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Combination of carrageenan and a neuraminidase inhibitor as superior treatment for influenza A

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Introduction:
The 2009 flu pandemic and the appearance of neuraminidase-inhibitor resistant H1N1 influenza strains highlight the need for treatment alternatives. One such option is the creation of a protective physical barrier in the nasal cavity. Consequently, we tested a novel combination of carrageenans together with the neuraminidase inhibitor Zanamivir in *in vitro* and *in vivo* infection experiments. This combination showed a high protection level against novel H1N1 influenza.

Results:
Treatment of mice infected with a lethal dose of influenza A PR/8/34 (H1N1) or A/HH/01/2009 (H1N1) virus resulted in a strong protection of infected animals.

Conclusions:
Novel treatment options for influenza are desperately needed. Based on these encouraging results in animals we suggest testing a nasal spray containing carrageenans in combination with neuraminidase inhibitors in a clinical trial for prevention or treatment of influenza A in humans.

In vitro effect of Carrageenan plus Zanamivir after infection with influenza A PR/8/34 (H1N1)

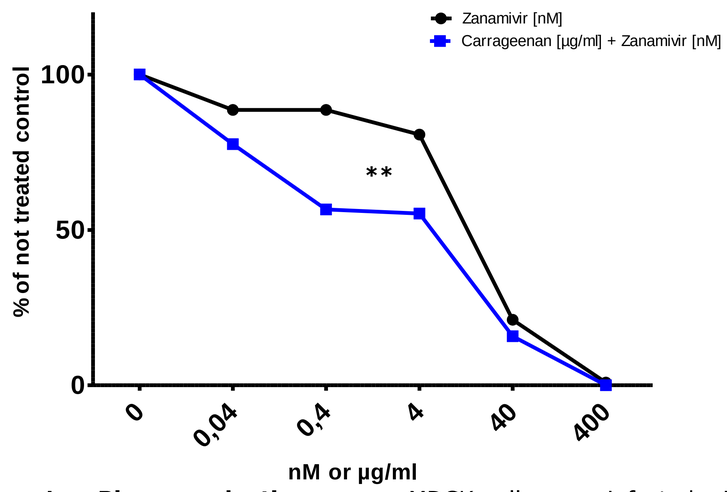

Figure 1. Plaque reduction assay: MDCK cells were infected with influenza A PR/8/34 (H1N1) at a MOI of 0,01. Percentage of plaque reduction is shown in regard to the non treated control. p values were calculated by a students t test. (Asterisk * *p<0.01)

In vitro effect of Carrageenan plus Zanamivir after infection with influenza A/HH/01/2009 (H1N1)

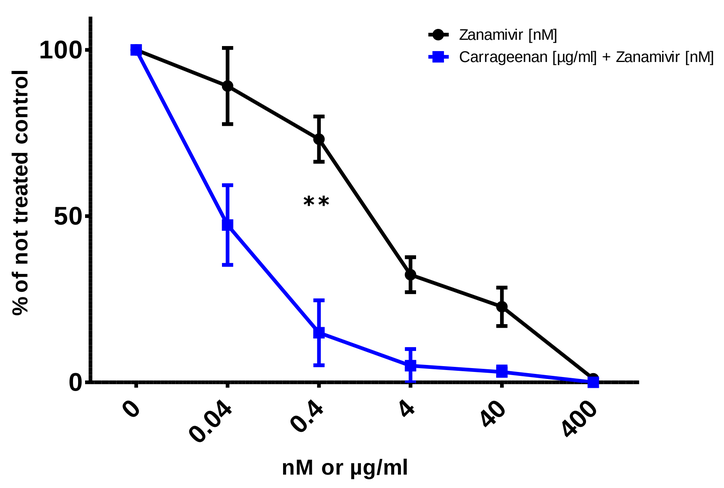

Figure 2. Plaque reduction assay: MDCK cells were infected with influenza A/HH/01/2009 (H1N1) at a MOI of 0,01. Percentage of plaque reduction is shown in regard to the non treated control. p values were calculated by a students t test. (Asterisk * *p<0.01)

In vivo infection with influenza A PR/8/34 (H1N1)

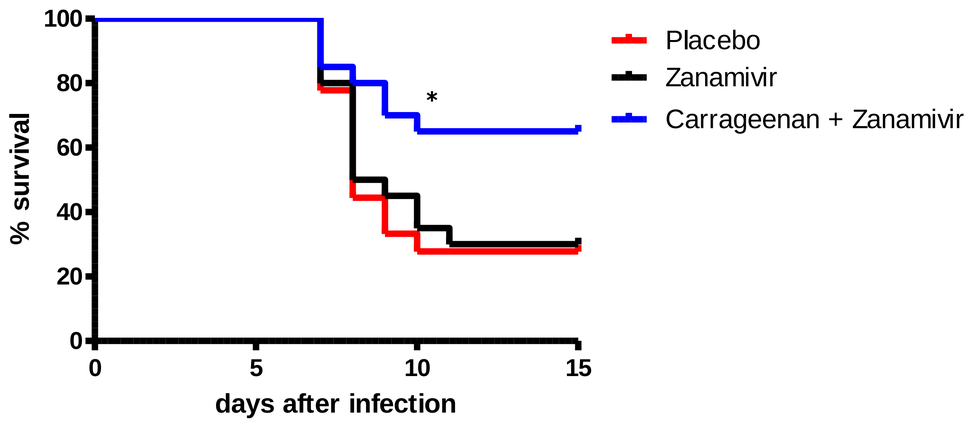

Figure 3. *In vivo* infection with influenza A PR/8/34 (H1N1):
Ten mice per group were intranasally infected with 6.3×10^3 PFU A/PR/8/34 (H1N1) viral particles at day 0. Intranasal therapy was performed with 240µg carrageenan preparation in 0.5% NaCl plus 0,5mg/kg Zanamivir (blue), 0,5mg/kg Zanamivir in 0,5% NaCl (black) or 0,5%NaCl (red) per day. Treatment started 48h post infection twice daily. P values were calculated by a Gehan Breslow Wilcoxon test. Asterisk * p<0.05

Figure 4. Red seaweed (natural source of carrageenans)

Methods:

For infection experiments influenza A PR/8/34 (H1N1) and influenza A/HH/01/2009 (H1N1nv) viruses were used. Influenza A/Hansa Hamburg/01/2009 (A/HH/01/2009) (H1N1) was kindly provided by the group of P. Stäheli[2]. Plaque reduction assays were performed with Madine Darbey Canine Kidney cells (MDCK). Cells were infected with an MOI of 0,01. *In vivo* infection experiments were performed using C57BL/6 mice infected intranasally, following FELASA guidelines, and survival was monitored over a 14 day period. Mice received $6,3x10^3$ PFU per animal for H1N1 PR8/34 and $2,3x10^2$ PFUfor novel H1N1 HH09, respectively. Animals were monitored and treated twice daily. Treatment consisted of a preparation containing carrageenans (isolated from red seaweed) in combination with Zanamivir or Zanamivir alone. Statistics were performed using graph pad prism software.

In vivo infection with influenza A/HH/01/2009 (H1N1)

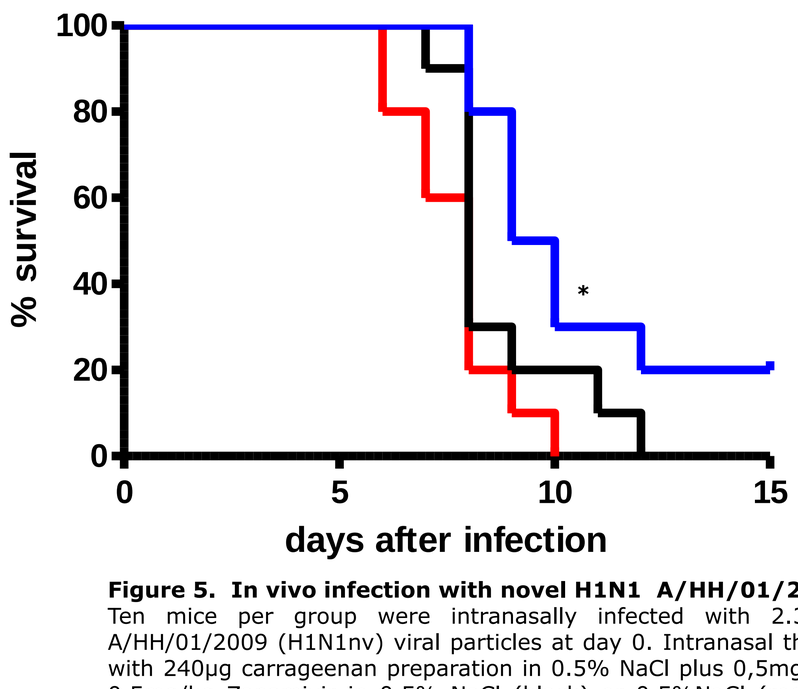

Figure 5. In vivo infection with novel H1N1 A/HH/01/2009:
Ten mice per group were intranasally infected with 2.3×10^2 PFU influenza A/HH/01/2009 (H1N1nv) viral particles at day 0. Intranasal therapy was performed with 240µg carrageenan preparation in 0.5% NaCl plus 0,5mg/kg Zanamivir (blue), 0,5mg/kg Zanamivir in 0,5% NaCl (black) or 0,5%NaCl (red) per day. Treatment started 24h post infection twice daily. P values were calculated by a Gehan Breslow Wilcoxon test. Asterisk * p<0.05

Discussion

We have already demonstrated the antiviral efficacy of carrageenan in infections with influenza A H1N1 PR/8/34 previously[1].
The present study shows that the combination of carrageenans with a neuraminidase inhibitor leads to superior protection of cells and mice compared with neuraminidase inhibitor treatment alone. Intranasal application delivers systemic levels of Zanamivir in sera of animals (data not shown). The combination of carrageenans and Zanamivir protects against infection with novel influenza A *in vitro* and *in vivo*. Taken together we propose a nasal spray containing carrageenans and Zanamivir as a novel treatment alternative for influenza A infections.

Reference:
1. Leibbrandt A, Meier C, König-Schuster M, Weinmüllner R, Kalthoff D, et al. (2010) Iota-Carrageenan Is a Potent Inhibitor of Influenza A Virus Infection. PLoS ONE
2. Seibert CW, Kaminski M, Philipp J, Rubbenstroh D, Albrecht RA, et al. Oseltamivir-resistant variants of the 2009 pandemic H1N1 inuenza A virus are not attenuated in the guinea pig and ferret transmission models. J Virology. 2010