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学位の種類	博士（医学）
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学位論文題目	Efficacy of neuraminidase inhibitors against H5N6 highly pathogenic avian influenza virus in a non-human primate model. （非ヒト霊長類モデルにおけるH5N6高病原性鳥インフルエンザウイルスに対するノイラミニダーゼ阻害薬の有効性）
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別紙様式３（課程博士・論文博士共用）

論　文　内　容　要　旨

※整理番号	904	氏名（ふりがな）	NGUYEN THANH CONG
学位論文題目	Efficacy of neuraminidase inhibitors against H5N6 highly pathogenic avian influenza virus in a non-human primate model. 非ヒト霊長類モデルにおけるH5N6高病原性鳥インフルエンザウイルスに対するノイラミニダーゼ阻害薬の有効性		

Purpose of study

Since first detected in humans in 1997, highly pathogenic avian influenza viruses (HPAIVs) such as H5N1 and H7N9 viruses have caused high human fatality rates. Currently, the ability of transmission among humans is low, but HPAIVs mutate and pose a significant public health threat.

H5N6 HPAIV is the first influenza virus carrying N6 neuraminidase (NA) causing human mortality. Previous report showed that this HPAIV had a high potential for avian-human, human-human transmission and wide dissemination. However, the characteristics of H5N6 HPAIV infection and the efficacy of antiviral drugs against this virus have not been well clarified.

The purpose of this study is to investigate the infectious disease induced by H5N6 HPAIV infection and the efficacy of current available and easily accessible antiviral drugs.

Methods

Cynomolgus macaques were challenged with H5N6 HPAIV and then treated from day 1 to day 5 with saline (control), oseltamivir, peramivir, and amantadine. Clinical signs of diseases in macaques were monitored every day with a clinical score system. Swab samples and blood samples were collected to examine virus replication and immune responses. Macaques were autopsied on day 7 post viral challenge to examine histology of lung tissues.

Results

1) H5N6 HPAIV infection led to high fever in cynomolgus macaques.
2) The lung injury caused by the viral infection was severe, with diffuse alveolar damage and infiltration of neutrophils and macrophages.
3) Levels of interferon alpha (IFN-α), interleukin-6 (IL-6), and monocyte chemoattractant protein 1 (MCP-1) were significantly increased on day 1 and then decreased on day 3. The increase in IFN-α was inversely correlated with virus titers in the trachea and bronchus on day 1.
4) Oseltamivir and peramivir (both are neuraminidase inhibitors), but not amantadine, early reduced virus titration in swab samples.

（備考）１．論文内容要旨は、研究の目的・方法・結果・考察・結論の順に記載し、２千字程度でタイプ等を用いて印字すること。
２．※印の欄には記入しないこと。

Discussion

1) The virus caused severe pneumonia and cytokine responses in macaques, comparable to the severe states in humans infected with H5N6 HPAIV.
2) Oseltamivir treatment and peramivir treatment were effective in early reduction of virus replication in swab samples. However, we detected virus in respiratory tract tissues of peramivir-treated macaques on day 7 without any reported antiviral resistant mutation. Treatment with peramivir more than 5 days should be considered in order to reduce completely the virus replication.
3) Amantadine treatment did not reduce virus titers in swab samples and no antiviral resistant mutation was found in the inoculum virus. This finding suggested that the efficacy of amantadine is also dependent on other factors than reported amantadine-resistant mutations. We detected lower virus titers in respiratory tissues of amantadine-treated macaques than in control macaques on day 7. Amantadine possibly induced reduction of virus in tissues via effect on immune responses of macaques.
4) Levels of lung inflammation were not greatly different among antiviral drug treated macaques and control macaques. Therefore, treatment of H5N6 HPAIV infection with current antiviral drugs within 48 hours as recommended widely may have limited effectiveness in level of lung inflammation until day 7. Combination of current antiviral drugs and other medications, and development of new antiviral therapies targeting into host factors are necessary.
5) Interferon alpha (IFN-α) significantly increased and was inversely correlated with virus titers, indicating that IFN-α may be a protective factor against H5N6 HPAIV infection. Early treatment with IFN-α might be a potential therapy.

Conclusion

Oseltamivir and peramivir, but not amantadine, were effective in reduction of H5N6 HPAIV replication. However, pathological findings of severe alveolar damage were not greatly different among antiviral-treated groups and the control group. Thus, the present study showed a need for close monitoring and further studies on viral pathogenicity and development of new antiviral therapies.

別紙様式９（課程博士・論文博士共用）

博士論文審査の結果の要旨

整理番号	９０４	氏　名	NGUYEN THANH CONG
論文審査委員			

（博士論文審査の結果の要旨）

本論文では、N6 ノイラミニダーゼをもつ最初のインフルエンザウイルスである H5N6 亜型高病原性鳥インフルエンザウイルス（H5N6 HPAIV）に着目し、カニクイザルモデルを用いて、このウイルスの病原性と抗ウイルス薬の有効性について検討を行い、以下の点を明らかにした。

1）H5N6 HPAIV を感染させたカニクイザルは高熱を発した。
2）感染 7 日後の剖検肺の組織学的検討では、びまん性肺胞傷害および好中球とマクロファージの浸潤を伴う重症肺炎を認めた。
3）血中 IFN-α、IL-6、MCP-1 は感染 1 日後に上昇し、IFN-α の上昇は気管と気管支スワブ検体のウイルス力価と逆相関した。
4）ノイラミニダーゼ阻害薬のオセルタミビルは、鼻腔、気管、気管支スワブ検体のウイルス力価を低下させ、ペラミビルは早い段階ではウイルス増殖と重症度を低下させた。
5）M2 阻害薬のアマンタジンのウイルス増殖に対する抑制効果は、in vitro でも in vivo でも認められなかった。
6）病理学的にはノイラミニダーゼ阻害薬投与による肺傷害の改善は認められなかった。

本論文は、非ヒト霊長類モデルでの H5N6 HPAIV 感染の病態と抗ウイルス薬の有効性について新たな知見を与えたものであり、また最終試験として論文内容に関連した試問を実施したところ合格と判断されたので、博士（医学）の学位論文に値するものと認められた。

（令和３年１月２８日）