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Review article about modified nucleosides (Pro-Tide) as potential anti-HCV Therapeutics

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ABSTRACT

Hepatitis C virus is among the most common causes of cirrhosis and chronic liver disease worldwide. As a result, many researchers are interested in designing and synthesis of a clinical treatment for hepatitis C virus. Nucleoside monophosphates and monophosphonates play an important role for treatments of incurable diseases such as hepatitis. For example, 2'-C-methyladenosine and 2'-C-methyl guanosine have shown activities against HCV in the replicon assay as well as against several members of the flavivirus family. However, their development as drug molecules has been hindered by the inherent poor drug-like properties of the monophosphate and monophosphonate groups. The monophosphate and monophosphonate groups have low bioavailability due to the inefficient cellular uptake, poor in vivo stability and poor intracellular metabolism, the latter drawback being most relevant to monophosphates than monophosphonates. These limitations can be addressed by using portide strategy, which able to help the nucleoside monophosphate delivered inside the cell. In this review, we discuss the different keys monophosphate and monophosphonate nucleoside prodrugs that entered clinical development. In addition, the role of the protide technology that highlighted the success in the discovery of nucleoside therapeutics.

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1. Introduction

1.1. Hepatitis

Hepatitis is a viral infection that has emerged as a major public health problem throughout the world affecting several hundreds of millions of people (Wasley and Alter, 2000). Viral hepatitis is a cause of considerable morbidity and mortality in the human population, both for acute (lasting less than six months) or chronic (lasting more than six months), such as hepatitis B, C and D, chronic active hepatitis and cirrhosis (Zuckerman, 1996). It can destroy liver tissue, spread from person to person, weaken the body's immune system, liver cancer (hepatitis B and C), and lead to Death (Wauchope et al., 2010). The increasing number of publications and patents devoted to HCV-related research shows the attempts of researchers to design an active antiviral against hepatitis.

1.2. Hepatitis C Virus (HCV) Infection

Hepatitis C virus, which has been discovered by Choo et al., (1989), is an RNA virus that belongs to the family Flaviviridae (Lauer and Walker, 2001). Infection with hepatitis C virus (HCV) has become a serious public health problem because it leads to cause chronic liver disease as well as the primary indication for liver transplantation (Wasley and Alter, 2000). In fact, more people are expected to die from HCV than from AIDS in the near future (Ly et al., 2016). The World Health Organization (WHO) estimates that about 3% of the world's population, i.e.170 million people, are currently infected with HCV, which is four times as many as those infected with human immunodeficiency virus (HIV) (Chen, 2006). A new study in 2015 demonstrated that the Central, East Asia, North Africa and the Middle East have the highest prevalence of hepatitis C virus (HCV) infection (Messina et al., 2015). In the fact, with the high risk of HCV infection, there is no vaccine is available for preventing infection. Furthermore, many of current conventional therapies such as ribavirin and pegylated interferon are unsatisfactory.

1.3. Therapy of HCV

The current therapy for HCV pegylated interferon and ribavirin (Fig.1) (Chander et al., 2002), show nonspecific inhibitors of HCV with limited efficacy with at least half of the patients (Gordon, 2005; Mcguigan et al., 2011). Moreover, pegylated interferon and ribavirin provide limited sustained virologic response (SVR) rates and can produce various undesirable side effects ranging from flu-like symptoms to severe adverse effects, including anemia, cardiovascular events, and psychiatric problems such as suicidal ideation (Lawitz, et al., 2013). In contrast, some nucleosides such as 2'-C-methyladenosine (Fig. 2b) and 2'-C-methyl guanosine (Fig. 2c) are more active against HCV with the absence of detectable cytotoxicity (Mcguigan et al., 2010). 2'-C methylguanosine triphosphate has been known as a potent inhibitor of HCV RNA polymerase for some time, but the parent nucleoside is only moderately active due to poor intracellular phosphorylation (Mcguigan et al., 2011). Consequently, more effective therapies with better tolerability profiles are urgently needed. The application of phosphoramidate ProTide technology has been used to bypass the rate-limiting initial phosphorylation of this nucleoside.

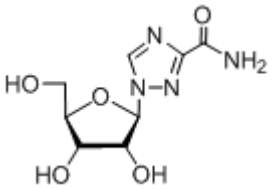

Fig. 1. Structure of ribavirin.

2. Nucleosides as Antivirals

The nucleosides play an important role for treatments of incurable diseases such as hepatitis, HIV and cancer. In order to be effective, all nucleosides require metabolic activation in their target cell to the bioactive phosphate "nucleotide" form (Mcguigan et al., 2011). Several families of nucleoside analogues (Fig. 2) have emerged with apparent selectivity for HCV (Mcguigan et al., 2011). However, this metabolic activation often is not very efficient, and thus the therapeutic potential for these nucleosides often is quite limited (Mcguigan et al., 1969). Pre-formation of the phosphate does not offer any advantage because of poor permeation of the phosphate through the cell membrane. The nucleoside monophosphate analogues are unstable in biological media where they are highly susceptible to dephosphorylation. They also show poor membrane permeation because of the associated negative charges at physiological pH. This barrier must be overcome in order to produce a marketable drug. One avenue of investigation has used phosphate prodrugs, which become known as the "Protide" approach. One of the protide approaches which has proved particularly effective to date is the use of aryloxy phosphoramidates (Fig. 3)(Mcguigan et al., 2011).

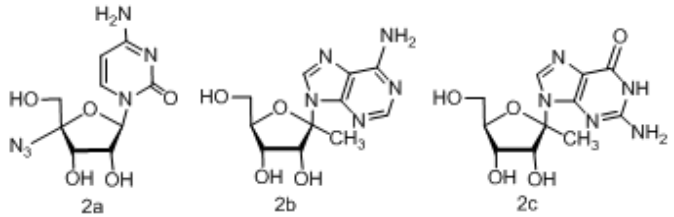

Fig. 2. Some anti-HCV nucleosides.

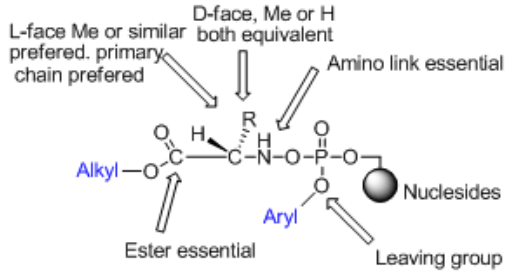

Fig. 3. Aryloxy phosphoramidate

3. Phosphoramidate (ProTide) prodrugs

Pronucleotides "ProTide" technology, has been discovered by the McGuigan's team at Cardiff University in 1996 (Mcguigan et al., 1969). It is a pro-drug strategy that masking of nucleoside phosphate and phosphonate groups by an aryl motif and an amino acid ester. These technologies have been used to overcome the limitations of the nucleoside monophosphate and monophosphonate therapeutics as mentioned before. Indeed, this technology has inspired the discovery of numerous ProTide entities have progressed to clinical trials as cancer, HIV and hepatitis C treatments, and much more are currently undergoing (pre)clinical development. The basic structure of a phosphoramidate motif is shown in (Fig. 4) (Mcguigan et al., 1969). The concept of pronucleotide (protide) analogue is a strategy that masks the charges of the nucleoside analogue monophosphates so that they penetrate the membrane and then selectively release the nucleoside analogue monophosphate inside the cell (Fig. 5).

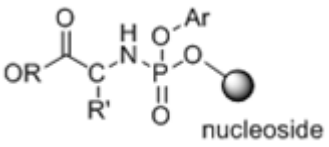

Fig. 4. A general ProTide structur

3.1 ProTides nucleoside against HCV

The Phosphoramidate (ProTide) approach led to the successful development of numerous of nucleoside phosphonate analogues, that are approved for clinical use against HCV such as, cidofovir (Fig. 6A)(Clercq et al., 1987), tenofovir (Fig. 6B) (Clercq et al., 1987), Sofosbuvir (Fig. 6C)(Sofia et al., 2010) which is now used to treat patients with HCV (Pawlotsky et al., 2013), and tenofovir alafenamide (Fig. 6D)(Chapman et al., 2001; Eisenberg et al., 2001).

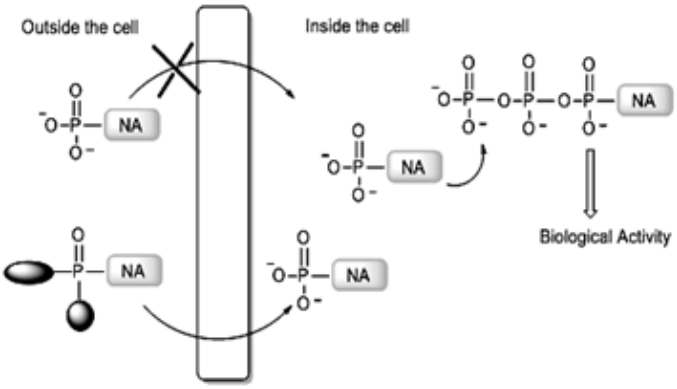

Fig. 5. General pronucleotide (protide) concept (NA: nucleoside analogue).

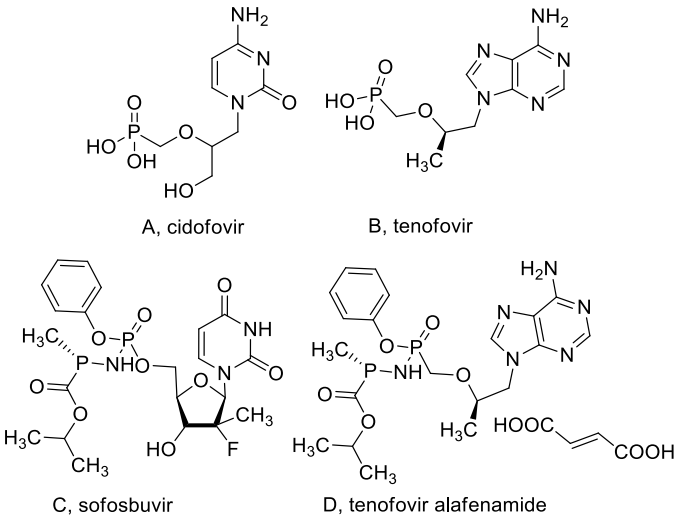

Fig. 6. New anti-hepatitis C virus (HCV) therapeutics continues as the current treatment.

Additionally, this particular prodrug approach was adopted to discover numerous other nucleoside ProTides entering clinical trials (Fig. 7) (Thornton et al., 2016).

NUC-1031 (Fig. 7A) (Slusarczyk et al.,2014) is an aryloxy triester phosphoramidate (ProTide) of the anticancer drug gemcitabine.

NUC-3373 (Fig. 7B), showed excellent preclinical properties (Mcguigan et al., 2011). McGuigan and co-workers reported the discovery in 2011 and highlighted its advantageous properties in terms of tumors reduction.

GS-5734 (Fig. 7C) (Warren et al., 2016) is currently the only C-nucleoside based ProTide undergoing clinical trials following the termination of the clinical development of the initial anti-HCV (Thornton et al., 2016).

Stampidine (Fig. 7D) (Venkatachalam et al., 1998) and **thymectacin** (Fig. 7E) (Lackey et al., 2001), both are anti-HIV ProTide clinical candidates.

3.2. The Hypothesis

As we have mentioned before, nucleoside analogues are structurally different from natural nucleosides. The phosphorylation that utilized to generate bioactive monophosphate in the cell has often limited efficiency. (Fig. 8A) (Stein et al., 2001). These limitations can be addressed by using a protide strategy that used to deliver the phosphorylated metabolites of these nucleoside analogues rather than relying analogue monophosphates, have shown success in delivering the nucleoside into the cell, where they converted to their active species inside the cell (Fig. 8C). A phosphoramidate ProTide generates lipophilic prodrugs of the monophosphate of the them on active transport to achieve better potency. However, as unmodified agents, nucleoside monophosphates (Fig. 8B) are unstable in biological media and highly susceptible to dephosphorylation and they are poor membrane permeation because of the associated negative charges at physiological pH (Thornton et al., 2016). This barrier must be overcome in order to achieve a marketable drug (Brenner, 2002). The discovered protide technology that masks the charges of the nucleoside nucleoside. Modification of the ester and amino acid moieties lead to make the ProTide nucleotide 500 times more potent than the parent nucleoside (Mcguigan et al., 2010).

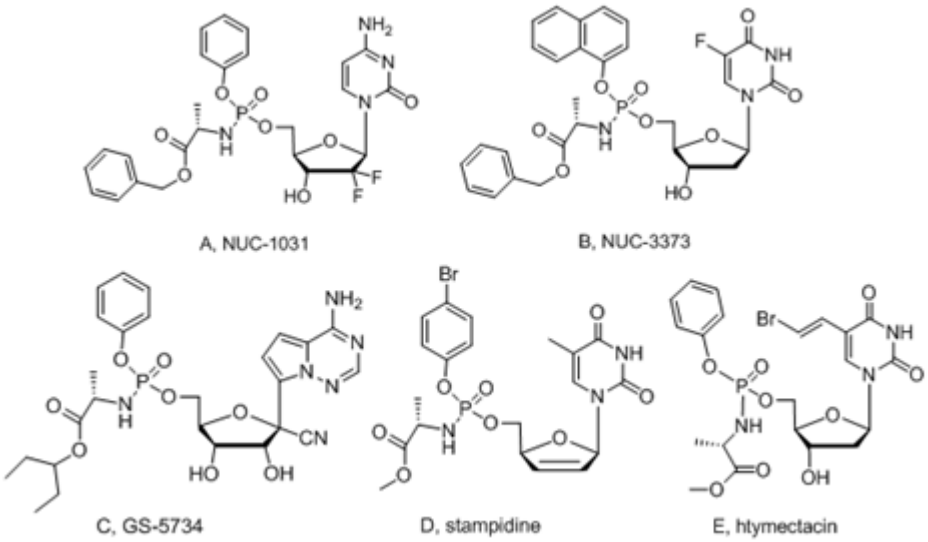

Fig. 7. Some nucleoside ProTides entering clinical trials.

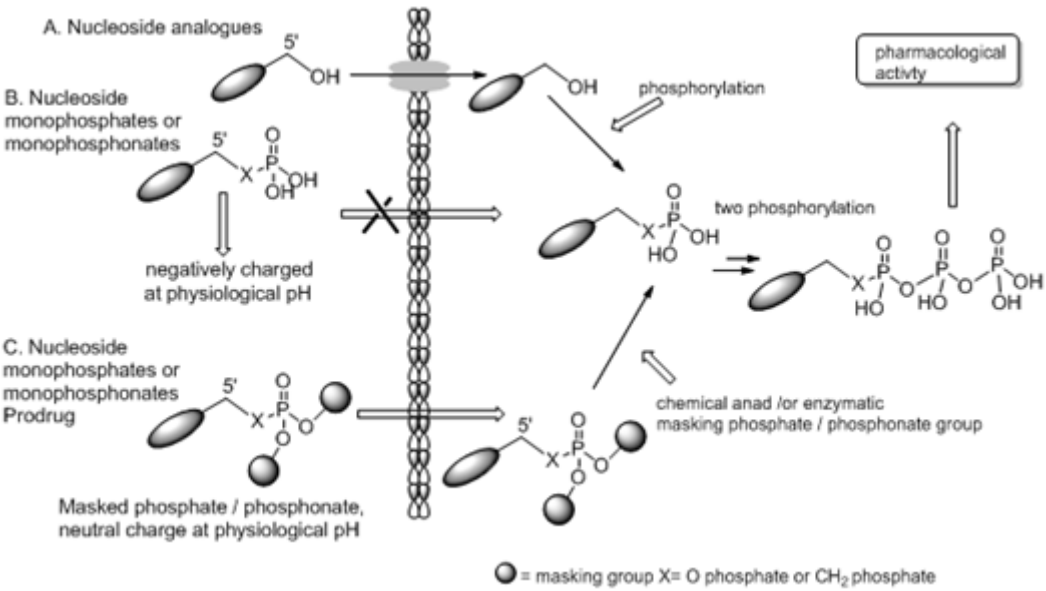

Fig. 8. (A) A general representation of the intracellular activation of nucleoside analogues (B) Nucleoside monophosphates and monophosphonates. (C) Masked nucleoside monophosphates and monophosphonates.

3.3. The mechanism of ProTide compounds action

The protide technology, discovered by the McGuigan team at Cardiff University, is a strategy that masks the charges of the nucleoside analogue monophosphates by an aryl motif and an amino acid ester groups; so that, they pass through the membrane (Fig. 9). After that, two enzymatic activation steps remove the masks in intracellular to release the nucleoside monophosphate. The first enzyme, esterases cleave the ester motif of the ProTide (ester hydrolysis) (Mehellou et al., 2009). Under physiological pH (< 7.4), the negatively charged carboxyl group carries out a nucleophilic attack on the phosphate or phosphonate group. As a result, the aryl motif leaves and formation a highly unstable five-

membered ring, with the speed of this process being dependent on the structure of the analogue (Mehellou et al., 2009). A water molecule attacks the released nucleoside and opens up the heterocyclic ring to give a phosphoramidate metabolite. A second enzyme is phosphoramidase-type enzyme mediates the cleavage of the P-N bond of this metabolite leading to release of the nucleoside analogue monophosphate or monophosphonate (Fig. 9) (Murakami, 2010).

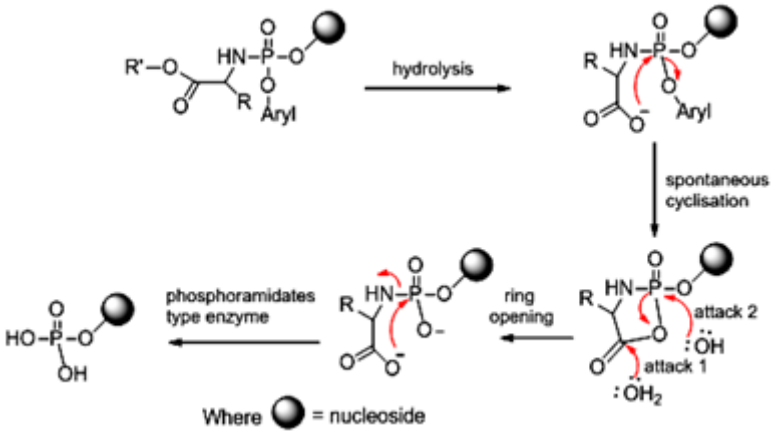

Fig.9. Mechanism of ProTides in vivo breakdown to release the nucleoside analogue monophosphate or monophosphonate.

4. Conclusion

This review was focused on the (ProTide) phosphate prodrug technologies that utilized to discover the nucleotide HCV therapeutics. ProTide technology, discovered by the McGuigan team at Cardiff University in the early 1990s, is one of the best areas improving drugs. In this review, one of these technologies, that known as the phosphoramidate protide method was described. These technologies aimed for intracellular delivery of nucleoside monophosphates into cells have proved to be effective in improving the therapeutic potential of antiviral and anticancer nucleosides. They embarked on designing phosphoramidate based protides following the concept that HCV protease might cleave a suitable oligopeptide from the phosphate moiety of a blocked nucleotide phosphoramidate. The protide approach has been well investigated and established as a viable method for the intracellular delivery of monophosphate nucleoside analogues. This approach has been proven to improve the antiviral and anticancer profiles of many nucleoside analogues. Clearly, the phosphoramidate triester pronucleotide technology has proved effectively as a tool for drug discovery, and so far numerous of phosphoramidate triester-based drugs are undergoing clinical trials. In addition, phosphoramidate-based compounds may be explored as treatments for a wide range of diseases. For example, Sofosbuvir a new antiviral drug Sofosbuvir (GS-7977), with the chemical name L-Alanine, N-[[P(S),2′R]-2′-deoxy-2′-fluoro-2′-methyl-P-phenyl-5′-uridylyl]-,1-methyl-ethyl ester, is a new prodrug, that shows a high potent activity against all HCV genotypes.

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