



Successful combinatorial therapy of sirolimus and neuraminidase inhibitors in a patient with highly pathogenic avian influenza A (H5N6) virus: a case report

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Background: Highly pathogenic avian influenza A (H5N6) virus poses a continuous threat to human health since 2014. Although neuraminidase inhibitors (NAIs) are prescribed in most patients infected with the H5N6 virus, the fatality remains high, indicating the need for an improved treatment regimen. Sirolimus, an inhibitor of the mammalian target of rapamycin (mTOR), has been reported to reduce viral replication and improve clinical outcomes in severe H1N1 infections when combined with oseltamivir. Here, we report the first case of severe H5N6 pneumonia successfully treated by sirolimus and NAIs.

Case Description: A 22-year-old man developed high fever and chills on September 24, 2018 (Day-0) and was hospitalized on Day-3. Influenza A (H5N6) was identified on Day-6 from a throat swab specimen. Despite the administration of NAIs and other supportive measures, the patient's clinical conditions and lung images showed continued deterioration, accompanied by persistently high viral titers. Consequently, sirolimus administration (rapamycin; 2 mg per day for 14 days) was started on Day-12. His PaO₂/FiO₂ values and Sequential Organ Failure Assessment (SOFA) score gradually improved, and imaging outcomes revealed the resolution of bilateral lung infiltrations. The viral titer gradually decreased and turned negative on Day-25. Sirolimus and NAIs were stopped on the same day. The patient was discharged on Day-65. Based on observations from a 2-year follow-up, the patient was found to be in a good condition without complications.

Conclusions: In conclusion, sirolimus might be a novel and practical therapeutic approach to severe H5N6-associated pneumonia in humans.

Keywords: Sirolimus; neuraminidase inhibitors; H5N6; severe pneumonia; case report

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Introduction

In 2014, scientists identified the first human infection caused by the highly pathogenic avian influenza A (H5N6) virus in China (1). In recent years, the H5N6 virus has circulated widely among poultry, reassorted with other avian influenza virus (AIV) subtypes (2), and caused sporadic

human infections with a high fatality rate (1). Although early antiviral therapy with neuraminidase inhibitors (NAIs) is associated with improved clinical outcomes in AIV infections (3), a significant number of patients have been lost to the H5N6 virus despite NAI treatment.

Drug repurposing, identifying new uses for existing

drugs approved as indications for other conditions, is an attractive solution for developing potential H5N6 treatments. Sirolimus, an inhibitor of the mammalian target of rapamycin (mTOR), has been reported to reduce influenza virus protein expression and virus replication (4). In combination with oseltamivir, it has been demonstrated to improve clinical outcomes of severe H1N1-associated pneumonia (5). Therefore, sirolimus may be a promising drug for H5N6 treatment. This report outlines the first case of severe H5N6-associated pneumonia that was successfully treated with the combinatorial therapy of sirolimus and NAIs. We present the following case in accordance with the CARE reporting checklist (available at <https://atm.amegroups.com/article/view/10.21037/atm-22-704/rc>).

Case presentation

The patient was a 22-year-old male without underlying medical conditions from Guangzhou in the Guangdong Province of China. He had a 5-year history of smoking (20 cigarettes per day) and was exposed to live poultry 2 days before illness onset. On September 24, 2018 (Day-0; *Figure 1A*), the patient developed high fever (40 °C) and chills. A visit to the local hospital led to the prescription of ibuprofen and azithromycin. However, his complaints progressed over the following days, necessitating a visit to the emergency department of Sun Yat-sen Memorial Hospital, Sun Yat-sen University, on Day-3 after illness onset.

Laboratory examination upon admission showed lymphopenia and thrombocytopenia, but white blood cell count and PaO₂/FiO₂ values were normal (*Table 1*). Plain computed tomography (CT) showed patchy shadows and consolidation in the lower lobe of the right lung, which progressed rapidly to both lobes within the next 2 days (*Figure 1B,1C*). The patient was treated with meropenem and moxifloxacin. Considering the possibility of viral pneumonia, a throat swab sample was obtained on Day-5, which was subsequently assessed using real-time reverse transcription-polymerase chain reaction. Oseltamivir was also administered at 75 mg twice a day. The PaO₂/FiO₂ value declined to 84 on Day-6, while the levels of aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, creatine kinase, and myoglobin elevated. The patient developed septic shock, and the Sequential Organ Failure Assessment (SOFA) score increased from 2 to 10. On the same day, the Guangdong Provincial Center for Disease Control and Prevention identified the H5N6 virus as the source of infection within this patient.

The patient was then transferred to the intensive care unit (ICU) and received invasive mechanical ventilation with a low tidal volume (5 mL/kg) and an open lung ventilation strategy. The bronchoalveolar lavage fluid (BALF) galactomannan (GM) test yielded a result of greater than 5 g/mL (normal range: <0.65 µg/mL). The dosage of oseltamivir was increased to 150 mg twice a day. In addition, peramivir, amphotericin B, and low-dose corticosteroid (20 mg per day for 14 days) were also administered. Nevertheless, mediastinal and subcutaneous emphysema quickly developed on Day-7. CT images acquired on Day-10 showed a rapid progression of lung consolidation (*Figure 1D*). Thus, venovenous (VV) extracorporeal membrane oxygenation (ECMO) was initiated, but the patient's condition continued to deteriorate.

On Day-12, the viral titer of the tracheal aspirate sample was still considered to be high (2.6×10^8 copies/mL), so sirolimus (2 mg per day for 14 days) was administered. Following this, the viral titers detected from the throat swab samples and the tracheal aspirate samples markedly decreased. On Day-16, the 4th day after sirolimus treatment was initiated, the H5N6 virus became undetectable in the throat swab specimens, and on Day-25, the 13th day after sirolimus treatment was initiated, the virus became undetectable in the tracheal aspirate samples. The patient's clinical condition gradually improved throughout the duration of sirolimus treatment, with the PaO₂/FiO₂ value increasing to 278, and the SOFA score decreasing to 3. The white blood cell count increased without signs of infection. The levels of aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, creatine kinase, and myoglobin gradually decreased. ECMO was stopped on Day-22, and the endotracheal tube was removed on Day-25. Sirolimus, NAIs, and antibiotics were discontinued at the same time. CT images on Day-28 and Day-52 (*Figure 1E,1F*) showed the resolution of the bilateral lung infiltrations.

The patient was discharged on Day-65. Based on observations from a 2-year follow-up, he was found to be in a good condition without complications. CT images (*Figure 1G*) revealed a few bilateral interstitial changes in the lungs, while a pulmonary function test showed normal ventilation and slightly decreased efficiency in diffusion. All procedures performed in this study were in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the ethics committee of Sun Yat-sen Memorial Hospital, Sun Yat-sen University (No. SYSEC-KY-KS-2018-123). Written informed consent was obtained

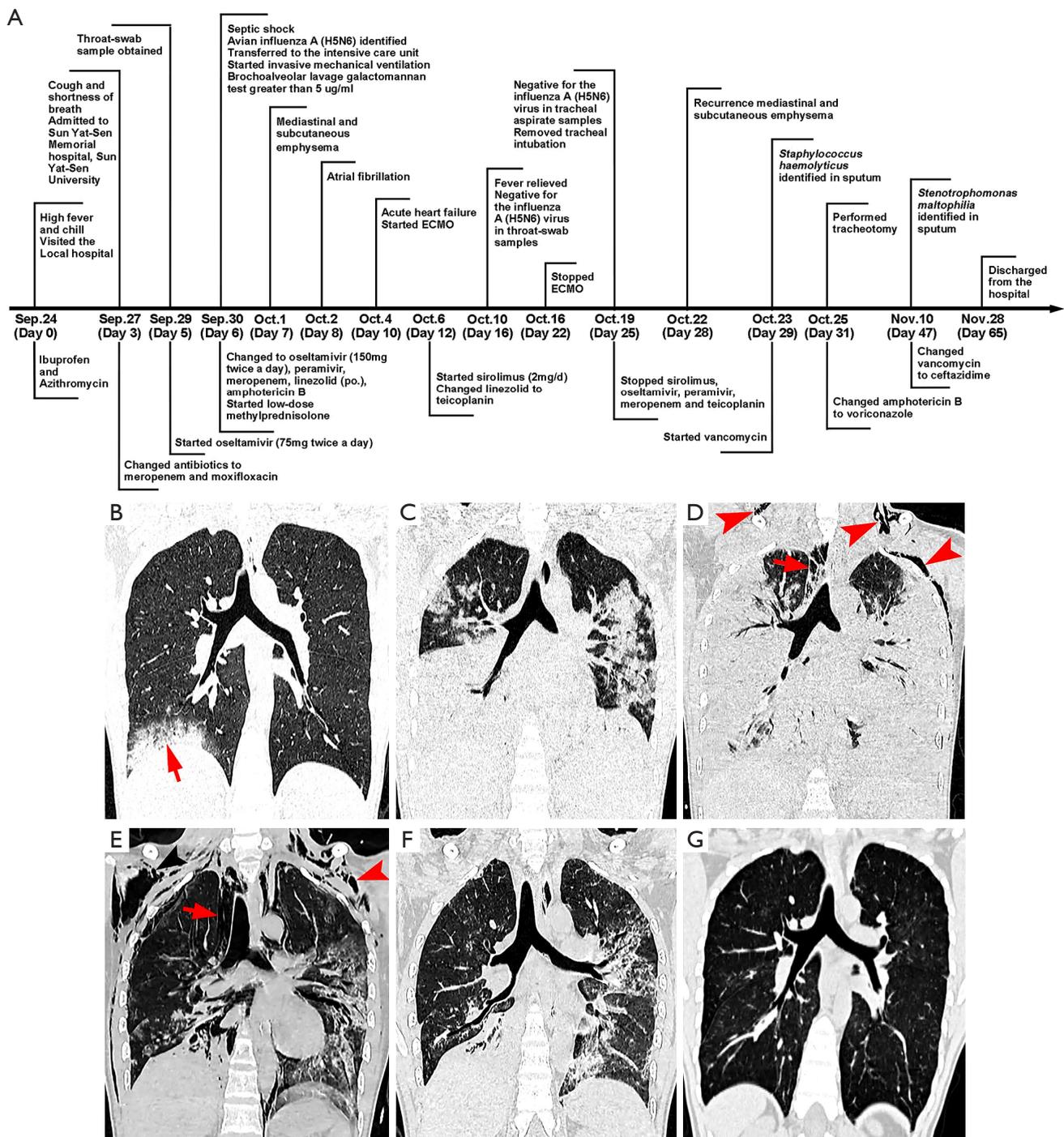


Figure 1 Timeline and imaging findings. (A) Timeline of the patient's clinical course. (B-G) Sequential reformatted coronal multiplanar CT images of the patient's chest (lung window). Patchy shadows and consolidation (arrow, B) were observed in the lower lobe of the right lung on Day-3 after illness onset (B), which then progressed rapidly to both lobes 2 days later (C). (D) The bilateral consolidation and bronchial functions in the lungs on Day-10; mediastinal (arrow, D) and subcutaneous emphysema (arrowhead, D) are indicated. The resolution of the bilateral lung infiltrations was observed both on Day-28 and Day-52 after illness onset (E,F). Mediastinal (arrow, E) and subcutaneous emphysema (arrowhead, E) were also observed on Day-28 but not on Day-52. (G) Bilateral interstitial changes in the lungs after 2 years. CT, computed tomography. ECMO, extracorporeal membrane oxygenation.

Table 1 Laboratory measurements of the patient with H5N6 infection

Parameters	Normal range	Days after the illness onset									
		3	6	7	12	15	19	22	25	42	64
White cells ($\times 10^9$ cells·L ⁻¹)	3.5–9.5	5.2	3.7	9.0	11.6	21.1	17.1	6.51	11.7	18.1	9.2
Neutrophil ratio (%)	40.0–75.0	81.6	87.8	86.8	88.5	77.1	84.3	85.1	87.9	85.7	75.3
Lymphocyte ratio (%)	20.0–50.0	14.8	9.5	9.8	7.3	13.0	8.4	8.0	6.4	6.2	12.6
Platelets ($\times 10^9$ cells·L ⁻¹)	125–130	116	141	172	232	303	243	231	288	305	246
C-reactive protein (mg·L ⁻¹)	<5.0	–	–	–	–	7.7	11.6	6.4	8.4	4.5	<5.0
Procalcitonin (ng·mL ⁻¹)	<0.05	–	3.33	3.75	0.35	0.13	<0.05	<0.05	<0.05	0.24	<0.05
Alanine aminotransferase (U·L ⁻¹)	9–50	17	48	29	198	85	69	64	97	122	61
Aspartate aminotransferase (U·L ⁻¹)	14–40	31	226	299	205	53	59	62	96	87	44
Lactate dehydrogenase (U·L ⁻¹)	108–252	–	1,825	6386	751	454	622	449	622	570	207
Creatine kinase (U·L ⁻¹)	126–174	–	8,534	6386	928	548	311	193	454	146	19
Myoglobin (μ g·L ⁻¹)	28.0–72.0	–	409.0	414.9	524.2	371.7	81.0	33.5	44.8	68.9	<21
Creatinine (μ mol·L ⁻¹)	4–133	97	86	86	94	81	81	75	66	58	92
PaO ₂ /FiO ₂ (mmHg)	>300	399	84	94	99	190	237	257	278	322	418
SOFA score	0	2	10	10	11	10	9	3	3	1	0
Throat swab virus titers (copies·mL ⁻¹)	ND	–	8.1 $\times 10^5$	9.0 $\times 10^5$	–	2.1 $\times 10^5$	ND	ND	ND	–	–
Tracheal aspirate virus titers (copies·mL ⁻¹)	ND	–	–	–	2.6 $\times 10^8$	1.4 $\times 10^8$	1.1 $\times 10^3$	2.2 $\times 10^3$	ND	–	–

SOFA, Sequential Organ Failure Assessment; ND, not detected.

from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

Discussion

Due to a lack of population immunity among humans and its ongoing viral evolution, the H5N6 virus poses an influenza pandemic risk if the virus were to develop more efficient transmission among humans (6). Up to 8 December 2021, a cumulative total of 53 laboratory-confirmed H5N6 patients have been reported globally, including 25 deaths (1). Among them, 49 patients required hospitalization, while 46 needed ICU treatment. The hospitalized patients usually presented with fever, sore throat, chills, cough, and myalgia as initial clinical symptoms. Their condition then quickly progressed to severe pneumonia, septic shock, and ultimately multiple

organ dysfunction syndrome (MODS). The high fatality rate and rapid disease progression indicate the need for an improved treatment regimen. Drug repurposing of existing approved drugs provides an attractive alternative drug discovery strategy to identify potential treatments for H5N6.

Sirolimus, which was approved by the US Food and Drug Administration on September 15, 1999, is an immunosuppressive agent that prevents organ rejection in patients receiving renal transplants. On the cellular level, sirolimus binds to an immunophilin named FK Binding Protein-12 to generate an immunosuppressive complex. This complex inhibits the activation of mTOR, an essential member of the phosphatidylinositol 3-kinase (PI3K) family of protein kinases (7). Several studies have shown that mTOR inhibitors exhibit *in vitro* antiviral activity against the influenza A virus, as well as reducing both the viral protein and viral messenger RNA (mRNA) levels (4,7,8).

In a lethal influenza A (H1N1 and H5N1) viral infection mouse model, the administration of everolimus (another mTOR inhibitor, 1 mg/kg/day for 14 days) significantly delayed death, in addition to reducing lung hemorrhage and increased lung weight, which are responses to infection (9). In another mouse infection model of the lethal influenza A (H1N1) pdm09 virus (pH1N1), Jia *et al.* showed that delayed oseltamivir-plus-sirolimus treatment protected mice by synergistically attenuating severe lung damage, reducing viral titer, and decreasing pH1N1-induced mTOR activation (10). Furthermore, in a prospective randomized, controlled study in patients with severe H1N1 pneumonia, a higher proportion of patients with sirolimus-corticosteroids-oseltamivir combinatory treatment achieved clearance of viral RNA within the airway after 7 days of treatment (75% *vs.* 33.3%; $P < 0.05$) compared to those in the non-sirolimus group (5). Patients who received the sirolimus-corticosteroids-oseltamivir combinatory treatment were also associated with improved clinical outcomes with respect to hypoxia, MODS, and duration of ventilator use (5). In our case, the acute symptoms and abnormalities in the thoracic radiographic findings progressed rapidly. The NAIs and ECMO did not result in symptomatic improvement, positive thoracic imaging findings, or viral titer relief until the prescription of sirolimus. Therefore, we propose that sirolimus might be a novel and practical therapeutic approach to severe H5N6-associated pneumonia in humans.

It is well-established that inflammation promotes the recruitment of immune cells during viral infections, while uncontrolled and exacerbated inflammation is associated with systemic edema and extensive tissue damage (11). One autopsy report found that when compared with pH1N1 fatal infection, H5N6 infection caused a more exacerbated immune response involving overt pulmonary inflammation (12). Similarly, Bi *et al.* tested 48 cytokines/chemokines and reported that H5N6 patients had significantly higher levels of cytokines/chemokines than those of healthy controls, H7N9 patients, and pH1N1 patients in 21, 11, and 5 parameters, respectively (13). In addition, the non-surviving H5N6 patients had higher concentrations of both pro-inflammatory and anti-inflammatory cytokines/chemokines than those of the survivors (13).

Sirolimus plays an essential role in both the innate and adaptive immune responses by inhibiting antibody production, B cell development, and T lymphocyte activation and proliferation that occur in response to

antigenic and cytokine [interleukin (IL)-2, IL-4, and IL-15] stimulation (14). Keating *et al.* showed that sirolimus treatment during primary H3N2 virus infection protected mice against a lethal, heterosubtypic secondary infection with influenza virus subtype H5N1, H7N9, or H1N1 (15). Moreover, sirolimus reduced class switching in B cells and altered the specificity patterns of immunoglobulins G (IgG) and M (IgM), which yielded a unique repertoire of antibodies (15). In addition, sirolimus may ameliorate the immunopathological injury to the lung by modulating dysregulated pro-inflammatory and anti-inflammatory cytokine production (10,16,17). In a study of H1N1 viral infection by Jia *et al.*, the groups receiving delayed oseltamivir-plus-sirolimus combinatory treatment exhibited significantly decreased concentrations of various pro-inflammatory cytokines and chemokines, as well as reduced inflammatory cell infiltration in lung tissue and BALF, in comparison with the untreated control or monotherapy groups (10). Sirolimus has also been suggested to optimize the treatment of coronavirus disease (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (18,19).

Our case is the first to report the effectiveness and safety of sirolimus and NAIs in improving clinical outcomes of patient with severe H5N6 infection. However, there are some limitations. First, we did not assess the immunologic impact, such as levels of serum or bronchoalveolar lavage cytokines/chemokines, during the infection or the intervention. Second, the role of corticosteroid could not be excluded, as low-dose systemic corticosteroid was also used in this patient. Studies without concomitant corticosteroid therapy should be conducted. To further investigate the effects of sirolimus and oseltamivir on the normalization of respiratory status, the changes to 10 cytokines/chemokines and pro-inflammatory mediators, viral clearance, and several other clinical endpoints in influenza patients, a new study will be performed by the Chinese University of Hong Kong (20).

In conclusion, the role of sirolimus in patients with severe viral (including the highly pathogenic AIV and SARS-CoV-2) pneumonia is a promising direction for further investigation.

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Footnote

Reporting Checklist: The authors have completed the CARE reporting checklist. Available at <https://atm.amegroups.com/article/view/10.21037/atm-22-704/rc>

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://atm.amegroups.com/article/view/10.21037/atm-22-704/coif>). The authors have no conflicts of interest to declare.

Ethics Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study were in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the ethics committee of Sun Yat-sen Memorial Hospital, Sun Yat-sen University (No. SYSEC-KY-KS-2018-123). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

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