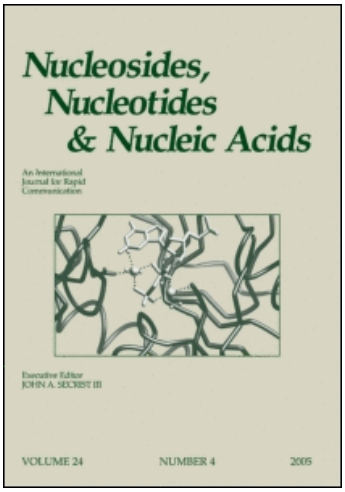

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Targeted Therapy of Respiratory Syncytial Virus by 2_5A Antisense

Hagen Cramer[a]; James R. Okicki[a]; Mei Kuang[a]; Zan Xu[a]
[a] Ridgeway Biosystems, Inc., Cleveland, Ohio, USA

To cite this Article Cramer, Hagen , Okicki, James R. , Kuang, Mei and Xu, Zan(2005) 'Targeted Therapy of Respiratory Syncytial Virus by 2_5A Antisense', Nucleosides, Nucleotides and Nucleic Acids, 24: 5, 497 — 501
To link to this Article: DOI: 10.1081/NCN-200061780
URL: http://dx.doi.org/10.1081/NCN-200061780

Nucleosides, Nucleotides, and Nucleic Acids, 24 (5–7):497–501, (2005)

ISSN: 1525-7770 print/ 1532-2335 online
DOI: 10.1081/NCN-200061780

Taylor & Francis
Taylor & Francis Group

TARGETED THERAPY OF RESPIRATORY SYNCYTIAL VIRUS BY 2-5A ANTISENSE

Hagen Cramer, James R. Okicki, Mei Kuang, and Zan Xu ▫ *Ridgeway Biosystems, Inc., Cleveland, Ohio, USA*

▫ *Respiratory syncytial virus is a leading cause of respiratory disease in infants, young children, immunocompromized patients, and the elderly. Previous work has shown that RNase L, an antiviral enzyme of the interferon system, can be recruited to cleave RSV genomic RNA by attaching tetrameric 2′ 5′-linked oligoadenylates (2 5A) to an antisense oligonucleotide complementary to repetitive intergenic sequences within the RSV genome (2 5A antisense). RBI034, a 2′-O-methyl RNA-modified analogue of the 2 5A anti-RSV compound, was found to have enhanced antiviral activity in cell culture studies while also cleaving RSV genomic RNA in an RNase L- and sequence-specific manner. RBI034s efficacy in suppressing RSV replication in cell culture is 50 to 100 times better than ribavirin, the only approved drug for RSV infection. Here we show that the activity of 2 5A antisense compound can be further enhanced by a combination treatment with interferon or ribavirin. The anti-RSV activity resulting from combination treatment is more potent than either treatment alone. We also demonstrate that RBI034 is effective against RSV in three different species: mice, cotton rats, and African green monkeys.*

INTRODUCTION

Respiratory syncytial virus (RSV) is a nonsegmented negative strand RNA virus belonging to the family Paramyxoviridae, subfamily Pneumoviridae.[1] Immediately after entering the cell, the RNA-dependent RNA polymerase transcribes 10 distinctive mRNA species by recognizing GS (gene starting) and GE (gene ending) elements of each gene.[2] The first nine RSV genes have a conservative intergenic GS element (AUUUGCCCC) in the 5′ region of each individual gene. Human RSV is a highly contagious virus, which infects all age groups. Outbreaks in the United States frequently reach epidemic proportions during the winter months. RSV causes severe respiratory disease in infants, children, and the elderly with

We thank Phil Wyde from Baylor College of Medicine, Ken Soike from Tulane, and Shyam Mohapatra from University of South Florida for performing cotton rat, African green monkey, and mouse studies, respectively. This work was in part supported by an NIH SBIR grant to H.C. (7R44-AI46079-03).

Address correspondence to Dr. Hagen Cramer, 9500 Euclid Ave., ND-50, Cleveland, OH 44195, USA; Fax: (216) 445-1344; E-mail: cramerh@ridgewaybio.com

weakened immune function.[3,4] Unfortunately, no effective treatment for RSV infections exists. The benefits of the only approved drug, ribavirin, have been questioned.[5] Therefore, the need for new anti-RSV drugs or therapeutic measures remains high.

We and others have previously described a promising antiviral strategy by targeting RSV infections with 2–5A antisense, thereby recruiting the cellular antiviral nuclease RNase L to specifically cleave RSV RNA.[6–9] RNase L is a ubiquitous cellular ribonuclease that is activated by 2′,5′-linked oligoadenylates (2–5A).[10] However, RNase L has little sequence specificity. In order to recruit RNase L for the sequence specific RNA cleavage, a 2–5A moiety was linked to a guide sequence complementary to the targeted RNA. Thereby, the 2–5A moiety binds and activates RNase L, while the antisense portion directs RNase L to the targeted RNA molecule, resulting in degradation of the RNA.[11–13]

RESULTS AND DISCUSSION

The most potent 2–5A anti-RSV antisense compounds discovered as of today are RBI034 and RBI245. Their antisense parts are entirely composed of 2′-*O*-methyl nucleosides including three phosphothioate internucleotide linkages at either end resulting in increased in vivo stability, improved affinity for the RSV genomic RNA, and enhanced cleavage activity. RBI034's 2–5A part is linked to the antisense part by two butane diol linkers, while RBI245's moieties are linked by one triethyleneglycol linker. Synthesis was performed similar to published procedures.[9]

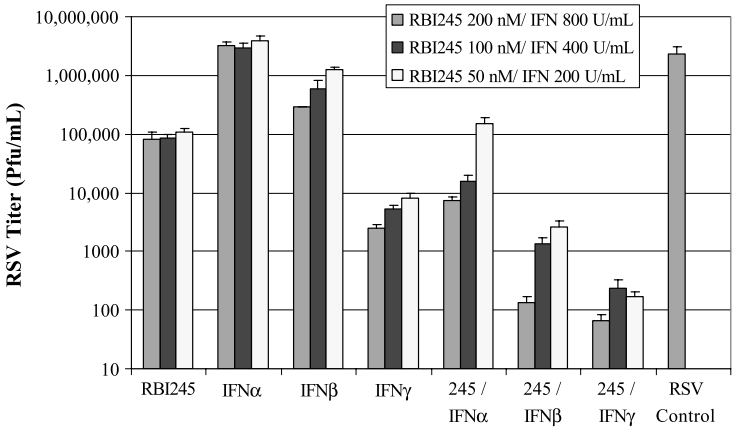

FIGURE 1 Synergistic anti-viral activity by combination treatment of RBI245 with interferon. HEp-2 cells were pretreated with IFNα2, β, or γ at concentrations between 200 and 800 U/mL. After 20 h, cells were infected with RSV at an m.o.i. of 0.01 and treated immediately with RBI245 as indicated. Progeny viral release was determined at day 4 after infection when there were no viable cells remaining in the RSV control. No cytotoxicity was observed for any of the compounds either alone or in combination using the CellTiter 96 AQueous Cell Proliferation Assay (Promega).

Combination treatment of ribavirin and RBI034 at low concentrations demonstrated a potent protection against RSV infection in cell culture, much more efficient than either treatment alone. The synergistic activity of combination treatment was confirmed by neutral red uptake assay, Taqman PCR, ELISA (fusion protein), and progeny virus titer measurement.[14]

Combination treatment of interferon and RBI245 at low concentrations demonstrated synergistic protection against RSV infection in cell culture. Activity was confirmed in neutral red assay, Taqman PCR, and viral titer measurements (data of viral titer measurements shown in Figure 1).

RBI034 and RBI245 are Reducing RSV Infections in Animal Models

RBI245 reduced viral titers from over 2000 to about 100 PFUs in mice when RBI245 was given intranasally at a concentration of 10 mg/kg every other day on day 1, 3, and 5 after and once 6 h before RSV infection (5×10^6 PFU by i.n.). Viral titers were determined from lung homogenates at day 6 after infection. RBI034/RBI245 reduced RSV infection in cotton rats when delivered by intranasal drops or by aerosol. When administered intranasally, RBI034 was effective in reducing viral titers at a dose, whereas ribavirin is completely ineffective (Figure 2).[15] RBI245 was effective in reducing viral titers in cotton rats when delivered by aerosol for 15 min/day using a reservoir concentration of 50 mg/mL RBI245 in water (Figure 3). In comparison, cotton rats need to be treated for a minimum of 2 h a day with ribavirin at such concentrations to be effective.[16–19] Similar results were obtained with RBI034 (not shown). RBI034 was able to reduce viral titers in African green

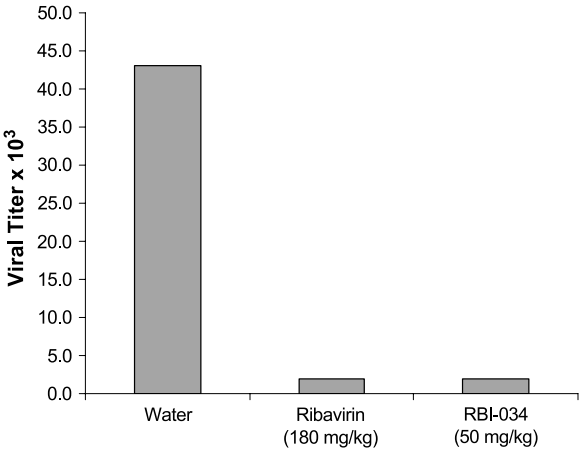

FIGURE 2 RBI034 is effective in reducing viral titers in cotton rats when delivered intranasally. Cotton rats were infected i.n. with virus (1×10^5 $TCID_{50}$). Cotton rats were treated with either 100 μL of water, 13.5 mg (180 mg/kg) of ribavirin, or 4 mg (50 mg/kg) of RBI034 in 100 μL water directly after, and on day 1, 2, and 3 after infection. On day 4 animals were sacrificed and RSV titers of lung fluids quantified.

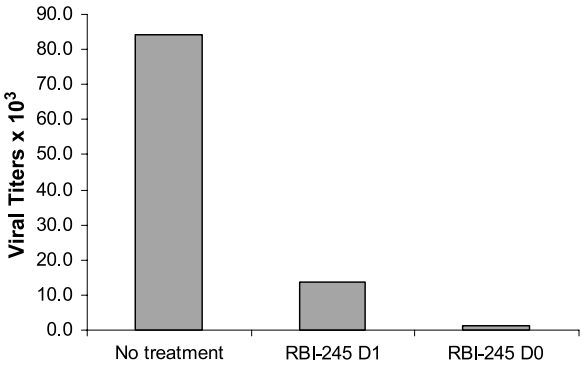

FIGURE 3 RBI245 is effective in reducing viral titers in cotton rats when delivered by aerosol. Cotton rats were infected i.n. with RSV (1×10^5 $TCID_{50}$). Cotton rats in group D0 received 15 min of aerosolized RBI245 (nose-only treatments, solution strength 50 mg/mL) immediately following infection and on day 1, 2, and 3. Cotton rats in group D1 received 15 min of aerosolized RBI245 on day 1, 2, and 3 after infection. On day 4 animals were sacrificed and the RSV titers in the lung fluids quantified. Particle size and aerosol concentration was determined using an Anderson 8-stage compactor: MMAD = 1.35 μm, aerosol concentration = 0.252 mg/L, estimated achieved dose = 1.2 mg/kg/day.

monkeys by up to 10,000-fold over control animals when administered intranasally.[9]

CONCLUSION

2–5A antisense compounds RBI034 and RBI245 were found to be effective anti-RSV agents suitable for clinical evaluation. Activity was demonstrated in three species using different routs of administration. No toxicity was apparent. The enhanced activity seen during combination treatments of RBI034 with interferons or ribavirin offers hope for a possible new treatment regiment against RSV infections in humans. Further in vivo efficacy and toxicity studies are ongoing.

REFERENCES

1. Collins, P.L.; Chanock, R.H.; McIntosh, K. Respiratory syncytial virus. In *Field's Virology*; 3rd Ed.; Fields, B.N., Knipe, D.M., Howley, P.M., Chanock, R.M., Melnick, J.L., Monath, T.P., Roizman, B., Straus, S.E., Eds.; Lippincott-Raven: Philadelphia, 1996; 1313–1351.
2. Collins, P.L.; Dickens, L.E.; Buckler-White, A.; Olmsted, R.A.; Spriggs, M.K.; Camargo, E.; Coelingh, K.V. Nucleotide sequences for the gene junctions of human respiratory syncytial virus reveal distinctive features of intergenic structure and gene order. Proc. Natl. Acad. Sci. U. S. A. **1986**, *83*(13), 4594–4598.
3. Wyde, P.R. Respiratory syncytial virus (RSV) disease and prospects for its control. Antivir. Res. **1998**, *39*(2), 63–79.
4. Falsey, A.R.; Walsh, E.E. Respiratory syncytial virus infection in adults. Clin. Microbiol. Rev. **2000**, *13*(3), 371–384.
5. Dominguez, K.D.; Mercier, R.C. Treatment of RSV pneumonia in adults—evidence of ribavirin effectiveness? Ann. Pharmacother. **1999**, *33*(6), 739–741.
6. Barnard, D.L.; Sidwell, R.W.; Xiao, W.; Player, M.R.; Adah, S.A.; Torrence, P.F. 2–5A-DNA conjugate inhibition of respiratory syncytial virus replication: effects of oligonucleotide structure modifications and RNA target site selection. Antivir. Res. **1999**, *41*(3), 119–134.

7. Cirino, N.M.; Li, G.; Xiao, W.; Torrence, P.F.; Silverman, R.H. Targeting RNA decay with 2′,5′ oligoadenylate-antisense in respiratory syncytial virus-infected cells. Proc. Natl. Acad. Sci. U. S. A. **1997**, *94*(5), 1937–1942.
8. Player, M.R.; Barnard, D.L.; Torrence, P.F. Potent inhibition of respiratory syncytial virus replication using a 2–5A-antisense chimera targeted to signals within the virus genomic RNA. Proc. Natl. Acad. Sci. U. S. A. **1998**, *95*(15), 8874–8879.
9. Leaman, D.W.; Longano, F.J.; Okicki, J.R.; Soike, K.F.; Torrence, P.F.; Silverman, R.H.; Cramer, H. Targeted therapy of respiratory syncytial virus in African green monkeys by intranasally administered 2–5A antisense. Virology **2002**, *292*(1), 70–77.
10. Silverman, R.H. 2–5A-dependent RNase L: a regulated endoribonuclease in the interferon system. In *Ribonucleases: Structures and Functions*; D'Alessio, G., Riordan, J.F., Eds.; Academic Press, 1997; 515–551.
11. Torrence, P.F.; Maitra, R.K.; Lesiak, K.; Khamnei, S.; Zhou, A.; Silverman, R.H. Targeting RNA for degradation with a (2′–5′)oligoadenylate-antisense chimera. Proc. Natl. Acad. Sci. U. S. A. **1993**, *90*(4), 1300–1304.
12. Torrence, P.F.; Xiao, W.; Li, G.; Cramer, H.; Player, M.R.; Silverman, R.H. Recruiting the 2–5A system for antisense therapeutics. Antisense Nucleic Acid Drug Dev. **1997**, *7*(3), 203–206.
13. Maran, A.; Maitra, R.K.; Kumar, A.; Dong, B.; Xiao, W.; Li, G.; Williams, B.R.; Torrence, P.F.; Silverman, R.H. Blockage of NF-kappa B signaling by selective ablation of an mRNA target by 2–5A antisense chimeras. Science **1994**, *265*, 789–792.
14. Xu, Z.; Kuang, M.; Okicki, J.R.; Cramer, H.; Chaudhary, N. Potent inhibition of respiratory syncytial virus by combination treatment with 2–5A antisense and ribavirin. Antivir. Res. **2004**, *61*(3), 195–206.
15. Wyde, P.R.; Ambrose, M.W.; Meyer, H.L.; Zolinski, C.L.; Gilbert, B.E. Evaluation of the toxicity and antiviral activity of carbocyclic 3-deazaadenosine against respiratory syncytial and parainfluenza type 3 viruses in tissue culture and in cotton rats. Antivir. Res. **1990**, *14*(4–5), 215–225.
16. Knight, V.; Gilbert, B.E.; Wyde, P.R.; Englund, J.A. High dose-short duration ribavirin aerosol treatment–a review. Bull. Int. Union Tuberc. Lung Dis. **1991**, *66*(2–3), 97–101.
17. Gilbert, B.E.; Wyde, P.R.; Ambrose, M.W.; Wilson, S.Z.; Knight, V. Further studies with short duration ribavirin aerosol for the treatment of influenza virus infection in mice and respiratory syncytial virus infection in cotton rats. Antivir. Res. **1992**, *17*(1), 33–42.
18. Gilbert, B.E.; Knight, V. Biochemistry and clinical applications of ribavirin. Antimicrob. Agents Chemother. **1986**, *30*(2), 201–205.
19. Wyde, P.R.; Wilson, S.Z.; Petrella, R.; Gilbert, B.E. Efficacy of high dose-short duration ribavirin aerosol in the treatment of respiratory syncytial virus infected cotton rats and influenza B virus infected mice. Antivir. Res. **1987**, *7*(4), 211–220.