Antiviral activity and molecular mechanism of an orally active respiratory syncytial virus fusion inhibitor

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BMS-433771 is an orally bioavailable respiratory syncytial virus (RSV) inhibitor, functioning through inhibition of viral F protein-induced membrane fusion. The compound is active against both A and B groups of RSV, with an average EC50 of 20 nM. BMS-433771 is also efficacious against RSV infection in two rodent models when dosed orally prior to infection. The compound possesses good pharmacokinetic properties, while maintaining a favourable toxicity profile. Consequently, BMS-433771 is well suited for further clinical evaluation in humans. Direct affinity labelling studies indicate that the compound binds in a hydrophobic cavity within the trimeric N-terminal heptad repeat. During the fusion process, this heptad repeat associates with a C-terminal heptad repeat to form a six helical coiled-coil bundle (or trimer-of-hairpins), and BMS-433771 presumably interferes with the functional association of these heptad repeats. The fusion protein of many other class 1 fusion viruses, such as HIV and influenza, form similar hairpin structures as a prelude to membrane fusion. The identification of BMS-433771 provides a proof of concept for small molecule inhibitors that target the formation of the six helical coiled-coil structure, which could be a prototype for the development of similar antivirals against other class 1 fusion viruses.

Keywords: respiratory syncytial virus, trimer-of-hairpins, heptad repeats, BMS-433771

Introduction

Respiratory syncytial virus (RSV) is a major cause of virus-induced lower respiratory tract disease in infants and young children. In addition, RSV is the most widespread virus detected in middle ear fluid of children with otitis media.1 Recent studies indicate that RSV infection is also a significant and underestimated aetiological agent in the elderly and in immunosuppressed adults.2 Moreover, RSV is one of the most widespread nosocomial infections and is a particularly significant pathogen in bone marrow transplant patients. The current clinically approved anti-RSV agents are ribavirin and a humanized monoclonal antibody, Synagis, both of which are restricted for use only in high-risk patients due to difficulty in administration or high cost. BMS-433771 is the first reported orally active, small molecule RSV antiviral agent with favourable pharmacokinetic properties, thus warranting further investigation in the clinic.

Evolution of BMS-433771: a potential clinical candidate

SP-78 (Figure 1) was initially identified as a potent inhibitor of RSV replication using a high throughput tissue culture screen.3 This benzotriazole-based chemotype was shown to be active against both A and B subtypes of RSV, but had no activity against the closely related Sendai and parainfluenza-3 viruses, or influenza virus, vesicular stomatitis virus, human immunodeficiency virus (HIV) or herpes simplex virus. Additional studies showed that SP-78 was a specific inhibitor of RSV–host cell membrane fusion, an essential step in virus entry. This prompted the initiation of a synthetic chemistry programme to probe the SP-78 pharmacophore, with the objective of improving potency and incorporating pharmacokinetic properties that would allow for a convenient oral dosing regimen. Structure–activity relationships were generated based upon an examination of the three key structural elements: (1) the benzimidazole moiety; (2) the benzotriazole heterocycle; and (3) the dialkylaminoethyl side chain.5,3 Although many early compounds displayed potent inhibitory activity in cell culture, with EC50s below 30 nM, most of these agents lacked in vivo antiviral efficacy due to poor metabolic stability and inadequate pharmacokinetic properties. Initial attempts to circumvent these problems and establish proof of concept for these novel RSV fusion inhibitors focused on the topical delivery of compounds to the site of RSV infection in cotton rat lungs via small particle aerosol. A series of water-soluble derivatives, of which BMS-315554 is representative, were prepared (Figure 1). In collaboration with Dr Phil Wyde at

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BMS-433771, all of which exhibited nearly identical biochemical profiles. Time-of-addition experiments indicated that BMS-433771 acts at an early stage of infection. Further studies demonstrated that BMS-433771 inhibits RSV at a stage where it remains susceptible to antibody neutralization. Additionally, it was shown that the compound can function at a late stage in the infection cycle by directly inhibiting syncytia formation when added subsequent to the establishment of a productive infection. Collectively, these data support inhibition of fusion as the mechanism of action for BMS-433771. Interfering with virus–host cell fusion is an antiviral mechanism that is being investigated for a number of different viruses, including HIV, where proof-of-principle has been demonstrated in a clinical setting using enfuvirtide (Fuzeon), a 36 amino acid peptide that interferes with the function of the fusion protein gp41.

Resistant viruses were generated using several structurally related inhibitors in this series in order to elucidate the molecular target. Gene sequencing of the resistant viruses revealed that amino acid changes were detected only in the F1 subunit. A single K394R mutation in the F1 protein inserted into an RSV A2 infectious clone conferred resistance to BMS-433771, further confirming the importance of the F1 polypeptide in the mode of action of these inhibitors. The F1 protein contains the hydrophobic fusion peptide at the N-terminus and two hydrophobic heptad repeat domains, one adjacent to the fusion peptide (HR-N) and one adjacent to the transmembrane-spanning domain (HR-C). A conformational change in the F1 protein exposes the fusion peptide, which then inserts into the opposing host cell membrane. Following a second rearrangement of F1, the two heptad repeat domains associate into a six-helix bundle structure, stabilizing a trimer-of-hairpins configuration in the F1 that catalyses the fusion of viral and host cell membranes. The peptide inhibitor enfuvirtide blocks this interaction by binding directly to the HR-N region of the HIV gp41 protein. Similar peptides have been identified for RSV, as well as other class I fusion proteins. BMS-433771 is a small molecule that presumably blocks the functional interaction of the HR-N to the HR-C peptide by binding tightly in a pocket formed in the HR-N region.

In order to demonstrate directly binding of the BMS-433771 chemotype to the F protein, a radiolabelled photoaffinity probe, \([^{1^2}\text{I}]\)-BMS-356188, was synthesized (Figure 1). Ultraviolet ray activation of this probe yields a highly chemically reactive carbene species that can covalently insert into proximal amino acids within the inhibitor binding site. The photoaffinity probe labelled the RSV F1 polypeptide exclusively in a reaction that could be prevented by the addition of a molar excess of BMS-433771. Peptide mapping indicated that the probe was binding within the N-terminal heptad repeat domain. Further photoaffinity labelling studies conducted with isolated peptides showed that the compound specifically reacted with the tyrosine residue at position 198 of the F protein. The structure of a key portion of the RSV fusion protein six-helix bundle has been solved using the N- and C-terminal heptad repeat peptides that associate into the fusogenic trimer-of-hairpins.

This crystal structure revealed a hydrophobic pocket in the HR-N trimer, where two key phenylalanines (F-483 and F-488) from the HR-C bind. Tyrosine 198 (Y-198) is present in this pocket and BMS-356188 can be readily modelled into this cavity in an orientation where the photo-reactive diazirine is directed towards this tyrosine. Based upon these results, we propose that these inhibitors...
interfere with the interaction of the key amino acids residues F-483 and F-488 from the HR-C with the hydrophobic cavity in the HR-N trimer, thereby destabilizing the trimer-of-hairpins structure required for fusion (Figure 2).

**BMS-433771 in vitro and in vivo anti-RSV activity**

BMS-433771 is a potent inhibitor of multiple laboratory strains of both A and B RSV subgroups, including the Long, A2 and B-Washington strains, as well as clinical isolates of both A and B strains, with EC_{50}s ranging from 9 to 50 nM in cell culture. In addition, BMS-433771 does not display significant cytotoxicity in any cell lines in which it was examined, including human hepatocytes.

BMS-433771 exhibited oral efficacy in two rodent models of RSV infection when administered 1 h prior to RSV inoculation. Significant reductions in viral titres were reproducibly achieved with a 5.0 mg/kg dose in RSV-infected mice. For infected cotton rats, a five-fold higher dose of 25 mg/kg of BMS-433771 was required to obtain an equivalent reduction in viral titre. No evidence for the selection of virus resistant to BMS-433771 was observed in either model. Although the exact basis for the disparity in efficacy levels between the two rodent models is not known, potential explanations may be related to the higher permissiveness of the cotton rat to RSV infection compared with the mouse, a pharmacodynamic divergence between the two rodent models, or differences in the pathogenesis and compartments of RSV infection in the respiratory tract of these rodents.

In order to establish that the mechanism of action of BMS-433771 in vivo is due to inhibition of virus fusion, a BMS-433771-resistant virus was used to infect BALB/c mice. Consistent with a fusion inhibitory mode of action in vivo, mice infected with resistant virus were insensitive to compound treatment at a dosage that effectively suppressed wild-type virus infection. BMS-433771 was also found to be effective against RSV in immunosuppressed mice, indicating that the host immune response does not contribute to the antiviral efficacy of the compound. In the mouse model, BMS-433771 was only marginally active when dosed in a therapeutic mode in which the drug is administered 1 h post-virus infection. This was consistent with the findings that a single oral prophylactic dose of BMS-433771 was as effective in reducing RSV lung titres as a multiple 4 day twice-daily dosing regimen in which only one dose was given before virus inoculation. The lack of therapeutic efficacy in mice may be related to the pathogenesis of the virus in this animal model or, alternatively, the mechanism of action of BMS-433771 as a fusion inhibitor may be a contributing factor. To that end, similar findings have been obtained for the topical efficacy of RSV fusion inhibitor, RFI-641 (CL387626) in cotton rats. RFI-641 was found to be highly active when dosed topically in cotton rats 4–5 days prior to infection, but was inactive when dosed via therapeutic regimens. However, RFI-641 has demonstrated therapeutic efficacy in an African Green Monkey infection model, where it reduced viral titres in both the nasal and throat compartments. RSV infection in African Green Monkeys results in a disease state similar to that seen in humans, whereas RSV infection in either mice or cotton rats does not cause overt symptoms. Interestingly, INJ 2408068 (R170591), another RSV fusion inhibitor, has been reported to exhibit both prophylactic and therapeutic activity in a cotton rat model of infection after delivery via small particle aerosol. The reason for the different inhibitory profiles of these topical inhibitors and the implications for clinical application is not clear, but could be a reflection of unknown dynamics associated with host–species–virus interactions. Moreover, since the small animal models of RSV infection poorly recapitulate the clinical syndrome, the efficacy of RSV inhibitors under prophylactic and therapeutic conditions will need to be determined in a Phase 2a study, where the timing of infection is carefully controlled. A third RSV fusion inhibitor has also been recently disclosed, although BMS-433771 is unique among the four, as it is the sole member of this class of fusion inhibitor, with significant oral bioavailability and the ability to inhibit virus infection in rodent models after oral administration. Another class of RSV inhibitors with good oral bioavailability, but an unknown mechanism of action, is also currently in clinical development, although no animal efficacy data have yet been presented.

![Figure 2](https://academic.oup.com/jac/article-abstract/55/3/289/758339/531x763)
Clinical experience with the influenza neuraminidase inhibitors may provide a blueprint for the development and clinical use of RSV inhibitors. Studies clearly show that this class of antiviral against influenza virus can have both prophylactic and therapeutic activity. However, maximum therapeutic activity against influenza-related disease is observed if treatment initiates soon after infection, making early diagnosis an important treatment factor. RSV usually begins with a 3–5 day upper respiratory tract infection before progressing to more serious lower respiratory tract illness. This upper respiratory tract incubation period may therefore provide a window, whereby therapeutic intervention with a small molecule inhibitor may be most effective. A key element of this is the need to diagnose and treat the infection before it can spread to the lower respiratory tract. Although diagnostic tests are currently available for RSV, additional studies are needed to determine whether these test kits would be sensitive enough to detect RSV infection in its early stages. In addition, for maximum effect, these kits should be available as a point-of-care tool for the practising physician.

Conclusions

Currently, there is no effective RSV vaccine and immunoprophylaxis is restricted to use in infants. In addition, ribavirin aerosol therapy is difficult to administer and its cost-effectiveness has been questioned. Thus, there is a clinical need for an effective and easy to administer antiviral agent for RSV. BMS-433771 is a member of a new class of RSV fusion inhibitors and may be a candidate for this unmet medical need, pending further clinical assessment.

References