NEWS AND VIEWS

Response to respiratory virus outbreak: Researchers lucky to catch flu

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With the increased public awareness and pandemic potential of avian influenza and other difficult to treat respiratory viruses such as SARS, pressure upon both researchers and medical organizations to deliver rapid protection and treatments for new outbreaks of infection has been greatly magnified. Although antiviral therapies for H5N1 avian flu have been rapidly developed, crystal structures of the avian virus neuraminidase suggest some scientific fortune was involved. Evaluation of the pharmacological screening strategy for SARS treatments is less kind, with many trials falling below the necessary scientific rigor to generate useful clinical data. Establishment of better designed and primed response protocols is important in ensuring that future outbreaks can be effectively tackled.

FLAWS IN SARS RESPONSE

The outbreak of the SARS virus prompted international panic; the virus rapidly spread around the world in 2002 and 2003, killing more than 700 and infecting a ten-fold higher number. Under pressure to act, a large number of potential beneficial drugs were screened for impact. However, faced with a potentially lethal disease, the ethical argument against creating comparison control groups led to the design of many studies which generated difficult to interpret, or clinically useless results.

Researchers at the US Centers for Disease Control and Prevention (CDC) (Stockman et al, 2006) collated clinical medical literature on six SARS treatments, predominantly antivirals, such as ribavirin, and corticosteroids that reduce immune activation. Of more than 54 studies of SARS-treatment outcomes: the majority were inconclusive and eight showed evidence of potential harm. Although some drugs may have been beneficial, the evaluation strategies were often poorly designed, and comparison between studies was difficult due to non-standard dose regimens and differences in health and age of the patients. Whilst some of the drugs tested inhibited SARS in experiments on infected tissues, other studies suggested that ribavirin and steroids actually harmed patients; ribavirin enhanced the risk of anaemia, and steroids are linked to bone deterioration and fungal infection.

H5N1 FORTUNE

Neuraminidase inhibitors seltamivir (Tamiflu) and zanamivir (Relenza) have been stockpiled in many countries to counter the pandemic threat of a human strain of the avian influenza virus H5N1. The effectiveness of these drugs depends upon the H5N1 neuraminidase surface structure, and crystal structures recently resolved by Russell et al (2006) suggest that greater consideration should be taken in designing assays to identify inhibitors of new neuraminidase subtypes.

H5N1 influenza virus belongs to the type A group, which sub-divided into nine subtypes dependent on the neuraminidase expressed. These neuraminidases subtypes themselves segregate into two phylogentically segregated groups groups, 1 and 2. The enzyme facilitates the spread of the virus during infection, and so antivirals that inhibit neuraminidase activity, limit the disease. Although designed against the crystal structures of neuraminidase group 2, the inhibitors are effective against group 1 enzymes also, leading to the assumption that the mode of binding to the active sites of the group-1 neuraminidases was the same as for group-2 enzymes (the amino-acid sequences of the active sites are similar for all the subtypes).

Russell et al have reported the structure of three group-1 enzymes have a significantly different three-dimensional structure to that of group-2 enzymes. The carboxylic amino acids Glu 119 and Asp 151 adopt an unexpected conformation, translating into differences in the '150-loop' that opens up an adjacent cavity. These amino acids also form critical interactions with neuraminidase inhibitors, and thus changes in these residues have the potential to destabilize their binding interaction. The crystal structure of N1 neuraminidase in a complex with an inhibitor (oseltamivir) reveals and 'induced fit' whereby the open polypeptide loop

adopts the conformation observed in the crystal structures of group-2 enzymes 3,4,5, explaining how the inhibitor manages to remain effective despite the slight structural differences. It is noted, however, that the active-site conformation may be affected by amino acid residues that lie outside of the active site of the two groups of neuraminidases, meaning that an enzyme inhibitor for one target will not necessarily be effective against another subtype with the same overall three-dimensional structure. In the case of H5N1 it is fortune rather than design that allows these anti-viral drugs to be currently effective.

Discoveries such as these remind researchers that protein structures can be unpredictably affected by small coding sequence changes, and that the binding affinity of neuraminidase inhibitors must also be evaluated when choosing the correct pharmacological agent to counter each new strain of influenza virus.

PROGRESS TOWARDS H5N1 VACCINES

Although person to person transmission of H5N1 is currently unlikely, efforts to generate vaccines for at risk individuals generate useful data that might be used in tailoring production of vaccines to future more virulent strain outbreaks. The European Union requires an acceptable response (a hemagglutinin-inhibition titer of 40 or more) in 70% of volunteers. Two recent studies offer promise that a vaccine for H5N1 will be soon available, and validate the methods used to generate vaccines for response to future spates of infection.

Bressel et al (2006) reported an adjuvanted 30 microg formulation that induced 67% haemagglutinin-inhibition seroconversion rate after two 15 microg vaccinations. The formulation induced an immune response in some individuals after only one does, and was well tolerated with no serious side effects. A Chinese study (Lin et al, 2006) more recently report a whole-virion vaccine made by Sinovac Biotech (Beijing, China) from an inactivated strain of H5N1 Vietnam/1194/2004. An antibody response was apparent after the first injection of as little as 1.25 microg and a highest response (78% seropositivity) observed after two doses of 10-microg. Whole-virion vaccines also offer a manufacturing advantage as 20% to 30% of vaccine antigen is commonly lost in the preparation of split-virion vaccines.

Although the researchers reports that the vaccines was well tolerated, whole-virion vaccines have been associated more severe side effects such as febrile reactions in children, and thus further safety testing is expected to follow.

KEEPING PACE WITH EVOLUTION

Progress in responding to the threats of SARS and H5N1 has been relatively swift, but by no means optimum. The rate of evolution of respiratory viruses requires constant monitoring of changes of the pathogen and adaptation of pharmacological treatment and preventative measures. Should avian influenza start spreading among people, hospitals must be primed to begin a coordinated set of pre-designed trials on experimental drugs and vaccines. The public and political pressure to respond quickly must be tempered by robust scientific method if the results are to be of therapeutic benefit. It is important that protocols are made available are of a national and international scope to ensure that outbreaks of these diseases can be tackled in the minimum of time and with the lowest human cost.

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