Excipient selection based on bioactivity: the case of inhaled interferon.

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Editorial

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In its earliest stages of infection, the SARS-CoV2 virus evades the body's innate immune response, which involves the secretion of interferons. The pre-emptive targeted administration of interferon into the lung, where the later stages of viral proliferation manifest as life-threatening severe acute respiratory syndrome (SARS) is therefore mechanistically logical. When administered prior to the emergence of symptoms, interferon's mechanism of action should theoretically keep patients from being hospitalized and recuperate faster from the disease. Administered in the later stages of the disease, however, it may exacerbate the cytokine storm. Several clinical trials using inhaled interferon are ongoing.

Synairgen plc is conducting clinical studies with inhaled nebulized interferon β (SNG001) for the treatment of SARS-CoV2 infection. In recently concluded phase II trials, patients receiving SNG001 had greater odds of improvement on the WHO ordinal scale for clinical improvement (OSCI), (OR 2·32 [95% CI 1·07–5·04]; p=0·033) on day 16. Due to the low hospitalization rate in the placebo arm of the home cohort, the prevention of severe lower respiratory tract illness attributable to the API could not be determined. A subsequent *ad hoc* analysis including only patients who were markedly or severely breathless at the time of treatment initiation, regardless of whether they were hospitalized or at home, showed that those treated with SNG001 (n=33) were 3.41 times more likely to recover than those on placebo (n=36) (HR 3.41 [95% CI 1.47- 7.94], p=0.004). In the ITT population, the odds of intubation or death were OR 0.42 [95% CI 0.09-1.83], p=0.246.

The most disappointing result from the phase II study was that a subset of patients (who were further along in disease progression) needed to be chosen to demonstrate tangible efficacy. The Phase III clinical study will now presumably target patients with a more advanced stage of disease than those with milder symptoms, i.e., those within days of being infected. This is disappointing because interferon β could justifiably have been thought of as a pan-virus prophylactic, being most efficacious at the earliest stage of infection, considering the mechanism by which SARS-CoV2 evades innate immunity. Interferon β would have been able to ward off the virus upon the first sign of symptoms (or even before). In hindsight, the Phase II study could have been designed to quantify more biomarkers such as viral RNA load, neutrophil to lymphocyte ratio (NLR), Interleukin-6 (IL-6) and C-reactive protein (CRP) to compensate for the small percentage of placebo patients who progressed to hospitalization within the

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studied time period. These are known to be prognostic risk-stratification biomarkers for patients infected with the SARS-CoV2 virus. Furthermore, formulating the API with efficacy potentiating excipients could have increased this stratification differentiation between the drug (formulation) arm and the placebo arm in these recently infected patients.

As a first approximation, excipients that reduce surface tension at the alveolar surface, upregulate Adenosine GPCRs' or S-adenosyl methionine (SAM) mimetics and molecules that decrease host immune response to the API should have been considered worthy of being incorporated into the interferon β inhaled formulation. According to the company, the current formulation is pH neutral, free of mannitol, arginine and human serum albumin.

A rheumatoid arthritis clinical study by Can-Fite biopharma found a high number of responders in the placebo arm. Further investigation revealed that the excipients polyoxyl 45 castor oil and Miglyol 812® upregulated the adenosine receptors, A_3 and A_2A which are known to mediate the effect of methotrexate, the API used in the study. Thus these excipients enhanced the anti-inflammatory effect of the API. Allosteric lipid interactions with the Adenosine A_2A receptor have been shown to potentiate the effects of endogenous adenosine or agonist ligands. The acyl chains of docosahexaenoic acid (DHA), present in the bilayer plasma membrane were similarly found to enhance the activation of G-proteins by the Adenosine A_2A receptor. It is, therefore not unreasonable to assume the ricinoleic acid and the caprylic/capric acid acyl chains from the Kolliphor® and Miglyol® excipients to possess Adenosine A_2A modulating effects. Significant benefits of inhaled adenosine in compassionate study protocols, small clinical studies or from anecdotal evidence have been published. These include significant anti-inflammatory effects as evidenced by a decrease in CRP, significant decrease in viral RNA and a significant increase in the PaO_2/FiO_2 ratio. The latter effect can be attributed in part to the fact that both these excipients are amphoteric molecules that act as surfactants to reduce the surface tension at the alveolar surface and thereby increase O_2 diffusion into the blood.

The majority of crystalline fructose is made up of its six-membered ring isoform, B-D-fructopyranose (BDF). The binding of viral nonstructural protein 16 (nsp16) to adenosine or SAM analogs is not nucleotide specific. BDF was shown to be bound to this site as well (PDB ID: 6W4H). Although it is not currently known if this binding affects viral 2'-o-methyltransferase activity, a SAM blocking molecule, or an allosteric inhibitor may well decrease the ability of the SARS-CoV2 to cap the 5' end of its virally encoded mRNA with methyl groups thereby restoring human innate immunity; including interferon secretion and potentiation. The utility of using fructose (or a SAM analog/nsp16-nsp10 complex inhibitor) as an excipient, with interferon as the API hence becomes evident. The former can be used to enhance the effect of the latter by decreasing the amount of methyl capped viral mRNA. Docking studies demonstrate that hesperidin, a citrus bioflavonoid, long used as a nutritional supplement, has among the highest binding affinities to the SAM pocket in nsp16, thereby making it exceptionally suitable as an inhibitor of viral 2'-o-methyltransferase. Since it is being investigated (administered orally) in ongoing SARS-CoV2 related clinical studies, using a GRAS orange extract, certified to >85% hesperidin could have circumvented patent issues.

The public domain has no data on whether Synairgen's formulation contains surfactants to prevent aggregation of the protein during its shelf life. Interferon β aggregation has been shown to reduce its efficacy by the development of host neutralizing antibodies when administered intranasally to mice. In that study, an alkylsaccharide surfactant, dodecyl maltoside, significantly reduced the immunogenicity of the drug. It may turn out that Kolliphor® and Miglyol® may also reduce protein aggregation, in addition to their Adenosine receptor modulation effects.

It must be emphasized that the formulation suggested in this editorial is a Gedankenexperiment. Issues such as stability, compatibility, toxicity, manufacturability, ADME and so on, would have needed to be addressed before clinical trials could have commenced. For example, Kolliphor® and Miglyol® have never been

used via the inhalation route and there is concern about hypersensitivity to these excipients. However, since the formulation is targeted, small quantities of these excipients would suffice. Furthermore, formulating as a DPI would probably significantly reduce the coughing reflex and hypersensitivity. Additionally, it would be worth investigating if tolerable amphoteric molecules with acyl chains would be effective Adenosine receptor modulators, so that they could replace Kolliphor® and Miglyol®. These solvable issues notwithstanding, it is nevertheless disconcerting that little or no thought is given to optimizing formulations with bioactive excipient armamentaria, relying instead on the bare-bones and anachronistic approach of incorporating buffers and tonicity agents to expeditiously get to the clinic. In the case of inhaled interferon (and others), the price paid is not only a formulation which may possess sub-optimal efficacy, but also one that does not meet its therapeutic rationale.

A decade ago, I wrote an editorial in this Journal entitled, "Excipients in formulations for clinical trials: Getting it right the first time ". The incorrect archaic notion that excipients are therapeutically nugatory, inactive appendages in formulations, still persists. In this regard, the long-drawn-out tragedy that is still unfolding, has as much to do with missed therapeutic and economic opportunities, as it does with the countless number of patients who may otherwise have benefited from an optimally formulated drug, but will now have to wait until either another 'more efficacious' API is discovered, or their disease progresses into the patient subset in which the existing poorly formulated drug has shown clinical efficacy.

DISCLOSURE

The author has an investment position in Synairgen plc.