HepDirect prodrugs for targeting nucleotide-based antiviral drugs to the liver

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HepDirect prodrugs represent a novel class of cytochrome P450-activated prodrugs capable of targeting certain drugs to the liver. In this review, the HepDirect prodrug concept and its use for the delivery of nucleotides to the liver for the treatment of viral hepatitis is summarized. Preclinical and clinical data for the most advanced HepDirect prodrug, pradefovir, highlight the liver-targeting capability of these prodrugs, and the potential benefit of liver targeting on drug efficacy, safety and viral resistance.

Keywords Adefovir, cytochrome P450, hepatitis B virus, HepDirect prodrug, liver-targeting, pradefovir

Introduction

A significant proportion of the world's population is chronically infected with either the hepatitis B virus (HBV) (400 million individuals) [1] or the hepatitis C virus (HCV) (170 million individuals) [2]. Inadequate treatment of infected patients results in chronic liver injury, which over decades can lead to liver cirrhosis [3,4] and an increased risk of liver failure and hepatocellular carcinoma. Interferon-based therapies produce a sustained viral response in some HBV patients and the majority of HCV patients, but these drugs are poorly tolerated. Oral therapies exist for HBV [5,6•], but these are associated with an indefinite treatment duration to maintain viral suppression and prevent liver disease progression. Moreover, drug-resistant viral variants frequently emerge over the course of therapy, which can lead to viral rebound, hepatic flares, liver decompensation and even death [7]. Consequently, there remains an urgent need for new drugs for the treatment of viral hepatitis that exhibit a lower incidence of viral resistance and a higher degree of viral suppression [8].

Nucleoside-based antiviral drugs

Nucleoside and nucleotide analogs are the most common classes of antivirals [9]. In general, antiviral activity requires a series of intracellular phosphorylations that convert the nucleoside to the corresponding nucleoside triphosphate (NTP), which then inhibits viral replication either by directly inhibiting the viral DNA or RNA polymerase, or by acting as a DNA or RNA chain terminator. Inadequate specificity for the viral polymerase over both nuclear and mitochondrial mammalian DNA polymerases can lead to cellular toxicity, and is likely to be the main reason that many nucleoside-based drugs exhibit a narrow therapeutic index and are associated with mitochondrial toxicity [10], carcinogenicity [11] and a variety of dose-limiting adverse events, including liver and gastrointestinal toxicities, neuropathy, nephropathy, anemia and myelosuppression.

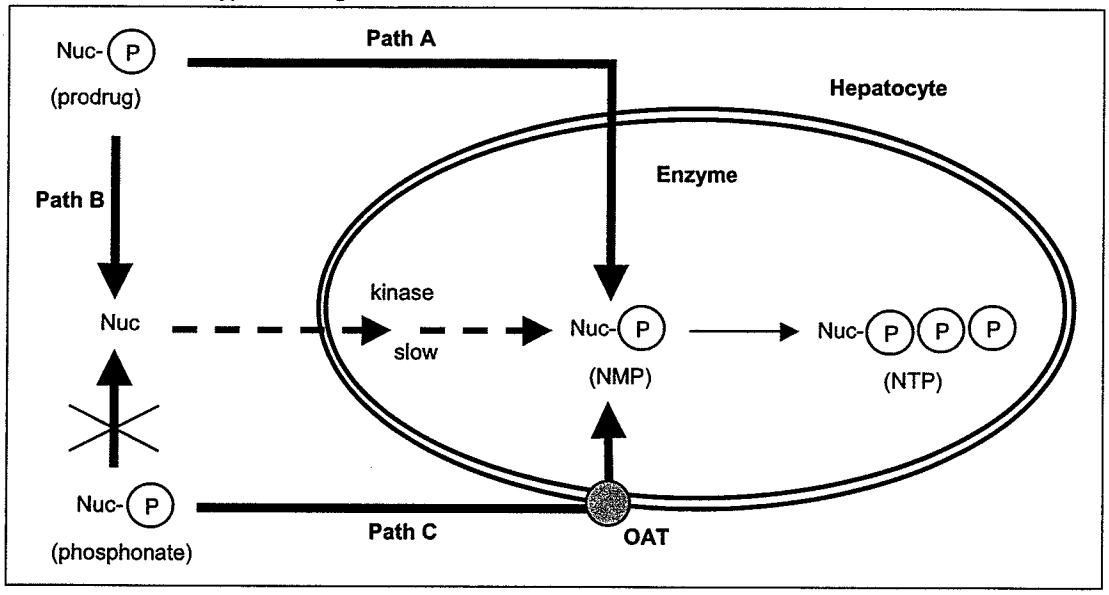
Efforts to discover nucleosides with improved safety profiles led to the identification of nucleosides that gain their specificity for viral replication either by using a viral kinase (eg, herpes simplex virus thymidine kinase) for conversion to the NTP [12], or by producing NTPs that inhibit the viral polymerase with high selectivity [13]. Unfortunately, most structural modifications that enhance NTP selectivity also impede conversion of the nucleoside to the corresponding NTP [14•]. Poor conversion is usually attributed to inefficient phosphorylation of the nucleoside to the nucleoside monophosphate (NMP) by a nucleoside kinase. Unlike the nucleotide kinases catalyzing the subsequent phosphorylations to the NTP, nucleoside kinases exhibit substantial intolerance for structural modifications and a more limited tissue distribution [15].

Nucleoside kinase bypass strategies

One strategy for enhancing the antiviral activity of nucleosides that are poorly converted to the NTP is to deliver the NMP directly to the cell, thereby bypassing the nucleoside kinase. Because NMPs are negatively charged and are readily dephosphorylated to the nucleoside by extracellular phosphatases, numerous strategies for enhancing phosphatase stability, cellular uptake and overall intracellular NMP delivery have been explored over the past two decades. Successful kinase bypass was achieved in cellular assays with several classes of phosphate prodrugs (Figure 1; Path A) [16,17•]. Accordingly, these prodrugs produced higher intracellular NTP levels and more potent antiviral activity than the corresponding nucleoside [18-20]. Unfortunately, in the whole animal, rapid prodrug cleavage by esterases in extrahepatic tissues, especially in the gastrointestinal wall (Figure 1; Path B), limits the availability of these prodrugs to the liver and, therefore, their ability to achieve *in vivo* kinase bypass [21].

An alternative kinase bypass strategy, first described by De Clercq *et al*, replaces the phosphate group with a phosphonate (Figure 1; path C) [22]. This simple change prevents dephosphorylation, but also often impedes intracellular conversion to the active form, that is, the phosphonate diphosphate (NTP equivalent). Nevertheless,

Figure 1. Nucleoside kinase bypass strategies.



Nucleoside kinase bypass is achieved using nucleoside monophosphate (**NMP**) prodrugs that enter hepatocytes and are converted by an intracellular enzyme to the NMP (Path A). Intracellular NMP production is reduced when the NMP prodrug undergoes extensive extrahepatic cleavage to the NMP followed by rapid dephosphorylation to the corresponding nucleoside (**Nuc**) (Path B). Kinase bypass is also achieved using certain phosphonate analogs of NMPs that enter hepatocytes (Path C), usually via a cell-surface organic anion transporter (**OAT**), and undergo conversion to the corresponding phosphonate diphosphate (nucleoside triphosphate (**NTP**) equivalent).

conversion does occur with certain acyclic nucleoside phosphonates enabling kinase bypass and good antiviral activity in humans [23,24•].

Liver-targeted NTP production

Organ-specific drug targeting is a well-recognized potential strategy for increasing drug efficacy and/or improving drug safety [25•,26]. Of all the organs, the liver has the greatest potential for organ-specific drug delivery, in part because of its fenestrated endothelium, which enables macromolecules to pass through the endothelial barrier and directly interact with hepatocytes and other liver-specific cells [27]. In addition, the liver is the organ primarily responsible for drug uptake and metabolism, and it therefore possesses a variety of liver-specific, cell-surface carrier and transport proteins as well as metabolizing enzymes [28,29•].

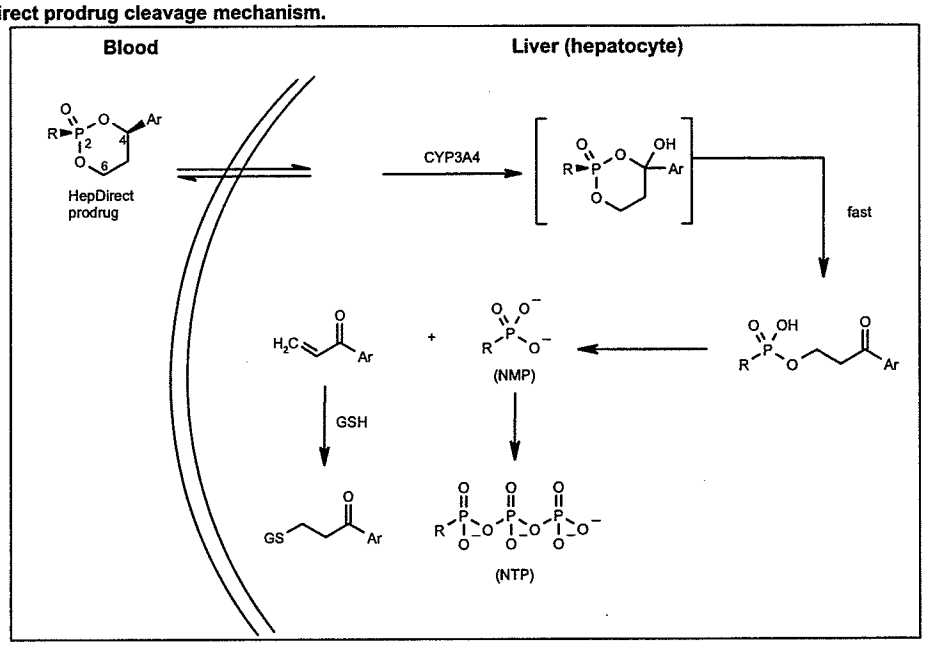
Over the past two decades, efforts to target NMPs to the liver have primarily used drug-conjugate strategies that target cell-surface receptors [30••] or carrier molecules [31,32] expressed predominantly on hepatocytes. Conjugates of NMPs that target the asialoglycoprotein receptor [33] exhibit increased liver targeting in animals [34] and an improved therapeutic index in humans [35]. Unfortunately, development of these conjugates is frequently hampered by low receptor levels in diseased liver, low rates of receptor internalization, poor loading capacities of the carrier molecules, and inefficient drug-conjugate cleavage inside hepatocytes. An alternative liver-targeting strategy is to use NMP prodrugs cleaved by an enzyme expressed in the liver. Ideally, the enzyme would be expressed exclusively in the liver but even when expressed more widely, sufficient liver targeting appears achievable via first-pass metabolism based on results reported for viramidine (ICN-3142; RibaPharm Inc), a prodrug of ribavirin [36], and a series of phospholipid-based prodrugs [37].

Successful targeting of drugs to the liver requires not only efficient uptake and conversion of the drug conjugate or prodrug to the biologically active drug form, but also sufficient retention of this form in the liver to enable drug accumulation to pharmacologically active levels. In addition, liver targeting, and its ability to reduce extrahepatic drug exposure and enhance safety, is dependent on whether liver activation alters the route of excretion (eg, enhances biliary excretion) or leads to increased metabolism to an inactive metabolite.

HepDirect prodrugs

Nucleoside kinase bypass and high liver-targeted NTP production have been achieved in animals using a novel class of prodrugs [101-105] called HepDirect, which undergo an oxidative cleavage reaction catalyzed by a cytochrome P450 (CYP) isozyme expressed predominantly in the liver [38•,39••]. Mechanistic studies indicate that prodrug cleavage is initiated by hydroxylation of the C(4)-methine, and that the hydroxylation reaction is catalyzed predominantly by the CYP3A4 isozyme in human liver microsomes. The hydroxylated prodrug subsequently undergoes rapid and irreversible ring opening followed by a β-elimination reaction to yield the NMP and an aryl vinyl ketone (Figure 2).

Figure 2. HepDirect prodrug cleavage mechanism.



HepDirect prodrugs of nucleoside monophosphates or phosphonates (**NMP**) undergo a CYP3A4-catalyzed oxidation of the C(4)-methine to produce a C(4)-hydroxylated product. Rapid and irreversible ring opening leads to the intermediate monoacid, which is converted to the NMP and an aryl vinyl ketone byproduct via a β-elimination reaction. The NMP is subsequently converted to the corresponding nucleoside triphosphate or equivalent (**NTP**), which typically results from a series of phosphorylations catalyzed by nucleotide kinases. The aryl vinyl ketone is trapped by intracellular glutathione (**GSH**) to form the corresponding glutathione conjugate.

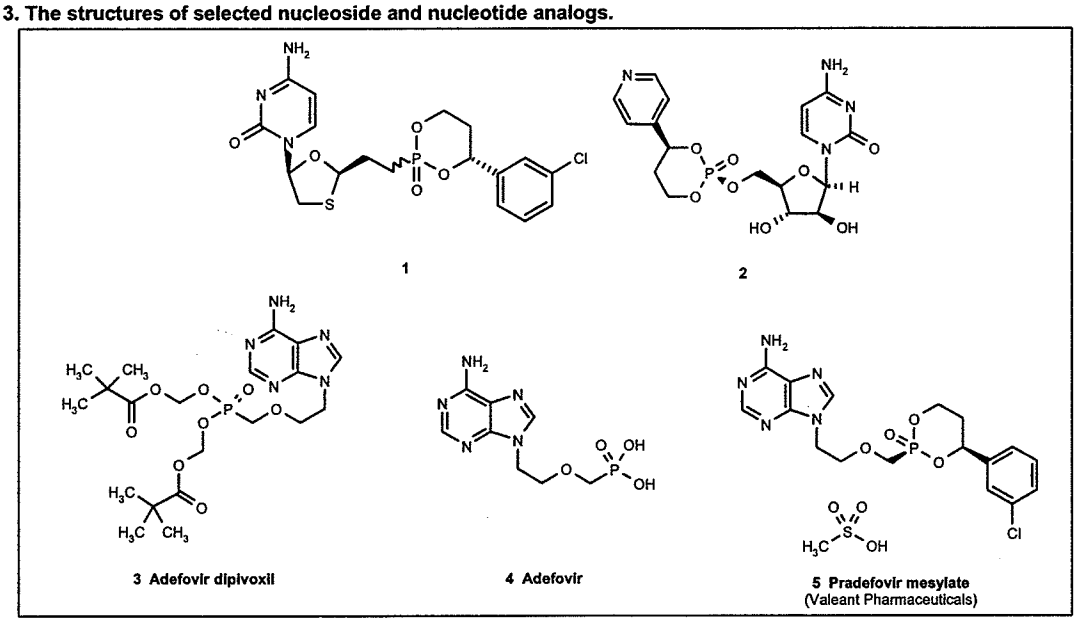
Liver-targeted NTP production has been observed for a variety of structurally different nucleoside and nucleotide analogs (Table 1 and Figure 3) [39••,40], and is primarily attributed to the high liver specificity of CYP3A4 expression [41] coupled with the remarkable stability of HepDirect prodrugs in aqueous solutions, blood and non-hepatic tissues other than the gastrointestinal tract [38•]. High prodrug stability enables the prodrug initially distributed into tissues to remain intact long enough to re-enter the circulation and travel to the liver. Liver targeting is attributed to the nucleoside moiety, especially when the nucleoside itself is poorly phosphorylated and, therefore, requires HepDirect prodrugs of the corresponding NMP to bypass the nucleoside kinase (eg, prodrugs of lamivudine (**1**; Figure 3) and cytarabine (**2**; Figure 3); Table 1). In this case, extrahepatic NTP production is low because HepDirect prodrug cleavage is limited outside the liver and because the nucleoside generated by intrahepatic dephosphorylation of the NMP is unable to undergo phosphorylation in extrahepatic tissues. Alternatively, liver targeting is achieved by HepDirect prodrugs through the ability of the hepatocytes to retain and accumulate negatively charged prodrug cleavage intermediates and products. Excretion of these products into the bile or into the systemic circulation after intrahepatic metabolism to an inactive metabolite enables high liver specificity and decreased extrahepatic drug exposure [39••].

Table 1. Tissue distribution of selected nucleoside and nucleotide analogs.

Compound	Dose (mg/kg)	Animal	Liver NTP (nmol.h/g)	Plasma Nuc (μM.h) or tissue NTP (nmol.h/g)	Tissue NTP (nmol.h/g)	Liver-targeting index[a]	Fold increase (HD/nuc[b])
Lamivudine	230 (ip)	Rat	AUC_{0-4} = 2.6	AUC_{0-4} = 617 (plasma)	ND	0.007 (liver/plasma)	-
Compound **1**	30 (ip)	Rat	AUC_{0-4} = 29.4	AUC_{0-4} < 12.8 (plasma)	ND	2.3 (liver/plasma)	> 320
Cytarabine	100 (ip)	Mouse	AUC_{0-4} = 19	AUC_{0-4} = 46.2 (BM)	ND	0.42 (liver/BM)	-
Compound **2**	100[c] (ip)	Mouse	AUC_{0-4} = 225	AUC_{0-4} < 11.7 (BM)	ND	20 (liver/BM)	> 48
Adefovir dipivoxil (**3**)	30 (po)	Rat	AUC_{0-24} = 284	AUC_{0-24} = 742 (kidney)	AUC_{0-24} = 3206 (GI)	0.38 (liver/kidney)	-
Pradefovir (**5**)	30[d] (po)	Rat	AUC_{0-24} = 884	AUC_{0-24} = 196 (kidney)	AUC_{0-24} = 118 (GI)	4.5 (liver/kidney)	12

[a]Liver-targeting index = area under the curve $(AUC)_{liver}/AUC_{tissue}$. [b]Fold increase in liver targeting = liver-targeting index HepDirect prodrug (**HD**)/liver-targeting index nucleoside (Nuc). [c]Cytarabine equivalent dose. [d]Adefovir equivalent dose. **BM** bone marrow, **GI** gastrointestinal tract, **ip** intraperitoneal, **ND** not determined, **NTP** nucleoside triphosphate, **po** oral administration.

Figure 3. The structures of selected nucleoside and nucleotide analogs.



Intracellular retention of the ring-opened, negatively charged prodrug cleavage intermediate (Figure 2) by cells expressing CYP3A not only enables liver targeting of the active drug, but also localizes production of the prodrug byproduct to these cells. This latter feature is especially important for HepDirect prodrugs that generate an aryl vinyl ketone byproduct, because vinyl ketones are generally associated with significant cytotoxicity and genetic toxicity as a consequence of their ability to alkylate essential proteins and DNA [42]. Fortunately, cells in the liver and intestine that express CYP3A also contain high levels of glutathione (3 to 5 mM) [43], a natural antioxidant used to prevent oxygen free radicals (produced in part from CYP-catalyzed oxidations) from damaging cellular proteins and membranes [44]. Vinyl ketones produced in these cells react instantaneously with glutathione to form a non-toxic glutathione conjugate that is excreted. Accordingly, acetaminophen (paracetamol), a drug metabolized in the liver to a highly reactive vinyl ketone, has a good safety profile provided that the acetaminophen dose does not exceed the recommended range (1 to 4 g/day) and reduce hepatic glutathione levels to < 0.5 to 1 mM (~ 20% of normal liver levels) [45]. On this basis, HepDirect prodrugs should also be devoid of byproduct-related toxicity, as the amount of vinyl ketone produced from doses within the projected human dose range (10 to 500 mg/day) would be substantially less than those produced by acetaminophen. Moreover, unlike acetaminophen, high doses of HepDirect prodrugs (1000 mg/kg) produce only transient reductions (25%) in hepatic glutathione levels and no increases in serum liver enzyme levels or changes in liver histology [39••]. While the absence of liver toxicity may reflect rapid byproduct detoxification by intracellular glutathione, it may also reflect an overall lower toxicity potential of aryl vinyl ketones as suggested by a study using glutathione-depleted hepatocytes [39••] and an embryotoxicity study with phenyl vinyl ketone [46].

Pradefovir mesylate

The most advanced HepDirect prodrug is pradefovir mesylate (5, ICN 2001-3, MB-06866, Valeant Pharmaceuticals International; Figure 3), which is currently undergoing phase II clinical trials. Pradefovir is a HepDirect prodrug discovered by Metabasis Therapeutics that is designed to target the HBV drug adefovir (4; Figure 3) to the liver [101,104].

Adefovir dipivoxil (3, ADV; Figure 3) is an esterase-activated prodrug of adefovir that is currently marketed for the treatment of HBV infection [24•]. Results from two 48-week, phase III clinical studies showed that administration of ADV to both hepatitis B e antigen (HBeAg)-positive and HBeAg-negative patients led to significant histological, virological, biochemical and serological improvement in their condition [47••,48]. In the HBeAg-positive patient study, two doses of ADV (10 and 30 mg/day) were compared with placebo. HBV patients administered the higher dose experienced a greater virological response than patients administered the lower dose, based on median serum HBV DNA levels (-4.76 versus -3.52 $\log_{10}$ copies/ml) and the percentage of patients with HBV DNA levels < 400 copies/ml (39 versus 21%) at the end of treatment [47••]. However, the higher dose also led to increased baseline serum creatinine levels (8% of patients with increases > 0.5 mg/dl) and decreased serum phosphate. These findings, coupled with earlier studies in HIV patients administered even higher doses of ADV (60 and 120 mg), suggested that the incidence and extent of the nephrotoxicity is dose and time dependent [49•] and is likely to be correlated with serum adefovir, which is almost exclusively eliminated by the kidneys [50]. Ultimately, these results led to the approval of the lower dose of ADV for the treatment of HBV.

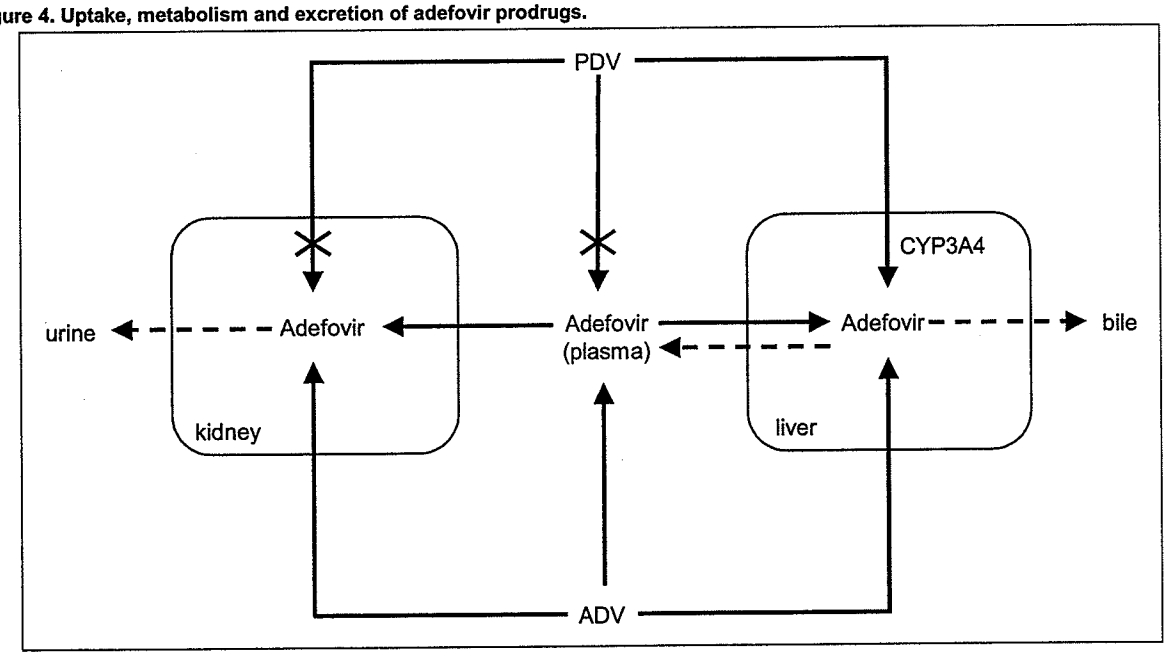
To regain the efficacy lost by using the lower dose (10 mg/day) of ADV, HepDirect prodrugs of adefovir were prepared and characterized in cellular and animal studies [38•,39••]. In both rats and monkeys, the lead compound

pradefovir mesylate produced a significant increase in the liver to kidney exposure of adefovir and adefovir mono- and diphosphates compared with ADV [39••,51••]. The increased liver targeting by pradefovir was attributed to the liver specificity of HepDirect prodrug cleavage (Figure 4). Esterase cleavage of ADV in extrahepatic tissues leads to higher circulating adefovir levels and greater kidney exposure. In contrast, adefovir produced inside hepatocytes is eliminated not only by the kidney, but also by the bile as a consequence of anion transporters [52] located on the hepatocyte sinusoidal and biliary canaliculus membranes [53]. Because adefovir is poorly absorbed across the gastrointestinal wall, no enterohepatic circulation occurs, meaning that increased prodrug activation in the liver leads to a net increase in biliary elimination and reduced kidney exposure. The liver targeting demonstrated in the pharmacokinetic studies was indirectly supported by results from both 28-day [51••] and longer-term (6 to 9 months) [Valeant Pharmaceuticals, unpublished data] toxicology studies in rats and monkeys, which showed that the 'no observed adverse effect dose' for pradefovir was at least 15-fold higher than that published for ADV [54].

Valeant Pharmaceuticals has reported results from several clinical trials demonstrating that pradefovir is converted to adefovir in humans and that pradefovir is safe and well tolerated [55,56,57••]. Moreover, an interim analysis at week 24 of a 48-week, randomized, open-label, phase II clinical study in 242 chronic HBV patients comparing four doses of pradefovir (5, 10, 20 and 30 mg/day) with ADV (10 mg/day) provided the first evidence that HepDirect prodrugs enhance liver targeting in humans [57••]. As shown in Table 2, pradefovir dose-dependently decreased the mean serum HBV DNA levels and increased the percentage of patients with < 400 copies/ml of HBV DNA. Viral suppression by the three highest doses was significantly better than that by ADV at 10 mg/day ($p < 0.05$). Circulating adefovir levels increased approximately dose proportionally in pradefovir-treated patients, but importantly remained less than the levels found in ADV-treated patients.

The combination of increased efficacy and decreased systemic adefovir exposure in pradefovir-treated HBV patients compared with ADV-treated HBV patients provides indirect evidence for liver targeting in humans and its potential for therapeutic benefit. Renal toxicity limited ADV therapy to a submaximally effective dose, which presumably led in some patients to incomplete viral suppression and, therefore, to an increased risk for liver disease progression and viral resistance [49•,58]. Accordingly, shifting adefovir exposure from the kidneys to the liver should increase the percentage of patients with adequate virological response. Results from the 24-week interim analysis (Table 2) show that pradefovir produced greater reductions in serum HBV DNA levels and a greater percentage of patients with undetectable levels of HBV DNA than ADV. Moreover, kidney exposure to adefovir following the 30-mg/day dose of pradefovir is only 75% of that for the 10-mg/day ADV dose, suggesting that pradefovir

Figure 4. Uptake, metabolism and excretion of adefovir prodrugs.



Adefovir dipivoxil (**ADV**) and pradefovir (**PDV**) are prodrugs of adefovir. ADV is rapidly hydrolyzed to adefovir by esterases that exist in plasma, liver, kidney and other tissues. PDV is stable in plasma and kidney, but undergoes rapid, CYP3A4-mediated oxidative hydrolysis to adefovir in the liver. Intracellular adefovir is reversibly converted to adefovir diphosphate (not shown), which results in inhibition of hepatitis B virus replication in the liver and toxicity in the kidney. Adefovir in the liver is excreted into the bile or into the circulation, whereas in the kidney it is excreted into the urine.

may be associated with a lower risk of nephrotoxicity from long-term drug therapy [49•]. Furthermore, the greater reduction in viral titers following pradefovir therapy may lead to lower rates of viral resistance than ADV, which is already low compared with many other HBV drugs [7,59] in both naïve [60] and lamivudine-resistant patients [61].

Table 2. Data summary for week-24 interim analysis of phase II study in patients with chronic HBV infection.

Parameter	Pradefovir mesylate (mg/day)				
	5	10	20	30	10
Mean HBV DNA reduction (log_{10} copies/ml)	-3.39	-4.22	-4.33	-5.02	-3.66
Patients with < 400 copies/ml (%)	17	24	29	38	16
Plasma adefovir AUC (ng.h/ml)	42	73	134	224	298

HBV hepatits B virus.

Pharmacokinetics of HepDirect prodrugs

The reliance of prodrug cleavage on CYP3A4 suggests that the efficacy and safety of HepDirect prodrugs may be compromised by characteristics commonly associated with CYP-metabolized drugs [62], including poor oral bioavailability, high inter-individual variability in drug levels, and drug-drug interactions. Drugs metabolized by CYP enzymes often undergo extensive first-pass metabolism in the liver and small intestine. Metabolism in the small intestine limits oral bioavailability and could result in intestinal-related toxicities, whereas metabolism in the liver reduces systemic prodrug exposure and enhances drug levels in the target organ for viral hepatitis. Studies with pradefovir in rats and monkeys suggest that intestinal metabolism is minor, especially compared with ADV [39,51••], and is not associated with any apparent toxicity. In contrast, uptake and metabolism by the liver is extensive, as demonstrated in portal-vein-cannulated rats and monkeys [51••].

Variability in drug and drug metabolite levels is another area of possible concern for CYP-metabolized drugs, especially if the therapeutic index is narrow [63]. Variability is particularly high for drugs metabolized by the CYP1 and CYP2 families because of genetic polymorphisms, but is still significant for CYP3A4, the predominant CYP enzyme in human liver [64•] and the isozyme that metabolizes HepDirect prodrugs [38•]. CYP3A4-linked variability is related to a range of dietary and environmental factors that induce or inhibit its activity. Some of the variability of CYP3A4-metabolized drugs can also be attributed to inconsistent metabolism by a closely related isozyme, CYP3A5, which, unlike CYP3A4, is predominantly found in the small intestine and is polymorphic [65]. Accordingly, HepDirect prodrugs that are specific for CYP3A4 or avoid prolonged intestinal exposure and, therefore, CYP3A5-mediated metabolism [66], may exhibit reduced variability.

Pharmacokinetic data from studies in humans suggest that pradefovir and adefovir plasma levels are approximately dose proportional over a dose range of 5 to 60 mg, with no evidence of differences in the pharmacokinetic profile following prolonged dosing [55,56]. Moreover, the inter-individual differences in pradefovir and adefovir exposure appear less than are commonly associated with highly variable drugs based on mean coefficients of variation [67,68•].

Drug-drug interactions represent a third possible CYP-related limitation for HepDirect prodrugs, as more than 50% of all marketed drugs are metabolized by CYP3A4 [62]. Of particular concern are interactions with drugs that have narrow therapeutic indices, especially if the co-administered drug is commonly used by the target patient population. Potent CYP inhibition is generally a greater drug-drug interaction concern than CYP induction, because inhibition often results in adverse events produced by higher circulating levels of the co-administered drug, whereas induction leads to diminished efficacy owing to increased metabolism to inactive metabolites. Importantly, pradefovir is neither a potent human CYP inhibitor (IC_{50} > 10 μM) nor CYP inducer [69]. Moreover, the products of pradefovir metabolism are negatively charged compounds that consequently display no CYP interactions.

Although these results suggest that pradefovir is unlikely to affect the pharmacokinetics of CYP-metabolized drugs, it does not rule out the possibility that drugs that interact with CYP3A4 might affect pradefovir/adefovir levels in the liver and plasma. Potent CYP inhibitors such as ketoconazole are expected to decrease pradefovir conversion [39••,70], whereas CYP inducers may increase conversion. However, because pradefovir is rapidly absorbed and efficiently converted within 1 h after oral administration [55,56], the effect of CYP3A4 inhibitors may be minimized by administering the drugs at separate times. The effect of CYP3A4 inducers may also prove to be clinically insignificant given the extent and speed in which pradefovir is converted in the absence of CYP3A4 induction [68•]. Nevertheless, drug-drug interaction studies are ongoing in order to address these potential concerns and their impact on the therapeutic use of pradefovir.

Conclusions

HepDirect prodrugs of NMPs increase NTP levels in the liver while decreasing NTP levels in extrahepatic tissues. Studies in rats and monkeys demonstrated that pradefovir targets adefovir and adefovir metabolites to the liver. In humans, indirect evidence for liver targeting was observed in a phase II clinical study in HBV patients, which showed that, compared with ADV, pradefovir resulted in both better efficacy and lower systemic adefovir exposure. Accordingly, HepDirect prodrugs may provide a valuable strategy for improving the efficacy and/or safety of nucleoside- and nucleotide-based drugs for patients with chronic viral hepatitis.

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