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Beneficial therapeutic effects of delayed intrapulmonary Peramivir administration during severe H1N1 influenza infection in ferrets

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Problem

Most patients with severe influenza disease seek hospital care many days after initial onset of symptoms, limiting the effective use of antivirals. The main goal in the present study was to compare therapeutic effects of delayed intrapulmonary and intramuscular delivery of Peramivir on severe infection outcome.

Materials and Methods

To recapitulate late stage, lower respiratory tract infection, aged ferrets were inoculated intrapulmonary with 106 EID50 influenza A/California/07/2009 H1N1. Peramivir was delivered at 3 and 5 dpi intrapulmonary (PP) or intramuscularly (PM). A group of animals was administrated intrapulmonary with only a vehicle (saline) for control purposes (VP).

Results

All animals in the VP group were euthanized due to severity of the diseases at 8 dpi. Survival in the PP group (75%) was 25% higher than in the PM group (**Figure 1**). In comparison to the control VP group, intrapulmonary and intramuscular Peramivir administration efficiently reduced viral titers in nasal washes. At 5 dpi, viral titers in lungs from the PP group were lower than in samples from other groups (PP – mean 3.37 TCID50/ml; PM – mean 4.55 TCID50/ml; VP – mean 6.44 TCID50/ml) (**Figure 2**). Reduced area of lung surfaces with pathology were observed in the PP group (PP – mean 19%; PM – mean 40%; VP – mean 72%) (**Figure 3**). Microscopic lesions associated with severe disruption of the lung architecture were found only in ferrets from the VP group and one ferret from the PM group (**Figure 4**).

Conclusions

Delayed intrapulmonary Peramivir administration in aged ferrets has benefits to recovery from severe influenza infection in comparison to delayed intramuscular Peramivir administration.

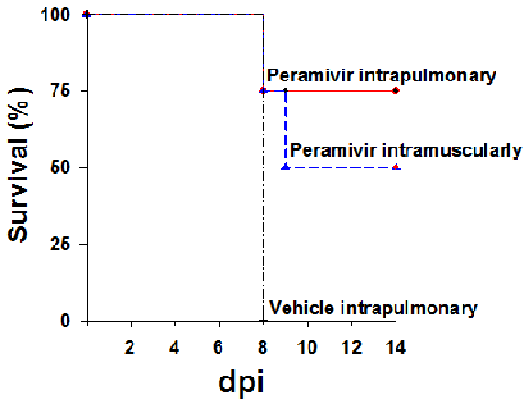

Figure 1 – Survival in aged ferrets challenged with H1N1 influenza virus intrapulmonary and administrated with Peramivir intrapulmonary, intramuscularly or with the vehicle intrapulmonary at 3 and 5 days post-virus inoculation (dpi). Kaplan-Meier survival analysis was used to generate the survival plot.

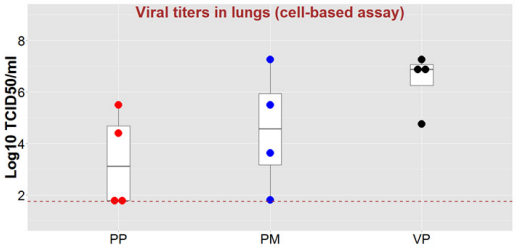

Figure 2 – Virological data of aged ferrets challenged with H1N1 influenza virus intrapulmonary and administrated with Peramivir intrapulmonary (PP), intramuscularly (PM) or with the vehicle intrapulmonary (VP) at 3 days post-virus inoculation (dpi). Lung samples were collected at 5 dpi. Viral titers at the level of the dashed line were below than assay detection limit (Log10 1.81 TCID50/ml). Each dot represents an individual ferret. Each box represents 25–75% of observations. The solid line within the box is mean. Whiskers below and above the box represent the 10th and 90th percentiles.

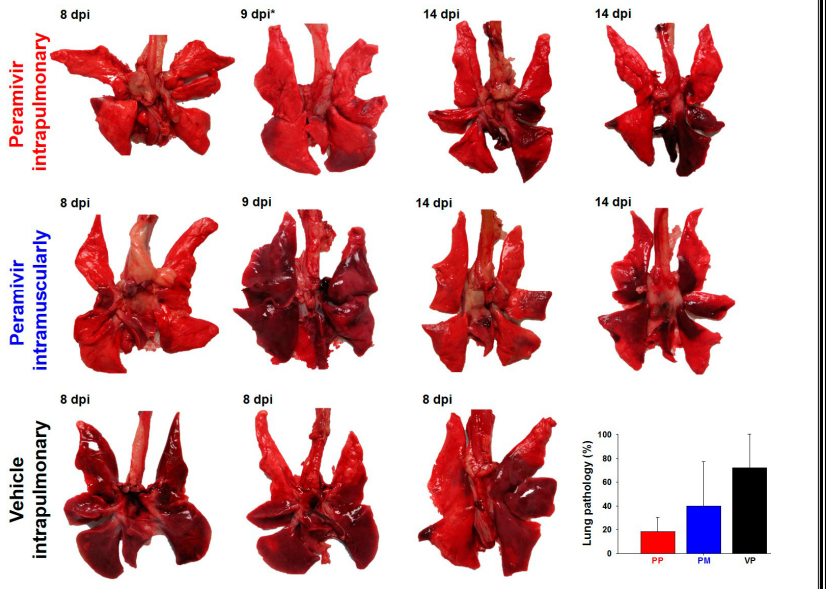

Figure 3 – Gross pathology in lungs of ferrets challenged with H1N1 influenza virus intrapulmonary and administrated with Peramivir intrapulmonary (PP), intramuscularly (PM) or with the vehicle intrapulmonary (VP) at 3 and 5 days post-virus inoculation (dpi). Lungs were collected when animals lost twenty or more percentage of baseline body weight (8 and 9 dpi) or at the end of the experiment (14 dpi). One ferret in the group administrated with Peramivir intrapulmonary was euthanized and sampled at 9 dpi (9 dpi*) to compare lung pathology. This animal was gaining weight and did not have signs of inactivity. The insert is a graph which represents the mean percentage and standard deviations of lung pathology in different groups. ImageJ software was used to select and calculate the total affected area with gross lesions from both lung surfaces.

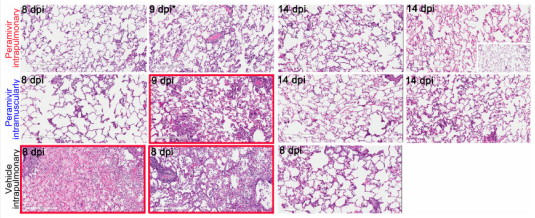

Figure 4 – Microscopic lesions in lungs of ferrets challenged with H1N1 influenza virus intrapulmonary and administrated with Peramivir intrapulmonary, intramuscularly or with the vehicle intrapulmonary at 3 and 5 days post-virus inoculation (dpi). The order of pictures is the same as in Figure 3. Lungs were collected when animals lost twenty or more percentage of baseline body weight (8 and 9 dpi) or at the end of the experiment (14 dpi). One animal in the group administrated with Peramivir intrapulmonary was euthanized and sampled at 9 dpi (9 dpi*) to compare lung pathology. This animal was gaining weight and did not have signs of inactivity. Most severe pathology was detected in the lungs of control ferrets administrated with the vehicle intrapulmonary and in one animal from the group administrated with Peramivir intramuscularly, outlined in red. Magnification ×100. The bar is 300 µm.