Tunicamycin – A born fighter against CORONA

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Abstract
One of the perilous threats to humans are Viruses. A greater risk of mortality and morbidity in mankind are caused by most of the viruses. Unfortunately, corona virus stood as a monster in the present scenario. The external envelope of the viruses are comprised of proteins namely glycoproteins. The primary target in viral replication is to interrupt the N-glycosidic linkage thereby obstruct the signals from endoplasmic reticulum to the envelope. This particular area has gained more scope in scientific research. The drug namely tunicamycin has grabbed scientist’s attention in prohibition of viral propagation by showing its effect on endoplasmic reticulum resulting in stoppage of the signals, envelope breaking and inhibiting the interactions with cellular membrane.

Keywords: Tunicamycin, Corona

Introduction
To a greater extent almost all the viruses are contagious and causing terrific mischief to human and animals. As viruses illustrate a wide variety regarding their structure and arrangement of envelope their differentiation occurs on envelope basis. Lipid bilayer and glycoproteins are components of viral envelope out of which glycoproteins consists of external hydrophilic part and internal hydrophobic segment by which the spikes are strongly associated with the lipid bilayer. (For review see compans and kien [1]). Viral spikes play an important role in the interaction between viral envelopes and cellular membrane. Here the primary initiation of the cellular level. It also been demonstrated that glycoproteins of this viruses are important determinants for the spread of infection and thus for pathogenicity. (For review see kienk et al. [25]). Most of the viral glycoproteins studied to date contain oligosaccharide side chain that are attached by N-glycosidic linkages to the polypeptide such as glycoprotein has recently been characterized in corona virus where it is presented together with another glycoprotein of the N-glycosidic type [2, 3]. The biosynthesis of viral glycoproteins with N-glycosidic linkages has been studied in detail It involves translation at membrane-bound ribosomes, insertion into the membrane of the rough endoplasmic reticulum, and transport to the site of virus assembly, which is usually the plasma membrane. In the course of transport, the glycoproteins are processed by glycosylation, proteolytic cleavage, and the covalent binding of fatty acids. At the cotranslational level, proteolytic cleavage in many instances removes the signal sequences required for insertion of the nascent polypeptides into the membrane of the rough endoplasmic reticulum. Posttranslational cleavage is involved in the processing of a series of these glycoproteins, such as the influenza hemagglutinin and the glycoproteins of paramyxoviruses, alphaviruses, and oncoviruses. In the case of the myxovirusglyco- proteins, posttranslational proteolytic cleavage was found to be a precondition for biological activity (for review, see Klenk and Rott [4]). The biosynthesis of glycoproteins can be inhibited by interfering with the intracellular transport, with proteolytic cleavage, and with glycosylation. This article will be confined to the inhibition of glycosylation. To date, only glycosylation inhibitors interfering with the biosynthesis of N-glycosidically linked oligosaccharides are known. Thus, only observations made on glycoproteins containing this type of side chains will be reviewed.

Note
Viral spikes play an important role in the interaction between viral envelopes and cellular membrane.
The biosynthesis and structure of carbohydrate side chains linked by N-glycosidic bonds to the polypeptide

The oligosaccharide side chains of the viral glycoproteins are assembled through the biosynthetic machinery of the host cell by the same general principles as the carbohydrate drapes of cellular glycoproteins. Glycosylation of glycoproteins with N-glycosidic linkages is initiated in the rough endoplasmic reticulum by the attachment of preformed oligosaccharides to asparagine residues in the polypeptide chain. The attachment sites have the sequence asparagine-X-threonine or asparagine-X-serine, with X being a variable amino acid. The oligosaccharides transferred to the nascent polypeptide are synthesized via the dolichol pathway of glycosylation which recently has been reviewed in detail elsewhere [5] and can be summarized as follows (Fig. 1).

![Fig 1: The biosynthesis of the dolichol-linked oligosaccharide precursors of the asparagine-linked carbohydrate side chains. Dol, dolichol; GlcNAc, N-acetylglucosamine; Man, mannose; G1c, glucose.](image)

The carbohydrate chains are assembled on the phosphate ester of the isoprenoid alcohol dolichol with UDP-N-acetylglucosamine, GDP-mannose, mannosylphosphoryldolichol, and glucosyl phosphoryldolichol as sugar donors. After the en bloc transfer of the oligosaccharide to the polypeptide and cleavage of the pyrophosphate linkage dolichol phosphate is available for a new assembly cycle. The oligosaccharide synthesized in this way is usually a tetradecasaccharide of the structure (glucosyl)3-(mannosyl)9-(N-acetylglucosamine)2. Under certain conditions to be described below a decasaccharide of the structure (glucosyl)-mannosyl-(N-acetylglucosamine)2 can be transferred. Glycosylatinn via the decasaccharide lipid is called the alternate pathway. The enzymes involved in the assembly of the lipid-linked oligosaccharides are membrane-bound and appear to occur mainly in the rough endo-plasmic reticulum (for a discussion, see Schwarz and Datema [6]). After the transfer to the polypeptide the oligosaccharide side chains are further processed (Fig. 2). First, the three glucose residues are removed by specific glucosidases to yield a mannose-rich carbohydrate side chain [7,8]. At this stage, processing of the oligosaccharide may cease. In most instances, however, it continues by a trimming process resulting in the release of all the mannoses but three. By the subsequent addition of N-acetylglucosamine, galactose, fucose, and neuraminic acid the complex oligosaccharides are formed [9]. The available evidence indicates that the enzymes involved in this last step in the glycosylation sequence are located in the Golgi apparatus for review, see Klenk and Rott [10].

![Fig 2: The processing of the protein-bound oligosaccharides. The structures of the complex (I) and of the mannose-rich (II) side chains found in mature glycoproteins are indicated. Dol, dolichol; N-Acetylsuccosaminidase (†); mannose (0); glucose (A); galactose (††); neuraminic acid (†††); fucose (a). (A-dapted from Kornfeld et al. 1411.)](image)

From this review on the carbohydrate synthesis it is clear that complex and mannose-rich side chains of the general structures shown in (Fig. 2) exist in mature viral glycoproteins and that both types are derived from a common precursor. It is also evident from what has been said above that the number and the position of the oligosaccharides on the polypeptide is determined by the presence of appropriate amino acid sequences that can act as attachment sites. The number of side chains attached to different glyco-proteins varies therefore over a wide range; e.g. the G protein of vesicular stomatitis virus has only two oligosaccharides [11,12], whereas as many as seven side chains can be observed on the influenza hemagglutinin. The studies on the influenza hemagglutinin revealed also that the many variants of this glycoprotein vary widely in the distribution of the glycosylation sites and the oligosaccharide types attached to them [13-16]. These results indicate that the structure of the polypeptide plays an important role in determining whether a mannose-rich or a complex oligosaccharide is attached to a given glycosylation site. Despite of the high variability of most glycosylation sites of the hemagglutinin, some of them appear to be conserved. It is interesting to note that according to the three-dimensional model of the hemagglutinin [17] all conserved side chains are located in close vicinity to each other at the base of the spike. This may indicate that these side chains have a special function in maintaining the proper implantation of the glycoprotein in the lipid bilayer [18].

**Inhibitor of glycosylation**

**Tunicamycin**

Corona virus (Fig-3) (virus group) Tunicamycin (inhibitor of glycosylation).

A nucleoside antibiotic extracted from Tamura’s group of micrococcus sylos/superfusicus was Tunicamycin (Fig-4) which is a tight-binding, competitive inhibitor of the enzyme transferring phosphoryl-N-acetylglucosamine from UDP-N-acetylglucosamine to phosphoryldolichol. Therefore N-acetylglucosaminylpyrophosphoryldolichol formation is inhibited [19-21].

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nearly observed. One study, Sturman Step
induced lactosamine need in focusing Anti
envelope breaking and; an. Annu. Rev. Biochem. 1981;
36x56 as a common step in the above 3 process which is inhibited by
diphosphate acetylhexosamine membrane. Translocation of a derivative of an
concentrations of drug in both the cells with and without
peptidoglycan. The above mechanisms are seen even at low
protein N
The major mechanism of tunicamycin includes inhibition of
Tunicamycin
0.5.
Intraperitoneally tunicamycin is administered at a dose of
widely recognized drug namely [nakamura and company
impact of nucleoside antibiotics on kidney are studied by
Both invitro effects of ER stress in various cell types and
biosynthesis of a coronavirus glycoprotein: demonstration of a novel type of viral glycoprotein.
Virology. 1981; 115:334-344.
3. Niemann H, Klenk HD. Coronavirus glycoprotein El, a
new type of viral glyco- protein. J Mol. Biol. 1981;
153:993-1010.
4. Klenk HD, Rott R. Cotranslafional and posttranslational
processing of viral glyco- proteins. Curt. Top. Microbiol.
Immunol. 1980; 90:19-48.
5. Hubbard SC, Ivatt RJ. Synthesis of the N-linked
oligosaccharides of glycoproteins: assembly of the lipid-
linked precursor oligosaccharide and its relation to
protein synthesis in vivo. Annu. Rev. Biochem. 1981;
50:555-583.

Fig 3: Structure of Corona Virus

Fig 4: Structure of Tunicamycin

In order to obtain glycosylation inhibition, maximum
concentrations of tunicamycin is required. Apart from this
other effect includes inhibition of glucosylphosphoryldolichol
formation and protein synthesis are seen in human fibroblasts.

Biological effects of glycosylation inhibitor tunicamycin
Tunicamycin forest alls the addition of core oligosaccharides
to nascent polypeptides by inhibiting n-linked glycosylation
and thereby blocks protein folding and transit through ER
from: methods in enzymology, 2011.

Nucleosides
Both invitro effects of ER stress in various cell types and
impact of nucleoside antibiotics on kidney are studied by
widely recognized drug namely Tunicamycin. Intrapertitoneally tunicamycin is administered at a dose of
0.5–1.0 mg/kg for 3 days to inhibit protein glycosylation [22].

Tunicamycin
The major mechanism of tunicamycin includes inhibition of
protein N- glycosylation, peptidoglycan biosynthesis and
binding of teichoic acids and teichuronic acids to
peptidoglycan. The above mechanisms are seen even at low
concentrations of drug in both the cells with and without
membrane. Translocation of a derivative of an N-
acetyhexosamine-1-phosphate residue from the uridine
diphosphate sugar to mono phosphoplyprenol is considered
as a common step in the above 3 process which is inhibited by
tunicamycin at minute doses. (Step 1, Fig. 4.8; step a, Fig.
4.13; and step 1, Fig. 4.15). Residues of uridine and N-
acetylglucosamine are linked by N-fatty acyl galactosamine
residues. It binds irreversibly to the translocase due to its
structural analogue with substrate.

Inhibition of peptidoglycan synthesis and N-glycosylation of
proteins marks this drug as an antibiotic and antiviral
respectively. Therapeutic usage of tunicamycin in animals is
avoided as it causes toxic to the cells due to inhibition of N-
glycosylation of essential glycoprotein. Certainly, biological
activities of different glycopolypeptides respond differently. The
sensitivity of cells from different sources to the antimetabolite varies markedly. Even though the
tunicamycin blocks glycosylation the production of transferrin
and VLDL apo-B-protein (both normally N-glycosylated) by
hepatocytes remains neutral whereas the low-density
lipoprotein receptor activity is declined. Along with these a
small change is observed in receptors that are present in
fibroblasts [23].

Effects on the susceptibility of viral glycoprotein to
proteolytic cleavage
The difference between the sugar analog and tunicamycin in
the effect on the proteolytic stability of the non
glycosylatedhemagglutinin it has to be pointed out.

Side effects and adverse effects of tunicamycin
GI symptoms, peripheral neuropathy, fatigue, and
thrombocytopenia are generally observed. One study
suggested that bortezomib-induced ER stress could be
involved in peripheral neuropathy, possibly by impairing
myelin synthesis [25].

Conclusions
As Viral infections are threatening human existence with
infections, there a urgent need in focusing Anti-Viral drugs.
The present paper is focused on the drug namely tunicamycin
which had grabbed scientist’s attention in prohibition of viral
propagation by showing its effect on endoplasmic reticulum
resulting in stoppage of the signals, envelope breaking and
inhibiting the interactions with cellular membrane.

This outbreak Investigations can lead to the development of
Novel Drugs.

References
1. Comps RW, Klenk HD. Viral Membranes. In: Comprehensive Virology, (Plenum Publishing Co., New
York. 1979; 13:293-407.
2. Holmes KV, Doller EW, Sturman 'LS. Tunicamycin
resistant glycosylation of a coronavirus glycoprotein: demonstration of a novel type of viral glycoprotein.
Virology. 1981; 115:334-344.
3. Niemann H, Klenk HD. Coronavirus glycoprotein El, a
new type of viral glyco- protein. J Mol. Biol. 1981;
153:993-1010.
6. Tkacz JS, Lampen JO. Tunicamycin inhibition of polyisoprenol, N-acetylgalco- saminy pyrophosphate formation in calf liver microsomes. Biochem. Biophys. Res. Commun. 1975; 65:248-257.

7. Grinn LS, Robbins PW. Glycoprotein biosynthesis. Rat liver microsomal glucosi- dases which process oligosaccharides. J Biol. Chem. 1979; 254:8814-8818.

8. Grinn LS, Robbins PW. Substrate specificities of rat liver microsomal glucosi- dases which process glycoproteins. J Biol. Chem. 1980; 255:2253-2258.

9. Kornfeld S, Li B, Tabas I. The synthesis of complex-type oligosaccharides. III. Characterization of the processing intermediates in the synthesis of the complex oligosaccharide units of the vesicular stomatitis virus G protein. J Biol. Chem. 1978; 253:7771-7778

10. Klenk HD, Rott R. Cotranslational and posttranslational processing of viral glyco- proteins. Curt. Top. Microbiol. Immunol. 1980; 90:19-48.

11. Etchison JR, Holland JJ. Carbohydrate composition of the membrane glycopro- tein of vesicular stomatitis virus. Virology. 1974; 60:217-229.

12. Schwarz RT, Rohrschneider JM, Sehmidt MFG. Suppression of glycoprotein formation of Semliki Forest virus by tunicamycin. J Virol. 1976; 19:782-791.

13. Klenk HD, Garten W, Keil W, Niemann H, Bosch FX, Schwarz RT. Processing of the influenza virus hemagglutinin. In: Genetic Variation among Influenza Viruses. Eds. Nayak, D. and Fox, C.F. Academic Press New York, 1981, 193-211.

14. Nakamura K, Compans RW. Host-cell and strain- dependent differences in oligo- saccharides of hemagglutinin glycoproteins of influenza A viruses. Virology. 1979; 95:8-23.

15. Schwarz RT, Klenk HD. Carbohydrates of influenza virus. IV. Strain-dependent variations. Virology. 1981; 113:584-593.

16. Ward CW. Structure of influenza virus hemagglutinin. Curl Top. Microbiol. Immunol. 1981; 94:t-74.

17. Wilson IA, Skehel JJ, Wiley DC. Structure of the haemagglutinin membrane glycoprotein of influenza virus at 3 ~ resolution. Nature. 1981; 289:366-373.

18. Klenk HD, Garten W, Keil W, Niemann H, Bosch FX, Schwarz RT. Processing of the influenza virus hemagglutinin. In: Genetic Variation among Influenza Viruses. Eds. Nayak, D. and Fox, C.F. (Academic Press New York), 1981, 193-211.

19. Lehle L, Tanner W. The specific site of tunicamycin inhibition in the formation of dolichol-bound N- acetylgalactosamine derivatives. FEBS Lett. 1976; 71:167-170.

20. Takatsuki A, Tamura G. Tunicamycine, a new antibiotic. II. Some biological pro- perties of the antitodal activity of tunicamycin. J Antibiot. 1971; 24:224-231.

21. Tkacz JS, Lampen JO. Tunicamycin inhibition of polyisoprenol, N-acetylgalco- saminy pyrophosphate formation in calf liver microsomes. Biochem. Biophys. Res. Commun. 1975; 65:248-257.

22. Cybulsky AV. Endoplasmic reticulum stress, the unfolded protein response and autophagy in kidney diseases. Nature reviews nephrology. 2017; 13(11):681-696.

23. Brockhausen I, Schachter H. Glycosyl transferases Involved in N–and O–Glycan Biosynthesis. Glycosciences: status and perspectives, 1996, 79-113.

24. Nakamura K, Compans RW. Effects of gincosamine, 2-deoxy-D-glucose, and tunic- mycin on glycosylation, sulfation and assembly of influenza viral proteins. Virology. 1978; 84:303-3792.

25. Flatters SJL, Dougherty PM, Colvin LA. Clinical and preclinical perspectives on chemotherapy-induced peripheral neuropathy (CIPN): a narrative review. BJA: British Journal of Anaesthesia. 2017; 119(4):737-749.