Antiviral Research 133 (2016) 14–22

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Review

Norovirus drug candidates that inhibit viral capsid attachment to human histo-blood group antigens

Eunüs S. Ali [a], Harinda Rajapaksha [b], Jillian M. Carr [c], Nikolai Petrovsky [a, b, *]

[a] School of Medicine, Flinders University, Adelaide, South Australia, Australia
[b] Vaxine Pty Ltd, Flinders Medical Centre/Flinders University, Adelaide, South Australia, Australia
[c] Department of Microbiology & Infectious Diseases, School of Medicine, Flinders University, Adelaide, South Australia, Australia

ARTICLE INFO

Article history:
Received 14 May 2016
Received in revised form
2 July 2016
Accepted 6 July 2016
Available online 13 July 2016

Keywords:
Norovirus
Human histo-blood group antigen
Norovirus capsid proteins
Binding inhibitors
Antiviral therapy

ABSTRACT

Human noroviruses are the leading causative agents of epidemic and sporadic viral gastroenteritis and childhood diarrhoea worldwide. Human histo-blood group antigens (HBGA) serve as receptors for norovirus capsid protein attachment and play a critical role in infection. This makes HBGA-norovirus binding a promising target for drug development. Recently solved crystal structures of norovirus bound to HBGA have provided a structural basis for identification of potential anti-norovirus drugs and subsequently performed *in silico* and *in vitro* drug screens have identified compounds that block norovirus binding and may thereby serve as structural templates for design of therapeutic norovirus inhibitors. This review explores norovirus therapeutic options based on the strategy of blocking norovirus-HBGA binding.

Contents

1. Introduction 15
 1.1. Human histo-blood group antigens as norovirus attachment receptors 15
 1.2. Norovirus classification 15
 1.3. HBGA structure and synthesis 16
 1.4. Norovirus-HBGA interaction 17
 1.5. Physical screening for compounds blocking norovirus host cell attachment 17
 1.6. *In silico* screening approaches for compounds with anti-norovirus activity 18
 1.7. Drug candidate screening *in vivo* 19
 1.8. Potential challenges 19
2. Concluding remarks 20
 Acknowledgments 20
 Supplementary data 20
 Financial support 20
 Conflict of interest 20
 References 20

List of abbreviations: HBGA, Human histo-blood group antigen; FBLD, Fragment based lead discovery; Kd, Dissociation binding constant; LIE, Linear interaction energy; MD, Molecular dynamics; Ki, Inhibition constant.

* Corresponding author. Department of Diabetes and Endocrinology, Flinders Medical Centre, Adelaide, South Australia, 5042, Australia.
E-mail address: nikolai.petrovsky@flinders.edu.au (N. Petrovsky).

http://dx.doi.org/10.1016/j.antiviral.2016.07.006
0166-3542/

1. Introduction

Noroviruses are single-stranded, positive-sense RNA viruses that are the leading cause of acute viral gastroenteritis worldwide, being responsible for nearly 60% of all foodborne illness outbreaks (Arias et al., 2013; Changotra et al., 2009; Kocher and Yuan, 2015; Prasad et al., 2016; Rocha-Pereira et al., 2014; Scallan et al., 2011; Thorne et al., 2016). The human histo-blood group antigens (HBGAs) and in particular the Lewis, Secretor and ABO HBGA families, have been shown to serve as norovirus attachment receptors (Dabelsteen, 2002; Marionneau et al., 2001) with norovirus genotype-specific sequences determining recognition of specific HBGAs (Donaldson et al., 2010; Tan et al., 2009). The large strain diversity in noroviruses presents a major challenge for vaccine development and there are currently no approved vaccines against norovirus (Arias et al., 2013; Karst et al., 2014). Although anti-viral approaches targeting norovirus replication (Arias et al., 2013; Rocha-Pereira et al., 2014) and monoclonal antibodies have been attempted (Chen et al., 2013; Lindesmith et al., 2012) this has been with limited success.

Recent determination of the recognition requirements between norovirus attachment protein VP1 and host receptors and, in particular, solving of the crystal structure of the complex of norovirus P domain and HBGAs (Cao et al., 2007) have provided a new opportunity to develop drugs targeting the first attachment step in norovirus infection (Huang et al., 2005; Koppisetty et al., 2010; Lundborg et al., 2013). The idea of targeting viral binding is well established in antiviral drug development. For example, Palivizumab is an approved monoclonal antibody used to treat children with Respiratory Syncytial virus (RSV) infection that targets the RSV F protein receptor (Subramanian et al., 1998). Similarly, Maraviroc, a CCR5 inhibitor, prevents binding of HIV gp120 and thereby provides broad protection against a wide range of HIV-1 strains (Bocket et al., 2016). Other drugs that block HIV viral fusion include Enfuvirtide (Fuzeon), which binds to HIV gp41 and interferes with its ability to approximate the two membranes (Chong et al., 2016; Zhu et al., 2015). Pleconaril, a capsid-binding antiviral, prevents rhinoviruses from attaching to the host cell and successfully reduced infection in human trials (Hayden et al., 2003).

This review describes current norovirus drug candidates directed at inhibition of HBGA attachment.

1.1. Human histo-blood group antigens as norovirus attachment receptors

Human HBGA's serve as norovirus attachment receptors and co-receptors (Hutson et al., 2002; Marionneau et al., 2002; Tan and Jiang, 2005, 2007, 2008, 2011, 2014). HBGA's are complex carbohydrates located on the surfaces of erythrocytes and intestinal epithelial cells (Ravn and Dabelsteen, 2000) and are important markers used for blood typing. Blood group trisaccharides A and B (Fig. 1) serve as the main attachment receptors for norovirus strain VA387 (Hutson et al., 2002). The binding modes of HBGA-A (BGTA) and -B (BGTB) trisaccharides to a representative norovirus capsid protein are shown in Fig. 2A and B, where, a predominant GII.4 norovirus VA387 strain has been characterized for its binding to HBGAs. Binding free energies of the HBGA A and B trisaccharides are −11.4 and −9.5 kcal $mol^{-1,}$ respectively (Lundborg et al., 2013). HBGAs with a terminal fucose serve as attachment receptors for human norovirus in the gastrointestinal tract (Rydell et al., 2011).

While the question of whether HBGA molecules act as functional receptors that internalize norovirus into cells remains debated, it is clear that they act as attachment receptors and that this binding interaction is a precondition to internalization for most strains. For example, an *in vitro* study employing norovirus virus-like particles (VLPs) showed that norovirus VLP attachment to HBGA resulted in VLP internalization into the cell (Marionneau et al., 2002). Moreover, several studies, using X-ray crystallography, demonstrated the interaction of norovirus capsid protein with HBGA, and the fact that such interactions affect host susceptibly in human volunteer studies indicate that HBGA act as attachment and/or internalization receptors determining host susceptibility (Cao et al., 2007; Hutson et al., 2002; Tan and Jiang, 2014). While several studies have reported that some human cell lines expressing HBGA did not support norovirus replication (Duizer et al., 2004; Karst and Wobus, 2015), this may simply be due to these cell lines lacking other factor or co-receptors required for viral infection and replication and does not diminish the importance of HBGA in viral infection (Duizr, E. et al., 2004). In addition to those drug candidates, serum HBGA blocking antibodies have recently been recognized as a correlate of protective immunity against norovirus-initiated illness (Czako et al., 2012; Reeck et al., 2010). In addition, virus-specific salivary IgA antibodies are also correlates of protection (Ramani et al., 2015). In a recent study, a monoclonal antibody, when incubated with virus prior to inoculation, was able to stop human norovirus infection in a chimpanzee (Chen et al., 2013). Nevertheless, secretor negative individuals remain susceptible to some norovirus strains, consistent with the existence of a non-HBGA receptor as well, a fact that needs to be consider when targeting HBGA binding as a norovirus antiviral strategy. Interestingly, human GII.4 norovirus VLP induces membrane invaginations during assembly of glycosphingolipids (Rydell et al., 2013); thus, binding to glycosphingolipids by human norovirus may also be involved in cellular entry (Karst and Wobus, 2015). Additional cofactors may also play a part in norovirus binding, entry and uncoating. For example, murine noroviruses bind to sialic acids and glycolipids (Taube et al., 2009, 2012) and recent studies suggest that enteric bacteria may bind human noroviruses (Miura et al., 2013) with human norovirus infection of human B cells shown to be expedited by enteric bacteria expressing a HBGA (Jones et al., 2014, 2015; Karst, 2015).

The fucosyltransferase gene is involved in catalyzing the addition of a terminal fucose to HBGA's. A polymorphic change in a single nucleotide (G428A) in the fucosyltransferase gene on chromosome 19 present in 20% of the Caucasian population reduces susceptibility to norovirus infection (Rydell et al., 2011). However, such protection is not complete, with some norovirus genotypes still capable of infecting people with this HBGA type (Nordgren et al., 2010). HBGA backbone structure is important for presenting the oligosaccharide antigen for recognition by norovirus (Lundborg et al., 2013; Tan et al., 2004; Tan and Jiang, 2005). The terminal residues and internal structures of HBGA's may assist norovirus-HBGA interaction (Harrington et al., 2004; Shirato, 2011). Different norovirus genotypes have variable HBGA-interaction patterns (Huang et al., 2005), due to the polymorphic characteristics of HBGA and the diverse recognition of HBGA receptors by noroviruses (Tan and Jiang, 2007). Because norovirus consists of antigenically diverse groups, identification of conserved norovirus-HBGA structures will be critical to the development of broadly-acting norovirus attachment inhibitors (Shirato, 2012).

1.2. Norovirus classification

Noroviruses are divided into genogroups I–VII depending on the identity of amino acids in the major structural protein (VP1) (Dang Thanh et al., 2016; Vinjé, 2015). Furthermore, based on >85% sequence similarity in the whole VP1 genome, noroviruses are further categorized into genotypes (Wang et al., 2005; Zheng et al., 2006). Noroviruses evolve over time through the changes in the