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Review

Resiquimod: a new immune response modifier with potential as a vaccine adjuvant for Th1 immune responses

Jashin J. Wu[a], David B. Huang[b,c,d], Stephen K. Tyring[e,f,*]

[a] *Department of Dermatology, University of California, Irvine, Irvine, CA, USA*
[b] *Division of Infectious Diseases, Department of Medicine, Baylor College of Medicine, Houston, TX, USA*
[c] *University of Texas at Houston School of Public Health, University of Texas Health Science Center at Houston, Houston, TX, USA*
[d] *Division of Infectious Diseases, Department of Medicine, University of Texas Health Science Center at Houston, Texas, USA*
[e] *Department of Dermatology, University of Texas Health Science Center at Houston, Houston, TX, USA*
[f] *Center for Clinical Studies, Houston, Texas, USA*

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Abstract

Genital herpes is one of the most common sexually transmitted diseases worldwide. Currently, there are three FDA-approved nucleoside analogs and other therapies such as foscarnet and cidofovir used to treat genital herpes. Resiquimod, the latest immune response modifier (IRM), has shown in vivo evidence of efficacy against herpes simplex virus (HSV) type 2. The first clinical trial involving resiquimod demonstrated that it reduced the recurrence rate of genital herpes, but phase III trials were suspended due to lack of efficacy. Resiquimod shows promise for other viral infections and as a vaccine adjuvant.

Keywords: Resiquimod; Immunomodulation; Genital herpes; Herpes simplex virus type 2

Contents

1. Introduction

Genital herpes is one of the three most common sexually transmitted diseases in the United States (Corey and Hands-

* Corresponding author. Tel.: +1 281 333 2288; fax: +1 281 335 4605.
E-mail address: styring@ccstexas.com (S.K. Tyring).

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field, 2000). This serious public health concern has exploded in the last few decades. A seroprevalence study reported that 22% of the United States adult population is herpes simplex virus (HSV) type 2 seropositive (Fleming et al., 1997), the type that more commonly causes recurrent genital herpes. This correlates to approximately 45 million Americans who are afflicted with genital herpes.

FDA-approved treatment for recurrent genital herpes comprises of a trio of nucleoside analogs: acyclovir, famiciclovir, and valacyclovir (Mertz et al., 1988; Mertz et al., 1997; Reitano et al., 1998). When taken at the onset of symptoms, these drugs can decrease the duration of viral shedding and the time to heal lesions (Fife et al., 1997; Sacks et al., 1996; Spruance et al., 1996; Tyring et al., 1998). Although chronic suppressive therapy can prevent most future attacks, it requires good compliance and long-term daily dosing. However, they have no long-term effect on genital herpes if the medication is stopped (Fife et al., 1994; Wagstaff et al., 1994). Valacyclovir has been shown to reduce transmission by decreasing asymptomatic shedding (Corey et al., 2004).

With the limitations of current therapy against genital herpes, the focus has shifted to a new class of drugs called the immune response modifiers (IRMs). This article reviews the development of the latest IRM, resiquimod. We present in vitro and in vivo results of resiquimod and its possible use in the future.

2. The immune response modifier resiquimod

An investigation of nucleoside analog structures in the 1980s resulted in the first IRM. The chemical family became known as the imidazoquinolines. It was found that all of the imidazoquinolines showed in vivo anti-HSV activity (Miller et al., 1999). Imiquimod was the first commercially available imidazoquinoline used for anogenital warts, actinic keratosis, and superficial basal cell carcinoma (Abramovits and Gupta, 2004).

Resiquimod (R-848, S-28463, 4-amino-2-ethoxymethyl-α,α-dimethyl-1*H*-imidazo[4,5-c]quinolin-1-ethanol) is a more potent and soluble analog of imiquimod (Garland, 2003; Jones, 2003). Resiquimod produces a 50- to 100-fold cytokine response compared to imiquimod (Tomai et al., 1995). IRMs do not seem to directly target viruses or their replication cycle. Rather, they induce immune cells to produce cell-mediated or T helper type 1 (Th1) cytokines.

3. The innate immune response from resiquimod

Resiquimod produces an environment conductive for the Th1 response (Brugnolo et al., 2003). IRMs stimulate immune cells through the Toll-like receptor 7 (TLR7)-MyD88-dependent pathway (Akira and Hemmi, 2003; Edwards et al., 2003; Hemmi et al., 2002; Lee et al., 2003) and TLR 8 (Sauder et al., 2003). TLRs play a role in innate immune response through recognition of foreign proteins, activation of plasmacytoid dendritic cells (Gibson et al., 2002), and the induction of apoptosis in human epithelial cells lines (Meyer et al., 2003). The TLR adaptor molecule MyD88 associates with TLRs and brings IL-1 receptor-associated kinase and tumor necrosis factor (TNF) receptor-associated factor 6 to the TLRs. This signaling pathway activates c-Jun NH_2 terminal kinase (Jnk) and NF-κB transcription factors. After treatment with IRMs, TLR7-deficient mice did not have increased inflammatory responses, and MyD88-deficient mice did not secrete inflammatory cytokines. In these knockout models, there was no activation of NF-κB or Jnk.

Both imiquimod and resiquimod induce the Th1 cytokine IFN-γ and antagonize the Th2 cytokines IL-4, IL-5, and IL-13 (Wagner et al., 1999). These effects are mediated by IFN-α and IL-12, which are produced by macrophages, monocytes, and dendritic cells in response to imiquimod or resiquimod (Ahonen et al., 1999; Wagner et al., 1999). After application of topical resiquimod on hairless mice and rats, IFN-γ and TNF-α concentrations are increased in the skin, confirming in vitro reports (Imbertson et al., 1998).

The most important cell type activated by resiquimod through the TLR7 is the dendritic cell, an important antigen-presenting cell. In response to resiquimod, dendritic cells secrete IL-6, IL-12, TNF-α, and IFN-α (Ahonen et al., 1999). Monocytes respond to resiquimod with the secretion of IL-1, IL-6, IL-8, IL-12, TNFα, and IFN-α (Wagner et al., 1999). Cultures of Langerhans' cells show a similar cytokine response to resiquimod (Burns et al., 2000). Similar to how the CD40 ligand CD154 stimulates B lymphocytes (Bishop et al., 2000), resiquimod stimulates B lymphocytes to produce antibodies and the class II surface receptors for antigen presentation (Bishop et al., 2001). IFN-α limits viral replication in infected cells, and, together with IL-12 and TNF-α, activates natural killer cells. Natural killer cells are part of the early phase of the innate immune response against viral infection.

IL-12, IFN-α, and dendritic cells are especially important in promoting CD4+ Th1 cells rather than CD4+ Th2 cells. Once developed, the Th1 response is characterized by the presence of CD4+ Th1 cells secreting IFN-γ and IL-2. The established Th1 response together with continued administration of resiquimod stimulates the activation of several effector mechanisms directed against viral-infected cells. CD8+ cytotoxic T lymphocytes are driven by activated dendritic cells, CD4+ Th1 cells, and IL-2. Macrophages are activated by resiquimod, TNF-β, and IFN-γ directly (Tomai et al., 1995; Wagner et al., 1999). Resiquimod also stimulates nitric oxide production from macrophages (Buates and Matlashewski, 1999), which promotes cytotoxicity.

4. Efficacy in animal models

Imiquimod is effective treatment for recurrent genital herpes in the guinea pig model (Harrison et al., 1994). Guinea

pigs that had recently recovered from primary HSV-2 genital herpes were administered imiquimod intravaginally either for 5 days or 21 days. Those in the 5-day treatment group had fewer recurrences only during therapy, but those in the 21-day treatment group had fewer recurrences for 8 weeks (Harrison et al., 1994). Over 10 weeks of observation, the 21-day regimen decreased the total number of recurrences by 67% compared to the 5-day regimen ($p < 0.0001$). This regimen also reduced the HSV antibody response and increased memory-dependent T-cell and cytokine responses.

Resiquimod has been found to be equally effective as imiquimod while at a less frequent dosage (Bernstein et al., 2001). Guinea pigs were given subcutaneous resiquimod daily, every other day, or once weekly for 3 weeks, and they showed a 65–75% reduction in recurrences compared with the controls. Weeks after the treatment stopped, the number of recurrences was significantly lower compared to pretreatment occurrence. The decrease in recurrence was correlated with an increase in the in vitro IL-2 produced by mononuclear cells from the peripheral blood. This suggests that the antiviral effect may stem from the induction of immunologic memory for HSV after IRM treatment is completed. In a sense, IRMs may enhance the immunologic response by using the reactivated virus as an endogenous vaccine.

5. Efficacy in human subjects

These results are consistent with a randomized, placebo-controlled study of topical application of resiquimod (Sauder et al., 2003). In this study of healthy adults, 0.25% resiquimod was applied 8 h, two times per week and shown to increase levels of mRNA for IL-6, IL-8, IFN-α, and Mx (an IFN-α-inducible protein) compared to individuals receiving placebo.

The favorable results in the guinea pig model extended to human subjects in early trials. In a phase II clinical study, resiquimod reduced recurrences of genital herpes even after the end of treatment (Spruance et al., 2001). In this randomized, double-blind, vehicle-controlled, cohort dose-escalation study, 52 patients with six or more genital herpes recurrences a year were treated for 3 weeks with either resiquimod 0.01% twice or thrice weekly, 0.05% once or twice weekly, or vehicle alone. The median time to first recurrence in the vehicle-treated group was 57 days compared to 169 days in the pooled resiquimod-treated group ($p = 0.0058$) as observed during the 6-month period after therapy. The percentage of patients without a recurrence during the observation period was 32% in the resiquimod-treated group compared with 6% in the vehicle-treated group ($p = 0.039$).

The more efficacious regimens appeared to be the resiquimod 0.01% twice and thrice weekly, with median times to first recurrence at 172.5 and >195 days, respectively. The median times to first recurrence for the resiquimod 0.05% once and twice weekly were >60 and 105 days, respectively. It was thought that efficacy may be related more to dose frequency than to dose concentration. More frequent dosing may increase the chances that cytokine induction coincides with available HSV antigen, which would augment the specific immune response.

Resiquimod 0.01% twice weekly was the best tolerated regimen. There was a non-statistically significant increase in local symptoms and signs at the location of drug application for the resiquimod 0.05% treatment groups and the more frequent dosing of resiquimod 0.01% thrice weekly. Dosing with resiquimod 0.05% twice weekly was not well tolerated. Two patients who received resiquimod and one patient who received vehicle discontinued treatment because of severe local skin symptoms and signs. No systemic side effects of resiquimod were observed.

In a press release on February 24, 2003, 3M announced that due to a lack of efficacy of resiquimod gel 0.01% in recurrent genital herpes as observed in a phase III study, the clinical trial was suspended. The 3M-Lilly joint development agreement of resiquimod for recurrent genital herpes was terminated on October 1, 2003. It is not known why the positive results seen in the prior phase II did not extend to the phase III study.

6. Use as an adjuvant

Vaccination is widely considered the most important method of significantly reducing the prevalence of most viral infections (Wu et al., 2004). A Th1 immune response is required for protection against various microorganisms and tumors. Vaccination against these pathologic agents requires a strong Th1 adjuvant such as Freund's adjuvant, which is not tolerated in humans (Claassen et al., 1992). Aluminum hydroxide (alum) is the only FDA-approved vaccine adjuvant, but it enhances a Th2-specific response (Hem and White, 1995). With vaccines that are poorly immunogenic, there is a need for a safe and effective adjuvant that can adequately stimulate cell-mediated immune (CMI) responses.

Although resiquimod failed in phase III trials for genital herpes, the role of IRMs as vaccine adjuvants requires further investigation. When given together with a herpes simplex virus glycoprotein, imiquimod increased protection both prophylactically and therapeutically against HSV challenge (Bernstein et al., 1995; Bernstein et al., 1993; Harrison et al., 2001). Imiquimod enhances the CMI response (Harrison et al., 1988; Harrison et al., 1994), which destroyed transplantable murine transitional cell carcinoma (FCB) tumor cells in mice and protected them against later challenge, implying the induction of immune memory (Vasilakos et al., 2000).

In a mouse model using resiquimod, IFN-γ-dependent production of Th1-specific IgG_2 was increased and the level of Th2-specific IgE was decreased (Vasilakos et al., 2000). The effects of resiquimod on production of these antibodies were supported by other studies (Frotscher et al., 2002; Tomai et al., 2000). The experiments of Vasilakos et al. involved the use of alum with resiquimod. Resiquimod also worked when

given only in secondary immunization, suggesting that it can skew an already existing Th2 response to a Th1 antibody response. By increasing the number and maturation of dendritic cells and IFN-γ production, resiquimod has recently been shown to be a modest adjuvant for DNA vaccination (Otero et al., 2004; Thomsen et al., 2004).

7. Conclusion

Although imiquimod has been approved for anogenital warts, actinic keratosis and superficial basal cell carcinomas, it and its more potent analog, resiquimod, have not been shown to be clinically effective in genital herpes (Schacker et al., 2002). However, resiquimod may play a role in the future for other viral infections such as human papillomavirus and molluscum contagiosum. IRMs may have efficacy in the treatment of conditions that are treated with IFN-α, such as Kaposi's sarcoma and chronic hepatitis C infection (Dockrell and Kinghorn, 2001). IRMs, especially resiquimod, have also shown activity against leishmaniasis due to nitric oxide synthesis in macrophages in an animal model of cutaneous leishmaniasis (Buates and Matlashewski, 1999).

Resiquimod has strong potential as a therapy and/or vaccine adjuvant for viruses and tumors that require a Th1 response as well as Th2 diseases such as asthma and other atopic diseases. However, it may have a harmful effect in Th1-associated autoimmune disorders (Liblau et al., 1995).

For unknown reasons, the IRMs have not shown consistent efficacy in recurrent genital herpes. Imiquimod does not alter the short-term natural history of recurrent genital herpes (Schacker et al., 2002). Although clinical trials for resiquimod gel as therapy for genital herpes have been suspended due to lack of efficacy, it may make a comeback as a vaccine adjuvant for viral infections that require a strong Th1 immune response. In the next few years, it is probable that resiquimod will be more heavily investigated as a vaccine adjuvant. If it is shown to have clinical efficacy in humans, IRMs in combination with nucleosides might dramatically reduce transmission and recurrence rates of viral infections.

As the prevalence of genital herpes markedly increases on a global scale, public health warrants the development of treatments that reduce the risk of transmission and the frequency of recurrence. By stimulating the innate immune system by driving the Th1 cytokines, resiquimod may provide the solution as an "endogenous vaccine" that may be equivalent to or surpass traditional vaccinations. Resiquimod has the potential to quell the painful relapses and even transmission of genital herpes.

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