CLINICAL IMPLICATIONS OF BASIC RESEARCH

# Silencing Herpes Simplex Virus with a Vaginal Microbicide

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The use of RNA interference (RNAi) as an experimental tool to analyze mammalian gene function has increased dramatically over the past five years. Its application to animal models of human disease is also on the increase. Palliser and colleagues recently provided an example: they used RNAi to prevent herpes simplex virus 2 (HSV-2) infection of the vaginal mucosa of mice,[1] opening up possibilities for the development of topical RNAi microbicides to block viruses as they enter the body.

RNAi was first described in plants and later found to be an endogenous process by which mammalian messenger RNAs (mRNAs) are inactivated.[2,3] Small interfering RNAs (siRNAs), which are about 20 bp long, are introduced into cells or are processed from longer double-stranded RNAs produced from DNA expression vectors. The siRNAs associate with a silencing complex, promoting sequence-specific hybridization with cytoplasmic mRNAs and selective degradation or inactivation of the mRNAs by arresting translation (Fig. 1). In cultured cells, siRNAs are effective (often reducing gene expression by more than 90 percent), nontoxic, readily applied, and specific, although "off-target" effects have been observed. For these reasons, approaches involving siRNAs have largely replaced the use of ribozyme and antisense techniques. On the downside, the delivery of siRNAs to certain solid tissues can be problematic and, although siRNAs are relatively stable in the blood, they are rapidly excreted. The use of chemically modified RNAs, virus vectors, or siRNAs or nanoparticles in complex with target-specific ligands may promote efficient and tissue-specific delivery and avoid toxic effects in other organs.[2]

Mammalian viruses are especially attractive targets for RNAi. Viral RNAs (whose sequences are unrelated to those of any host RNAs) are produced in the cytoplasm, and siRNAs can effectively silence the expression of viral genes and block viral replication. Partial or incomplete inhibition of this process can have profound effects: even a fairly small delay in viral replication can allow the immune system to catch up and effectively control the spread of the virus.

Palliser et al. joined anti–HSV-2 siRNAs to cationic lipids and applied the complex to the vaginal epithelia of mice (Fig. 1). The siRNAs prevented viral infection and shedding and protected mice against a lethal HSV-2 challenge. The authors made several interesting observations. First, the use of a combination of two siRNAs, even when delivered hours after exposure to the virus, reduced the risk of infection and death. Second, the antiviral responses were durable, lasting for nine days or longer. Third, viral shedding into vaginal fluids was scant, averaging less than 1 percent of that in control mice, and the majority of siRNA-treated animals did not shed detectable amounts of virus. There was no indication that the targeted genes mutated to avoid this enemy. (This finding is, perhaps, not unexpected given that replication of HSV DNA is of higher fidelity than, for example, that of human immunodeficiency virus [HIV] or influenza virus.) Moreover, there was no evidence of siRNA-induced cytokine responses. The findings are clear: siRNAs silenced the expression of the HSV-2 gene in the vaginal epithelium, blocking replication and spread of the virus.

Delivery of siRNAs into epithelial tissues, obviously, has major advantages because viruses can be intercepted as they enter the body. The cornea may also be amenable to this type of therapy. HSV keratitis is still a leading cause of blindness. Regardless, the findings of Palliser et al.[1] raise the exciting prospect of the use of siRNAs as vaginal microbicides, either to prevent HSV

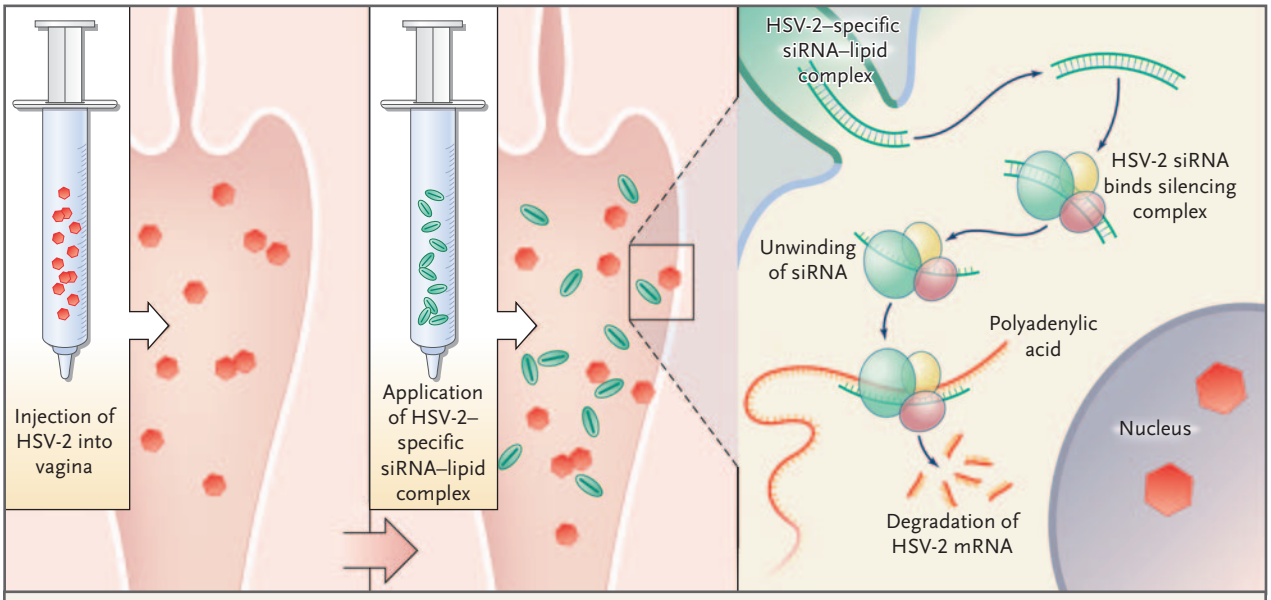


**Figure 1. Assaulting Herpes Simplex Virus 2 (HSV-2) in Mice.**

A recent study by Palliser et al.[1] shows that topical application of a small interfering RNA (siRNA) specific to an HSV-2 gene protects mice against infection with and death from the virus. The siRNA recruits a silencing complex complementary to the messenger RNA (mRNA) encoded by HSV-2. The complex binds the target HSV-2 mRNA and degrades it, thus preventing protein synthesis and HSV-2 replication. This proof-of-principle experiment suggests a prophylactic strategy by which to counter HSV-2 infection in humans.

infection or to reduce the pathology of the virus in recurrent disease. Complexes of siRNAs and lipids or other carriers could be included in spermicidal gels. One could argue that developing such treatments is not worthwhile, given the existence of effective drugs against HSV. However, microbicides are cheap and preventative, and a topical agent has enormous advantages over systemic use of a drug. The authors extend this notion to the prevention of HIV infection in genital tissues, but here, the hurdles mount quickly. HIV can infect cells that are moving targets, such as macrophages and T cells that travel into the mucosa and may be harder to hit. Moreover, there is relatively high sequence variability among HIV strains — and HIV mutates more rapidly than HSV — rendering their escape from an siRNA more likely. The use of multiple siRNAs targeting different HIV strains and genes may solve these problems. However, countering infection with HSV may also be achieved in part through countering infection with HIV. As the authors note, by blocking access to the blood through HSV lesions, anti-HSV microbicides may reduce the chances of infection with HIV.

No potential conflict of interest relevant to this article was reported.

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