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Review

The use of sialidase therapy for respiratory viral infections

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ABSTRACT

DAS181 is an inhaled bacterial sialidase which functions by removing sialic acid (Sia) from the surface of epithelial cells, preventing attachment and subsequent infection by respiratory viruses that utilize Sia as a receptor. DAS181 is typical of bacterial sialidases in cleaving Sia α2-3 and Sia α2-6 linkages, and it also has a demonstrated effect against acetylated and hydroxylated forms of Sia. The potency of the compound has been enhanced by coupling the active sialidase with an amphiregulin tag, allowing a longer duration of action and minimizing spread to the systemic circulation. DAS181 is now in Phase II development for the treatment of influenza, and it has also demonstrated activity in individual cases of parainfluenza in immunosuppressed patients. Continued evaluation of the roles and activities of bacterial sialidases is required to expand the range of successful antiviral therapies targeting Sia or its derivatives.

Contents

1. DAS181: a bacterial sialidase for influenza prevention and therapy

Influenza is an infection of the respiratory tract caused by influenza A and B viruses. To infect epithelial cells, the virus must first penetrate the overlying mucus barrier, then bind to the cell surface

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– an interaction involving viral polypeptides or attachment proteins and cognate cell-surface receptors. DAS181 is the first antiviral compound in Phase II development that functions by blocking this pathogen–host interaction, by destroying the influenza host-cell receptor, sialic acid (Sia), on the surface of respiratory epithelial cells. In this paper, we provide background information on Sia and sialidases; discuss the potential role of bacterial sialidases as antiviral agents; review the *in vitro* and Phase II evaluation of DAS181 for the treatment of influenza; and note evidence that the drug would also be useful against parainfluenza virus infections.

Current antiviral strategies focus on prevention of viral replication within the cell or inhibition of release of newly formed virions. Neuraminidase (NA) inhibitors primarily act at this step, but they play a minor role in modulating viral entry (Matrosovich et al., 2004). The first step of viral binding often involves close contact between the viral attachment protein and host receptor. Before the development of DAS181, studies in the 1980s demonstrated that natural compounds present in serum, in particular alpha 2-macroglobulin, inhibited virus adsorption to surfaces (Hanaoka et al., 1989; Rogers et al., 1983). Later studies identified Sia as being the key determinant of binding for these compounds (Pritchett et al., 1987; Pritchett and Paulson, 1989). Lentz proposed a number of strategies to prevent cell entry by blocking the host receptor (Lentz, 1988, 1990), but these were mainly directed at developing an antibody-mediated approach, rather than receptor destruction.

In 1988, Weis and colleague used X-ray crystallography to map the haemagglutinin–Sia interaction (Weis et al., 1988). With this structure solved, it was envisioned that it might be possible to develop synthetic analogs of Sia to block binding (Pritchett et al., 1987; Pritchett and Paulson, 1989), though it was acknowledged that such drug design would have a number of structural obstacles (Weis et al., 1988). This route of anti-influenza therapy was not pursued in as much detail as blocking the viral neuraminidase site, but there have been two recent reports on developing drug design by blocking the HA binding site, using databases of commercially available compounds (ZINC) (Li et al., 2011; Nandi, 2008) and other techniques (Al-qattan and Mordi, 2010a,b; Blessia et al., 2010).

2. Evidence that influenza virus uses sialic acid as a receptor

2.1. An introduction to sialic acid

In 1958, Springer and Ansell found that influenza viruses and bacteria had a common enzyme property: the ability to remove receptors from red blood cells and prevent haemagglutination (Springer and Ansell, 1958). To investigate this requires an understanding of the nature of Sia, its presence in the respiratory tract and the structure and function of bacterial sialidase. Sialic acid is the term used for a family of nine-carbon monoscaccharides found in animals and certain bacteria (Chen and Varki, 2010). They are typically found at the terminal portions of oligosaccharide chains attached to proteins and lipids to form glycoproteins and glycolipids, respectively. There are over 40 naturally occurring variants of these nine-carbon keto-sugars based on a neuraminic acid (Neu) or a 2-keto-3-deoxynononic acid (Kdn) backbone (Schauer, 1973; Varki, 1997). The discovery of neuraminic acid by Klenk and colleagues has been reviewed by Schaeur (Schauer, 1973) with the term sialic acid proposed by Blix, Gottschalk and Klenk based on the Greek word sialion (salivea) as these carbohydrates were isolated from the mucin of submaxillary glands.

2.2. Sialic acid: structure and function

2.2.1. Sialic acid structure

The diversity of Sia present in nature is due to the substitution at the 4–9 carbons by O-acetyl, sulfate, methyl and lactate groups and by glycosidic linkage by the anomeric C_2 to the 3 or 6 hydroxyl group of galactose (Gal) (referred to as the α2-3 or α2-6), N-acetyl galactosamine (GalNAc) or N-acetyl glucosamine (GlcNAc) (Varki and Varki, 2007) (Fig. 1). Eleven different types of sialic acid have been identified on the human erythrocyte membrane, using gas chromatography and mass spectrometry (Bulai et al., 2003). GalNAc is typically present on O-glycans, and GlcNAc is normally a component of N-glycans. The binding of Sia to Gal or GalNAc requires a family of enzymes called sialyltransferases (ST), with each ST producing a specific linkage (Harduin-Lepers et al., 2005; Paulson and Rademacher, 2009; Xia et al., 2005). In some instances, multiple Sia may be linked together to form polysialic acid, most commonly in a α2-8 configuration (Hildebrandt et al., 2010). The linkage of Sia to the adjacent sugar is in an α configuration, which means the bond is acid-labile and is readily destroyed by enzymes known as sialidases. It should be noted that in solution, unbound Sia is mainly in the ß-configuration, with the C2 hydroxyl positioned axial to the ring and in equilibrium with the minor α-form (Vimr, 1994).

2.2.2. Sialic acid function

The acidic nature of Sia is due to the carboxylate at C_1, which is ionized at physiological pH and has a pK of 1.8–2.6, giving the Sia a number of useful features, as highlighted by Schauer (Schauer,

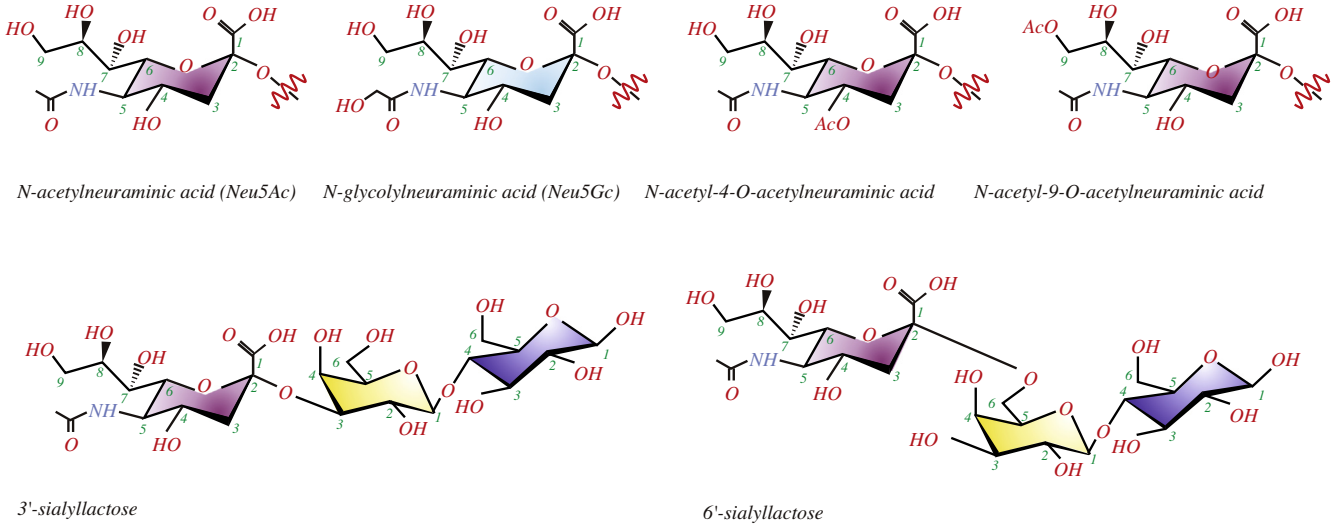

Fig. 1. Structures of different sialic acid derivatives. The numbering of carbons is listed in green; AcO refers to acetylation. Two common linkages of Sia with galactose to form 3′sialyllactose (Siaα2-3Galß1-4Glu) and 6′sialyllactose (Siaα2-6Galß1-4Glu) are shown.