

Review

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A rationale for using steroids in the treatment of severe cases of H5N1 avian influenza

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Acute hypercytokinaemia represents an imbalance of pro-inflammatory and anti-inflammatory cytokines, and is believed to be responsible for the development of acute respiratory distress syndrome and multiple organ failure in severe cases of avian (H5N1) influenza. Although neuraminidase inhibitors are effective in treating avian influenza, especially if given within 48 h of infection, it is harder to prevent the resultant hypercytokinaemia from developing if the patient does not seek timely medical assistance. Steroids have been used for many decades in a wide variety of inflammatory conditions in which hypercytokinaemia plays a role, such as sepsis and viral infections, including severe acquired respiratory syndromes and avian influenza. However, to date, the results have been mixed. Part of the reason for the discrepancies might be the lack of understanding that low doses are required to prevent mortality in cases of adrenal insufficiency. Adrenal insufficiency, as defined in the sepsis/shock literature, is a plasma cortisol rise of at least $9 \mu\text{g dl}^{-1}$ following a $250 \mu\text{g}$ dose of adrenocorticotropin hormone (ACTH), or reaching a plasma cortisol concentration of $>25 \mu\text{g dl}^{-1}$ following a $1\text{--}2 \mu\text{g}$ dose of ACTH. In addition, in the case of hypercytokinaemia induced by potent viruses, such as H5N1, systemic inflammation-induced, acquired glucocorticoid resistance is likely to be present. Adrenal insufficiency can be overcome, however, with prolonged (7–10 or more days) supraphysiological steroid treatment at a sufficiently high dose to address the excess activation of NF- κ B, but low enough to avoid immune suppression. This is a much lower dose than has been typically used to treat avian influenza patients. Although steroids cannot be used as a monotherapy in the treatment of avian influenza, there might be a potential role for their use as an adjunct treatment to antiviral therapy if appropriate dosages can be determined. In this paper, likely mechanisms of adrenal insufficiency are discussed, drawing from a broad background of literature sources.

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Introduction

Although an H5N1 influenza A global pandemic is by no means a certainty, the odds are sufficiently high enough that many experts have warned us to be prepared (Osterholm, 2005; Webster & Govorkova, 2006). In such a situation, or for individual cases of severe avian influenza, the preferred treatment of choice remains the use of the neuraminidase inhibitors oseltamivir and zanamivir (Moscona, 2005). Despite their effectiveness, however, the combination of late arrival of patients at medical facilities and the severity of illness, which can produce high viral loads and cytokine imbalances (de Jong *et al.*, 2006; Kandun *et al.*, 2006; Yuen & Wong, 2005), still results in case fatality levels of 50–75%.

Both severe acute respiratory syndrome (SARS) and avian influenza have a number of gross pathologies in common (Ng *et al.*, 2006), and can swiftly progress to acute respiratory distress syndrome and multiple organ failure.

Research to date has suggested that hypercytokinaemia, an acute increase in cytokines and chemokines, plays a large role in such situations (Chan *et al.*, 2005; Ng *et al.*, 2006; To *et al.*, 2001) although the change in cytokine profile is likely to be different in each case.

Several drugs and nutraceuticals have been proposed as immunomodulators for avian influenza strains, including the statins, macrolide antibiotics, pentoxifylline, resveratrol, Lyprinol and cinnamaldehyde (Fedson, 2006; Guo *et al.*, 2006; Rainsford, 2006). Their use is predicated on the assumption that if hypercytokinaemia can be mitigated, and a better cytokine balance achieved, this will buy time, reduce the severity of the disease and allow concomitant antiviral drugs to clear the virus from the body. However, little has been published on the use of steroids in such a context. In this paper, I review the theoretical and experimental evidence for immunomodulation by steroids, and discuss a rationale for their use in the clinical treatment of severe cases of avian influenza.

Literature retrieval

Relevant articles were identified by searching PubMed using the key phrases hypercytokinaemia, inflammation, steroids or corticosteroids, in combination with the terms influenza, avian influenza or H5N1. In addition, the drug terms methylprednisolone and hydrocortisone, in combination with influenza terms were also searched, as well as the terms sepsis and steroids, and cortisol, adrenocorticotropin hormone (ACTH) and influenza. Papers cited from articles retrieved in the initial search were also reviewed.

Hypercytokinaemia

Cytokine families and functions

Cytokines are signalling proteins involved in the response to pathogens and subsequent inflammatory processes. Of the six classes, the interferons (IFNs), interleukins (ILs) and tumour necrosis factors are the most important. A clinical and experimentally useful classification system divides cytokines into those that promote the proliferation and function of T-helper type 1 cells and those that do the same for T-helper type 2 cells. Examples of Th-1 cytokines include IL-1 and IFN- γ , and examples of Th-2 cytokines include IL-4, IL-10, IL-13 and TGF- β .

The IFN family comprises several types: type I, of which IFN- α and IFN- β are the most well known, have antiviral activity and are associated with the regulation of natural immunity, while type II IFNs, such as IFN γ , are associated mainly with cellular immunity (Sladkova & Kostolansky, 2006). More recently, a type III family has been described that is evolutionarily distinct from type I IFNs but appears to have a similar function to them, and whose members are structurally related to IL-10 cytokines (Onoguchi *et al.*, 2007).

Dozens of IL entities have been described, of which the most important are IL-1 (α and β), IL-6, IL-10, IL-11, IL-12 and IL-18. When viral infection occurs, the action of these entities can be generally described as pro-inflammatory (with the exception of IL-10), and sometimes overlapping in effects, which can be local or systemic. Conversely, depending upon the phase of the infection (early or late), some of these cytokines, such as IL-18, can act in an anti-inflammatory capacity through feedback to prevent excessive cytokine production (Liu *et al.*, 2004; Sareneva *et al.*, 1998). In some instances, however, this feedback breaks down, and dysfunctional regulation occurs. The result is an imbalance that is thought to lead to chronic hypercytokinaemia in such conditions as rheumatoid arthritis, Crohn's disease and other autoimmune syndromes, or acute hypercytokinaemia and life-threatening events when potent viruses are involved (Hussell *et al.*, 2001; Palucka *et al.*, 2005; To *et al.*, 2001). A demonstration of the power of acute hypercytokinaemia was recently illustrated in a clinical phase 1 trial of the anti-CD28 mAb TGN1412 in six young healthy males (Suntharalingam *et al.*, 2006). Induction of TNF- α , IFN- γ , IL1- β , IL-2, IL-10 and other ILs occurred within 1–2 h, and resulted in pulmonary

infiltrates, lung injury, renal failure and disseminated intravascular coagulation 12–16 h after infusion that required immediate and extensive medical treatment.

Effect of influenza viruses on cytokines

Certain strains of influenza viruses can also elicit acute hypercytokinaemia. For example, using mice infected with recombinant influenza viruses containing the haemagglutinin and neuraminidase genes from pandemic 1918 influenza strains, Kash *et al.* (2004) demonstrated that dozens of genes associated with cytokines, apoptosis, lymphocytes, activated T cells, macrophages and oxidative damage were upregulated with lethal consequences.

The mechanism by which influenza viruses can effect hypercytokinaemia is poorly understood. Cells sense the presence of viral material through a variety of pattern recognition receptors, such as the Toll-like receptors (TLR). Both TLR7 and TLR8 are involved in the recognition of single-stranded RNA, and TLR3 in the recognition of double-stranded RNA (Garcia-Sastre, 2006; Matikainen *et al.*, 2006). Induction of IFN- α/β follows recognition, although influenza A viruses are able to prevent this via the antagonistic action of the NS1 protein (Garcia-Sastre, 2006), which also inhibits adaptive immunity by attenuating human dendritic cell maturation (Fernandez-Sesma *et al.*, 2006).

In the case of H5N1, both the induction of TNF- α and IFN- β/γ have been observed in alveolar and bronchial epithelial cells (Yuen & Wong, 2005). Induction of IL-6, IP-10 (a macrophage attractant) and RANTES, a member of the IL-8 family and a selective attractant for memory T lymphocytes and monocytes, has been additionally witnessed in cell culture experiments (Chan *et al.*, 2005) and patients infected with H5N1 (de Jong *et al.*, 2006). Moreover, de Jong *et al.* (2006) reported that the highest levels of IL-6, IL-10, MIG and MCP-1 (the latter two cytokines are also monocyte and macrophage attractants) were found in those patients who subsequently died, highlighting the concentration–response relationship.

The production of these early response cytokines then causes the infiltration of macrophages, and attraction of monocytes, eosinophils, basophils and T cells. Transcription factor NF- κ B also seems to play a central role in development of the cytokine cascade, as well as upregulation of the mitogen-activated protein kinases, which are strongly associated with the production of TNF- α at the transcriptional and posttranscriptional levels (Lee *et al.*, 2005).

Autopsy findings confirm TNF- α involvement in hypercytokinaemia induced by H5N1 avian influenza strains, with its presence in the lungs (Uiprasertkul *et al.*, 2005). In addition, in influenza-associated (non-H5N1) encephalopathy, serum levels of both IL-8 and TNF- α were found to be significantly higher than for controls (influenza, but no encephalopathy). However, serum cytochrome c levels were nearly 90-fold higher, which seems indicative of

apoptosis in several organs, including the cerebrum and liver (Nunoi *et al.*, 2005).

While these studies show that H5N1 can increase pro-inflammatory activity, the virus can lower the cell's ability to produce antiviral responses. For example, it appears that TNF- α can depress generation of plasmacytoid dendritic cells (a source of IFN $\alpha\beta$), as well as suppressing IFN α secretion by immature cells of this type (Palucka *et al.*, 2005). It should be pointed out in this context that while IFNs are generally regarded as pro-inflammatory, they exert a powerful antiviral effect (Sladkova & Kostolansky, 2006). Therefore, suppressing IFN gene expression would be counter-productive to clearing viral infections. In this regard, it should be noted that corticosteroids can augment the action of exogenously given IFNs because they help suppress the NF κ B-mediated downregulation of IFN gene expression (Loutfy *et al.*, 2003; Wei *et al.*, 2006). Interestingly, low-dose steroids were also found to counter the influenza-like side effects of exogenously administered IFN (IFN β -1b) in patients suffering from relapsing–remitting multiple sclerosis (Rio *et al.*, 1998). In addition, dexamethasone was found to selectively suppress the pro-inflammatory gene expression induced by IFN γ , including cyclo-oxygenase-2 expression in human bronchial epithelial cells (Pawliczak *et al.*, 2005).

The H5N1 virus can also affect both non-specific and specific immunity. Thus, Hsieh and Chang (2006) found that perforin expression appears to be suppressed, and the cytotoxicity of CD8+T cells is reduced in the presence of H5-bearing cells. Furthermore, this led to lymphoproliferation, the overproduction of IFN γ and macrophage activation.

Rationale for corticosteroid use

Hypothalamic–pituitary–adrenal (HPA) axis failure

Under normal circumstances, corticotropin-releasing hormone is produced in the pituitary gland in response to a variety of factors. In turn, corticotropin-releasing hormone stimulates ACTH production in the anterior pituitary, and this hormone stimulates cortisol secretion in the adrenal glands. Increased cortisol levels can then modify the original response. In critically ill patients, cortisol levels can be extremely high and mirror the severity of the disease, but often the response to the stressor is inadequate, leading to what is termed HPA axis failure or adrenal insufficiency. What constitutes adrenal insufficiency is still a matter for debate. One definition suggests that 30–60 min following a 250 μ g dose of ACTH, blood cortisol levels should rise at least 9 μ g dl⁻¹. If they do not, this would constitute adrenal insufficiency (Annane *et al.*, 2000). However, some investigators have criticized the supraphysiological-dose ACTH test and believe that the amount of ACTH administered should be near physiological (1–2 μ g), or that a random plasma cortisol sample of <25 μ g dl⁻¹ under situations of extreme, sepsis, illness

or infection is a more realistic marker of adrenal insufficiency (Marik & Zaloga, 2002).

The HPA axis and cytokines

In cases of infection by viruses or bacteria, corticosteroids are produced in response to pro-inflammatory cytokines, such as IL-1 and IL-6, and downregulate these entities, ensuring a controlled feedback loop (Padgett *et al.*, 2000). An extensive review of early work (mostly cell culture studies) concerning the effect of cytokines, such as IL-1 (α and β), IL-6 and TNF α , showed stimulation of the HPA axis in the majority of cases (Besedovsky & del Ray, 1996), but some investigations have demonstrated that inhibition of the axis can also occur (Bateman *et al.*, 1989; Gaillard *et al.*, 1990; Jaattela *et al.*, 1990, 1991; Mastorakos *et al.*, 1993).

Chronic inflammation, which is the result of high, long-term cytokine levels, is one example in which suppression of the HPA axis is likely. For example, rheumatoid arthritis patients, who demonstrate secondary adrenal insufficiency, also show IFN γ /IL-10 ratio peaks during the early morning hours when cortisol levels are at their lowest (Cutolo *et al.*, 2006). In other words immunomodulation of the IFN γ does not occur, because of HPA axis failure.

In acute hypercytokinaemia resulting from H5N1 influenza, as evidenced by the raised levels of IFN- γ , IL-6, IL-8, IL-10 and other factors in blood, which are correlated with viral load (de Jong *et al.*, 2006), what happens to the HPA axis? Initially we might suspect that the axis would be strongly activated. However, since this influenza strain causes such a high case fatality rate, we might presume that either the suppression induced by the elevated cortisol levels is insufficient to depress the high cytokine levels, or the HPA axis collapses to produce adrenal insufficiency. In order to understand the possibilities, insight can be gained by studying the sepsis/shock literature, in which similar parallels take place.

Steroid treatment in sepsis and shock

For many decades, steroids have been empirically used to treat patients with shock or sepsis to reduce mortality, with unclear results. By 2002, however, it was becoming apparent to some researchers that low, rather than high doses of steroids, might be the correct way to treat patients with demonstrated adrenal insufficiency. Not all investigators agreed. In summing up the state of research in 2002, Bernard (2002) said, 'For now, the data indicate that we should not administer steroids to patients who are in shock'. However, a systematic review and meta-analysis conducted in 2004 showed that for post-1995 reports separated into trials that used (a) short courses of high-dose steroids (6 trials, $n=511$), and (b) long courses of low-dose steroids (5 trials, $n=465$), there was a difference (Annane *et al.*, 2004); mortality remained the same for group (a) but was significantly reduced for group (b). In conclusion, Annane *et al.* (2004) recommended giving

Table 1. Matches of HA amino acid residues from selected H5N1 strains with ACTH peptide sequence 25–39

HA sequences were aligned using standard markers (WHO, 2005). Antigenically important amino acid positions proposed by Wheatland (2004) for the human ACTH sequence and matches in influenza virus sequences are shown in bold.

Strain	Sequence match	GenBank accession no.
A/Egypt/2782-NAMRU3/2006	ACTH	NGAE DES AEAFPLEF
	50	LILRDCSVAGWLLGN
	92	PGDFNDY EEL KHLLS
	416	DGFLDVW TYNA ELLV
	454	LQLRDN AKEL GNGCF
A/Hong Kong/483/97	ACTH	NGAE DES AEAFPLEF
	50	LILRDCSVAGWLLGN
	92	PGNFNDY EEL KHLLS
	416	DGFLDVW TYNA ELLV
	454	LQLRDN AKEL GNGCF
A/Indonesia/CDC1047S/2007	ACTH	NGAE DES AEAFPLEF
	50	LILKDCSVAGWLLGN
	92	PGSFNDY EEL KHLLS
	416	DGFLDVW TYNA ELLV
	454	LQLRDN AKEL GNGCF
A/Indonesia/CDC523/2006	ACTH	NGAE DES AEAFPLEF
	50	LILRDCSVAGWLLGN
	92	PGSFNDY EEL KHLLS
	416	DGFLDVW TYNA ELLV
	454	LQLRDN AKEL GNGCF
A/Thailand/3(SP-83)/2004	ACTH	NGAE DES AEAFPLEF
	50	LILRDCSVAGWLLGN
	92	PGDFNDY EEL KHLLS
	416	DGFLDVW TYNA ELLV
	454	LQLRDN AKEL GNGCF

patients 200–300 mg hydrocortisone daily for 5–11 days when adrenal insufficiency was demonstrated (typical percentage of patients ~50%, condition for adrenal insufficiency was a rise of $\leq 9 \mu\text{g dl}^{-1}$ after ACTH administration or a random cortisol level of $\leq 15 \mu\text{g dl}^{-1}$).

Should a daily 250 mg dosage of hydrocortisone be considered physiological? Studies conducted by Samuels (2000) suggested that in primary adrenal insufficiency, 19 mg hydrocortisone per day is a sufficient replacement dosage. Taking into account the increase in cortisol of 200–300% often observed following a systemic response to stress (Marik & Zaloga, 2002), 250 mg hydrocortisone in the sepsis-shock model is clearly supraphysiological, and not a replacement level, even if it is regarded as a low dose.

Mechanisms of HPA axis failure

Wheatland (2004) proposed that SARS and influenza A cause adrenal insufficiency, because portions of viral amino acid sequences mimic human ACTH sequences (amino acid residues 25–39) less conserved between species. This may result in the host producing specific antibodies against these viral epitopes, also resulting in lower ACTH levels. A

search of influenza A H5N1 HA gene GenBank sequences showed similar amino acid identities as detected by Wheatland (2004) with various influenza A non-H5N1 isolates and the 1997 Hong Kong H5N1 isolate (see Table 1).

Wheatland (2004) cites additional evidence for his hypothesis from data reported by Jefferies *et al.* (1998), who demonstrated that influenza-infected patients (H3N2) had normal cortisol levels but lowered levels of ACTH 1–3 days after onset of illness (cortisol levels in infected patients $13.7 \pm 1.4 \mu\text{g dl}^{-1}$; cortisol levels in controls $10.8 \pm 1.0 \mu\text{g dl}^{-1}$, P =not significant; ACTH levels in infected patients $13.5 \pm 2.1 \text{ pg ml}^{-1}$; ACTH levels in controls $23 \pm 3.2 \text{ pg ml}^{-1}$, $P=0.02$). However, ACTH levels rebounded to normal approximately a week later. Wheatland (2004) argues that the data are the result of auto-antibodies generated during the influenza infection that compete with assay antibodies in the immunoassay, causing erroneously low ACTH readings. Moreover, he suggests that experiments in which influenza-infected mice were treated with antilymphocyte serum, which resulted in 50% less mortality (Suzuki *et al.*, 1974), are indicative that the T lymphocytes induce antibodies to ACTH. This interpretation is questionable, since it can take up to 10 days to

generate an immune response, although it does not invalidate the hypothesis itself. This leaves us with the puzzling data of Jefferies *et al.* (1998), which shows a transitory depression of ACTH but no substantial effect on cortisol. Normally, stimulation by the influenza infection of both ACTH and cortisol levels would be expected. However, since this is not the case (assuming there were no methodological problems with the measurement of the ACTH), it has to be assumed that the virus has induced suppression of ACTH but not cortisol. As no other investigators have reported ACTH or cortisol data for influenza-infected patients, this unexpected finding must be accounted for when developing a rationale for steroid use.

An interesting report that sheds further light on the subject is the case of a 32-month child who was treated for persistent reactive airway disease with long-term systemic and inhaled steroids. One day after influenza vaccination, the child presented with acute adrenal insufficiency, confirmed by plasma cortisol levels before and after ACTH administration ($0.6 \mu\text{g dl}^{-1}$ and $8.4 \mu\text{g dl}^{-1}$, respectively) (Kennedy *et al.*, 2002). Following hydrocortisone replacement therapy and tapering fluticasone treatment, the child completely recovered. In this immunocompromised child, we can speculate that influenza antigens further reduced an already low ACTH level and cortisol levels. However, it is clear from the timescale that this must have involved a cytokine response, rather than a humoral (adaptive auto-antibody generation) response of the kind suggested by Wheatland (2004). In fact, from the time of influenza infection, it could take up to 10 days for the kind of auto-antibodies that Wheatland (2004) suggests to form, and given the timeline of H5N1 cases presented in the literature (Chotpitayasunondh *et al.*, 2005; Hien *et al.*, 2004; Kandun *et al.*, 2006), it is certain that in many cases of H5N1, any adrenal insufficiency would have to result from hypercytokinaemia. This case of the child also points out the hazard of using prolonged high-dose corticosteroids, which can cause direct HPA axis failure.

In reviewing acute respiratory distress syndrome cases that fail to resolve, Meduri & Yates (2004) suggest an alternative mechanism based on excess activation of NF- κ B compared to the anti-inflammatory transcription factor glucocorticoid (GC) receptor alpha. This imbalance causes an inability to downregulate the transcription of inflammatory cytokines despite elevated cortisol levels, which the authors term 'systemic inflammation-induced acquired GC resistance'. However, the imbalance can be alleviated via prolonged GC administration (Meduri & Yates, 2004). Other explanations for this condition, which also appears to be the peripheral GC resistance encountered in the sepsis/shock literature (Ali *et al.*, 1991; Chadda & Annane, 2002; Meduri & Chrousos, 1998; Molijn *et al.*, 1995), include dysfunctional transcription of the GR gene and dysregulation of the mineralocorticoid receptor enzyme, 11- β -HSD (Pretorius *et al.*, 2006). Activator protein AP1, a

regulator of cell proliferation, inflammation and immune response similar to NF- κ B, also appears to be repressible by corticosteroids via GC receptors (Reily *et al.*, 2006).

While the GC resistance mechanism appears to provide a better rationale for the use of supraphysiological low-dose steroid levels, there are caveats. For example, repeated cycles of restraint stress – an exogenous stressor used in mice and rat models – can produce high enough corticosterone levels to compromise the overall immune response to an influenza infection, perhaps by oversuppressing cytokine production (Padgett *et al.*, 2000). Furthermore, differential suppression of cytokines can also occur, as was witnessed in a murine influenza A/PR8 infection following restraint stress that suppressed IL-1 α , but not IL-6 (Konstantinos & Sheridan, 2001). This is interesting, because both IL-1 and IL-6 can stimulate adrenal GC production.

If stress-mediated responses are present in the early stages of influenza-infected patients, and the doses of corticosteroids are employed are too high, the result might be oversuppression of the immune response and a further cytokine imbalance, essentially a forward feedback that leads to fatality. Such scenarios might explain apparent steroid treatment failure in some severe cases of SARS (Li *et al.*, 2004; details cited in Stockman *et al.*, 2006; Poutanen *et al.*, 2003) and avian influenza (Hien *et al.*, 2004). Conversely, in the absence of an a priori stress-mediated response and no exogenous steroids, a virulent influenza strain can prevent corticosteroid production with the result of uncontrolled hypercytokinaemia and poor prognosis.

Influenza and steroid treatment

Few animal studies have been reported regarding steroid use in influenza. Ottolini *et al.* (2003) used daily dosages of 1, 4 and 16 mg triamcinolone kg^{-1} to treat rats infected with a strain of H3N2 (A/Wuhan/359/95). The two highest dosages were effective in reducing pulmonary lesions and suppressing IFN γ mRNA, which led to shutdown of macrophage activation. However, the lowest dose was ineffective. While all the doses are supraphysiological – 1 mg kg^{-1} translates to 70 mg per day for a 70 kg patient, or approximately 350 mg hydrocortisone daily – this experiment appears to demonstrate that there is a relatively high threshold steroid concentration necessary for successful treatment. This is a key observation. However, what we cannot extrapolate from these experiments is the threshold steroid dosage for humans infected with H5N1 strains even though the cotton rat model is a good influenza model compared to mice and ferrets (Ottolini *et al.*, 2005).

Reports of the use of steroids in human avian influenza treatment are limited, and the case numbers few (Table 2). Confounding factors also render analysis extremely difficult. Although steroid treatment has not appeared to alter mortality rates, there are several factors that must be borne

Table 2. Case reports of patients with confirmed H5N1 in which steroid treatment was given

Country/year/reference	No. of cases	Steroid treatment	Outcome	Remark
Hong Kong/1997/Yuen <i>et al.</i> (1998); Beigel <i>et al.</i> (2005)	5	Probably given late in the course of the disease for ARDS, no other details published	2/5 survived	Amantadine (1g 3 × daily) given to some patients 1–8 days after admission.
Indonesia/2005/Kandun <i>et al.</i> (2006)	3	Patient 1 fluticasone, patient 2 budesonide, dexamethasone, patient 3 MP	0/3 survived	Antibiotics but no oseltamivir, except for patient 3 given on day 10 (death on day 11).
Thailand/2003-2004/Chokephaibulkit <i>et al.</i> (2005); Chotpitayasonondh <i>et al.</i> (2005); Beigel <i>et al.</i> (2005)	8	Generally 2 mg MP kg ⁻¹ daily typically given for ARDS late in the course of the disease for 2–5 days.	2/8 survived	Oseltamivir given in 6/8 (including two survivors), survivors were given antiviral earlier, 6/8 aged ≤13 years.
Vietnam/2003-2004/Hien <i>et al.</i> (2004)	7	Four patients (group 1) 5 mg MP kg ⁻¹ daily, three patients (group 2) 1–2 mg MP kg ⁻¹ (4 × daily) for 1 or 3 or 4, days. Probably initiated relatively shortly after admission.	1/7 survived	Antibiotics on admission, two patients group 1 given ribavirin (800 mg 3 × daily, 400 mg 3 × daily), three patients (two in group 2) given oseltamivir (75 mg daily, two patients 150 mg daily) 5–12 days after start of illness.
Vietnam/2005/Beigel <i>et al.</i> (2005)	5	0.4 mg dexamethasone kg ⁻¹ for 5 days	1/5? survived	All patients received oseltamivir. Steroid treatment part of randomized trial.

ARDS, Acute respiratory distress syndrome; MP, methylprednisolone.

in mind. First, the steroids were often given in high doses, some 7–8 times higher relative to the recommendation of Annane *et al.* (2004). For example, Hien *et al.* (2004) treated four H5N1-infected patients with 5 mg methylprednisolone kg⁻¹ daily. Another three patients were treated with 6 mg methylprednisolone kg⁻¹ daily. Second, to be effective in an immunomodulation capacity, steroid treatment must be initiated relatively early and for 10 days minimum. This was illustrated in SARS patients in whom it was found that 7–10 days of treatment was needed to alter cytokine levels (IL-6, IL-8 and IL-10; Ng *et al.*, 2004). Third, steroid monotherapy cannot clear the virus from patients but must be used in conjunction with an antiviral, such as oseltamivir, in a timely fashion. This did not occur in many patients (Hien *et al.*, 2004; Kandun *et al.*, 2006). Thus it has to be concluded that in published reports of patients treated with steroids in H5N1 cases, steroids were administered in a far-from-optimal manner.

SARS and steroid treatment

With many more cases of SARS than H5N1, of whom a substantial proportion were treated with steroids, one might think that more substantial conclusions would have been reached regarding steroid treatment. Unfortunately, this is not the situation (Gomersall *et al.*, 2004). Recently, Stockman *et al.* (2006) reviewed the literature to date on

the subject and decided that because of confounding factors, and the fact that no large randomized clinical trials were conducted, it was not possible to reach any conclusions. The study of Zhao *et al.* (2003) is illustrative. These investigators describe patients being randomly allocated to one of three groups (a later group of 60 patients was created). Group A received ribavirin and cefoperazone/sulbactam, group B received fluoroquinolone, azithromycin, IFN α and restricted steroids (80–160 mg methylprednisolone per day) 14 days after treatment was initiated, group C received the same antibiotics as group B but only some had IFN, and the same steroid regimen was only given for 2–3 days in the most severe cases, group D received levofloxacin and azithromycin with 75% being given IFN, and an unknown number methylprednisolone for 5–14 days at a dose of 160–1000 mg per day (individual patients received varying dosages depending upon condition). Although group D fared the best, it is impossible to draw from the study the contribution of the steroids.

Conclusions

There appears to be weak evidence of both a theoretical and experimental nature that suggests steroids might have a role to play as an adjunctive therapy to antiviral agents. Although this should advocate a randomized controlled trial of corticosteroids, such a design is difficult to execute

in practice, and is unlikely to be realized in the near future. Therefore, there are two choices: refrain from using steroids in avian influenza cases, or attempt to use steroids in a rational, meaningful way.

If we assume that avian influenza initially activates the HPA axis, a clinician admitting an H5N1 patient is likely to find one of three situations: (a) high cortisol levels and minimal GC resistance, (b) high cortisol levels and GC resistance, and (c) low cortisol levels (adrenal insufficiency). Superimposed on these situations might be a finding of resistance to ACTH, which is another indication of adrenal insufficiency. Besides the issue of testing (time, resources) is the fact that we cannot routinely test for GC resistance. However, does that preclude treating patients? For example, we could argue that steroid therapy is appropriate in case (b) and (c), but in case (a) additional steroids might be counterproductive and steroid treatment contra-indicated. If hypercytokinaemia is present, suppression to the extent of bringing cytokines back into balance is desirable, but total suppression, if it could be achieved, is not. This highlights the need not only for randomized controlled trials of steroids, but also for measurement of ACTH, cortisol and cytokine levels in H5N1 patients.

The implication for treatment based upon the aforementioned discussion is for clinicians to proceed cautiously. In terms of practicalities, if adjunctive steroid therapy is decided upon, it should be at a low dose and administered for a sufficient time (7–10 days). There is no evidence that higher dosages are useful from both a theoretical or experimental point of view, and moreover, the side effects of high dosages can be substantial, even if the patient recovers from the infection.

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