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Original Articles

A preliminary study for the treatment of cervical colposcopic lesions with the biological compound AV2

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Summary

Objective: To evaluate the efficacy of AntiViral 2 (AV2) in the regression of moderate and severe colposcopic lesions, when compared to placebo. *Materials and Methods:* Women, aged over 18 years with a colposcopic diagnosis of moderate to severe dysplasia were randomized to receive either two applications of AV2 or placebo within four days. Both examining physician and patients were blinded to the treatment option. Follow-up colposcopy was performed on days 11, 21, and 60. *Results:* A total of 50 patients were enrolled in this study. There was no statistically significant difference in screening entry criteria between the two groups. The results showed that the application of AV2 yielded a reduction of more than 50% for 21 out of 28 (75%) patients who received the active treatment versus a 0% for the comparable placebo group ($p < 0.001$). *Conclusions:* The authors conclude that AV2 can have a place in the treatment of colposcopically-detected cervical lesions. Due to the proven broad spectrum antiviral activity of AV2, a plausible explanation is that the lesions regress due to deactivation of the virus. Further trials with larger numbers and detailed cytology and histology are needed to confirm these results.

Key words: Cervical lesion; Colposcopy; Human papillomavirus; AV2; Essential oil; Treatment.

Introduction

Persistent human papillomavirus (HPV) infections cause virtually all of the more than 530,000 cases of invasive cervical cancer per year worldwide [1]. The 250,000 deaths from cervical cancer reported in 2008 make it the third leading cause of cancer death in women [2].

Cervical cancer is associated with poverty even in high-income countries, and 90% of deaths from cervical cancer worldwide occur in developing countries where cytologic or virologic screening is not available. In this setting with limited resources, screening by visual inspection with or without a colposcope can be offered [3-5].

Colposcopy is the critical diagnostic step for women with cervical cytological abnormalities. It is the standard for primary evaluation of cervical epithelial abnormality and also constitutes an essential follow-up modality. Current approaches to cervical cancer prevention interpose colposcopy as a triage test to define better which women require treatment [6, 7].

Recently, Cesa Alliance, an international collaboration of researchers, laboratories, and universities has developed a broad-spectrum antiviral drug called AV2 that can be used in the treatment of cervical HPV-related lesions at an affordable cost [8]. AV2 is a combination of FDA approved organic compounds (natural essential oils: carvone, eugenol, geraniol, and nerolidol), having a broad spectrum anti-viral effect, both topically and orally. AV2 deactivates infectious virions outside the cell and case studies have shown that both topical and oral treatment of AV2 can lead to shrinkage of vaginal and penile papilloma warts and diminish outbreaks of herpes labialis [9, 10]. The present authors hypothesize that applications of AV2 by viral inactivation can diminish cervical HPV linked lesions. The aim of this preliminary study was to evaluate the effect of AV2 on colposcopically-detected cervical lesions as secondary prevention in a setting without cytology nor virology.

Materials and Methods

This study was a single center, randomized, double-blind, placebo-controlled phase 2 trial comparing the clinical efficacy of AV2 and placebo over a 60-days period. The protocol was approved by the Hospital de la Familia Institutional Review Board. Written informed consent was obtained from all eligible participants before enrollment in the study. All subjects were recruited from the general population attending the Hospital de la Familia. Potential candidates for this study were recruited through educa-

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Table 1. — *The study protocol after initial recruitment.*

Number of visits	Time (day)	Follow-up	Purpose
1	0		Presentation made, consent obtained
2	1	Colposcopy	Application 1
3	4	Colposcopy	Application 2
4	11	Colposcopy	
5	21	Colposcopy	
6	60	Colposcopy	Exit interview with doctor
7	60-90	Report	

tion classes offered by medical personnel and advertisements. At the center colposcopy is offered as a screening method, also outside of this study. Women were eligible if they did not meet any exclusion criteria. All sexually-active women aged between 18 and 45 years, with a menstrual cycle length of at least 25 days, and who had no previous history of cervical intraepithelial neoplasia and local or systemic anti-wart therapy were included in the study. They were enrolled after giving their informed consent to comply with all the study procedures.

Pregnant women or those with unpredictable or irregular menstrual cycle, women who had no uterus, women who reported history of drug abuse, and those with a diagnosis of carcinoma in situ, adenocarcinoma in situ or invasive cervical cancer were excluded from the study. Inclusion and exclusion criteria were confirmed by a trained colposcopist (MG) who then verified for the presence of suspicious cervical lesions infection using standard colposcopy techniques. All colposcopies were performed according to the official Mexican guidance, *NPM-014-SSA2-1994* for prevention, detection, diagnosis, treatment, control, and epidemiology of uterine cervical cancer. The cervix was painted with 5% acetic acid and then examined using a binocular colposcope for the presence of suspicious lesions.

Table 1 summarizes the study protocol. After the screening, interview and initial colposcopic examination, patients entered the intent-to-treat (ITT) group, were randomized to one of the two arms (AV2 or placebo), and entered the 60-day treatment and observation period. The treatment consisted of administration of AV2 or placebo on days 1 and 4. The observation cycle was scheduled on days 11, 21, and 60 (for this reason patients with irregular cycles were excluded). A follow-up contact was made on day 90 to discuss the results of the intervention with the patients.

Randomization was separated from the recruitment process. Once eligible for the study and after providing informed consent to participate, subjects were randomly assigned to one of two treatment groups on a two-to-one basis based on a computer generated randomization list. The concealed allocation was assured by the distribution of study medication by an administrator of the Hospital de la Familia. The medical staff and the participants were all blinded to treatment arm assignments. Unblinding took place after all participants completed the 60-day observation cycle. All patients were notified of which treatment arm they were in along with the preliminary study results.

The clinical trial material consisted of AV2 or a placebo. The appearances of the two products were similar as both products were presented in identical glass containers with identical labeling. The containers were dispensed by administrative personnel such that neither the patients nor the investigators were aware of the treatment assignments.

AV2 is a blend of four very well-known and extensively-used natural essential oils mixed together in specific quantities diluted 50% in olive oil (olea europaea). Two of those oils are single component essential oils (eugenol and geraniol), the other two are racemic mixtures (carvone and nerolidol).

The placebo consisted of 10% lemon (citrus limon) and 10% lime (citrus aurantifolia) essential oils in 80% olive oil. The oils were included to provide a fragrance to the placebo.

AV2 or placebo were administrated as a direct topical spray to the cervix while the patient was in the lithotomic position and fitted with speculum. The manual spray applicator was positioned just inside the anterior of the vagina and two pumps were applied. Each push delivered 100 μL of solution.

This trial aimed at evaluating the clinical efficacy of AV2 in reducing the size of colposcopic-detected cervical lesions. To establish a comparative tool, the investigators created an ad hoc evaluation scale from 1 to 20 grading severity of the lesion and expressed sizes of lesions as the percentage of the cervical surface that showed changes suggestive for dysplasia. All the outcomes of the study were measured using this evaluation, and the baseline for success was established at a minimum reduction of 20% of the lesion's original size, or a reduction of 2 to 4 degrees on the 1-20 scale.

Differences in clinical outcomes between AV2 treatment and placebo were compared using a Chi-square or a Fisher's Exact Test or all statistical comparisons, and a p-value of less than 0.05 was considered statistically significant.

Results

The study was carried out during the period from December 2009 to February 2010. A total of 321 participants were screened for eligibility as shown in Figure 1. Among these, 125 did not meet the pre-colposcopy inclusion/exclusion criteria, 61 participants declined to participate, and 85 were deemed ineligible after the colposcopic examination. Finally, 50 participants were enrolled and randomized; 37 were assigned to the AV2 group and 13 to the placebo group. During the 60-day trial, six participants withdrew from the study group: four from AV2 group and two from the placebo group. Additionally, one patient completed the study and observation but attended scheduled visits outside the prescribed schedule. The average age of women was 32 years in the AV2 group and 31 years in the placebo group.

Thirty-seven of the 44 patients who completed the study exhibited a positive overall response to treatment in the trial. More patients in the AV2 group showed a positive clinical response than those in the placebo group (31/33 *vs*. 6/11) and the difference was statistically significant (Table 2). Ten of the 11 patients who had been included with extremely small lesions (at 5% or below) exhibited a complete regression.

The present authors concluded that the combination of the treatment and the repeated administration of acetic acid during the colposcopic examinations caused the eradication of the very small lesions. Accordingly, to avoid a statistical skew in the results, the evaluation of the data before and after controlling for the results of patients with the

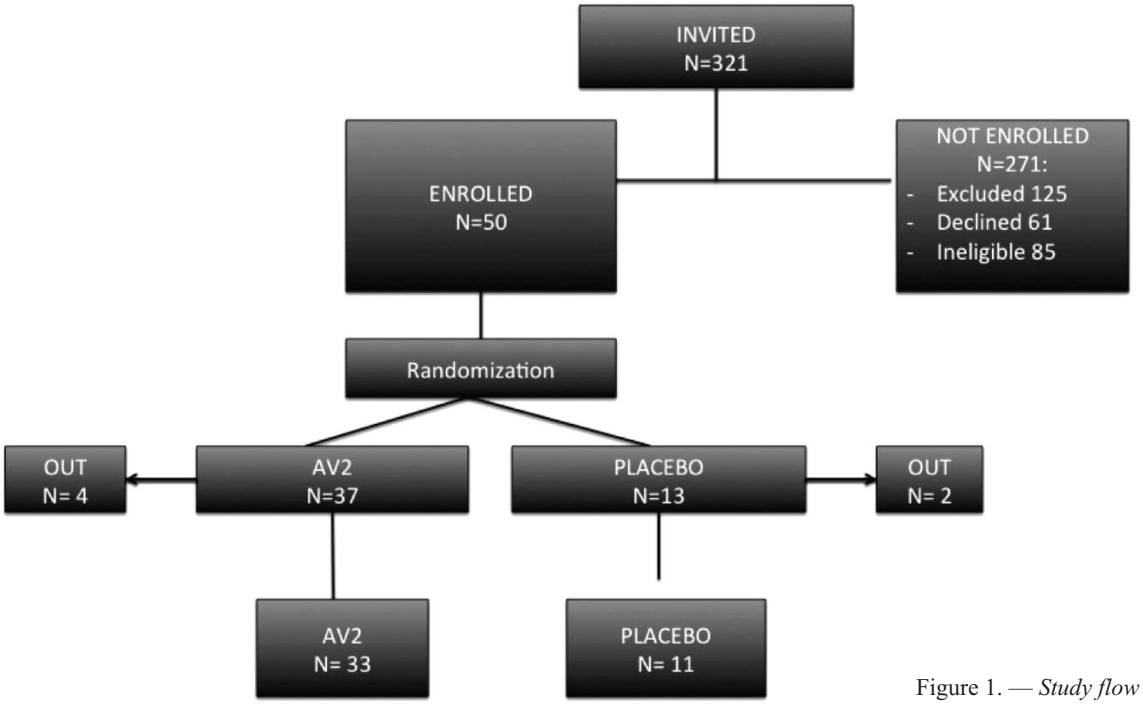

Figure 1. — *Study flow chart.*

Table 2. — *Overall response to treatment (relative reductions of the lesion on a 1-100% scale).*

Group	Response		Total	*p*
	No	Yes		
AV2	2 (6%)	31 (94%)	33 (100%)	
Placebo	5 (45.4%)	6 (54.5 %)	11 (100%)	0.002
Total	7 (15.9 %)	37 (84%)	44 (100%)	

Table 3. — *Evaluation of activity of AV2 during the clinical trial basic pool.*

	Patients with AV2 treatment	Patients with Placebo treatment	Total	*p*
Applicable pool	33 (100.0)	11 (100.0)	44 (100.0)	
Complete regression 100%	13 (39.4)	5 (45.5)	18 (40.9)	
Partial regression > 50%	13 (39.4)	0 (0)	13 (29.5)	0.011
Partial regression > 30%	3 (9.1)	1 (9.1)	4 (9.1)	
Partial regression > 20%	2 (6.1)	0 (0.0)	2 (4.5)	
No change or deterioration	2 (6.1)	5 (45.5)	7 (15.9)	

Table 4. — *Evaluation of activity of AV2 during the clinical trial controlled pool. Results after the elimination of the small lesions.*

	Patients with AV2 treatment	Patients with Placebo treatment	Total	*p*
Applicable pool	28 (100)	5 (100)	33 (100)	
Complete regression 100%	8 (28.6)	0 (0)	8 (24.2)	
Partial regression > 50%	13 (46.4)	0 (0)	13 (39.4)	0.002
Partial regression > 30%	3 (10.7)	1 (20)	4 (12.1)	
Partial regression > 20%	2 (7.1)	0 (0)	2 (6.1)	
No change or deterioration	2 (7.1)	4 (80)	6 (18.2)	

Table 5. — *Success in reduction of four grades on 1-20 scale.*

Success in reduction	Patients with AV2 treatment	Patients with placebo treatment	*p*
No	7 (25%)	5 (100%)	
Yes	21 (75%)	0 (0%)	0.001
Total	28 (100%)	5 (100%)	

small lesions that had disappeared was done as presented in Tables 3 and 4.

Application of AV2 yielded a reduction of more than 50% for 21 out of the 28 (75%) patients who received the active treatment versus a 0% for the comparable placebo pool. At the other end of the scale, only two (7%) AV2 participants failed to respond positively compared to 80% of the placebo group (Table 4).

In addition to the results presented above, the authors performed a Chi-Square analysis to evaluate the absolute regressions at the minimum levels of four grades (Table 5) and on the 1-20 scale. Table 5 presents the success in reduction of at least four grades on the 1-20 scale that was used.

No participant reported serious adverse events. A total of six participants withdrew (four AV2 and two placebo). None

of the patients who decided to withdraw from the study referred discomfort associated with their treatment. Both AV2 and placebo were safely administered and well-tolerated.

Discussion

The primary aim of this small-scaled study was to evaluate the efficacy of AV2 on colposcopically detected cervical lesions placebo in a setting where HPV testing nor cytology are available. The authors found a clear advantage of AV2 over placebo. This small preliminary study does not provide any proof of virucidal effect nor of any change in histology after application.

Other weaknesses of this study include the small number of patients enrolled and the necessity to control for the 11 patients who were included with very small lesions. However, although the authors only enrolled 50 patients, they were able to achieve adequate statistical power to show a statistical difference in response rates between the two therapies. One other weakness of this study was the subjective nature of the evaluation of the response, as both the 1-20 scale and the evaluation of the surface of the lesions were operator dependent. The fact that the same operator, who was blinded for treatment and that performed all colposcopies, still validates the differences in outcomes.

Those patients receiving AV2 were significantly more likely to have a decrease in the grade and size of their cervical dysplasia. The present authors hypothesize that this is due to the deactivation of HPV, although an influence on the local immune response can also be considered as an alternative explanation. They had no idea how many times a patient should be treated to deactivate the virus. A four-week interval was their first guess. They realize that the short interval between examinations and the lack of long term follow-up (no data are available whether the results are reversible or not) are weaknesses, therefore further research is required to control this.

As the present authors did not test for the presence of HPV and had no cytological nor histological diagnosis, this study does not provide any data on the immediate effect on the virus nor on histologic dysplasia. Further research on this is continuing on https://clinicaltrials.gov/ct2/show/NCT02346227

The medication had no side effects and the present authors observed a 75% response rate in this cohort of patients, which was much higher than the placebo group. They later discovered that one placebo patient who was erroneously treated with AV2 the first time and with placebo three times thereafter reacted very well to the treatment (accelerated lesion regression). The patient was excluded, but the information was very valuable.

Further studies are necessary to obtain data on such factors as the optimal timing and time interval of the spray, and if two doses are necessary or if only one is sufficient. This study does however provide data to justify further research on the virological, cytological, histological, and immunological effects of AV2 as it might play a role in secondary prevention of cervical cancer, for which different options are now being developed as reviewed by Guido *et al.* [11]. Recently one of the components of AV2 (eugenol) has been used as en adjunctive in cervical cancer [12].

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