Antiviral Effects of Nitric Oxide-Releasing Drug Candidates in Suppressing Productive Infection of HPV-18 in an Organotypic Epithelial Raft Culture Model System


ABSTRACT

HPV can cause persistent infections of mucosal and cutaneous epithelia in patients. There has not been an effective pharmaceutical agent to treat benign infections reliably. Nitric oxide (NO), a key component of the host immune response, could be in principle act in vivo to curb HPV infection. Novan has been developing a novel approach as a potential treatment of HPV lesions by stably storing nitric oxide while maintaining (A) the same total amount of nitric oxide released over time. (B) the nitric oxide releasing properties of the compounds, and (C) the same total amount of nitric oxide released over time. This approach, applied to HPV-18 raft cultures, showed that NO-loaded nanoparticles (NVN) were able to effectively inhibit HPV-18 DNA amplification in organotypic raft cultures of primary human keratinocytes (PHKs) harboring whole genomic HPV replicons to assess the efficacy of two of these agents against HPV infection. We also measured selective toxicity to infected cells compared to uninfected PHKs. The compounds were applied topically once a day for up to a week, each time for one hour, and then removed. Our results showed significant inhibition of HPV-18 DNA amplification in these cultures despite an absence of immune cells. Thus, other mechanisms are believed to be responsible. We performed in situ assays of key biomarkers as well as western immunoblots of raft culture lysates. Informative alterations in a number of viral and host proteins were identified. These changes likely contributed to the inhibitory effects observed with the NO-releasing agents.

RESULTS

Novan Compounds Efficiently Inhibit HPV-18 DNA Amplification

Relative copy numbers of HPV-18 DNA extracted from raft cultures determined by real-time PCR in a subsequent study. HPV-18 DNA copy number was reduced to non-detectable levels following 6 daily exposures to 1.0 mg/ml NVN4000 (data not shown). This result was confirmed by fluorescence in situ hybridization on the 2nd and 3rd set of experiments (see below).

CONCLUSIONS

- Both Novan compounds, fast (NVN1000) and slow (NVN4000) releasing, applied at 1.5 mg/ml or higher concentration inhibited HPV-18 DNA amplification and HPV-18 induced suprabasal host DNA replication.
- E6 and E7 proteins, both of which contain zinc finger motives, are potential targets of NVN compounds.
- E6 protein levels are dramatically reduced by NVN4000, leading to a sharp increase of p53. At this high concentration, it also induces apoptosis in HPV-18 raft cultures, reducing expression and activity of E6 and E7. At this high concentration, it also induces apoptosis in HPV-18 raft cultures.
- E6 expression is reduced and its activity is compromised by NVN4000, as deduced from reduced expression and activity of E6 and E7. At this high concentration, it also induces apoptosis in HPV-18 raft cultures.
- The level of p53, a target of E7 for down-regulation, did not change significantly. However, hypophosphorylated forms of pRB increased.
- The level of E6 protein was significantly reduced.
- The E6 protein target, p53, was significantly elevated.
- high p53AX and extensive apoptosis were detected in cultures treated with NVN4000 at 2.0 mg/ml, suggesting the reduction of viral DNA replication.}

Key References: