Galanthus Nivalis Agglutinin (GNA)

A Broad-Spectrum Virus Binding Agent
Table of Contents

• Description of the GNA-based Hemopurifier (#3)

• GNA background information (#4)

• Science journal abstracts of GNA antiviral properties (#5-17)

• GNA-based Hemopurifier® virus capture validations (#18-22)
The Aethlon Hemopurifier® is a first-in-class medical technology that prevents viruses from infecting host cells. Residing within the device is a remarkable antiviral binding agent known as galanthus nivalis agglutinin (GNA). GNA binds high-mannose carbohydrate structures that form a protective glycan shield that cloaks infectious viruses from being recognized by the immune system. GNA has a specificity to bind a broad-spectrum of viral pathogens, yet has limited interactions with most human proteins. The resulting mechanism establishes the Hemopurifier® as an immune response ally to combat life-threatening viral infections.
GNA is derived from the common snowdrop

GNA derives naturally from the common snowdrop (galanthus nivalis), which is one of the most popular of all cultivated bulbous plants, and its flowering is traditionally seen to herald the end of winter. Galanthus nivalis was described by the Swedish botanist Carl Linnaeus in his Species Plantarum in 1753, and given the specific epithet nivalis, meaning snowy (Galanthus means with milk-white flowers). This narrow-leaved snowdrop, with its delicate white hanging flowers, has become very popular in cultivation and is commonly planted in gardens and parks.

The common snowdrop occurs throughout Europe, from the Pyrenees eastward to the Ukraine, and from Germany and Poland southwards to southern Italy, Albania and northern Greece.
Third party journal publications describing the virus binding properties of GNA
Abstract

Carbohydrate binding agents (CBAs), including natural lectins, are more and more considered as broad-spectrum antivirals. These molecules are able to directly inhibit many viruses such as Human Immunodeficiency Virus (HIV), Hepatitis C Virus (HCV), Dengue Virus, Ebola Virus or Severe Acute Respiratory Syndrome Coronavirus through binding to envelope protein N-glycans. In the case of HIV, it has been shown that CBAs select for mutant viruses with N-glycosylation site deletions which are more sensitive to neutralizing antibodies. In this study we aimed at evaluating the HCV resistance to CBAs in vitro. HCV was cultivated in the presence of increasing *Galanthus nivalis agglutinin* (GNA), Cyanovirin-N, Concanavalin-A or Griffithsin concentrations, during more than eight weeks. At the end of lectin exposure, the genome of the isolated strains was sequenced and several potential resistance mutations in the E1E2 envelope glycoproteins were identified. The effect of these mutations on viral fitness as well as on sensitivity to inhibition by lectins, soluble CD81 or the 3/11 neutralizing antibody was assessed. Surprisingly, none of these mutations, alone or in combination, conferred resistance to CBAs. In contrast, we observed that some mutants were more sensitive to 3/11 or CD81-LEL inhibition. Additionally, several mutations were identified in the Core and the non-structural proteins. Thus, our results suggest that in contrast to HIV, HCV resistance to CBAs is not directly conferred by mutations in the envelope protein genes but could occur through an indirect mechanism involving mutations in other viral proteins. Further investigations are needed to completely elucidate the underlying mechanisms.
Targeting N-glycan cryptic sugar moieties for broad-spectrum virus neutralization: progress in identifying conserved molecular targets in viruses of distinct phylogenetic origins.


Abstract

Identifying molecular targets for eliciting broadly virus-neutralizing antibodies is one of the key steps toward development of vaccines against emerging viral pathogens. Owing to genomic and somatic diversities among viral species, identifying protein targets for broad-spectrum virus neutralization is highly challenging even for the same virus, such as HIV-1. However, viruses rely on host glycosylation machineries to synthesize and express glycans and, thereby, may display common carbohydrate moieties. Thus, exploring glycan-binding profiles of broad-spectrum virus-neutralizing agents may provide key information to uncover the carbohydrate-based virus-neutralizing epitopes. In this study, we characterized two broadly HIV-neutralizing agents, human monoclonal antibody 2G12 and Galanthus nivalis lectin (GNA), for their viral targeting activities. Although these agents were known to be specific for oligomannosyl antigens, they differ strikingly in virus-binding activities. The former is HIV-1 specific; the latter is broadly reactive and is able to neutralize viruses of distinct phylogenetic origins, such as HIV-1, severe acute respiratory syndrome coronavirus (SARS-CoV), and human cytomegalovirus (HCMV). In carbohydrate microarray analyses, we explored the molecular basis underlying the striking differences in the spectrum of anti-virus activities of the two probes. Unlike 2G12, which is strictly specific for the high-density Man9GlcNAc2Asn (Man9)-clusters, GNA recognizes a number of N-glycan cryptic sugar moieties. These include not only the known oligomannosyl antigens but also previously unrecognized tri-antennary or multi-valent GlcNAc-terminating N-glycan epitopes (Tri/m-Gn). These findings highlight the potential of N-glycan cryptic sugar moieties as conserved targets for broad-spectrum virus neutralization and suggest the GNA-model of glycan-binding warrants focused investigation.
Structural analysis and binding properties of isoforms of tarin, the GNA-related lectin from Colocasia esculenta.

Pereira PR1, Winter HC2, Verícimo MA3, Meagher JL4, Stuckey JA5, Goldstein IJ6, Paschoalin VM7, Silva JT8.

Abstract

The lectins, a class of proteins that occur widely in animals, plants, fungi, lichens and microorganisms, are known for their ability to specifically bind to carbohydrates. Plant lectins can be classified into 12 families including the Galanthus nivalis agglutinin (GNA)-related lectin superfamily, which is widespread among monocotyledonous plants and binds specifically to mannose, a behavior that confers remarkable anti-tumor, anti-viral and insecticidal properties on these proteins. The present study characterized a mitogenic lectin from this family, called tarin, which was purified from the crude extract from taro (Colocasia esculenta). The results showed that tarin is a glycoprotein with 2-3% carbohydrate content, composed of least 10 isoforms with pIs ranging from 5.5 to 9.5. The intact protein is a heterotetramer of 47kDa composed of two non-identical and non-covalently associated polypeptides, with small subunits of 11.9kDa and large subunits of 12.6kDa. The tarin structure is stable and recovers or maintains its functional structure following treatments at different temperatures and pH. Tarin showed a complex carbohydrate specificity, binding with high affinity to high-mannose and complex N-glycans. Many of these ligands can be found in viruses, tumor cells and insects, as well as in hematopoietic progenitor cells. Chemical modifications confirmed that both conserved and non-conserved amino acids participate in this interaction. This study determined the structural and ligand binding characteristics of a GNA-related lectin that can be exploited for several different purposes, particularly as a proliferative therapeutic molecule that is able to enhance the immunological response.
Inhibition of hepatitis C virus by the cyanobacterial protein Microcystis viridis lectin: mechanistic differences between the high-mannose specific lectins MVL, CV-N, and GNA.


Abstract

Plant or microbial lectins are known to exhibit potent antiviral activities against viruses with glycosylated surface proteins, yet the mechanism(s) by which these carbohydrate-binding proteins exert their antiviral activities is not fully understood. **Hepatitis C virus (HCV)** is known to possess glycosylated envelope proteins (gpE1E2) and to be potently inhibited by lectins. Here, we tested in detail the antiviral properties of the newly discovered Microcystis viridis lectin (MVL) along with cyanovirin-N (CV-N) and Galanthus nivalis agglutinin (GNA) against cell culture HCV, as well as their binding properties toward viral particles, target cells, and recombinant HCV glycoproteins. Using infectivity assays, CV-N, MVL, and GNA inhibited HCV with IC50 values of 0.6 nM, 30.4 nM, and 11.1 nM, respectively. **Biolayer interferometry analysis demonstrated a higher affinity of GNA to immobilized recombinant HCV glycoproteins compared to CV-N and MVL.** Complementary studies, including fluorescence-activated cell sorting (FACS) analysis, confocal microscopy, and pre- and post-virus binding assays, showed a complex mechanism of inhibition for CV-N and MVL that includes both viral and cell association, while GNA functions by binding directly to the viral particle. Combinations of GNA with CV-N or MVL in HCV infection studies revealed synergistic inhibitory effects, which can be explained by different glycan recognition profiles of the mainly high-mannoside specific lectins, and supports the hypothesis that these lectins inhibit through different and complex modes of action. Our findings provide important insights into the mechanisms by which lectins inhibit HCV infection. **Overall, the data suggest MVL and CV-N have the potential for toxicity due to interactions with cellular proteins while GNA may be a better therapeutic agent due to specificity for the HCV gpE1E2.**
Anti-tumor and anti-viral activities of Galanthus nivalis agglutinin (GNA)-related lectins.

Wu L1, Bao JK.

Abstract

Galanthus nivalis agglutinin (GNA)-related lectin family, a superfamily of strictly mannose-binding specific lectins widespread among monocotyledonous plants, is well-known to possess a broad range of biological functions such as anti-tumor, anti-viral and anti-fungal activities. Herein, we mainly focused on exploring the precise molecular mechanisms by which GNA-related lectins induce cancer cell apoptotic and autophagic death targeting mitochondria-mediated ROS-p38-p53 apoptotic or autophagic pathway, Ras-Raf and PI3K-Akt anti-apoptotic or anti-autophagic pathways. In addition, we further discussed the molecular mechanisms of GNA-related lectins exerting anti-viral activities by blocking the entry of the virus into its target cells, preventing transmission of the virus as well as forcing virus to delete glycan in its envelope protein and triggering neutralizing antibody. In conclusion, these findings may provide a new perspective of GNA-related lectins as potential drugs for cancer and virus therapeutics in the future.
Broad antiviral activity of carbohydrate-binding agents against the four serotypes of dengue virus in monocyte-derived dendritic cells.

Alen MM, De Burghgraeve T, Kaptein SJ, Balzarini J, Neyts J, Schols D.

Abstract

BACKGROUND:
Dendritic cells (DC), present in the skin, are the first target cells of dengue virus (DENV). Dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) is present on DC and recognizes N-glycosylation sites on the E-glycoprotein of DENV. Thus, the DC-SIGN/E-glycoprotein interaction can be considered as an important target for inhibitors of viral replication. We evaluated various carbohydrate-binding agents (CBAs) against all four described serotypes of DENV replication in Raji/DC-SIGN(+) cells and in monocyte-derived DC (MDDC).

METHODOLOGY/PRINCIPAL FINDINGS:
A dose-dependent anti-DENV activity of the CBAs Hippeastrum hybrid (HHA), Galanthus nivalis (GNA) and Urtica dioica (UDA), but not actinohivin (AH) was observed against all four DENV serotypes as analyzed by flow cytometry making use of anti-DENV antibodies. Remarkably, the potency of the CBAs against DENV in MDDC cultures was significantly higher (up to 100-fold) than in Raji/DC-SIGN(+) cells. Pradimicin-S (PRM-S), a small-size non-peptidic CBA, exerted antiviral activity in MDDC but not in Raji/DC-SIGN(+) cells. The CBAs act at an early step of DENV infection as they bind to the viral envelope of DENV and subsequently prevent virus attachment. Only weak antiviral activity of the CBAs was detected when administered after the virus attachment step. The CBAs were also able to completely prevent the cellular activation and differentiation process of MDDC induced upon DENV infection.

CONCLUSIONS/SIGNIFICANCE:
The CBAs exerted broad spectrum antiviral activity against the four DENV serotypes, laboratory-adapted viruses and low passage clinical isolates, evaluated in Raji/DC-SIGN(+) cells and in primary MDDC.
Fundamental difference in the content of high-mannose carbohydrate in the HIV-1 and HIV-2 lineages.

Stansell E1, Desrosiers RC.

Abstract

The virus-encoded envelope proteins of human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) typically contain 26 to 30 sites for N-linked carbohydrate attachment. N-linked carbohydrate can be of three major types: high mannose, complex, or hybrid. The lectin proteins from Galanthus nivalis (GNA) and Hippeastrum hybrid (HHA), which specifically bind high-mannose carbohydrate, were found to potently inhibit the replication of a pathogenic cloned SIV from rhesus macaques, SIVmac239. Passage of SIVmac239 in the presence of escalating concentrations of GNA and HHA yielded a lectin-resistant virus population that uniformly eliminated three sites (of 26 total) for N-linked carbohydrate attachment (Asn-X-Ser or Asn-X-Thr) in the envelope protein. Two of these sites were in the gp120 surface subunit of the envelope protein (Asn244 and Asn460), and one site was in the envelope gp41 transmembrane protein (Asn625). Maximal resistance to GNA and HHA in a spreading infection was conferred to cloned variants that lacked all three sites in combination. Variant SIV gp120s exhibited dramatically decreased capacity for binding GNA compared to SIVmac239 gp120 in an enzyme-linked immunosorbent assay (ELISA). Purified gp120s from six independent HIV type 1 (HIV-1) isolates and two SIV isolates from chimpanzees (SIVcpz) consistently bound GNA in ELISA at 3- to 10-fold-higher levels than gp120s from five SIV isolates from rhesus macaques or sooty mangabeys (SIVmac/sm) and four HIV-2 isolates. Thus, our data indicate that characteristic high-mannose carbohydrate contents have been retained in the cross-species transmission lineages for SIVcpz-HIV-1 (high), SIVsm-SIVmac (low), and SIVsm-HIV-2 (low).
Differences in the mannose oligomer specificities of the closely related lectins from Galanthus nivalis and Zea mays strongly determine their eventual anti-HIV activity.

Hoorelbeke B1, Van Damme EJ, Rougé P, Schols D, Van Laethem K, Fouquaert E, Balzarini J.

Abstract

BACKGROUND:
In a recent report, the carbohydrate-binding specificities of the plant lectins Galanthus nivalis (GNA) and the closely related lectin from Zea mays (GNAmaize) were determined by glycan array analysis and indicated that GNAmaize recognizes complex-type N-glycans whereas GNA has specificity towards high-mannose-type glycans. Both lectins are tetrameric proteins sharing 64% sequence similarity.

RESULTS:
GNAmaize appeared to be ~20- to 100-fold less inhibitory than GNA against HIV infection, syncytia formation between persistently HIV-1-infected HuT-78 cells and uninfected CD4+ T-lymphocyte SupT1 cells, HIV-1 capture by DC-SIGN and subsequent transmission of DC-SIGN-captured virions to uninfected CD4+ T-lymphocyte cells. In contrast to GNA, which preferentially selects for virus strains with deleted high-mannose-type glycans on gp120, prolonged exposure of HIV-1 to dose-escalating concentrations of GNAmaize selected for mutant virus strains in which one complex-type glycan of gp120 was deleted. Surface Plasmon Resonance (SPR) analysis revealed that GNA and GNAmaize interact with HIV IIIB gp120 with affinity constants (KD) of 0.33 nM and 34 nM, respectively. Whereas immobilized GNA specifically binds mannose oligomers, GNAmaize selectively binds complex-type GlcNAcβ1,2Man oligomers. Also, epitope mapping experiments revealed that GNA and the mannose-specific mAb 2G12 can independently bind from GNAmaize to gp120, whereas GNAmaize cannot efficiently bind to gp120 that contained prebound PHA-E (GlcNAcβ1,2man specific) or SNA (NeuAca2,6X specific).

CONCLUSION:
The markedly reduced anti-HIV activity of GNAmaize compared to GNA can be explained by the profound shift in glycan recognition and the disappearance of carbohydrate-binding sites in GNAmaize that have high affinity for mannose oligomers. These findings underscore the need for mannose oligomer recognition of therapeutics to be endowed with anti-HIV activity and that mannose, but not complex-type glycan binding of chemotherapeutics to gp120, may result in a pronounced neutralizing activity against the virus.
Molecular modeling, docking and dynamics simulations of GNA-related lectins for potential prevention of influenza virus (H1N1).

Xu HL, Li CY, He XM, Niu KQ, Peng H, Li WW, Zhou CC, Bao JK.

Abstract

The Galanthus nivalis agglutinin (GNA)-related lectin family exhibit significant anti-HIV and anti-HSV properties that are closely related to their carbohydrate-binding activities. However, there is still no conclusive evidence that GNA-related lectins possess anti-influenza properties. The hemagglutinin (HA) of influenza virus is a surface protein that is involved in binding host cell sialic acid during the early stages of infection. Herein, we studied the 3D-QSARs (three-dimensional quantitative structure-activity relationships) of lectin- and HA-sialic acid by molecular modeling. The affinities and stabilities of lectin- and HA-sialic acid complexes were also assessed by molecular docking and molecular dynamics simulations. Finally, anti-influenza GNA-related lectins that possess stable conformations and higher binding affinities for sialic acid than HAs of human influenza virus were screened, and a possible mechanism was proposed. Accordingly, our results indicate that some GNA-related lectins, such as Yucca filamentosa lectin and Polygonatum cyrtonema lectin, could act as drugs that prevent influenza virus infection via competitive binding. In conclusion, the GNA-related lectin family may be helpful in the design of novel candidate agents for preventing influenza A infection through the use of competitive combination against sialic acid specific viral infection.
Synergistic antiviral effect of Galanthus nivalis agglutinin and nelfinavir against feline coronavirus.

Hsieh LE, Lin CN, Su BL, Jan TR, Chen CM, Wang CH, Lin DS, Lin CT, Chueh LL.

Abstract

Feline infectious peritonitis (FIP) is a fatal disease in domestic and nondomestic felids caused by feline coronavirus (FCoV). Currently, no effective vaccine is available for the prevention of this disease. In searching for agents that may prove clinically effective against FCoV infection, 16 compounds were screened for their antiviral activity against a local FCoV strain in Felis catus whole fetus-4 cells. The results showed that Galanthus nivalis agglutinin (GNA) and nelfinavir effectively inhibited FCoV replication. When the amount of virus preinoculated into the test cells was increased to mimic the high viral load present in the target cells of FIP cats, GNA and nelfinavir by themselves lost their inhibitory effect. However, when the two agents were added together to FCoV-infected cells, a synergistic antiviral effect defined by complete blockage of viral replication was observed. These results suggest that the combined use of GNA and nelfinavir has therapeutic potential in the prophylaxis and treatment of cats with early-diagnosed FIP.
The carbohydrate-binding plant lectins and the non-peptidic antibiotic pradimicin A target the glycans of the coronavirus envelope glycoproteins.

van der Meer FJ, de Haan CA, Schuurman NM, Haijema BJ, Verheije MH, Bosch BJ, Balzarini J, Egberink HF.

Abstract

OBJECTIVES:
Many enveloped viruses carry carbohydrate-containing proteins on their surface. These glycoproteins are key to the infection process as they are mediators of the receptor binding and membrane fusion of the virion with the host cell. Therefore, they are attractive therapeutic targets for the development of novel antiviral therapies. Recently, carbohydrate-binding agents (CBA) were shown to possess antiviral activity towards coronaviruses. The current study further elucidates the inhibitory mode of action of CBA.

METHODS:
Different strains of two coronaviruses, mouse hepatitis virus and feline infectious peritonitis virus, were exposed to CBA: the plant lectins Galanthus nivalis agglutinin, Hippeastrum hybrid agglutinin and Urtica dioica agglutinin (UDA) and the non-peptidic mannose-binding antibiotic pradimicin A.

RESULTS AND CONCLUSIONS:
Our results indicate that CBA target the two glycosylated envelope glycoproteins, the spike (S) and membrane (M) protein, of mouse hepatitis virus and feline infectious peritonitis virus. Furthermore, CBA did not inhibit virus-cell attachment, but rather affected virus entry at a post-binding stage. The sensitivity of coronaviruses towards CBA was shown to be dependent on the processing of the N-linked carbohydrates. Inhibition of mannosidases in host cells rendered the progeny viruses more sensitive to the mannose-binding agents and even to the N-acetylglucosamine-binding UDA. In addition, inhibition of coronaviruses was shown to be dependent on the cell-type used to grow the virus stocks. All together, these results show that CBA exhibit promising capabilities to inhibit coronavirus infections.
Antiviral activity of carbohydrate-binding agents against Nidovirales in cell culture.

van der Meer FJ1, de Haan CA, Schuurman NM, Haijema BJ, Peumans WJ, Van Damme EJ, Delputte PL, Balzarini J, Egberink HF.

Abstract

Coronaviruses are important human and animal pathogens, the relevance of which increased due to the emergence of new human coronaviruses like SARS-CoV, HKU1 and NL63. Together with toroviruses, arteriviruses, and roniviruses the coronaviruses belong to the order Nidovirales. So far antivirals are hardly available to combat infections with viruses of this order. Therefore, various antiviral strategies to counter nidoviral infections are under evaluation. Lectins, which bind to N-linked oligosaccharide elements of enveloped viruses, can be considered as a conceptually new class of virus inhibitors. These agents were recently evaluated for their antiviral activity towards a variety of enveloped viruses and were shown in most cases to inhibit virus infection at low concentrations. However, limited knowledge is available for their efficacy towards nidoviruses. In this article the application of the plant lectins Hippeastrum hybrid agglutinin (HHA), Galanthus nivalis agglutinin (GNA), Cymbidium sp. agglutinin (CA) and Urtica dioica agglutinin (UDA) as well as non-plant derived pradimicin-A (PRM-A) and cyanovirin-N (CV-N) as potential antiviral agents was evaluated. Three antiviral tests were compared based on different evaluation principles: cell viability (MTT-based colorimetric assay), number of infected cells (immunoperoxidase assay) and amount of viral protein expression (luciferase-based assay). The presence of carbohydrate-binding agents strongly inhibited coronaviruses (transmissible gastroenteritis virus, infectious bronchitis virus, feline coronaviruses serotypes I and II, mouse hepatitis virus), arteriviruses (equine arteritis virus and porcine respiratory and reproductive syndrome virus) and torovirus (equine Berne virus). Remarkably, serotype II feline coronaviruses and arteriviruses were not inhibited by PRM-A, in contrast to the other viruses tested.
Validations of the GNA-based Hemopurifier®
to capture infectious viral pathogens
Hemopurifier® in vitro capture validations

Chronic & Latent Viruses

- Human Immunodeficiency Virus (HIV)
- Hepatitis C Virus (HCV)
- Cytomegalovirus (CMV)
- Epstein-Barr Virus (EBV)
- Herpes Simplex Virus-1 (HSV-1)
Hemopurifier® in vitro capture validations

**Bioterror & Pandemic Threat Viruses**

- ☑ Ebola
- ☑ Lassa
- ☑ MERS-CoV
- ☑ Smallpox (based on Monkeypox & Vaccinia models)

*The Hemopurifier after treating an Ebola patient*
Hemopurifier® in vitro capture validations

Mosquito-Borne Viruses

☑ Chikungunya
☑ Dengue
☑ West Nile
☑ Zika
Hemopurifier® in vitro capture validations

Pandemic Influenza Viruses

☑ H1N1 Swine Flu
☑ H5N1 Bird Flu
☑ Spanish Flu of 1918 (reconstructed)

Actual Spanish Flu of 1918 pandemic resulted in approximately 50 million deaths worldwide.
This summary overview has been provided for informational purposes only. It may contain predictions and other forward looking statements that involve risks and uncertainties, including whether and when our products are successfully developed and introduced; market acceptance of the Aethlon Hemopurifier® and other product offerings; regulatory delays, manufacturing delays, and other risks detailed in our SEC filings, which are accessible at www.sec.gov or on our website: www.AethlonMedical.com