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REPORTS

Enhancement of Regression of Cervical Intraepithelial Neoplasia II (Moderate Dysplasia) With Topically Applied All-*trans*-Retinoic Acid: a Randomized Trial

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Background: **Retinoids enhance differentiation of most epithelial tissues. Epidemiologic studies have shown an inverse relationship between dietary intake or serum levels of vitamin A and the development of cervical dysplasia and/or cervical cancer. Pilot and phase I investigations demonstrated the feasibility of the local delivery of all-*trans*-retinoic acid (RA) to the cervix using a collagen sponge insert and cervical cap. A phase II trial produced a clinical complete response rate of 50%. *Purpose:* This randomized phase III trial was designed to determine whether topically applied RA reversed moderate cervical intraepithelial neoplasia (CIN) II or severe CIN. *Methods:* Analyses were based on 301 women with CIN (moderate dysplasia, 151 women; severe dysplasia, 150 women), evaluated by serial colposcopy, Papanicolaou cytology, and cervical biopsy. Cervical caps with sponges containing either 1.0 mL of 0.372% β-*trans*-RA or a placebo were inserted daily for 4 days when women entered the trial, and for 2 days at months 3 and 6. Patients receiving treatment and those receiving placebo were similar with respect to age, ethnicity, birth-control methods, histologic features of the endocervical biopsy specimen and koilocytotic atypia, and percentage of involvement of the cervix at study. Treatment effects were compared using Fisher's exact test and logistic regression methods. Side effects were recorded, and differences were compared using Fisher's exact test. *Results:* RA increased the complete histologic regression rate of CIN II from 27% in the placebo group to 43% in the retinoic acid treatment group (P = .041). No treatment difference between the two arms was evident in the severe dysplasia group. More vaginal and vulvar side effects were seen in the patients receiving RA, but these effects were mild and reversible. *Conclusions:* A short course of locally applied RA can reverse CIN II, but not more advanced dysplasia, with acceptable local side effects. *Implications:* A derivative of vitamin A can reverse or suppress an epithelial preneoplasia, lending further support to the notion that chemoprevention of human cancer is feasible.** [J Natl Cancer Inst 86:539-543, 1994]

Vitamin A and its natural and synthetic derivatives (retinoids) are potent stimulators of cellular response and affect the biological function of a diversity of organs and tissues (*1*). Both in vitro and in vivo, retinoids modulate the growth of normal epithelial cells, in general slowing or inhibiting proliferation and enhancing differentiation and maturation of cells and tissues (*2,3*). A large number of epidemiologic studies have demonstrated a protective effect of vitamin A against smoking-related tumors (*4*) and, in the last decade, against cervical dysplasia and cancer (*5*). These observations supported the notion that vitamin A may affect the natural history of cervical intraepithelial neoplasia (CIN) and cervical malignancy.

We have conducted a series of preclinical studies and phase I and II trials (*6-10*) in CIN and demonstrated the feasibility of chemoprevention of cervical cancer. Our initial studies were directed to developing an optimal local delivery device to the cervix for all-*trans*-retinoic acid (RA). The device consisted of a cervical cap and collagen sponge insert within which the RA was placed (*6,7,11*). A phase I trial was done, and a toxic dose was defined (*8*). Based on this experience, the next lower dose, which was well tolerated, was chosen for use in a subsequent phase II investigation. The distribution of responses in the phase I study suggested a dose–response effect (*9*). A phase II trial among 20 patients demonstrated an encouraging complete clinical response rate of 50% in CIN (*10*). On the basis of these results, we designed a randomized phase III trial of topical RA in placebo cream versus placebo cream in patients with moderate and severe cervical dysplasia.

Patients and Methods

Patient Eligibility

Criteria for eligibility were as follows: (*a*) biopsy-proven moderate or severe dysplasia; (*b*) complete colposcopic delineation of the lesion after biopsy specimens were taken; (*c*) endocervical curettage negative for severe dysplasia, carcinoma in situ, or invasive carcinoma of the cervix; and (*d*) negative pregnancy test and plan not to become pregnant while under treatment. All patients

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received oral contraceptives, used an intrauterine device, or were placed on an effective form of barrier contraception. Patients who had had prior treatment by cauterization, cryosurgery, laser, or conization were eligible as long as this treatment was administered 6 or more months prior to study entry.

Criteria for exclusion included the following: (*a*) prior or concomitant genital malignancy, (*b*) previous pelvic irradiation, (c) in utero diethylstilbestrol exposure, or (*d*) prior retinoid treatment for any reason (>25 000 IU vitamin A/d or the equivalent for 3 months).

Written informed consent was obtained from each patient in adherence to the guidelines of the Institutional Review Board of the University of Arizona. All patients were specifically warned of the potential teratogenic effects of the RA and that the use of this compound in this manner was experimental.

Cervical biopsy specimens (and accompanying Papanicolaou smears) classified at local centers as moderate or severe squamous dysplasia identified patients who were potentially eligible for the study. Entry in the trial occurred only after biopsy confirmation by the project pathologist (J. R. Davis). The confirmation was initiated by the coordinator by submitting slides and the patient data form with code number. Slides (biopsy specimens and smears) were retained in the Department of Pathology for the duration of the project.

Histologic Classification of Dysplasia

The classification proposed by the World Health Organization was used (*12*). The various subgroups of cervical dysplasia were classified according to 1) the degree to which the cervical squamous epithelium was replaced by neoplastic cells and 2) the degree of atypia and maturation. Thus, lesions in which the neoplastic cells showed minimal atypism and extended one quarter or one third of the way from the basal layer to the surface were classified as mild dysplasia. Lesions in which the neoplastic cells showed greater atypism and extended through one half to two thirds of the epithelial layer were classified as moderate dysplasia, and those involving three quarters to 90% of the epithelium but showing some surface maturation were classified as severe dysplasia. Lesions demonstrating full-thickness replacement by neoplastic cells with marked atypism and no evidence of maturation were classified as carcinoma in situ; patients with these lesions were not eligible for study entry.

Histologic description included the following: (*a*) the degree of retention of nuclear polarity, based on the normal tendency of the squamous cells to assume a parallel rather than a perpendicular position with reference to the basement membrane, graded as mild, moderate, or marked; (*b*) the degree of loss of cellular maturation, based primarily on a nuclear–cytoplasmic ratio, graded as mild, moderate, or marked; (*c*) the presence of so-called "koilocytotic atypia," characterized by the presence of large cells with relatively small, irregular hyperchromatic nuclei and surrounded by clear, transparent cytoplasm (this cell has also been referred to as a koilocyte); and (*d*) extension into endocervical "glands" also specified and the extent graded as mild, moderate, or marked.

Cytologic description of dysplasia included the following: (*a*) mild dysplasia (CIN I)—mature, polygonal superficial, and intermediate squamous cells; clean background; small percentage of abnormal cells; nuclei slightly enlarged; and hyperchromatic with a finely granular chromatin pattern. (Nucleoli were not seen.) (*b*) moderate dysplasia (CIN II)—clean background; increased number of abnormal cells; intermediate cells; increased nuclear–cytoplasmic ratio; and nuclei hyperchromatic, usually with a finely granular chromatin pattern. (Nucleoli were not seen.) (*c*) severe dysplasia—clean background; increased number of abnormal cells, often occurring in sheets but maintaining definite cytoplasmic borders; immature, round, or oval parabasal cells; increased nuclear–cytoplasmic ratio; and increased hyperchromasia with finely granular chromatin pattern. (Nucleoli were not seen.)

Biopsy–Cytology Evaluation Reporting

Each biopsy specimen was evaluated separately by the project pathologist, and a written report with morphologic description and diagnosis was sent by the project pathologist to the coordinator. Each cytology specimen was evaluated separately by the project pathologist, and the results were also sent to the coordinator.

A Pathology Data Flow Record was maintained by the project pathologist. It captured the types of study (biopsy and cytology), the accession numbers of the study materials, dates of acquisition and position in the study, and diagnoses. In all instances of persistent dysplasia of moderate or severe degree, comparisons were made with prior study materials. The Pathology Data Flow Record captured all pathology data for use of review at project completion.

Patients had prestudy Papanicolaou cytology, colposcopic mapping of the cervix, biopsy of cervical lesion most involved by colposcopy, complete blood cell counts, and renal and liver function studies.

Therapeutic Regimen

Patients were stratified by degree of dysplasia (moderate or severe). They were randomly assigned to receive placebo cream or RA in the placebo cream.

Drug Requirements and Delivery

The drug characteristics and delivery system are described extensively elsewhere (*7,8*). The free chemical and cream-based vehicle were provided by George Weber (Ortho Pharmaceuticals, Raritan, N.J.), stored at –20 °C, and protected from light. RA was dissolved in a proprietary cream-based vehicle, which contained polyethylene glycol 400, butylated hydroxytoluene, and 55% alcohol. The retinoid was incorporated into the cream under special minimal UV lighting (Sylvania Red F96T12-R tubes) to prevent photoisomerization and decomposition. The uniformity and content of RA were confirmed by reverse-phase high-pressure liquid chromatography with the use of a previously published assay method (*7*). The batches of prepared creams were also negative for endotoxin pyrogens according to the *Limulus* amebocyte lysate method (Pyrogent; Mallinckrodt Inc., St. Louis, Mo.). On the basis of our phase I trial and acceptable results with side effects in our phase II study, we selected 1.0 mL of 0.375% RA (0.375 mg RA/100 g of cream by weight) administered daily as the treatment dose.

Delivery System

The delivery system consisted of a cervical cap within which a collagen sponge was inserted. 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 (*11*). 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, 80-1400 Å). The sponges were cut into thin, round wafers approximately 3- to 4-mm thick and 7 mm in diameter.

Schedule of RA Administration

One milliliter of a 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 was applied to a new sponge, and a fresh delivery device was reapplied. A new collagen sponge–cervical cap device with RA was inserted daily for 4 days. Patients returned at 3 and 6 months for follow-up and for maintenance treatment consisting of RA at a concentration of 0.372% inserted daily for 2 days. The frequency and duration of maintenance treatment were selected on the basis of the following factors (*11,13*): Two days of treatment was the minimum time at which cervical changes were evident; re-treatment for 2 but not for 4 days was acceptable to most patients; responses in the phase I trial lasted 3-4 months; and this frequency of re-treatment was deemed acceptable by most patients in a poll conducted during planning of the phase II study. This regimen was subsequently shown to be acceptable in our phase II trial (*10*).

The dropout rates during therapy (first 6 months) were similar in both arms, both for true dropouts and dropouts for protocol-specified reasons. True dropouts included 34 patients (22%) in the therapy arm and 35 patients (23%) in the placebo arm. Reasons for dropouts were as follows: patient ineligible (therapy arm, n = 1; placebo arm, n = 2), patient refused further follow-up (*7,9*), patient lost to follow-up (*14,15*), physician's decision (*2,3*), and other reasons (*4,5*). If severe dysplasia was evident at any time (including months 3 and 6 of maintenance treatment), a biopsy was performed; if the biopsy was indicative of severe disease, the patient was removed from the study. Ten patients in the treatment arm and 21 patients in the placebo arm had persistent severe biopsy-proven dysplasia at 3 or 6 months, and they were removed from the study. In addition, the moderate dysplasia progressed in four patients in the treatment arm to severe biopsy-

proven dysplasia in the first 6 months, while it did so in five patients in the control group. No patient had disease that progressed to carcinoma in situ during this trial.

Follow-Up

Patients had follow-up at intervals of 9, 12, 15, 21, and 27 months after study initiation with Papanicolaou smears and colposcopy. At 15 months (9 months after maintenance treatment), the designated primary end point of the study, all patients were evaluated for response by Papanicolaou smear, colposcopy, and biopsy. Some patients had persistent severe disease at 9 and 12 months and therefore had had a biopsy and were removed from the study. If patients missed their 15-month evaluation time point, they were evaluated at the 21- or 27-month follow-up point. The study design was to use a single experienced pathologist (J. R. Davis), who was blinded to the treatment assignment. Entry biopsy specimens were compared with interval biopsy specimens to facilitate recognition of change. Patients were considered to have achieved a complete response only if the Papanicolaou smear and the biopsy specimen were negative 15 months after the start of treatment.

Toxicity

Toxicity was evaluated on days 1 through 4 and 8 of the initial treatment and at the completion of each maintenance treatment by clinical evaluation, colposcopy, and colpophotographs.

We have described our system for systemic toxicity of retinoids elsewhere. Clinical and colposcopic assessments for cervical, vaginal, and vulvar side effects were evaluated at each visit, as previously described and summarized below (*10*). The adverse local effects were compared in patients treated with RA and in those treated with placebo and are as follows: grade 0, *none*; grade 1+ (*mild*), cervical erythema (vaginal burning, irritation, itching, or discharge not bothersome to patient); grade 2+ (moderate), cervical erythema (vaginal burning, itching, or irritation bothersome to patient), erythema or increased vascularity of vaginal mucosa, or both; and grade 3+ (severe), cervical ulceration (vaginal burning, itching, irritation, or discharge leading to discontinuation of treatment), ulceration or bleeding, or both.

Statistical Considerations

In the analysis, we included all patients with a follow-up biopsy at 15, 12, 9, 21, or 27 months. The primary null hypothesis of the study was that there would be no difference between the retinoid and placebo groups in the complete biopsy frequency at 15 (or 12, 9, 21, or 27 months) months. We used Fisher's exact test to compare the frequency of complete biopsy response between groups; complete response of the follow-up biopsy indicated no atypia. We also used logistic regression methods to compare the frequency of response and to simultaneously adjust for potential confounding or risk modification factors, including all characteristics listed in Table 1, plus treatment by age, treatment by quadrant, and age by quadrant. The LOGIST procedure in the SAS statistical package was used. Treatment comparisons were based on one-sided tests, since the hypothesis of increased complete response and of greater toxicity in the RA treatment arm was of interest.

Comparisons of toxicity rates between the treatment arms were based on Fisher's exact test. All eligible patients were included in the analyses of toxicity.

Results

Patient Characteristics

A total of 301 patients (moderate dysplasia, 151; severe dysplasia, 150) from the University of Arizona were entered in the study and were randomly assigned to treatment between November 19, 1985, and March 27, 1989. Three patients (1%) were subsequently found to be ineligible and did not receive a 15-month follow-up biopsy; 14 were excluded by the physician; and 52 were lost to follow-up, did not receive a follow-up biopsy, and were excluded from analysis. Therefore, a total of 69 patients were excluded from follow-up, leaving 141 patients with CIN II and 99 patients with CIN III available for analysis of efficacy.

The treatment arms were fairly well balanced according to various patient characteristics including age, ethnicity, birth-control methods, and prior treatment (Table 1). Smoking histories of the patients were not recorded. The distribution of disease characteristics at study entry between the two groups as measured by colposcopic appearance and histologic characterization of the lesion was equivalent. Colposcopic characteristics included degree of quadrant involvement, epithelial appearance (inflammation, abnormal vessels, white epithelium, condyloma, mosaicism, leukoplakia, and punctation), and degree of involvement. Histologic characteristics of the lesion included Papanicolaou smear evaluation, biopsy, endocervical biopsy, and endocervical curettage koilocytotic atypia. Among these factors, only mosaicism was associated with treatment outcome. Its presence predicted that a lesion would be less likely to regress ($P = .016$).

Participant Recruitment and Follow-Up

Three hundred one patients were accrued and were randomly assigned to receive their initial 4-day treatment. The patterns of loss to follow-up were similar in the two treatment arms (Table 2). At the primary evaluation point of 15 months, 81 participants remained in the placebo group and 88 in the RA treatment group.

Table 1. Patient characteristics

Characteristic	Placebo*	RA*
Age, y		
≤24	69	57
25-29	38	41
≥30	44	53
Ethnicity		
Non-Hispanic white	113	114
Hispanic white	32	24
Other	6	12
Birth-control method		
Oral contraceptives	83	69
Other	61	62
None	7	9
Birth control		
Barrier	29	28
Nonbarrier	121	118
Unspecified other	1	4
Prior treatment		
No	134	134
Yes	17	16

*Values = number of patients.

Table 2. Participant follow-up and loss*

Duration in the study, mo	Placebo		RA	
	Seen	Lost	Seen	Lost
Randomization	151	—	150	—
3	130	20	133	16
6	97	52	107	39
9	60	56	63	48
12	63	59	70	50
15	81	64	88	56
21	25	118	23	123
27	25	126	21	129

*Seen = participants were seen at that particular follow-up. Lost = participants were not seen at that particular follow-up and were never seen again. If a participant did not attend an earlier follow-up, then reappeared at a later date, she is not included in either the "seen" or "lost" columns.

Comparison of End Point to Entry Biopsy

Biopsy results at the end of the trial were compared with those at study entry (Table 3). For the patients with moderate dysplasia, 32 (43%) demonstrated complete regression by biopsy in the treatment arm compared with 18 (27%) in the placebo arm ($P = .041$). In contrast, no treatment effect was evident for patients with severe dysplasia.

We used the logistic regression model including treatment, quadrant, age, age

Table 3. Comparison of end point to entry biopsy in participants with CIN

Dysplasia	Intervention	Regression*	Other†
Moderate	RA	32 (43%)	43
	Placebo	18 (27%)	48
Severe	RA	10 (25%)	30
	Placebo	16 (31%)	35

*Regression = complete biopsy response (or endocervical curettage if biopsy not available) at 15, 9, 12, 21, or 27 months as defined in the text.

P = .041 for moderate dysplasia and P = .33 for severe dysplasia.

†Other = no response or CIN upstaged.

Table 4. Adverse local events during initial 4-day medication administration*

	Placebo, mo in the study†			RA, mo in the study†		
Event	0	3	6	0	3	6
Cervical						
Inflammation	9	5	4	28	5	2
Vascularity	2	1	0	5	1	0
Vaginal inflammation	3	1	1	10	2	1
Vaginal						
Burning	1	1	0	5 (3,0)	1	1
Itching	3	1 (1,0)	0	6 (4,0)	2	1
Irritation	4	0 (2,0)	0	9 (5,0)	1	2 (0,1)
Discharge	44 (14,10)	18 (20,8)	10 (13,5)	32 (26,14)	26 (13,4)	13 (11,3)
Vulva						
Burning	1	0	0	13 (9,3)	0	4
Itching	4	1	1	11 (9,1)	2 (1,0)	2
Irritation	3	1	0	13 (10,3)	2 (1,0)	4

*Table shows the side effects as categorical yes/no.

†Numbers in columns represent the number of patients with the designated toxicity during the initial 4-day treatment. Number of patients with greater than mild toxicity is shown in parentheses (moderate, severe).

by quadrant, treatment by age, and treatment by quadrant. All adjustment factors were statistically significant (P<.10), with a significant treatment difference (P = .002).

Toxic Effects

No systemic side effects were seen by participants. Local adverse events experienced by the participants during the initial 4-day treatment phase are shown in Table 4. These side effects were recorded as occurring during the treatment periods, in general during the application of the medication or after completing the initial 4-day (0-month) treatment. These side effects were mild for the most part and did not result in study discontinuation. There was slightly greater colposcopically determined cervical inflammation reported in the RA treatment group (P = .001), but this was clinically not significant. There was also a higher instance of vaginal inflammation at time 0, but this was not as significant (P = .01). In both arms, there was an increased vaginal discharge in a substantial number of the patients. This discharge was equivalent in the two treatment arms and therefore likely due to the placebo cream. This side effect was not dose limiting. The only significant difference in toxic effects between the two groups was increased vulva burning, itching, and irritation during the initial treatment (P = .001). Local adverse side effects during the maintenance phase were uncommon and mild (data not shown).

Discussion

This study suggests that locally applied RA may favorably alter the natural history of CIN if the lesion has not progressed too far. In patients with CIN II, there was a greater than 50% increase in the biopsy-proven complete regression rate in the RA treatment arm compared with the placebo arm. No such differences were evident in patients with severe dysplasia. Although these effects were modest, these changes represent the first time that any therapeutic medication has been shown in a randomized trial to alter the natural history of CIN. What might be the biologic basis for such an effect?

RA has been demonstrated to be an effective inhibitor of growth and a stimulator of differentiation in many epithelial and nonepithelial tissues, including cervical tissues (*1*). In randomized trials, clinical studies (*13,16*) have also demonstrated that malignant secondary recurrences in patients with upper aerodigestive malignancies or in patients with early lung cancer can be inhibited by oral 13-*cis*-retinoic acid or retinol, respectively. There has also been an encouraging experience with retinoids and carotenoids in the treatment of oral precancer, a lesion somewhat similar to that seen in the cervix (*17*). A number of studies (*18*) have shown clinical activity against oral leukoplakia, and a large randomized trial has demonstrated efficacy in a phase III trial for relatively high-dose 13-*cis*-retinoic acid. The effect seen may reflect a generalized response of early disease to a differentiation agent.

Can biologic studies provide us with insight into the activity of RA in CIN? Several different pathways may be involved. (*a*) Human papillomavirus (HPV) has been shown to be involved in the development of CIN and cervical carcinoma (*14*), and RA markedly decreases the HPV-immortalized rate of human keratinocytes and decreases production of E6 and E7 (*19*); (*b*) vitamin A deficiency produces a series of abnormalities of keratin expression in mouse cervical epithelium (*15*); and (*c*) RA induces secretion of latent transforming growth factor β_1 and β_2 in normal and HPV type 16-immortalized keratinocytes (*20*). RA may well be effecting its response via a number of different mechanisms. Further detailing of the interactions of vitamin A, RA, and cervical keratinocytes should be informative.

How is RA effecting its response? Vitamin A can produce innumerable biologic effects on cells, including indirect immunologic and inflammatory responses and direct and indirect biochemical responses. Many of the responses were accompanied by inflammation. However, only CIN II and not severe dysplasia responded in the current trial. If the response had been effected chemically, then CIN III should have responded as well. Nuclear retinoic acid receptors have been recognized as the natural ligand for RA, the proximate metabolite of vitamin A and the natural effector of vitamin A action (*21*). This finding suggests that RA may cause many of its effects through modulation of a transcription signal, but the role of RA receptors in transcriptional control of cervical cells is unexplored.

Why is the complete response rate in the placebo group so high (27% for moderate dysplasia; 31% for severe dysplasia)? In planning for sample-size calculations for the phase III trial, we anticipated that a "spontaneous remission rate" between 15% and 20% might occur, based on a prior extensive literature review. We also recognized that the placebo cream itself might induce or enhance responses, particularly since the butylated hydroxytoluene contained in the cream is a known chemopreventive agent.

Why did CIN II, but not severe dysplasia, respond to the RA? Our presumption is that CIN represents a continuum and that with further progression the tissue and/or cells in the lesion become less responsive. This predicts that CIN I would be responsive to RA. We do not know, but because of the low risk of CIN I and its high spontaneous regression rate and the teratogenicity of RA, the premise would be difficult to test. Also, we would predict that RA should be ineffective against malignant disease. However, 13-*cis*-retinoic acid combined with interferon α appears to be effective against cervical cancer (*22*). An understanding of why this might be so would be quite informative.

What is the next step? Further testing of RA would not seem warranted. Practitioners will need to decide the clinical indications vis-à-vis the risk or benefit for RA and CIN. The testing of less toxic agents would seem worthwhile. Folic acid has been tested in a phase III trial and found to be ineffective (Chu J: personal communication). An impressive amount of epidemiologic data suggests that both beta carotene and vitamin C protect against CIN (*23*). Trials of these agents are ongoing. Also a non-teratogenic retinoid (oral) 4-hydroxyphenylretinamide has produced responses in oral leukoplakia (*24*), and several studies in CIN are now being conducted. Results from these trials are eagerly anticipated. The results of the current trial provide further evidence for the feasibility and efficacy of chemoprevention trials in epithelial malignancies. The series of studies leading to the results presented here have spanned over 14 years. We can anticipate that advances in the future will proceed more rapidly.

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Notes

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