



Tunicamycin Anticancer Drug May Reliable to Treat Coronavirus Disease-19

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Abstract

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BACKGROUND: SARS-CoV-2 outbreaks remains a medical and economic challenge, due to the lack of a suitable drug or vaccine. The glycan in some proteins plays an important role in protein folding, sorting, transport, and oligomerization, so the hindering of N-linked glycosylation of glycoproteins will prevent assembly of the virion. Tunicamycin anticancer drug inhibits the N-linked glycan.

AIM: This study aimed to find out the mechanism action of tunicamycin on the viral glycoproteins.

RESULTS: The growth of the virus in the presence of tunicamycin conducted in the production of non-infectious and absence of spike protein (spikeless virions). Tunicamycin inhibits E2, S, and M glycoproteins of coronaviruses. Tunicamycin has also diminished glycosylation of PTMs such as HE, and 8ab of SARS-CoV. Finally,

CONCLUSION: This study recommends using this drug to treat the SARS-CoV-2.

Introduction

Since the last December, a new coronavirus has been a challenge for all of the world. The reason of elevation number of infections and fatality that the researchers failed to find suitable drug or vaccine to stem the outbreaks [1]. A few studies focused on the effect of N- and O-glycosylation through the process of virion assembly. N-linked glycosylation is the attachment of an oligosaccharide (glycan), to a nitrogen atom an asparagine (Asn) residue of a protein. This linkage is important for the structure and function of some eukaryotic proteins. Severe acute respiratory syndrome coronavirus (SARS-CoV) is one of viruses that contain N-linked glycoproteins which are glycosylated by the transfer of core oligosaccharides from a dolichol pyrophosphate carrier to asparagine residues on the polypeptide [2]. O-linked glycosylation is the attachment of a sugar molecule to the oxygen atom on serine (Ser) or threonine (Thr) residues in a protein. O-glycosylation is a post-translational modification (PTM) that occurs after the protein has been synthesized. It occurs either in the endoplasmic reticulum or Golgi apparatus and it influences on the stability and regulation of protein [3]. The main function of O-glycan is allowing recognition of foreign

material and controlling cell metabolism. The changes in O-glycosylation are important in many diseases including cancer, diabetes Alzheimer's, as well as some viral infections [4]. Because glycosylation and transport of viral proteins depend on cellular processes, prevention this process depends on the type of the viral protein and the activity of the substrate to diminish it. Coronavirus glycoproteins may be glycosylated by a different mechanism. This glycoproteins serve as a useful model for the study of N- and O-linked glycoproteins [5].

The Role of Tunicamycin (TM)

TM, an antibiotic, was produced by *Streptomyces clavuligerus* and *Streptomyces lysosuperficus* bacteria along with several other species and a proposed biosynthetic pathway was characterized. TM is a white crystalline powder which is soluble in alkaline water, pyridine, and hot methanol, slightly soluble in ethanol and *n*-butanol and insoluble in acetone, ethyl acetate, chloroform, benzene, and acidic water [6].

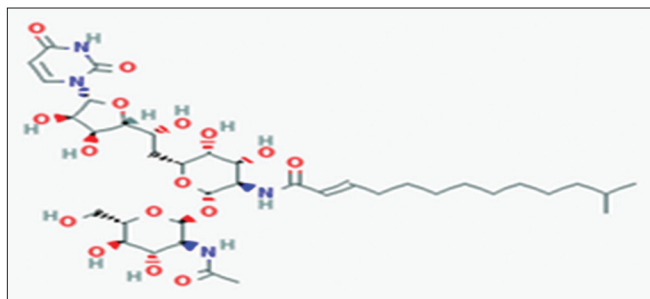


Figure 1: The structure of tunicamycin drugbank

TM, Figure 1, is an analog of UDP-N-acetylglucosamine, interfered with the formation of dolichol pyrophosphate-acetylglucosamine which acts as a carrier for N-glycosidic linkage of core oligosaccharides to asparagine (Asn) residues on glycoproteins. The bacteria utilize the enzymes in the *tun* gene cluster (TunA-N) to make TMs. Several of cellular glycoproteins contain glycosylation linkage such as immunoglobulins, fetuin, thyroglobulin, and proteoglycan. Tunicamycin inhibits the first step in the biosynthesis of N-glycosylation linked in the ER resulting produced many misfolded proteins [7]. When the antibiotic blocks glycosylation of N-glycans, the cell cycle arrests at the G1 phase in human cells. The previous studies suggested that TM may work as a therapeutic drug against cancer cells as it has been shown to sensitize human colon and prostate cancer cells to TRAIL-induced apoptosis. The inhibited glycosylation of the structural glycoproteins was showed in alphaviruses, bunyaviruses, herpesviruses, and myxoviruses and all other viruses possess glycoprotein envelopes. Han *et al.* proposed that inhibition of the N-linked glycan biosynthesis by TM may be a promising therapeutic strategy for enhancing the sensitivity of cancer cells to trastuzumab [8].

Mechanism of Action of TM

TM inhibits the reverse reactions in the first step of the biosynthesis of N-linked oligosaccharides in cells. It prevents the formation of UDP-N-acetylglucosamine from N-acetylglucosaminylpyrophosphoryl dolichol. The inhibition is increased by pre-incubating the enzyme with antibiotic for up to 5 min before addition the substrate. The addition of phosphatidylcholine at the concentration up to 20 mM does not affect the inhibition regardless of whether it was added during the pre-incubation or at the same time at the substrate. TM binds to the heat denatured microsomal particles of aorta as shown by the fact that pre-incubation of the antibiotic with these particles prevented the inhibition of the N-acetylglucosamin-1-phosphate transferase [9].

The Glycosylation of E1 and E2 Glycoproteins of Coronavirus

The transmembrane glycoprotein E1 composed of three domains: A glycosylated domain projects from the envelope. The second domain lies within the membrane, and the third domain interacts with the nucleocapsid inside the viral envelope. E1 may be an O-linked glycoprotein [10]. Glycosylation of E1 appears post-translational event. The glycoprotein E2 forms the large petal-shaped peplomers characteristic of the coronavirus envelope. E2 is a 180 kDa which can be cleaved by trypsin to yield two 90 kDa components. It has been recorded that TM inhibits formation of the E2 glycoprotein but does not prevent synthesis or glycosylation of the glycoprotein E1 through the formation or releasing of virion from the infected cell. Moreover, the shift from the non-glycosylated 20 kDa form to the glycosylated 23 kDa form does not inhibit by TM [11], [12]. This provides indirect evidence that E1 is not an N-linked glycoprotein but may be an O-linked glycoprotein. Although E1 glycoproteins are more negatively charged than those of E2, these glycoproteins differ in carbohydrate composition, electrophoretic patterns of glycopeptides, and response to the antibiotic [13]. These data suggested that E1 is an O-linked glycoprotein while E2 is an N-linked glycoprotein. In contrast, the effects of TM on the synthesis and glycosylation of the two SARS-CoV-E glycoproteins have permitted tentative assignment of functions to these proteins [14].

The Glycosylation of Coronavirus S Protein

Since the 1980s, the N-linked glycosylation S protein of coronavirus was defined for hepatitis virus MHV. S protein in the rough ER was found to acquire high mannose oligosaccharides. It has been found that the Golgi transport blocker (monensin) inhibited the transport of S protein from trans-Golgi network to the cell surface [15]. Other studies revealed that MHV S protein also modified the N-linked glycosylation of bovine coronavirus BCoV, alpha-coronavirus transmissible gastroenteritis virus (TGEV), and gamma-coronavirus infectious bronchitis virus (IBV). The high mannose oligosaccharides of SARS-CoV S protein were trimerized as early as 30 min post-entry into ER, before the acquisition of complex glycans in the Golgi apparatus using pulse-chase experiments coupled with fractionation. Then, the maturation status of S protein can be monitored by its sensitivity to endoglycosidase H. This enzyme hydrolyzes the high mannose glycans [16]. After that, the structure of N-linked glycosylation of S protein was determined using mass spectrometry. This linked was enriched with high mannose, hybrid and complex glycans with

or without bisecting *N*-acetyl-galactosamine (GalNAc), and core fucose. A 12 out of 23 putative glycosylation sites detected of SARS-CoV S protein were actually glycosylated [17].

The SARS-CoV S protein has two domains S1 and S2. When the S1 domain of bovine-CoV S protein was cloned and expressed in insect cells, the mature S protein was glycosylated and bound by neutralizing monoclonal antibodies. On the other hand, the infected cells with TGEV in the presence of TM, the antigenicity of both S and M protein, were significantly reduced [18]. This may confirm that TM is an inhibitor factor of the glycosylation proteins. It has been demonstrated that the inhibition of N-linked glycosylation by TM or removal of N-linked glycans by PNGase F reduced TGEV-induced IFN- α production. Therefore, the N-linked glycans on SARS-CoV S protein may be a pathogen associated molecular pattern recognized by host pattern recognition receptors. These receptors are activated downstream antiviral innate immune response. The growth of coronavirus in the presence inhibitor TM resulted in the production of spikeless, non-infectious virions which were devoid of S protein [19], [20].

The Glycosylation of Coronavirus M Protein

M protein of coronaviruses is the most abundant protein comprising of 220–260 amino acids. It plays a central role of the viral assembly. M protein is a multipass transmembrane protein with a short N-terminal ectodomain, three hydrophobic TM domains, and a large C-terminal endodomain. O-linked glycosylation of the mouse hepatitis virus M protein was first revealed in 1981. It was noted that in the presence of TM, M protein was still normally produced and glycosylated, resulting in the formation of non-infectious virions containing normal amounts of N and M protein, but lacking S completely [21], [22].

Distinct from the O-linked glycosylation perceived in the M protein of bovine-coronavirus BCoV, human-coronavirus HCoV-OC43, alpha-coronavirus TGEV, gamma-coronavirus IBV, and turkey enteric coronavirus is all modified by N-linked glycosylation. This linked of M protein is sensitive to endoglycosidase H and can be inhibited by TM. The N-linked glycosylation sites were mapped to N3 and N6 of IBV. M protein of betacoronaviruses in other lineages is also N-linked glycosylated [23]. For example, SARS-CoV M protein contains a single N-glycosylation site at N4. When transiently transfected as a C-terminally FLAG-tagged protein, SARS-CoV M protein was found to obtain high mannose N-glycans and was modified into complex N-glycans in the Golgi. Although the glycosylation of the coronavirus M protein is a strongly conserved feature, this glycosylation is not important for virus assembly or replication [24], [25].

The Glycosylation of Coronavirus Non-structural Proteins nsp3 and nsp4

Some of the luminal domains of nsp3 coronaviruses proteins undergo N-linked glycosylation in the ER. For instance, MHV nsp3 is inserted into ER cotranslationally and glycosylated at N1525. Glycosylation of nsp4 was detected in IBV at N48 residue while for the nsp4 of MHV, two glycosylation sites were predicted at N176 and N237 residues. Till now, no study supports that TM inhibits N-linked glycans either in nsp3 or nsp4 [26], [27], [28].

The Glycosylation of Coronavirus PTMs Proteins

Coronavirus genome encodes various accessory proteins called apart from the structural and non-structural proteins (PTMs), most of which share no homology to any known proteins. However, some of the PTMs accessory proteins are incorporated in mature virions, others have been concerned in the modulation of host immune response and *in vivo* pathogenesis. One of the PTM proteins is HE protein which is a part of beta-coronaviruses S protein. The HE protein of bovine CoV was also shown to be glycosylated when expressed using human adenovirus vector. Furthermore, HE protein of MHV was found to be modified by N-linked glycosylation and was inhibited by TM but not monensin. The importance of N-linked glycosylation of coronavirus HE protein has not been fully characterized [14], [29].

The O-linked glycosylation of SARS-CoV 3a protein and M shares the same N-exo/C-endo membrane topology. The both proteins contain three TM domains. O-linked glycans of the SARS-CoV protein 3a are resistant to the treatment of PNGase F, and pulse-chase analysis suggested that the oligosaccharides were acquired post-translationally. Protein 3a has been implicated in modulating host immune response [30], [31].

The sgRNA8 of SARS-CoV encodes a single protein 8ab. A 29-nt deletion in the center split open reading frame ORF8 into two smaller frames, encoding proteins 8a and 8b, respectively. The 8ab protein is cotranslationally smuggled into the ER and is N-linked glycosylated at N81. The 8b protein is synthesized in the cytosol and not modified. Both proteins 8b and 8ab were shown to interact and modified by ubiquitination. The glycosylation at N81 stabilized 8ab protein and protected it from proteasomal degradation. Protein 8b is unstable and undergoes rapid proteasomal degradation. The ubiquitinated 8b and 8ab may mediate rapid degradation of IRF3 and regulate host antiviral innate immunity. The inhibition N-linked glycosylation of SARS-CoV 8ab protein by TM is not completely understood [32], [33].

Conclusions

The transmembrane structural proteins S, E, M, nsp3, nsp4, and accessory proteins (HE, 3a, and 8ab) of the most coronavirus family are modified by glycosylation. Although the M proteins of beta-coronavirus adopt the special O-linked glycosylation, the M proteins of the other coronaviruses are modified by N-linked glycosylation. It has been registered that glycosylation of coronavirus S protein is essentially N-linked. The folding and intracellular trafficking N-linked glycans of the coronavirus S protein also constitute a significant part of conformation of mature protein. TM inhibits formation of the coronavirus E2 glycoprotein but does not prevent synthesis or glycosylation of the glycoprotein E1. The antigenicity of TGEV S and M protein was significantly reduced in the presence of TM. M protein of the most *Coronaviridae* is sensitive to endoglycosidase H and can be inhibited by TM. Lack information about the effect TM on SARS-CoV N-linked glycans ns3 and ns4. HE and 8ab proteins of SARS-CoV glycosylation are inhibited by TM. Although TM inhibits N-linked glycosylation of coronaviruses glycoproteins, no drug available to inhibit O-linked glycosylation has been identified yet.

We concluded that TM inhibits E2, S, and M glycoproteins of coronaviruses. TM is also diminished glycosylation of PTMs such as HE and 8ab of SARS-CoV. Since TM has long been used as an anticancer and has the ability to inhibit glycoproteins of coronaviruses, we recommend to use this drug to treat the SARS-CoV-2.

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