				Endocervical curettage	Deep/ stroma	Epithelial cervix	Endocervical curettage	Deep/ stroma	Epithelial cervix
1	0.05	0.5000	0.0917	4.13	70.90	112.25	4.90	69.81	70.77
2	0.05	0.5000	0.0917	1.16	106.71	152.65	0.26	14.45	32.65
3	0.05	0.5000	0.0917	1.29	8.97	266.00	NA[b]	0.90	3.16
4	0.05	0.5000	0.0917	1.41	226.03	251.02	NA	2.05	60.77
5	0.05	0.5000	0.0917	0.38	77.18	22.05	52.56	3.59	NA
			Mean	1.67	97.96	160.79	19.24	16.02	44.90
			(SD)	(0.64)	(35.75)	(45.21)	(16.71)	(13.46)	(21.07)
6	0.372	3.7200	0.0917	392.12	392.53	1,306.64	101.24	3.73	NA
7	0.372	3.7200	0.0917	3.32	242.32	94.19	0.42	17.43	0.42
8	0.372	3.7200	0.0917	NA	809.96	2,675.52	1.25	19.50	74.27
9	0.372	3.7200	0.0917	9.54	8,406.22	4,721.99	12.03	90.87	50.62
10	0.372	3.7200	0.0917	4.15	541.49	968.88	0.83	95.85	155.19
			Mean	102.28	2,078.50	1.953.44	23.15	45.48	70.13
			(SD)	(96.60)	(1,584,70)	(807.18)	(19.64)	(19.75)	(32.27)
Mann-Whitney P-value:				0.028	0.009	0.076	0.882	0.059	0.724

[a] TRA – All-trans-retinoic acid provided by Ortho Pharmaceuticals, Raritan, New Jersey.
[b] NA: Sample not available.

Results

We studied five patients after both low (0.05%) and high (0.372%) dose administration of TRA. The cervical tissue TRA uptake results are summarized in Table 1. Tissue concentrations of TRA were calculated on the basis of ng of ^{3}H-TRA equivalents per g wet weight of curettage or biopsy sample. At the 4-h sampling time point, the endocervical and deep stroma samples obtained from the five patients administered 0.372% TRA had significantly higher concentrations of ^{3}H-TRA equivalents than the five patients administered the 0.05% TRA dose.

Mean concentrations of ^{3}H-TRA equivalents were not significantly higher ($p = 0.076$) in the epithelial cervix biopsies following the higher versus lower concentrations of TRA; however, these results were affected by the inordinately low tissue concentration of TRA in patient number seven. In contrast to these 4 h data differences, concentrations of ^{3}H-TRA equivalents in cervical tissues did not differ significantly between the low and high doses of TRA at the 24 h sampling time point. The concentration of ^{3}H-TRA equivalents in cervical tissues were significantly lower at 24 h as compared to concentrations of 4 h after administration of the 0.372% doses, suggesting a relatively short elimination half-life of TRA in cervical tissues.

The measurement of TRA in post-treatment blood samples indicated that there was no systemic absorption of TRA after local cervical administration for up to 24 h post-treatment.

Discussion

Chemoprevention of human cancers by the modulation or suppression of preneoplasia is a relatively new concept (1–7). The use of TRA delivered via a collagen sponge-cervical cap device in human subjects with intraepithelial neoplasia of the cervix is similarly unique (8). Initial phase I studies of TRA administered via a cervical cap with a collagen sponge insert have validated this novel drug delivery method and identified a maximally tolerated dose (11), which was used in our phase II trials (12).

The results of the present disposition studies document the uptake of ^{3}H-TRA equivalents into various cervical tissues after direct application. The uptake of TRA into cervical tissues 4 h after administration was significantly increased at the maximally tolerated TRA dose. This dose-response relationship adds credibility to the collagen sponge-cervical cap method of localized drug delivery in patients with cervical dysplasia and provides additional rationale for phase III trials. The rapid decrease in cervical tissue concentrations of TRA at the 0.372% dose between 4 and 24 h after local application suggests that optimal therapeutic results may require daily TRA dosing schedules at least for induction of remission in patients with cervical dysplasia.

The lack of measurable TRA concentrations in plasma samples of patients treated even at the highest doses of topically applied TRA correlates well with the results of the phase I and II clinical trials which revealed no evidence of significant drug-related systemic toxicity. Thus, this novel drug delivery device is associated with high local concentrations of TRA without causing systemic symptoms, a result which was needed to validate the future use of this new drug delivery method.

Acknowledgments

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