PART I

HUMAN IMMUNODEFICIENCY VIRUS
INTRODUCTION
Atazanavir is an azapeptide inhibitor of HIV-1 protease that prevents the formation of mature virions in HIV-1-infected cells by inhibiting the cleavage of gag and gag-pol polyproteins. Atazanavir was developed to address a number of concerns with existing protease inhibitors: high pill burden, high frequency of dosing, and metabolic side effects such as hyperlipidemia. The development program focused initially on dosing atazanavir without coadministering a pharmacokinetic enhancer such as ritonavir. Ritonavir inhibits hepatic CYP3A4 and the metabolism of drugs (such as atazanavir) utilizing this pathway, thereby increasing the blood levels of these drugs. The emerging practice of “boosting” with low-dose ritonavir led to the evaluation of this alternative dosing strategy, initially in treatment-experienced subjects who had previously received and failed multiple antiretroviral regimens, then in antiretroviral-naive patients.

DISCOVERY
Atazanavir (BMS-262362, Scheme 1) was identified from studies of a series of pseudosymmetric azapeptide substrate analogs of HIV-1 protease in which fundamental inhibitory activity relies on replacement of the hydrolyzable amide bond by a hydroxyethylene isostere [1–3]. Although the initial examples of this chemotype demonstrated favorable in vitro and in vivo properties [1,2], further optimization for in vitro HIV-1 inhibitory potency and in vivo exposure was required to identify atazanavir [3,4]. As shown in Scheme 1, the two closely related lead compounds 1 and 2 demonstrated disparate properties: The isoamyl derivative (1) is a potent HIV-1 protease inhibitor, IC₅₀ = 16 nM, with good antiviral activity in cell culture, EC₅₀ = 2.7 nM, but exhibits poor oral bioavailability, while the cyclohexylmethyl analog (2) is a 10-fold weaker protease inhibitor, IC₅₀ = 177 nM, and antiviral agent, EC₅₀ = 55 nM, that demonstrates good bioavailability following oral administration to mice [3]. Based on x-ray crystallographic information of the early lead CGP-53820 (3) co-crystallized with HIV-1 protease [5], the potential of introducing larger P₁’ substituents was examined, with the anticipation that potency would be enhanced while preserving the physicochemical properties conferring good absorption. From a drug design perspective, the effort sought to establish additional favorable contacts between the inhibitor and protease residues Arg8, Phe153, Gly148, and Gly149 located at the periphery of the S₁’ pocket [4]. The synthesis and evaluation of a derivative in which the cyclohexylmethyl substituent of 2 was replaced by a biphenyl moiety established the validity of the hypothesis since the new compound potently inhibited HIV-1 protease, IC₅₀ = 35 nM, demonstrating tolerance for a large P₁’ element, while expressing excellent antiviral activity in cell culture, EC₅₀ = 1.8 nM. Replacing the two valine residues with tert-Leu resulted in CGP-75355 (4), which showed...
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![Chemical structures]

SCHEME 1  Discovery of atazanavir.

a further improvement in cell culture potency, \( EC_{50} = 0.7 \) nM. More important, protease inhibitors that incorporated \( tert\)-Leu residues were generally well absorbed in mice following oral administration.

With the structural elements critical for potent antiviral activity established, attention was directed toward improving pharmaceutical properties, with a focus on increasing solubility [4]. To this end, heterocycles designed to increase hydrophilicity were introduced to replace the distal phenyl of the biphenyl moiety. This exercise identified the 2-pyridyl as the optimal element, providing atazanavir (5, BMS-262632, CGP-73547) as a potent HIV-1 protease inhibitor, \( IC_{50} = 2.6 \) nM, that retained potency toward HIV-1 protease resistant to saquinavir. Atazanavir (5) exhibits excellent antiviral properties in cell culture, \( EC_{50} = 1.4 \) nM and \( EC_{90} = 3 \) nM, with a good therapeutic index, \( CC_{80} = 21.8 \) \( \mu \)M and \( CC_{90} = 31.8 \) \( \mu \)M. Following oral administration to mice and dogs, atazanavir (5) showed good plasma bioavailability, establishing blood levels in excess of the \( EC_{90} [4] \).

SYNTHESIS

The strategic approach to the preparation of atazanavir developed by the discovery chemistry team, summarized in Scheme 2, has largely been followed by the process synthesis depicted in Scheme 3 [4], with several modifications designed to facilitate the preparation of multikilogram quantities of the active pharmaceutical ingredient [6]. The discovery synthesis was satisfactory for the preparation of material for preliminary toxicological evaluation but was deemed to be less than optimal for the synthesis of the significantly larger quantities of drug required for longer-term toxicological studies and clinical trials. The process chemistry strategy was focused on improving the synthesis of the two key intermediates, hydrazine (9) and epoxide (11), that were used in the discovery synthesis. With access to these molecules in hand, optimization of the reagents and conditions used for their coupling, followed by improvements in the final elaboration to the product, were completed.

In the discovery synthesis (Scheme 2), a nickel-catalyzed cross-coupling reaction between 2-bromopyridine and the Grignard reagent (7) derived from a protected form of 4-bromobenzaldehyde (6) was used to prepare the aldehyde (8) [4]. This procedure required the use of disobutyllaluminium hydride, a problematic reagent when used in large-scale reactions. Access to (9) was improved considerably by coupling 2-bromopyridine with commercially available 4-formylbenzenearboronic acid (13) under Suzuki conditions (Scheme 3), a process mild enough to obviate the
SCHEME 2  Discovery synthesis of atazanavir.

SCHEME 3  Optimized process for the large-scale preparation of atazanavir.
Alternative synthesis of atazanavir.

Scheme 4: Alternative synthesis of atazanavir.

Preclinical Pharmacokinetics

The nonclinical safety profile of atazanavir has been evaluated extensively in pharmacokinetic/absorption, distribution, metabolism, and excretion (ADME), and toxicology studies. Sensitive and specific bioanalytical methods, high-performance liquid chromatography/ultraviolet, and liquid chromatography/mass spectrometry were used to determine the plasma and urine concentrations of atazanavir, respectively. The concentrations of radioactivity in plasma and urine samples from radiolabeled studies were determined by
rats following oral administration of a 100-mg/kg dose of atazanavir was distributed extensively into and decayed from all tissues in the total body water (0.67 L/kg in the rat and 0.60 L/kg in dogs (1.62 L/kg) and dogs (0.76 to 2.45 L/kg) is greater than the total body water. The steady-state volume of distribution of atazanavir in rats (1.62 L/kg) and dogs (0.76 to 2.45 L/kg) is greater than the total body water (0.67 L/kg in the rat and 0.60 L/kg in the dog), indicating extravascular distribution and/or tissue protein binding. Accordingly, drug-related radioactivity was the dog), indicating extravascular distribution and/or tissue protein binding. Accordingly, drug-related radioactivity was the dog), indicating extravascular distribution and/or tissue protein binding. Accordingly, drug-related radioactivity was administered and expressed as a percentage of the drug administered. The recovery of a significant amount of unchanged drug in rat (39%) and dog (79%) after oral administration suggested incomplete absorption and/or biliary elimination of atazanavir in both species. When compared to the decline in plasma concentrations after intravenous dosing, the slow decline in plasma levels after achieving peak plasma levels (at 2 h) in nonfasted rats and dogs following oral administration suggested prolonged oral absorption [9].

Distribution

The ADME profiles of atazanavir in rats and dogs indicated that these species were appropriate for the toxicity evaluation of atazanavir. The oral toxicity of atazanavir was well characterized in a comprehensive nonclinical toxicology program that included single- and repeat-dose toxicity, reproductive and developmental toxicity, in vitro and in vivo genotoxicity, carcinogenicity, and immunotoxicity studies. The safety assessment included comparisons of plasma exposures to atazanavir in animals to those in humans following the recommended clinical dosing regimens of 400 mg/day atazanavir without ritonavir, or 300 mg/day atazanavir and 100 mg/day ritonavir.

The metabolic pathways of atazanavir in rats and dogs, and humans are similar and involve monooxygenation, dioxygenation, glucuronidation, N-dealkylation, hydrolysis, and oxygenation with dehydrogenation. The multiple monohydroxylated, dihydroxylated, and trihydroxylated metabolites demonstrated that the oxygenation of atazanavir can occur on the phenylmethyl ring, the pyridinylphenylmethyl ring system, and at different positions on the pentaazatetradecane dioic acid dimethyl ester part of the molecule. The major elimination pathway appeared to be CYP3A4-mediated conversion to these oxygenated metabolites and excretion in the bile as either free or glucuronidated metabolites. Additional minor metabolic pathways observed consisted of N-dealkylation metabolites and hydrolytic cleavage of the carbamate moiety. In rat and dog plasma, the major circulating components were atazanavir and 4(2-pyridyl)benzoic acid (M2), along with small amounts of an unidentified keto metabolite. In addition, traces of an N-dealkylated metabolite, M14, were observed in dog plasma. These metabolites were also identified in humans and are depicted in Scheme 5 [9,10].

Elimination

Atazanavir is an intermediate- to high-extraction drug in animals; the elimination half-life in the rat and dog is 0.94 and 0.45 h, respectively. The minimal recovery of radioactivity in urine (>7%) and substantial recovery in feces (>67%) after intravenous administration in rats and dogs suggested that the major route of excretion of atazanavir and its metabolite(s) is via the bile.

Single-Dose Toxicity

Single-dose oral toxicity studies were conducted in mice and rats at doses of 200 to 1600 mg/kg. Atazanavir demonstrated a low order of acute toxicity in both species. In mice, the minimal lethal doses were 1600 and 800 mg/kg in males and females, respectively. Deaths occurred from 1 to 5 days after dosing and were not associated with any drug-related gross or histopathologic changes. Clinical signs, considered to be agonal, were present just prior to or concomitant with death. Nonspecific clinical signs were also observed after dosing in surviving animals at high doses. Atazanavir was well tolerated in mice up to 400 mg/kg. In rats, the minimal lethal dose was greater than 1600 mg/kg. The greater sensitivity of mice to the acute effects of atazanavir compared to rats may be related to higher systemic exposure to atazanavir, as demonstrated in subsequent toxicity studies [9].
Repeat-Dose Toxicity

Repeat-dose toxicity of atazanavir was evaluated in studies in rats, dogs, and mice of up to 9 months' duration. Atazanavir-related findings in all three species were generally confined to the liver. In rats, the toxicity of atazanavir was evaluated at doses up to 1200 mg/kg per day in the 2-week study and up to 900 mg/kg per day in the 6-month study with a 3-month interim evaluation and a 2-month postdose recovery period. Atazanavir was generally well tolerated. Liver changes consisted of increased serum total bilirubin at ≥300 mg/kg per day; increased liver weights and associated minimal to mild hepatocellular hypertrophy at ≥100 mg/kg per day; that were considered an adaptive response consistent with hepatic enzyme induction; pale livers at 900 mg/kg per day; and minimal to moderate hepatocellular cytoplasmic lipid vacuolation at 100 mg/kg per day. None of the hepatic changes were accompanied by elevations in serum transaminases or microscopic evidence of cholestasis or degenerative liver changes. The hepatic alterations generally did not progress between 3 and 6 months of dosing and, with the exception of increased liver weights at 900 mg/kg per day, were reversible. Systemic AUC exposures at the well-tolerated dose of 900 mg/kg per day for 6 months were 1.2 to 4.0 times the exposure at the recommended clinical dose of 400 mg/day atazanavir or 0.5 to 1.8 times the exposure at the recommended clinical dose of 300 mg/day atazanavir with ritonavir.

In dogs, the toxicity of atazanavir was evaluated at doses up to 360 mg/kg per day in 2-week studies and up to 180 mg/kg per day in the 9-month study. Because of clinical toxicity at 90 to 360 mg/kg per day in the initial 2-week dog study, a second 2-week study was conducted at lower doses (10 to 75 mg/kg per day). In the 9-month study in dogs, the low dose (10 mg/kg per day) was increased to 180 mg/kg per day after 3 months due to the absence of drug-related findings at 10 mg/kg per day. In the initial 2-week study, hepatic changes consisted of minimal to moderate increases in serum total bilirubin, liver enzymes (alanine and aspartate aminotransferases, γ-glutamyltransferase, and alkaline phosphatase), total cholesterol, and triglycerides, and decreases in protein and/or albumin at all doses. In the second 2-week study, there were no drug-related changes. In the 9-month study, hepatic changes were limited to minimally increased serum total bilirubin and γ-glutamyltransferase at ≥30 mg/kg per day, minimally to moderately increased serum alkaline phosphatase at ≥90 mg/kg per day in individual animals, and minimally increased liver weights at ≥90 mg/kg per day. No drug-related gross or microscopic liver changes or evidence of cholestasis were observed in any of the studies in dogs. Additional findings of minimally altered water balance (increased water consumption at 180 mg/kg per day) and minimally decreased heart weights at 30 and 90 mg/kg per day at 3 months only were not associated with any functional impairment or gross or microscopic...
organ changes, and were considered to be toxicologically insignificant. At the well-tolerated dose of 180 mg/kg per day for 6 months, systemic AUC exposures were 1.7 to 7.9 times the human exposures at 400 mg/day atazanavir or 0.8 to 3.1 times the human exposure at 300 mg/day atazanavir with ritonavir.

In mice, doses of 40 mg/kg per day in males and 160 mg/kg per day in females were generally well tolerated after 3 months with no effects seen at the lower doses of 20 and 40 mg/kg per day in males (M) and females (F), respectively. Similar to the liver changes noted in rats and/or dogs, mild increases in serum total bilirubin at 80 (M) and 640 (F) mg/kg per day; minimal-to-marked increases in liver weights, hepatocellular hypertrophy, and lipid vacuolation at ≥ 40 (M) and 160 (F) mg/kg per day; and pale livers at 80 (M) and ≥ 160 (F) mg/kg per day were also observed in mice. In addition, clinical and microscopic evidence of hepatotoxicity at 80 (M) and 640 (F) mg/kg per day was present and was characterized by minimal-to-moderate elevations in serum transaminases (M and F) and a low incidence of minimal hepatocellular single-cell necrosis (F only). Increased cellular glycogen [80 (M) and 640 (F) mg/kg per day] was also noted only in mice. Systemic AUC (area under the plasma concentration–time curve) exposures at the well-tolerated doses 40 (M) and 160 (F) mg/kg per day) were 0.4 and 4.1 times the exposure in humans given 400 mg/day atazanavir or 0.2 to 1.8 times the exposure in humans given 300 mg/day atazanavir with ritonavir. Other drug-related findings in female mice included mild-to-moderate decreases in platelet counts at ≥ 160 mg/kg per day; minimal-to-mild increases in leukocytes, neutrophils, and lymphocytes at 640 mg/kg per day; and morphological changes in erythrocytes (i.e., poikilocytosis, anisocytosis, polychromasia) at 640 mg/kg/day. Increased spleen weight and size and histological evidence of increased splenic and hepatic extramedullary hematopoiesis in females ≥ 160 mg/kg per day were interpreted as secondary effects related to the reduced platelet counts rather than direct drug effects. However, a clinical or morphological basis for the reduction in platelets was not determined. All of these changes, which were limited to female mice, were considered to have no established clinical relevance, as systemic exposures at doses producing these alterations were relatively high (1.8 to 12 times human exposure at the recommended doses), and similar consistent changes were not observed in rats and dogs treated chronically with atazanavir. Importantly, there have been no reports of thrombocytopenia in clinical trials of patients treated with atazanavir.

Reproductive and Developmental Toxicity

A complete battery of reproductive toxicity studies was conducted with atazanavir to assess potential effects on fertility, reproductive function, gestation, parturition, and lactation of the parental generation in rats; on embryonic and fetal development in rats and rabbits; and on growth, development, and reproductive performance of progeny in rats. Systemic exposures supporting the reproductive studies were established in the interim 3-month toxicity study in rats and supportive 7-day toxicokinetic studies in pregnant rats and rabbits. High doses of atazanavir in the reproductive studies in rats were based on a maximum feasible dose (azanavir concentration and PEG-400 dose volume limitations based on study duration) or results of previous reproductive or repeat-dose toxicity studies. The high dose in the embryo-fetal development study in rabbits was based on toxicity in a range-finding study.

In the study of fertility and reproductive performance in rats (100, 375, and 1400 mg/kg per day), drug-related effects were limited to prolonged diestrus with abbreviated estrus and metestrus in females at all doses. Minimally lower fertility was seen in female rats at 1400 mg/kg per day; however, fertility was only marginally below that observed historically in control rats. This finding was not duplicated in a second fertility study at 1400 mg/kg per day; and therefore, the reduced fertility was considered not to be drug related. There were no effects of atazanavir on early embryonic development or reproductive performance, including mating, at doses up to and including 1400 mg/kg per day. In the absence of any adverse effects on mating, fertility, or reproductive performance or morphological changes in the ovaries and female reproductive tract in toxicity studies, the perturbation of estrous cyclicity in female rats was considered to be of limited toxicological significance. Systemic AUC exposures in male and female rats at 1400 mg/kg per day, which had no effect on fertility, were at least 1.4 (M) and 3.4 (F) times human exposure at 400 mg/day atazanavir or 0.6 (M) and 1.5 (F) times the human exposure with 300 mg/day atazanavir with ritonavir.

Systemic AUC exposure in pregnant rats at 1400 mg/kg per day, which produced no effects on reproductive performance or early embryonic development, was 0.9 or 1.9 times the human exposure with 300 mg/day atazanavir with ritonavir, or 400 mg/day atazanavir dose, respectively.

In the embryo-fetal development studies, atazanavir produced no adverse embryonic or fetal effects at maternally toxic doses (up to 1920 mg/kg per day in rats and 60 mg/kg per day in rabbits). Maternal AUC exposures at the fetal no-effect doses were in rats 0.8 or 1.8 times and 0.4 or 1.0 times the human exposure at 300 mg/day atazanavir with ritonavir or 400 mg/day atazanavir, respectively.

In a study of pre- and postnatal development in rats exposed to atazanavir (50, 220, and 1000 mg/kg per day), findings were limited to mean body weight gain suppression in the F1 generation from 4 days of age through the early postweaning growth period at the maternally toxic dose of 1000 mg/kg per day. This finding was considered likely to be secondary to the maternal body weight reductions rather than a direct effect of atazanavir. At the highest no-effect dose
(220 mg/kg per day), systemic AUC exposure to atazanavir was 0.5 times the human exposure at the recommended daily dose of atazanavir with ritonavir and 1.1 times the human exposure at the recommended daily dose of atazanavir alone. Atazanavir had no effect on the reproductive performance in the F3 generation.

In summary, atazanavir demonstrated no selective developmental toxicity and no effects on reproductive function or fertility at exposures generally equivalent to that in humans at the recommended daily dose of atazanavir or ritonavir.

Genetic Toxicity
The genotoxic potential of atazanavir was evaluated in a battery of in vitro and in vivo test systems. All in vitro assays were conducted with and without metabolic activation (rat liver S9 fraction). Atazanavir was not mutagenic in either the bacterial mutagenicity screening assay or in the definitive Ames reverse-mutation study. In the definitive Ames assay, cytotoxicity was observed in each of the Salmonella and Escherichia coli strains at the highest concentration evaluated (2500 µg/plate), both with and without metabolic activation. In the in vitro chromosomal aberration test in primary human lymphocytes, atazanavir was clastogenic at cytotoxic concentrations of 30 µg/mL in the absence of metabolic activation and of 240 µg/mL in the presence of metabolic activation. In contrast, atazanavir was not clastogenic in the in vivo micronucleus test in rats at doses up to 2000 mg/kg for 3 days. As positive in vitro results and a negative bone marrow micronucleus test were obtained, it was necessary to perform a second in vivo test based on the International Conference on Harmonization guidelines. In vivo/in vitro unscheduled DNA synthesis (UDS) assays in both male and female rats were conducted with single oral doses up to 2000 mg/kg, and atazanavir did not induce UDS in the liver. Since the UDS endpoint may not have been sensitive for detection of any clastogenic effect, as such damage is not readily repaired by UDS mechanisms, an alternative assay, the single-cell gel electrophoresis (Comet) assay, with a more appropriate endpoint, was conducted with assessments of liver and duodenum. Although no valid data could be obtained from livers of atazanavir-treated rats (assay failure due to lack of positive control response), clear negative results for DNA damage were obtained in the duodenum at single oral doses up to 2000 mg/kg.

Carcinogenicity
The carcinogenic potential of atazanavir was evaluated in mice and rats by oral gavage administration for two years. In mice administered atazanavir at doses of 20, 40, or 80 mg/kg per day in males and 40, 120, or 360 mg/kg per day in females, an increased incidence of benign hepatocellular adenomas was observed in females given the highest dose of atazanavir. Systemic exposure at the high dose in females was three times the exposure in humans given 300 mg/day atazanavir with 100 mg/day ritonavir and seven times the exposure in humans given 400 mg/day atazanavir. No increased incidence of tumors was observed in females at lower doses or in males at any dose. Exposures at the nontumorigenic doses in males and females ranged from 1.8 to 4.2 times the human exposure with the two clinical dosing regimens. Based on the nonneoplastic liver findings and the overall lack of genotoxicity, the hepatocarcinogenic effect in high-dose female mice was considered secondary to atazanavir-related cytotoxicity and lacking in clinical relevance because of the relatively high dose and exposure associated with this finding.

In rats administered atazanavir at doses of 100, 350, or 1200 mg/kg per day for two years, there were no statistically significant positive trends in the incidence of neoplasms at any dose. Exposures in rats at the high dose of 1200 mg/kg per day were 0.7 (males) and 2.5 (females) times the exposure in humans at the recommended dose of atazanavir with ritonavir and 1.6 (males) and 5.7 (females) times the human exposure at the recommended dose of atazanavir without ritonavir.

Based on the results of the mouse carcinogenicity study, additional investigational studies were conducted in mice which strongly supported the hypothesis that the female mouse-specific increased incidence in hepatocellular tumors was due to increased hepatocellular proliferation secondary to cytotoxicity, based on 5-bromo-2’deoxyuridine (BrdU) staining and terminal deoxynucleotidyl transferase-mediated dUTP-X nick end labeling (TUNEL), respectively. Increased cell proliferation secondary to drug-related cytotoxic changes is a well-recognized nongenotoxic (epigenetic) mechanism of tumor development in rodents [11–14]. In addition, the absence of a carcinogenic effect of atazanavir in other tissues or organs in the mouse carcinogenicity study and the absence of any carcinogenic effect in the companion rat carcinogenicity study further support the nongenotoxic nature of the hepatocellular tumorigenic effect since mutagens are more likely than nonmutagens to induce tumors in multiple organs in a single study and in more than one animal species [15]. Moreover, the collective results of the genotoxicity and carcinogenicity studies and of the investigation of atazanavir-induced hepatic effects support a threshold nongenotoxic, cytolethal-proliferative mechanism of tumor development that does not occur at clinically relevant exposures in mice. Together, the result from the genotoxicity and carcinogenicity studies support that atazanavir is not a genotoxic carcinogen and does not pose a carcinogenic risk to humans at therapeutic doses and exposures.

In conclusion, the toxicity of atazanavir was adequately evaluated in a comprehensive battery of GLP (good laboratory practices)–compliant nonclinical toxicology studies. Based on the results of these studies, only the liver was identified as a potential target organ, with microscopic evidence of hepatotoxicity observed only in female mice. Atazanavir...
demonstrated no selective developmental toxicity, no effects on reproductive function or fertility, and no evidence of immunotoxicity. The results of the genotoxicity, carcinogenicity, and investigative studies support the fact that atazanavir is not a genotoxic carcinogen and does not pose a carcinogenic risk to humans. Therefore, there were no nonclinical findings that precluded the safe administration of atazanavir for treatment of HIV infection in humans.

**MICROBIOLOGY AND RESISTANCE**

Atazanavir exhibits anti-HIV-1 activity with a mean 50% effective concentration (EC_{50}) in the absence of human serum of 2 to 5 nM against a variety of laboratory and clinical HIV-1 isolates grown in peripheral blood mononuclear cells, macrophages, CEM-SS cells, and MT-2 cells. Atazanavir has activity against HIV-1 group M subtype viruses A, B, C, D, AE, AG, F, G, and J isolates in cell culture. Atazanavir has variable activity against HIV-2 isolates [9].

In vitro studies were conducted to assess the development of resistance in three strains (RF, LAI, and NL4-3) of HIV-1 virus in cell culture in the presence of increasing concentrations of atazanavir for up to 4.8 months. Genotypic and phenotypic analysis indicated that an N88S substitution in protease appeared first during passage of the RF and LAI viruses. The LAI virus exhibiting a 93-fold reduced susceptibility to atazanavir contained five amino acid changes in protease (L10F, I50L, L63P, A71V, and N88S), while the NL4-3 virus showing a 96-fold decrease in susceptibility contained four amino substitutions (V32I, M46I, I84V, L89M). The NL4-3 variant has a well-documented resistance substitution (protease I84V) located near the active site, whereas the resistance phenotype of LAI is attributed to the appearance of I50L and N88S substitutions, in combination with other secondary substitutions at residues 10, 63, and 71. Atazanavir-resistant virus remained susceptible to saquinavir while showing varying levels (0.06- to 71-fold change) of cross-resistance to nelfinavir, indinavir, ritonavir, and amprenavir. Atazanavir-resistant LAI viruses (harboring the unique protease I50L substitution) exhibited increased susceptibility to ritonavir and amprenavir.

Analysis of the genotypic profiles of 943 of the 950 protease inhibitor susceptible and resistant clinical isolates from atazanavir clinical trials identified a strong correlation between the presence of the specific protease amino acid changes at I50L/F, 53I/R/M, 74V, 34I/F/V, 36I/L/V, 46L, 48V, 54A/V, 63R, 71V/T/I, 73C/S/T/A, 82A/F/S/T, 84V, and 90M and decreased susceptibility to atazanavir. Although no single substitution or combination of substitutions is predictive of atazanavir resistance, the presence of at least five of these substitutions correlated strongly with loss of atazanavir susceptibility [16].

**PRECLINICAL SAFETY ASSESSMENTS**

**Electrophysiology and Cardiac Conduction Studies**

The ECG effects of atazanavir were assessed in rabbit Purkinje fibers. Atazanavir minimally increased action potential duration (13% at 30 µM). This concentration is approximately four times the mean C_{ssmax} and 17 times the mean C_{ss} in humans given atazanavir 400 mg/day. Weak inhibition of sodium currents (IC_{50} > 30 µM) and moderate inhibition of calcium currents (IC_{50} of 10.4 µM) were also identified in vitro. Atazanavir was also evaluated in the in vitro IKr (hERG) and IKs potassium channels, where it produced weak inhibition of IKr current and no significant inhibition of the IKs current. The clinical significance of these weak inhibitory effects on several ion channels, including hERG, and modest prolongations of the action potential duration is not clear. In in vivo studies, no direct drug-related effects on cardiac function were noted in dogs treated with atazanavir for up to 9 months. Plasma concentrations of atazanavir at the high dose of 180 mg/kg per day in the 9-month toxicity study, which produced no ECG changes, were up to three times the C_{ssmax} and seven times the AUC in humans given atazanavir 400 mg/day.

**Metabolic Effects**

Preclinical findings showed that atazanavir exhibited markedly less interference with lipid metabolism in cell culture models of hepatocytes and adipocytes than did other protease inhibitors. Atazanavir exhibited lesser effects on expression of lipogenic genes in hepatocyte and adipocyte models. Direct assays of human 20S proteasome in vitro showed that atazanavir is a weak inhibitor of proteasome activity with IC_{50} values several times higher than typical plasma C_{ssmax}, while several other protease inhibitors inhibited proteasome activity with IC_{50} values below reported plasma C_{ssmax} values.

Additionally, data from in vitro studies demonstrated that atazanavir has little or no effect on GLUT-4 or GLUT-1 glucose transporters in cell culture models of adipocytes and myocytes. These glucose transporters are critical for glucose uptake in fat and muscle tissues. Because of the role of glucose metabolism in lipid biosynthesis and its regulation, the lack of inhibition of GLUT-4 or GLUT-1 activity by atazanavir may contribute to its neutral lipid profile.

**Hyperbilirubinemia**

Elevation in bilirubin is the most frequent laboratory abnormality observed in atazanavir clinical trials. In a heterologously expressed human UGT1A1 in vitro system, atazanavir—at clinically relevant concentration—inhibits the glucuronidation of bilirubin. This has also been observed in
human liver microsomes. The majority of elevations in total bilirubin are indirect (unconjugated) and not associated with liver function test elevations. Bilirubin elevations are reversible upon discontinuation or interruption of atazanavir.

PHASE I–III SAFETY AND EFFICACY

Atazanavir clinical safety and efficacy were demonstrated through a clinical development program in antiretroviral therapy-naive and experienced patients. Two randomized controlled dose-ranging phase II studies compared unboosted atazanavir at doses of 200 to 600 mg against nevirapine [17,18], and two comparative phase III studies compared unboosted atazanavir at a dose of 400 mg against efavirenz [19] in treatment-naive patients and lopinavir/ritonavir in treatment-experienced patients [20]. Atazanavir 300 mg with ritonavir 100 mg used as a pharmacokinetic enhancer was compared to unboosted atazanavir in treatment-naive patients [21] and studied against lopinavir/ritonavir in both treatment-naive [22] and treatment-experienced patients [23]. The clinical development program of atazanavir has resulted in its approval in combination with other antiretroviral drugs for treatment-naive patients with either boosted or unboosted dosing of atazanavir, and for treatment-experienced with boosted atazanavir dosing.

CLINICAL PHARMACOLOGY

Pharmacokinetics

Most atazanavir pharmacokinetic studies were conducted in HIV-uninfected subjects. Atazanavir is rapidly absorbed after oral administration, reaching peak plasma concentrations after 2 to 3 h. Steady state is reached by around day 6 with once-daily dosing. Nonlinear pharmacokinetics were demonstrated, resulting in greater than dose-proportional increases in bioavailability over a dose range of 200 to 800 mg daily in HIV-uninfected subjects [24].

Administration of atazanavir with food enhances its bioavailability and reduces pharmacokinetic variability [25, 26]. The absolute oral bioavailability of atazanavir was not determined in humans; however, the bioavailability of atazanavir capsules relative to a solution was 68% in humans. Peak concentrations of atazanavir in humans were achieved in 1.5 h after a single oral capsule dose in the fed state, with no evidence of a plateau effect after peak levels, indicating that the absorption is rapid. Consistent with the findings in animals, approximately 79% of the administered radioactivity was recovered in the feces of humans, suggesting biliary elimination and/or incomplete absorption. Renal elimination was a minor pathway in humans, since only 13% of the radioactivity administered was recovered in the urine after a single 400 mg oral dose, with unchanged drug accounting for approximately half of that radioactivity. In humans, females have modestly higher exposures to atazanavir compared to males, but the difference ( < 25%) is considered not to be of clinical relevance. Scheme 5 shows the chemical structures of the proposed human metabolites. Atazanavir was the major circulating component in human plasma. Additionally, M2, M14, and the unidentified keto metabolite, each constituting approximately 10% of the plasma radioactivity, were present in human plasma. M2 and M14 possess no anti-HIV activity.

Atazanavir is ≥ 86% protein bound in human plasma (86% to albumin and 89% to α1-acid glycoprotein). Atazanavir is a substrate of human cytochrome P450 3A4 (CYP3A4) in vitro. Consistent with these in vitro data, ritonavir (a potent CYP3A4 inhibitor) increases [27] and efavirenz (a potent CYP3A4 inducer) decreases the exposure to atazanavir [28] following concomitant administration in humans. The inhibitory effect of low-dose ritonavir on CYP3A4 substantially increases atazanavir concentrations. Compared to the atazanavir 400 mg once daily at steady state, atazanavir AUC and Cmax for atazanavir/ritonavir 300/100 mg once daily is 4- and 12-fold greater, respectively [29]. Atazanavir inhibits, but does not induce, human CYP3A4 in vitro, and is a moderate inhibitor of uridine diphosphate glucuronosyl transferase 1A1 (UGT1A1) in vitro. In clinical studies, elevations in endogenous serum bilirubin, a UGT1A1 substrate, are observed but rarely result in treatment discontinuation.

Atazanavir requires acidic pH for dissolution. Therefore, concomitant use of medications that raise gastric pH, such as antacids, histamine (H2) blockers, and proton pump inhibitors, reduce atazanavir bioavailability. Atazanavir boosting with ritonavir and concomitant or temporal separation with acid-reducing agents decrease the effect of acid-reducing agents on atazanavir pharmacokinetics [30–32].

Pharmacodynamics

Seven non-placebo-controlled clinical pharmacology studies to evaluate the effect of atazanavir exposure on ECG parameters were completed in healthy subjects. Doses of unboosted atazanavir at 200 through 800 mg once daily were studied. ECG parameters were measured prior to and during study drug administration. Equivocal effects of atazanavir on the QTc interval were observed in these non-placebo-controlled studies. A dose- and concentration-dependent effect on PR interval was seen in these initial non-placebo-controlled studies [9].

A definitive, placebo-controlled double-blind three-treatment three-period crossover study in 72 healthy subjects was designed to determine the effect of unboosted atazanavir (400 and 800 mg) on ECG parameters, specifically the QTcB and PR intervals. While boosted atazanavir (atazanavir 300
atazanavir results in concentrations intermediate between atazanavir 400 and 800 mg [9]. In this study, atazanavir had neither a clinically significant nor a statistically significant concentration-dependent effect on the QTcB interval. Fourteen percent of patients taking atazanavir 400 mg/day developed PR intervals greater than 200 ms (normal range 120 to 200 ms). The PR prolongations was dose related, and their frequency and magnitude increased with increasing dose of atazanavir. PR prolongations at the 400-mg dose of atazanavir were all mild (≤ 250 ms). All prolongations of the PR interval were asymptomatic and not associated with significant clinical findings. No episodes of 2- or 3- AV block were observed.

Studies with other protease inhibitors demonstrated that C_{trough} (trough) concentrations of HIV protease inhibitors correlate with efficacy. Atazanavir trough concentrations and inhibitory quotient using the clinical dosing regimens of atazanavir 400 and 300 mg with ritonavir were determined in patients naive to antiretroviral therapy [33]. The inhibitory quotient was defined as the atazanavir C_{trough}/individual’s HIV-1 protein binding-adjusted EC_{50} for atazanavir against HIV and was available from 32% of subjects at baseline. In these antiretroviral-naive subjects, C_{trough} in both regimens was associated with the probability of virologic suppression at 48 weeks; however, even in the lowest C_{trough} quartile, 88% of subjects achieved virologic suppression. Total bilirubin levels and the development of jaundice were positively associated with atazanavir C_{trough} and a regimen of atazanavir 300 mg with ritonavir were associated with greater increases in fasting triglycerides and total cholesterol. Despite a statistically significant regimen effect for fasting triglycerides and total cholesterol elevations, only a weak positive correlation of atazanavir C_{trough} was noted, suggesting a stronger association of these lipid elevations with ritonavir administration rather than atazanavir C_{trough} values. Hepatic transaminase elevation (AST or ALT) and the probability of diarrhea or nausea were not associated with atazanavir C_{trough}. The relationship of atazanavir C_{trough} and total bilirubin, lipid profile, and liver transaminase changes was confirmed further in study AI424-138 (CASTLE) pharmacokinetic/pharmacodynamic analysis [34].

Clinical Trials in Antiretroviral-Naive Patients

Study AI424-007 [17] was a 48-week dose-ranging phase II study comparing the safety and efficacy of atazanavir in doses of 200, 400, and 500 mg once daily to nelfinavir 750 mg three times a day in combination with didanosine and stavudine in 420 antiretroviral naive patients. The study was blinded to atazanavir dose but included an open-label comparison to nelfinavir. Subjects received atazanavir monotherapy for 2 weeks followed by the combination regimen for 46 weeks. After 48 weeks, mean change from baseline in plasma HIV-1 RNA (−2.57 to −2.33 log_{10} copies/mL), the proportion of subjects with plasma HIV-1 RNA < 400 copies/mL (56 to 64%) and < 50 copies/mL (28 to 42%), and mean increases in CD4+ T-cell count (185 to 221 cells/mm^3) were comparable across treatment groups. Diarrhea was two to three times more common in the nelfinavir group (61% of subjects) than in the atazanavir groups (23 to 30% of subjects), and jaundice occurred only in atazanavir-treated subjects (6, 6, and 12% in the 200-, 400-, and 500-mg groups, respectively). There was minimal effect on blood lipids parameters at all atazanavir doses, in contrast to the nelfinavir arm, which experienced an increase from baseline in total cholesterol, triglycerides, and fasting low-density lipoprotein (LDL) cholesterol levels.

Atazanavir 400 and 600 mg once daily were compared to nelfinavir 1250 mg twice a day in combination with lamivudine and stavudine in another phase II study (AI424-008) in 467 antiretroviral-naive patients [18]. At 48 weeks, mean changes in plasma HIV-1 RNA (log_{10} copies/mL) from baseline to 48 weeks were −2.51, −2.58, −2.31; HIV-1 RNA < 400 copies/mL were 64, 67, and 53%; and HIV-1 RNA < 50 copies/mL were 35, 36, and 34% in atazanavir 400 mg, 600 mg, and nelfinavir arms, respectively. CD4+ T-cell count increases were similar across all arms. Adverse events were similar across treatments with the exception of diarrhea (more frequent with nelfinavir) and jaundice (more frequent with atazanavir). Unconjugated hyperbilirubinemia was reported more frequently in the higher-dose atazanavir (600 mg) arm. Similar to study AI424-007, both atazanavir arms had minimal effects on lipids compared to nelfinavir. Based on these data and the risk–benefit profile, atazanavir 400 mg once daily was chosen as the recommended dose of unboosted atazanavir.

Study AI424-044 was a rollover study of patients completing the earlier nelfinavir comparative phase of AI424-008 with plasma HIV-1 RNA fewer than 10,000 copies/mL [35]. Patients were maintained on atazanavir, or (if they received nelfinavir) switched to atazanavir and followed for 24 weeks after completing 48 weeks of study AI424-008. Atazanavir was well tolerated and effective during extended use and also in patients who switched from nelfinavir. In virologically suppressed, nelfinavir-treated patients switched to atazanavir, virologic suppression continued, whereas nelfinavir-induced lipid elevations were reversed within 12 weeks, approaching pretreatment values. Extended atazanavir use in patients who received atazanavir previously resulted in continued virologic suppression and lipid changes that were not clinically relevant.

Study AI424-034 [19] was a phase III randomized double-blind double-dummy active-controlled two-arm study in 810 patients comparing the antiviral efficacy and safety of atazanavir 400 mg administered once daily vs. efavirenz 600 mg administered once daily in combination with open-label fixed-dose zidovudine plus lamivudine twice daily in...
treatment-naive patients. At week 48, plasma HIV-1 RNA levels were lower than 400 copies/mL in 70% of patients receiving efavirenz. Immunological responses were similar in both treatment arms. Atazanavir-treated patients did not demonstrate significant increases in total cholesterol, fasting LDL cholesterol, fasting triglycerides, fasting glucose or insulin levels, and atazanavir-related bilirubin elevations resulted in treatment discontinuation in less than 1% of participants.

Boostered atazanavir (atazanavir 300 mg with ritonavir 100 mg) was compared to atazanavir 400 mg in antiretroviral naïve patients in a phase II study, AI424-089, which randomized 200 patients (95 to boosted atazanavir and 105 to unboosted atazanavir). Response rates of plasma HIV-1 RNA < 400 copies at week 48 were 86% and 85% on the boosted atazanavir and unboosted atazanavir arms, respectively. At 48 weeks, 78% of those on atazanavir and 76% of those on lopinavir/ritonavir achieved the primary endpoint of plasma HIV-1 RNA fewer than 50 copies/mL, demonstrating the noninferiority of atazanavir/ritonavir to lopinavir/ritonavir.

There were 3 and 10 patients with virologic failure in the boosted atazanavir and unboosted atazanavir groups, respectively. Plasma lipid elevations were low with both regimens. At 96 weeks, discontinuation rates were similar in both arms, but the response rates were slightly higher in the boosted atazanavir arm, with fewer virological failures than in the unboosted atazanavir arm. Increases in plasma lipids in both regimens were similar; however, there were higher rates of hyperbilirubinemia on the boosted atazanavir arm [36]. The CASTLE study (AI424-138) was a large, open-label randomized phase III study comparing atazanavir/ritonavir (300/100 mg once daily) to lopinavir/ritonavir (400/100 mg twice daily) in antiretroviral-naïve patients. The study randomized 440 and 443 patients to atazanavir/ritonavir and lopinavir/ritonavir arms, respectively. At 48 weeks, 78% of those on atazanavir/ritonavir and 76% of those on lopinavir/ritonavir achieved the primary endpoint of plasma HIV-1 RNA fewer than 50 copies/mL, demonstrating the noninferiority of atazanavir/ritonavir to lopinavir/ritonavir. Similar increases in CD4+ T-cell count were noted in both arms. The rates of discontinuation were 9 and 13% in the atazanavir/ritonavir- and lopinavir/ritonavir-containing arms, respectively. The atazanavir/ritonavir arm was associated with significantly lower increases in total cholesterol, triglycerides, and non-high-density lipoprotein cholesterol.

Atazanavir/ritonavir demonstrated higher rates of hyperbilirubinemia and lower rates of gastrointestinal events than those of lopinavir/ritonavir [22]. Noninferiority of atazanavir/ritonavir to lopinavir/ritonavir was also confirmed at week 96. Virological failure rates were low with both regimens. Atazanavir/ritonavir had a better lipid profile, such that 2% of subjects on atazanavir/ritonavir vs. 9% on subjects on lopinavir/ritonavir initiated lipid-lowering drugs on study. Additionally, atazanavir/ritonavir had a more favorable gastrointestinal tolerability than lopinavir/ritonavir [37]. Similar results were demonstrated regardless of the patients’ race or gender [38,39].

Clinical Trials in Antiretroviral-Experienced Patients

BMS studies AI424-043 and AI424-045 were conducted in treatment-experienced patients. In study AI424-045 [23], 358 patients who had failed two or more prior antiretroviral regimens were randomized to receive atazanavir/ritonavir 300/100 mg once a day, atazanavir/saquinavir 400/1200 mg once a day, or lopinavir/ritonavir 400/100 mg twice a day, each with tenofovir and another nucleoside reverse transcriptase inhibitor. The primary endpoint of time-averaged reductions in plasma HIV-1 RNA from baseline for atazanavir/ritonavir and lopinavir/ritonavir were comparable at 48 weeks (1.93 and 1.87 log10 copies/mL, respectively). Mean CD4+ T-cell count increases were 100 and 121 cells/mm3 for atazanavir/ritonavir and lopinavir/ritonavir, respectively. The efficacy of atazanavir/saquinavir was lower than lopinavir/ritonavir by both these parameters and this arm was discontinued at 24 weeks. Declines in total cholesterol and fasting triglycerides were greater with atazanavir/ritonavir and atazanavir/saquinavir than with lopinavir/ritonavir, with lipid-lowering agents used more frequently in the lopinavir/ritonavir arm than in the atazanavir arms. Hyperbilirubinemia was more common on the atazanavir/ritonavir arm, and diarrhea was more common in lopinavir/ritonavir arm, which also had greater use of anti diarrheal agents. In the extended follow-up period, to 96 weeks, once-daily atazanavir/ritonavir demonstrated durable safety and efficacy and was associated with significant reductions in total cholesterol and fasting triglycerides [40].

In study AI424-043 [20], 300 patients failing protease inhibitor–based antiretroviral therapy were randomized to receive atazanavir 400 mg once a day without ritonavir or lopinavir/ritonavir 400/100 mg twice daily, each with two nucleoside reverse transcriptase inhibitors. Lopinavir/ritonavir resulted in significantly greater reduction in plasma HIV-1 RNA than that of unboosted atazanavir at 48 weeks. However, both regimens were equally effective in patients who had no baseline nucleoside reverse transcriptase inhibitors mutations. Atazanavir resulted in either no change or decreases in fasting LDL cholesterol, total cholesterol, and fasting triglycerides, whereas lopinavir/ritonavir resulted in increases in these lipid parameters. Based on these results, unboosted atazanavir is not recommended for use in treatment-experienced patients.

Clinical Trials in Pediatric Patients

The PACTG 1020A (AI424-020) study was designed as a phase III open-label pharmacokinetics and safety study of atazanavir (powder and capsules) in combination regimens in antiretroviral-naïve and antiretroviral-experienced HIV-1-infected infants, children, and adolescents. Consistent with the adult experience, the results of AI424-020 support the fact...
that atazanavir/ritonavir is generally safe and well tolerated in pediatric populations. Compared with adults, no new safety findings related to atazanavir/ritonavir were identified in the pediatric population. The results of this study have led to the approval of pediatric dosing recommendation in adolescents and children above the age of 6 in the United States [41–43].

FUTURE DIRECTIONS

Atazanavir in combination with two nucleosides has been studied extensively in both antiretroviral treatment-naive and experienced patients. However, since nucleoside reverse transcriptase inhibitors may be associated with long-term toxicities or development of extensive nucleoside cross-resistance, studying atazanavir in new treatment regimens without nucleoside reverse transcriptase inhibitors is warranted. With the introduction of new classes of antiretroviral therapy with new mechanism of action, the use of atazanavir with these new compounds is being explored, including ongoing studies with integrase inhibitors (raltegravir [44] and elvitegravir [45]), CCR5 inhibitors (maraviroc [46] and vicriviroc [47]), as well as with new nonritonavir pharmacokinetic enhancers (such as GS 9350) [48].

Studies of boosted atazanavir monotherapy as a potential option for maintenance therapy after initial virological suppression with a full antiretroviral regimen have also been conducted. These studies have shown mixed results regarding the efficacy of boosted atazanavir monotherapy and the value of this strategy [49–51].

CONCLUSIONS

Atazanavir was developed as the first potent once-a-day protease inhibitor. The efficacy, safety, and tolerability of the compound have been demonstrated in both treatment-naive and treatment-experienced patients, and atazanavir is now recommended as part of a preferred regimen in many HIV treatment guidelines [52–54]. Atazanavir is currently approved uboosted for antiretroviral-naive patients who cannot tolerate ritonavir and boosted with ritonavir for both antiretroviral-naive and antiretroviral-experienced patients. Atazanavir is being evaluated in new treatment paradigms as antiretroviral therapy, and patients’ medical needs continue to evolve.

REFERENCES


REFERENCES


