Clinical and Molecular Responses in High-Grade Intraepithelial Neoplasia Treated with Topical Imiquimod 5%

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ABSTRACT

Objective: To assess the clinical and molecular response of patients with recurrent high-grade vulvar, vaginal, or cervical intraepithelial neoplasia treated with topical 1–2(2-methylpropyl)-1H-imidazo [4,5-c] quinolin-4-amine (imiquimod) cream 5%, an immune response modifier with known efficacy in the treatment of external genital warts.

Methods: This is the first case series in the peer-reviewed literature reporting the use of imiquimod in high-grade intraepithelial neoplasia of the lower genital tract. Eight patients with high-grade intraepithelial neoplasia were treated with imiquimod in the gynecological oncology clinic and the HIV gynecology clinic at The University of Texas Medical Branch at Galveston. Frozen biopsies were available for RNA extraction on four patients before and after therapy. Using semiquantitative reverse transcription-PCR, we measured RNA levels of IFNs α and γ, 2′,5′-oligoadenylate synthetase, as well as CD4 and CD8 lymphocyte markers.

Results: Of the patients treated, four had complete responses, two had partial responses, one progressed, and one did not tolerate the therapy. Of the four complete responders, two remained disease-free (mean follow-up, 33 months). 2′,5′-Oligoadenylate synthetase RNA expression showed an increased trend after therapy.

Conclusions: These results obtained in this small and heterogeneous group merit further study in the use of topical 5% imiquimod use in the treatment of intraepithelial neoplasia. An important mechanism of action of imiquimod may involve 2′,5′-oligoadenylate synthetase antiviral activity.

INTRODUCTION

Standard therapy for high-grade genital intraepithelial neoplasia is surgical, either ablative or excisional. Morbidity associated with the procedure includes pain, bleeding, scarring, and uncommonly, cervical stenosis or incompetence. Furthermore, scarring from previous procedures can make future colposcopic evaluations inadequate.

Medical therapy is evolving in the management of intraepithelial neoplasia. Therapies used previously include IFNs, topical 5-fluorouracil, and retinoids (2, 3) with variable results. Imiquimod is a low molecular weight imidazoquinoline that acts as an immune response modifier and induces cytokines that promote a T-helper 1 or cell-mediated immune response (2, 4). Imiquimod 5% cream has been demonstrated to be safe and effective in the treatment of external genital warts caused by various low-risk HPV types.

Similarly, high-risk HPV types cause carcinoma of the anogenital tract and its precursor lesion, high-grade neoplasia of the anal canal, vulva, vagina, and cervix. It is reasonable to study an agent that has known efficacy in the treatment of HPV-associated warts on high-grade intraepithelial neoplasia.

Our aim is to assess the clinical response of patients with high-grade vulvar, vaginal, or cervical intraepithelial neoplasia treated with topical imiquimod cream 5% and to corroborate it with the levels of local immune markers in the lesional tissues before and after treatment.

MATERIALS AND METHODS

This is a case series with a review of the medical records of eight patients who had received topical imiquimod 5% for treatment of recurrent genital high-grade intraepithelial neoplasia between 1997 and 1999 in our gynecological oncology clinic and HIV gynecology clinic at The University of Texas Medical Branch in Galveston.

Patients with a history of failed standard therapy with a biopsy-proven high-grade intraepithelial lesion in the vulva, vagina, or cervix were offered medical therapy with topical imiquimod as an option to standard therapy. All patients had a grossly or colposcopically visible lesion after initial biopsy. Patients with positive endocervical curettage or a lesion suspicious for invasion were excluded. A colposcopic exam was done in the standard fashion by one of the authors (C. D.-A.) before starting the study and again at 6 and 16 weeks. All biopsy specimens were interpreted separately by the study pathologists (T. V. D. and Z. G.).

The lesions were monitored for response clinically with colposcopy and confirmed histologically. Clinical and pathological data were obtained by review of the medical records of all
patients (approved by the University of Texas Medical Branch Institutional Review Board 00-086). A complete response was defined clinically by total colposcopic resolution of the lesion for at least 1 month and histopathologically by absence of dysplasia or carcinoma. A partial response was defined as a decrease in lesion size by at least half or a histopathological change from high-grade to low-grade for at least 1 month. All patients received treatment with imiquimod 5% cream digitally self-applied to the vulvar, vaginal, or cervical lesion at bedtime three times weekly for 6–16 weeks. No douching was recommended. The median follow-up was 30.5 months (5).

Frozen tissue, matching the formalin-fixed biopsies, was available only from 4 patients. This tissue corresponded to the diagnostic biopsies before and after therapy. Total RNA was isolated using Tri-Reagent (Molecular Research Center, Inc., Cincinnati, OH), and a comparative reverse transcription-PCR method was used to semiquantitatively assess the transcripts of the biological marker.

For each experimental sample, control amplification was included using α-tubulin. Target mRNA levels were normalized to α-tubulin and presented as target gene:α-tubulin ratios. The primer pairs for α-tubulin were purchased from Clontech (Palo Alto, CA). 2',5'-Oligoadenylate synthetase, an IFN-inducible protein, was designed using Gene Runner 3.0 software (Hastings Software, Inc.) and synthesized as described.

Only descriptive statistics are appropriate because of the nature of this study.

RESULTS

Of the eight patients with high-grade dysplasia of the genital epithelium, two patients had cervical dysplasia, two had vaginal dysplasia, and four had vulvar dysplasia. These patients were at high-risk for recurrence because they had failed previous therapies (Table 1). The median age was 47 years, with a range of 33–60 years; 3 (37.5%) were HIV positive with a mean CD4 count of 118 (7–640) and a mean viral load of 11624.23 (50–67130). Five (63%) were tobacco users, and 6 (75%) of the patients were Caucasian. Of the eight patients treated, four had a complete clinical response within the 16-week regimen; three were histologically confirmed, and the fourth clinical complete responder refused biopsy. Two patients had a partial response; one patient, who did not use the medication because of local toxicity, had stable disease; and one patient had disease progression to microinvasive carcinoma of the vaginal cuff (Table 2). Of the four complete responders, two remained disease free after a mean follow-up of 33 months; two patients, both HIV infected, had recurrences after a 3- and 15-month disease-free interval.

The patients who recurred after an initial response were retreated with imiquimod applied three times a week per the original regimen and achieved a complete response again. Among the HIV-infected patients, no association was noted between clinical response and viral load, CD4 counts, or use of highly active antiretroviral therapy.

The toxicity of this regimen was acceptable: grade 1 for 5 patients (pain, erythema, and pruritus all relieved spontaneously; Ref. 6). One patient did not tolerate the treatment secondary to severe burning immediately after application of the cream to a periclitoral lesion.

Using a semiquantitative reverse transcription-PCR technique, we compared the IFN-inducible protein 2',5'-oligoadenylate synthetase levels in tissues before and after treatment. Levels of 2',5'-oligoadenylate synthetase mRNA increased in expression after therapy in four of five patients (P = not significant; Fig. 1).

DISCUSSION

Despite a success rate of >90% for surgical therapy for patients with high-grade intraepithelial neoplasia, the patients included in this study all had already failed standard therapy with as many as four previous surgical procedures.

Although this study is limited by its nonrandomized nature, small sample size, and heterogeneity of diseases sites, it is the first report in the peer-reviewed literature to use imiquimod 5% cream in high-grade intraepithelial neoplastic lesions of the genital tract. A total of six responders (combined partial or complete responders) in this population of treatment failures without causing further surgical morbidity appear promising for successful treatment.

The three recurrences were retreated and again achieved a complete response with reapplication of the topical imiquimod. To date, with a median 22.5-month follow-up, none of the responders have required surgical management. One patient progressed from vaginal intraepithelial neoplasia 3 to a lesion invading the stroma 0.5 mm. Natural history studies show that the relative risk of carcinoma in situ progressing to invasive carcinoma is 2.5 (7).

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Dx</th>
<th>HIV</th>
<th>Hx of intraepithelial neoplasia (yr)</th>
<th>No. of previous procedures</th>
<th>Procedure (yr from study entry)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HGVAIN</td>
<td>No</td>
<td>14</td>
<td>3</td>
<td>Cryotherapy (13,12); hysterectomy (11)</td>
</tr>
<tr>
<td>2</td>
<td>HGVAIN</td>
<td>No</td>
<td>1</td>
<td>1</td>
<td>Upper vaginectomy (1)</td>
</tr>
<tr>
<td>3</td>
<td>HGVIN</td>
<td>No</td>
<td>18</td>
<td>3</td>
<td>Laser vulva (3); wide local excision (2) (1)</td>
</tr>
<tr>
<td>4</td>
<td>HGVIN</td>
<td>No</td>
<td>13</td>
<td>4</td>
<td>Laser vulva (13); vulvectomy (14); wide local excision, laser, and clitoplasty y (5); WLE (1)</td>
</tr>
<tr>
<td>5</td>
<td>HGVIN</td>
<td>No</td>
<td>1</td>
<td>1</td>
<td>Wide local excision of vulva (1)</td>
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<tr>
<td>6</td>
<td>HGVIN</td>
<td>Yes</td>
<td>3</td>
<td>2</td>
<td>Wide local excision and laser vulva (3); laser vulva (2)</td>
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<tr>
<td>7</td>
<td>HGCIN</td>
<td>Yes</td>
<td>2</td>
<td>1</td>
<td>Loop electrosurgical excision procedure (2)</td>
</tr>
<tr>
<td>8</td>
<td>HGCIN</td>
<td>Yes</td>
<td>20</td>
<td>1</td>
<td>Cryotherapy (7)</td>
</tr>
</tbody>
</table>

* Dx, diagnosis; Hx, histopathology; HGVAIN, high-grade vaginal intraepithelial neoplasia; HGVIN, high-grade vulvar intraepithelial neoplasia; HGCIN, high-grade cervical intraepithelial neoplasia; WLE, wide local excision.
in general. Treatment of warts with imiquimod increased the picornaviruses and plays a major role in antiviral activity of IFNs. This cation by-product that leads to inhibition of protein synthesis. This induced enzyme activated by double-stranded RNA, a viral repli-
treatment in most patients (Fig. 1). This molecule is an IFN-
stroma. This problem with heterogeneity of dysplastic tissues can
inflammation in tissue samples masked the actual response of the
fact that we were testing dysplastic lesions, not warts (7).
We were unable to identify a correlation between pretreat-
ment cytokine levels and response to treatment. The difference
between the current results and those in the published literature
regarding cytokine expression levels may be attributable to the
level of 2\textsuperscript{,5}'-oligoadenylate synthetase RNA normalized to
\(\alpha\)-tubulin in biopsies of four patients before and after treatment with
The 2\textsuperscript{,5}'-oligoadenylate synthetase levels increased after
treatment in most patients (Fig. 1). This molecule is an IFN-
induced enzyme activated by double-stranded RNA, a viral repli-
cation by-product that leads to inhibition of protein synthesis. This
inhibition is known to be the main antiviral action of IFN on
picornaviruses and plays a major role in antiviral activity of IFNs in
general. Treatment of warts with imiquimod increased the 2\textsuperscript{,5}'-
oligoadenylate synthetase RNA (8); therefore, it may be important
in the IFN-induced antiviral response seen with imiquimod use.
We were unable to identify a correlation between pretreat-
ment cytokine levels and response to treatment. The difference
between the current results and those in the published literature
regarding cytokine expression levels may be attributable to the
fact that we were testing dysplastic lesions, not warts (7).
Another possible explanation is that the mixed cell popu-
lation in tissue samples masked the actual response of the
epithelial cells. We found that epithelial cells, stromal cells, and inflam-
atory cells, among others, are found in the biopsy.
Compared with dysplastic lesions, external genital warts have
a thicker epithelium, making it easier to sample relatively less stroma. This problem with heterogeneity of dysplastic tissues can
be overcome with the laser capture microdissection, a new method
used to select and procure cell clusters from tissue sections. This
new method would allow extraction of nucleic acids only from the
epithelial cells selected by the investigator for microdissection (9).
In conclusion, topical imiquimod cream 5% is well toler-
ated and appears efficacious in the treatment of high-grade intraepithelial neoplasia in a small group of high-risk patients.
Our next step is a Phase II B cancer chemoprevention trial.

ACKNOWLEDGMENTS

We thank 3M Pharmaceuticals (St. Paul, MN) for providing imi-
quimod (Aldara) cream, used by all patients.

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Table 2 Follow-up after imiquimod treatment

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Dx\textsuperscript{a}</th>
<th>Response\textsuperscript{b}</th>
<th>F/u (mo)</th>
<th>RFI (mo)</th>
<th>Recurrence</th>
<th>Lesion size</th>
<th>DX at F/u</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>HGVAIN</td>
<td>Complete</td>
<td>24</td>
<td>24</td>
<td>No</td>
<td>Focal</td>
<td>No dysplasia</td>
</tr>
<tr>
<td>2</td>
<td>HGVAIN</td>
<td>Progression</td>
<td>40</td>
<td>0</td>
<td>N/A</td>
<td>Extensive upper vagina</td>
<td>HGVAIN</td>
</tr>
<tr>
<td>3</td>
<td>HGVIN</td>
<td>Complete</td>
<td>30</td>
<td>15</td>
<td>Yes</td>
<td>Multifocal extensive</td>
<td>HGVIN</td>
</tr>
<tr>
<td>4</td>
<td>HGVIN</td>
<td>None</td>
<td>22</td>
<td>0</td>
<td>N/A</td>
<td>Multifocal</td>
<td>HGVIN</td>
</tr>
<tr>
<td>5</td>
<td>HGVIN</td>
<td>Partial\textsuperscript{c}</td>
<td>31</td>
<td>3</td>
<td>Yes</td>
<td>Multifocal</td>
<td>HGVIN</td>
</tr>
<tr>
<td>6</td>
<td>HGVIN</td>
<td>Complete</td>
<td>42</td>
<td>42</td>
<td>No</td>
<td>Extensive</td>
<td>No dysplasia</td>
</tr>
<tr>
<td>7</td>
<td>HGCIN</td>
<td>Partial</td>
<td>31</td>
<td>0</td>
<td>N/A</td>
<td>4 quadrant</td>
<td>HGCIN</td>
</tr>
<tr>
<td>8</td>
<td>HGCIN</td>
<td>Complete</td>
<td>16</td>
<td>3</td>
<td>Yes</td>
<td>2 quadrant</td>
<td>LGCIN</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Dx, diagnosis; F/u, follow-up; RFI, recurrence-free interval; HGVAIN, high-grade vaginal intraepithelial neoplasia; HGVIN, high-grade vulvar intraepithelial neoplasia; Pap. Papanicolaou smear.

\textsuperscript{b} Complete response, total clinical resolution of the lesion for at least 1 month. Partial response, decrease in lesion size by at least one-half or a change from high-grade to low-grade for at least 1 month.

\textsuperscript{c} Partial response after initial 16-week treatment and complete response after additional 12-week treatment.

Fig. 1 Levels of 2\textsuperscript{,5}'-oligoadenylate synthetase RNA normalized to \(\alpha\)-tubulin in biopsies of four patients before and after treatment with imiquimod. P = not significant.

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