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Anti-Hepatitis B Virus Drugs in Clinical and Preclinical Development

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Abstract: Up to date, there are two types of drugs approved to treat hepatitis B: interferons and nucleos (t) ide analogues. However, the therapies are limited in the clinical context because of the negative side effects of interferon-α and the development of substantial viral resistance to nucleos (t) idic inhibitors. Therefore, new drugs with novel structures and mechanisms are needed. In this article, the drugs approved by FDA or the European Commission for treating chronic hepatitis B virus infection, as well as those under clinical trials, and several compounds in preclinical studies are reviewed. Additionally, some potential targets and strategies to combat chronic hepatitis B virus infection are discussed.

Key words: Anti-HBV drugs; Immunomodulatory agents; HBV

Hepatitis B virus (HBV) infection is a serious global public health problem. To date, despite efficient vaccination campaigns against HBV, two billion people worldwide still carry markers of HBV infection. Of these, more than 350 million people are chronic carriers and this number is still expanding. Among these, 75% live in Asia and the west Pacific regions (10). China has one of the highest rates of prevalence of HBV infection in the world. According to a recent survey, about 9.09% of the population (in number: 120 million individuals) are chronically infected with HBV (18). Around 15% to 40% of chronic hepatitis B patients will develop and die from the sequelae of the disease, such as liver failure, cirrhosis or hepatocellular carcinoma (21). Therefore, there is an urgent need to treat chronic hepatitis B patients to prevent the disease progression.

Until 2007, there were two types of drugs available in the clinics to treat hepatitis B: interferons, which boost the immune system to fight the HBV infection, and antiviral medications, which deliberately interfere with the virus's DNA synthesis and therefore block the viral replication. Below, a review is given for drugs that are currently approved by the U.S. Food and Drug Administration (FDA) or the European Commission to treat hepatitis B, and the up-and-coming drugs under research and clinical testing.

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DRUGS ACTING ON THE IMMUNE SYSTEM

Interferon-α

The first drug approved for the treatment of chronic

HBV infection was interferon-α (IFN-α) in 1992. Interferon has an "immunomodulatory effect", which means that it urges the immune system to activate lymphocytes that either bind the virus' antigens directly by antibodies, or they attack the HBV-infected liver cells. Such like B lymphocytes produce antigen-specific antibodies to neutralize virus particles and cytotoxic T cells recognize and kill HBV infected liver cells. An interesting process is the enhanced cell surface expression of Class I Major Histocompatibility (MHC): interferon enhances the expression or presence of certain antigens on the surface of HBV infected liver cells, which can help T cells to find them more easily and destroy the infected cells.

IFN-α can also exhibit direct antiviral function that is different from nucleos (t) ide analogues. It can directly induce antiviral states in uninfected cells. The activated antiviral pathways by interferon include (2'-5')-oligoadenylate synthetase and the dsRNA-dependent protein kinase (2), or other pathways (29). However, IFN-α has limited efficacy and low response rate below 40%, and it is associated with many adversary effects, such as flu-like symptoms, anemia, leucopenia, thrombocytopenia, anorexia. and depression. For these reasons, it is only suitable for a small number of patients (27). Due to the limited efficacy and side effects of IFN-α, other immunomodulators emerge because of demand.

Peg-IFN (Pegasys)

The second interferon is called pegylated interferon developed by Chinese University of Hong Kong and Roche, it requires only one injection each week, and has a time-release formula to achieve a consistent immune boosting level. It can distribute more efficiently in the liver and reduce HBeAg, HBV DNA and ALT level more efficiently (4), its large size leads to a more than 100-fold reduction in renal clearance compared with conventional IFN-α (40). The European Commission has approved its use for the treatment of chronic hepatitis B in the European Union in March 2005.

Zadaxin (thymosin α1)

This compound has been developed by SciClone Pharmaceuticals and is a safe and effective treatment for chronic hepatitis B when used alone or in combination with interferon. Nie *et al* report that 6 months treatment with thymosin α1 (1.6 mg twice weekly) almost tripled the sustained response rate (38%) compared with controls (13%). Compared with IFN-α, thymosin α1 has fewer side effects, is better tolerated and seems to induce a gradual and more sustained normalization of ALT and loss of HBV DNA (23). Thymosin α1 can stimulate the immune system by stimulating T cells and NK cells, which makes it as a promising drug for a wide variety of clinical conditions (39). It has been approved for sale in more than 30 countries, including France, German and Italy.

IMMUNOMODULATORS UNDER DEVELOPMENT

Immune stimulator

HE2000. HE2000 is an immune regulating hormone and was developed by Hollis-Eden Pharmaceuticals. It has received approval to initiate a Phase II clinical trial in HBV infected patients at Mt. Elizabeth Medical Centre in Singapore. The clinical study will evaluate the safety, tolerance, clinical and biological effects of HE2000 in young chronic HBV-infected patients. Hollis-Eden also announced that it has begun dosing with HE2000 in several malaria-infected

patients in a Phase II clinical trial in Thailand (31).

Antibodies

HepeX-B (XTL-001). HepeX-B, developed by XTL Biopharmaceuticals and Cubist Pharmaceuticals, is a combination of two fully human monoclonal antibodies targeting HBV surface antigens, selected using XTLbio's preclinical Trimera (TM) model. It is currently in evaluation for the prevention of infection by HBV in liver transplant patients who have been maintained on hepatitis B immune globulin. HepeX-B (TM) has already been granted Orphan Drug Status in both the U.S. and the European Union (1).

Hepatitis B therapeutic vaccine

EHT 899. EHT 899 has been discovered by Enzo Biochem Incorporation and is a proprietary formulation of an HBV viral protein designed to eliminate the undesirable immune response elicited by the HBV infection. It also apparently enhances a secondary immune response to suppress the viral infection, resulting in reduction in liver damage and decrease in viral load. It is reported that a formulation of EHT 899 was administered orally to a total of 42 patients with chronic active hepatitis three times a week for 20-30 weeks. 46% of subjects showed a decrease in HBV viral load and improvement in liver function tests. 33% of subjects showed a decrease in inflammation seen on liver biopsy (9). Now it is in a phase II clinical trial.

DRUGS ACTING ON THE VIRUS DIRECTLY

The development of specific therapy for HBV infections has been focused on the development of compounds interfering with the viral RT/polymerase. Given the crucial role of this enzyme in the HBV replicative cycle, and the success obtained with DNA polymerase inhibitors and reverse transcriptase inhibitors in the treatment of herpesviruses and retrovirus infections, a variety of nucleos (t) ide analogues that proved to be effective against either herpes simplex virus, varicella-zoster virus, cytomegalovirus or HIV were further examined for their anti-HBV potential. Several of these compounds turned out to be effective against HBV replication, and now some of them are being clinically used.

There are four FDA-approved antiviral medications available for HBV-infected patients: lamivudine, approved for adults in 1998 and for children in 2000; adefovir, approved for adults in 2002 and currently in clinical trials for children; entecavir, approved for adults in 2005; and telbivudine, approved in October 2006 for adults.

Lamivudine (Epivir HBV)

Lamivudine is a synthetic dideoxy analogue of cytidine and was developed by Biochem Pharmaceutical industries and marketed by Glaxo-SmithKline. It inhibits viral reverse transcriptase activity, i.e. elongation of viral minus strand DNA, as it is a competitive inhibitor of its natural substrate, dCTP, and furthermore, after its incorporation in nascent viral DNA strand, it inhibits the addition of the next nucleotide and act as a DNA chain terminator (41). When daily administered, it generally produces normal ALT levels and undetectable HBV DNA in about 65% of the adults who take it. However, it is not a permanent or complete cure: it has the problem of inducing drug resistant HBV after 6-12 months of therapy,and an associated risk of an increased viraemia during the therapy appeared with HBV patients. Once the use of drug is stopped, relapse rates are high: long-lasting virological and biochemical responses

have been maintained in approximately 20% of patients, and a 74% relapse rate has been reported at 12 months after therapy withdrawal (12, 15).

Adefovir dipivoxil (Hepsera)

Adefovir, developed by Gilead Sciences, is an ester prologue of adefovir [9-(2-phosphonylmethoxyethyl) adenine], a nucleotide analogue of adenosine monophosphate. Adefovir inhibits the priming of reverse transcription and inhibits viral minus strands DNA elongation (41). It has been proven to be effective in the therapy of infections with lamivudine-resistant HBV *in vitro* and *in vivo* (5). It might be a future therapeutic option for its good tolerability profile and few resistant HBV mutants have been identified to date. However, it is poorly absorbed, and associated with dose-limiting renal toxicity, so it was approved only at suboptimal doses. Additionally, just as lamividine, relapse rates are also serious because the nucleoside analogues cannot eradicate the covalently closed circular DNA (cccDNA) pool of HBV.

Entecavir (Baraclude)

Entecavir was developed by Bristol/Myers Squibb and is a carbocylic analogue of 2'- deoxyguanosine with selective activity against HBV. It is phosphorylated to the active triphosphate form and competes with the substrate dGTP to inhibit HBV polymerase. It can inhibit both the priming and elongation of viral minus strand DNA. Treatment with entecavir produces greater viral load reduction compared to adefovir at 48 weeks in HBeAg positive patients (8). It also showed the ability to reduce viral cccDNA levels. After withdrawal of therapy, there was a biphasic pattern with an initial fast increase in viral replication back to baseline (37).

Telbivudine (Tyzeka)

Telbivudine, developed by Idenix Pharmaceuticals, is a synthetic thymidine nucleoside analogue with activity against HBV DNA polymerase. It is phosphorylated by cellular kinases to the active triphosphate form, and then inhibits HBV DNA polymerase (reverse transcriptase) by competing with the natural substrate, dTTP. Incorporation of telbivudine 5'-triphosphate into viral DNA causes DNA chain termination, resulting in inhibition of HBV replication. Telbivudine is an inhibitor of both HBV first strand and second strand synthesis (25). Patients with chronic hepatitis B treated with telbivudine exhibited significantly greater virologic and biochemical responses compared with lamivudine. Clinical results with the combination regimens were similar to those obtained with telbivudine alone (16).

ANTIVIRALS UNDER DEVELOPMENT

Heteroaryldihydropyrimidine (HAP)

Bay41-4109, Bay38-7690 and Bay 39-5493. These new compounds were developed by Bayer and are non-nucleosidic inhibitors of HBV nucleocapsid maturation that possess *in vitro* and *in vivo* antiviral activity. These inhibitors have potential for future therapeutic regimens to treat chronic hepatitis B. HAP drugs act as allosteric effectors that induce an assembly-active state, and at high concentration, preferentially stabilize noncapsid polymers of core protein. They may have multiple effects *in vivo* stemming from inappropriate assembly of core protein (7, 33). The three compounds showed better inhibition than lamivudine. Although they have been withdrawn from clinical trials for their bad bioavailability, they are still expected to be potent valuable addition to anti-HBV therapy.

Phenylpropenamide derivatives

AT-61 and AT-130. AT-61 and AT-130 were developed by Avid Therapeutics and were found to be a highly selective and potent inhibitor of human HBV replication. AT-61 was able to reduce the amount of HBV cccDNA by >99%, and had little effect on the amount of viral RNA found within the cytoplasms of induced HepAD38 cells. However, it reduced the number of immature virions which contained pregenomic RNA by >99%, thus, the antiviral activity of AT-61 is specific for HBV replication and most likely occurs at one of the steps between the synthesis of viral RNA and the packaging of pregenomic RNA into immature core particles (14). Although the AT-61 and AT-130 were not as potent as lamivudine (100-300 folds lower), they are active against lamivudine-resistant HBV (6).

Nucleic acid drug

Ampligen. Ampligen, developed by Hemispherx Biopharma, is a mismatched double-stranded RNA that acts by inducing interferon production and by activating an intracellular enzyme (RNase-L) against viral RNA transcripts. Hemispherx has conducted phase I/II trials in patients with chronic hepatitis B, patients with severe thermal injury and patients with metastatic melanoma. (24). To date, it has caused no dangerous adverse effects.

Small molecule

NOV-205. Nov-205 acts as a hepatoprotective agent with immunomodulating and anti-inflammatory properties. It is approved for use in the Russian Federation under the trade name Molixan® and is commercialized in Russia. Clinical studies in 178 Russian hepatitis patients have demonstrated that NOV-205 is effective and safe. The Russian clinical studies in hepatitis B and C patients showed that after relatively short treatment periods (1 to 2 months) with NOV-205, viral load was undetectable in a high proportion of patients, and serum biochemical markers of liver damage were significantly decreased. Overall, more than 700 Russian hepatitis patients have been treated successfully with NOV-205. Preclinical studies also support NOV-205's antiviral and hepatoprotective effects (26). In the United States, NOV-205 is in Phase II/III clinical trials.

Nucleoside analogue

Tenofovir (Viread). Tenofovir was developed by Gilead Sciences and is an acyclic nucleoside phosphonate analogue of adenosine monophosphate. Tenofovir diphosphate inhibits HBV DNA polymerase by competing with dATP for incorporation into nascent DNA, resulting in premature chain termination. Tenofovir has activity against HBV and lamivudine-resistant HBV (36). In clinical trials, all tenofovir-treated patients showed a strong and early suppression of HBV DNA within a few weeks whether they were coinfected with HIV or were without comorbidity individually. No severe side effects were noticed in either group, and tenofovir may become an effective alternative for the treatment of patients with lamivudine-resistant HBV infection (35). Now Tenofovir is on phase III clinical trials for HBV treatment.

Pradefovir. Due to the poor absorption and dose-limiting renal toxicity of adefovir dipivoxil, a novel series of phosphate and phosphonate prodrugs of adefovir called HepDirect prodrugs were designed that specifically target the liver and thus bypass the risk of nephrotoxicity. Pradefovir mesilate (developed by Valeant Pharmaceuticals) emerged from this series as the lead compound. Pradefovir is activated via

oxidation mediated by cytochrome P-450 (CYP) 3A4, which is predominantly expressed in the liver. The novel prodrug is highly stable in both plasma and tissues and demonstrated potent preclinical and clinical anti-HBV activity. Now it is undergoing phase II development for the treatment of chronic hepatitis B (30). Pradefovir produced greater reductions in HBV DNA levels, with more patients achieving undetectable levels after 24 weeks. Efficacy was also demonstrated in patients who did not have an adequate response to previous therapy, and in patients who were HBeAg positive (34).

Clevudine. Clevudine is produced by Gilead. It is a nucleoside analogue with an unnatural L-configuration. In a phase I/II clinical trial, once daily doses ranging from 10 to 200 mg for 28 days were well tolerated, and produced significant antiviral activity. In another clinical trial, clevudine showed potent antiviral activity during therapy and induced a sustained post treatment antiviral effect for 6 months after a 12-week treatment period. The antiviral effect was associated with a sustained normalization of ALT levels (17).

Valtorcitabine. Valtorcitabine, developed by Idenix Pharmaceuticals, is an L-nucleoside analogue, has proven to be an extremely specific and selective anti-HBV agent and has exhibited an exceptional safety profile (13). Valtorcitabine has demonstrated no activity against HIV and other viruses that cause human diseases, which may permit the treatment of co-infected persons with other viruses like HIV, without an increased risk of resistance development for these other viruses. Additionally, valtorcitabine target the positive strand of HBV DNA, in contrast to lamivudine, which targets the negative strand. The targeting of the positive strand may be associated with a slower development of resistance mutations (22). Now the study for combination of telbivudine and valtorcitabine in patients with chronic hepatitis B is in phase II clinical trial.

Non-nucleoside analogue

Benzimidazole derivatives. These compounds were synthesized by Shanghai Institute of Materia and Medica and evaluated for their anti-HBV activity and cytotoxicity *in vitro*. Strong activity against HBV replication and low cytotoxicity were generally observed in these benzimidazoles. The most promising compounds were 12a and 12b, with similar high antiviral potency (IC_{50} = 0.9 and 0.7 μM, respectively) and remarkable selectivity indices (>1111 and 714, respectively). They were selected for further evaluation as novel HBV inhibitors (20).

Helioxanthin analogue. Helioxanthin analogue is a natural product that inhibits the replication of a number of viruses. Among these, 8-1 exhibited potent anti-HBV activity with little cytotoxicity: it suppressed HBV RNA and protein expression, as well as DNA replication of both wild type and lamivudine-resistant virus. More experiments show that 8-1 suppresses HBV replication by post transcriptional down-regulation of critical transcription factors in HBV-producing cells, thus diminishing HBV promoter activity and blocking viral gene expression and replication (38). This mechanism is unique and different from other anti-HBV compounds previously described, and it may be a potent novel anti-HBV inhibitor.

SOME POTENTIAL ANTI-HBV DRUG TARGETS

HBxAg

HBxAg has been shown to be a promiscuous trans-activating protein that may alter the expression of both viral and cellular genes. Further work has shown that HBxAg stimulates HBV gene expression and replication, which is important to help maintain the chronic carrier state. In addition, HBxAg alterations in the patterns of cellular gene expression play important roles in the pathogenesis of hepatocellular carcinoma, which is a late sequela of chronic HBV infection (19). The key function of HBxAg suggests that it is an important target for antiviral drug discovery and for the development of novel approaches against hepatocellular carcinoma, and it may provide multiple opportunities to develop chemopreventative approaches that may alter the course of chronic HBV infection.

TLM

TLM is a cell-permeable peptide [translocation motif (TLM)] identified within the surface protein of human HBV. Surface proteins of all hepadnaviruses contain conserved functional TLMs. It is a general feature of all hepadnaviruses and plays a role in the viral life cycle. Processing of surface protein by endosomal proteases induces their exposure on the virus surface, and mediates translocation of viral particles across the endosomal membrane into the cytosol, which is a prerequisite for productive infection. It means that after the virus internalization by receptor-mediated endocytosis, processing of surface protein in endosomes followed by. This processing activates the function of TLMs, which are essential for viral particle translocation through the endosomal membrane into the cytosol and productive infection (32). The key role of TLM in the entry of the HBV also suggests it will be a good target for anti-HBV drug discovery.

cccDNA

Viral cccDNA is a critical target for anti-HBV therapy. On infection, the partially double-stranded open circular genomic DNA is transported to the hepatocyte nucleus, where host-cell enzymes convert it to a covalently closed circular molecule. The HBV cccDNA remains in the cell nucleus and serves as the transcriptional template for HBV RNA production. After the nucleocapsids assembled, they can follow either of two pathways. They can associate with the envelope proteins to produce virions and be secreted from the cell, or they can be recycled back to the nucleus as part of a regulatory pathway to maintain a pool of cccDNA molecules. These two pathways result in the formation of a steady-state population of 5–50 cccDNA molecules per infected hepatocyte. This reservoir of HBV cccDNA in the nucleus poses a difficult hurdle for antiviral therapy to overcome. As HBV replication does not employ a semi-conservative mechanism, any nucleos(t)ide analogue-based therapy would not be expected to affect directly the pre-existing cccDNA template (3).

FUTURE DIRECTIONS

Four of the five anti-HBV drugs reviewed are nucleoside analogues, the other three are immunomodulators, so clearly new drugs with different structures and mechanisms of action are needed. Because the current anti-HBV treatments with nucleoside drugs easily induce HBV mutants, alternative strategies and new drugs are being sought to combat this disease. Nowadays, looking for the novel non-nucleoside anti-HBV drugs has become hot in global pharmaceutics, such as heteroaryldihydropy-rimidine

(HAP) and phenylpropenamide derivatives. Although HAP has a fairly bad bioavailability, it has a strong anti-HBV activity *in vitro*. Its unique mechanism suggests that it is worthy for further developments.

Additionally, there is currently a large and ever-expanding patient group that prefers the use of natural products in treating and preventing medical problems. This has pushed many pharmaceutical companies to produce new antiviral formulations extracted from plants or herbs. Many traditional medicinal plants have been reported to have strong antiviral activity and some of them have already been used to treat animals and people who suffer from viral infection, including HBV infection (11, 28). More novel anti-HBV drugs may come out of them.

Moreover, computer-aided design of molecules including molecular mechanics and molecular dynamics are developing rapidly. It is a convenient technique to study the biological activities of the molecules and develop the structure-activity relationships so as to design further novel molecules and evaluate their biological activities. Its appliance into the drug design and discovery may accelerate the course of finding good compounds to inhibit HBV.

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