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ISIS-301012

Antisense Inhibitor of Apolipoprotein B
Treatment of Lipoprotein Disorders

Antisense oligonucleotide targeting the 3120-3140 nucleotide residues of the coding sequence of the *APOB* gene

20-Mer antisense chimeric phosphorothioate oligonucleotide whose sequence is 5'-GCCTCAGTCTGCTTCGCACC-3' and in which nucleotides 6-15 are 2'-deoxynucleotides, nucleotides 1-5 and 16-20 are 2'-*O*-methoxyethyl (2'-MOE) nucleotides and all cytidine residues are 5-methylcytidines

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Abstract

ISIS-301012 is a second-generation antisense drug developed by Isis Pharmaceuticals for the treatment of hypercholesterolemia based on its ability to inhibit apolipoprotein B-100 (apoB-100). Preclinical studies in Hep 3B, Hep G2, human and cynomolgus monkey hepatocytes revealed that ISIS-301012 inhibited apoB-100 mRNA expression by 85% at 150 nM. Further preclinical studies indicated that ISIS-301012 reduced human apoB mRNA and serum apoB-100 protein levels in a concentration- and time-dependent manner. Plasma pharmacokinetics of ISIS-301012 were characterized by a rapid distribution phase and a prolonged elimination phase, and no pharmacokinetic interactions were observed between oral hypolipidemic agents and intravenous ISIS-301012. Administration of ISIS-301012 led to prolonged, dose-dependent reductions in both apoB and low-density lipoprotein (LDL) cholesterol. Early results from phase II clinical trials of ISIS-301012 in patients with high cholesterol demonstrated that it produced rapid, dose-dependent and prolonged reductions in apoB-100, LDL cholesterol, very-low-density lipoprotein (VLDL) cholesterol, total cholesterol and triglyceride levels, and was safe and well tolerated. Phase II clinical trials of ISIS-301012 in patients with hypercholesterolemia are ongoing.

Background

Cardiovascular disease is the leading cause of death in the United States. Studies have indicated that high cholesterol levels, which can lead to hardening of the arteries, are strongly correlated with cardiovascular disease. According to the American Heart Association, over 100 million American adults have high cholesterol and about 37 million American adults have levels of 240 mg/dl or above, which is considered high risk (1).

Low-density lipoprotein (LDL) cholesterol is a proven risk factor for cardiovascular disease and is the primary target of lipid-lowering therapies, including statins, which are among the most widely prescribed drugs in the world. Clinical studies of statins in large populations have shown positive result in terms of reducing cardiovascular disease rates. However, currently available lipid-lowering agents are ineffective in preventing cardiovascular events in a significant proportion of patients, and others cannot tolerate current therapies because of side effects. Alternative LDL cholesterol-lowering agents with mechanisms different from current lipid-lowering agents, or combination therapies with drugs that have different or complementary mechanisms, are therefore needed (1, 2).

Produced in the liver, apolipoprotein B (apoB) is an important component of all atherogenic lipoproteins, including LDL cholesterol and its precursors, intermediate-density lipoprotein (IDL) cholesterol and very-low-density lipoprotein (VLDL) cholesterol. ApoB-100 is therefore a promising target for the development of new treatment options to lower LDL cholesterol (1-3).

Developed by Isis Pharmaceuticals for the treatment of hypercholesterolemia, ISIS-301012, a 20-mer phosphorothioate oligonucleotide (5'-GCCTCAGTCT-GCTTCGCACC-3'), is a second-generation antisense drug designed to inhibit apoB-100. The agent binds to the coding region of human apoB-100 mRNA by Watson and Crick base pairing, leading to RNase H-mediated degradation of the cognate mRNA and thereby inhibiting the translation of the apoB protein (2, 4).

In 2006, the FDA granted ISIS-301012 orphan drug status for the treatment of homozygous familial hypercholesterolemia (FH), a rare genetic disorder that causes extremely high cholesterol levels and early cardiovascular disease. Phase II clinical trials of ISIS-301012 in patients with hypercholesterolemia are ongoing.

Y. Wang. IBC, 8106 Runnymeade Dr., Frederick, MD 21702, USA.

Preclinical Pharmacology

In Hep 3B, Hep G2, human and cynomolgus monkey hepatocytes, ISIS-301012 inhibited apoB-100 mRNA expression by 85% at 150 nM. Its potency was further tested in human apoB-100 transgenic mice. After twice-weekly administration at a dose of 25 mg/kg i.p., ISIS-301012 decreased human apoB-100 serum protein and hepatic mRNA levels by at least 80% for 8 weeks. However, administration of the drug had no effect on murine apoB-100 mRNA or protein (5).

To determine if selective reduction of human apoB-100 would affect the development of antherosclerosis, human apoB-100 transgenic mice were administered ISIS-301012 (20 and 50 mg/kg/week i.p.) for 14 weeks. In the study, ISIS-301012 reduced hepatic human apoB mRNA and serum apoB-100 in a dose- and time-dependent manner. At the higher dose, the antisense oligonucleotide suppressed hepatic apoB mRNA and serum apoB-100 by about 90% and 70%, respectively. Aortic sinus plaque volume was concomitantly reduced in these animals by 76% at the higher dose (6, 7).

Pharmacokinetics and Metabolism

The pharmacokinetics of ISIS-301012 administered via s.c. injection, i.v. bolus and i.v. infusion were evaluated in preclinical studies conducted in mice (2-50 mg/kg s.c. every other day x 4), rats (5 and 24 mg/kg i.v.) and monkeys (2-12 mg/kg by 1-h i.v. infusion and 20 mg/kg s.c. every other day x 4, then every fourth day), and also in a dose-escalating (50-400 mg by 2-h i.v. infusion every other day, then once weekly s.c. for 3 weeks) phase I clinical trial conducted in healthy volunteers. Characterized by a rapid distribution phase ($t_{1/2}$ < 1 h in animals and approximately 1.26 h in humans) and prolonged elimination phase ($t_{1/2}$ = 4.7 days in rats after 5 mg/kg by i.v. bolus administration; 16 days in monkeys after 4 mg/kg by 1-h i.v. infusion; 23 ± 1 days and 31 ± 11 days after 50 and 200 mg by 2-h i.v. infusion, respectively, in humans), the plasma pharmacokinetics of ISIS-301012 were generally similar across the species tested. In monkeys, the mean absolute bioavailability after s.c. injection was 82.8%, indicating nearly complete systemic absorption. In the clinical trial, peak plasma concentrations after the 2-h i.v. infusion (4.8-21.5 μg/ml) and s.c. administration (1.0-2.7 μg/ml) were also dose-dependent, and both routes also gave similar plasma AUC values. ISIS-301012 was rapidly distributed to tissues and mainly eliminated slowly in the urine (2, 4, 8). Across the concentration range tested (7.6-152 μg/ml), the majority (at least 85%) of ISIS-301012 was bound to plasma proteins in all species studied; the extent of plasma binding was greatest in humans (at least 90%) and lowest in mice (80-90%). Allometric comparison of clearance indicated that plasma clearance scaled well across species as a function of body weight alone, and the correlation improved when clearance was corrected for plasma protein binding (4).

Numerous metabolites, produced mainly via endonuclease-mediated metabolism, were detected in monkey and human urine collected within the first 24 h after dosing. These metabolites were shortened oligonucleotides with nucleotide length ranging from 7 to 14 nucleotides. Since the same metabolites were detected in tissues analyzed by using capillary gel electrophoresis, it was suggested that they were generated in tissues and subsequently excreted in urine (4).

The pharmacokinetics of oral or s.c. ISIS-301012 administered daily were also evaluated in dogs and mice over 13 weeks. Local and systemic exposure was reported in both species. In dogs, 3-10% of the dose was absorbed systemically. Consistent with previous preclinical studies, the highest tissue concentration of ISIS-301012 was observed in the kidney in both species, with a long half-life (> 14 days); slightly lower concentrations were reported in the liver. The biodistribution of the drug after oral and s.c. administration was identical, and histopathological studies indicated intracellular localization of the drug (9).

To guide dose and regimen selection for a phase II study of ISIS-301012 in patients with hypercholesterolemia, pharmacokinetic/pharmacodynamic (PK/PD) modeling and dose regimen simulation of ISIS-301012 were further studied. According to the PK/PD modeling and dose regimen simulations, the mean trough plasma levels at 2 weeks after a 13-week treatment at 50, 100 and 200 mg weekly would be approximately 5.9, 9.8 and 15.1 mg/ml, respectively, and the corresponding mean apoB reduction 17.2%, 28.7% and 40.4%, respectively (10).

Clinical Studies

The efficacy of ISIS-301012 in humans was first studied in a double-blind, randomized, placebo-controlled, dose-escalating phase I trial in 36 subjects with mild dyslipidemia (fasting cholesterol < 300 mg/dl) and a body mass index (BMI) of 30 kg/m^2 or less. The individuals were randomized to receive a single s.c. dose of ISIS-301012 (50, 100, 200 or 400 mg) or placebo, followed by a 4-week observation period, and they then received multiple doses of ISIS-301012 or placebo at the same assigned dose levels. Administration of ISIS-301012 led to dose-dependent and prolonged reductions in both apoB and LDL cholesterol, with maximum reductions from baseline of 50% and 35%, respectively, in the 200-mg group. After the last dose of ISIS-301012, the apoB and LDL cholesterol levels remained below baseline for 90 days. The AUC and the percentage reduction in apoB and LDL cholesterol were closely correlated. Total cholesterol showed a maximum reduction of 27% and 40%, respectively, in the 200- and 400-mg dose groups. Maximum reductions in triglycerides and VLDL cholesterol were 27% and 30%, respectively, in the 200-mg dose group and 43% and 60%, respectively, in the 400-mg dose group. No significant change was observed in HDL cholesterol. It appeared that a reduction in small, dense atherogenic LDL particles might be responsible in

large part for the reduction in LDL cholesterol. No serious drug-related adverse events were reported. Twenty-one of the 29 (72%) subjects who received ISIS-301012 experienced mild, painless injection-site reactions that resolved spontaneously after a median of 5 days. Drug-related asymptomatic alanine aminotransferase (ALT) elevations were reported in 4 of the 29 (14%) subjects. One of the 4 subjects had transaminase elevations > 3 times the upper limit of normal values, but values returned to normal within 2 weeks. A direct correlation between maximum ALT and maximum apoB reduction was reported in 26 subjects who completed the study. No abnormal changes in vital signs, ECGs or urinalysis were observed (2, 11-14).

The effect of dose and schedule on the efficacy and safety of ISIS-301012 was also evaluated in subjects with mild hypercholesterolemia (LDL cholesterol > 130 mg/dl). In the study, subjects (n=10 per group) received multiple doses of ISIS-301012 s.c. during a 13-week treatment period with or without loading. The first two groups received four loading doses (200 mg) of ISIS-301012 on days 1, 4, 8 and 11, followed by 100 or 200 mg every other week, and the other groups received 200, 300 or 400 mg/week without loading; 2 subjects in each group received placebo. The preliminary results (8 weeks) suggested that ISIS-301012 was effective when administered every other week. Median reductions of 12%, 22% and 42%, respectively, in LDL cholesterol and of 22%, 23% and 47%, respectively, in apoB were obtained on 100 mg every other week, 200 mg every other week and 200 mg every week; initial data for the group receiving 300 mg/week showed respective reductions of 42% and 41%. Mild erythema at the injection site was the major adverse event (15-17).

The safety and efficacy of ISIS-301012 were further evaluated in patients with hypercholesterolemia who were already on moderate stable doses of statins but failed to meet their target LDL cholesterol levels. In this double-blind, randomized, placebo-controlled, dose-escalating study, 50 patients (LDL cholesterol of 100-220 mg/dl) on a stable dose of atorvastatin or simvastatin were randomized to receive ISIS-301012 (30, 100, 200, 300 or 400 mg s.c. for 4 weeks) or placebo. Marked reductions in LDL cholesterol and apoB were observed at a dose of 200 mg. Non-HDL cholesterol was reduced by 47% in the 300-mg group. Changes in the placebo group were not significant. Again, erythema at the injection site was the most common adverse event (18, 19).

Phase II clinical trials of ISIS-301012 are ongoing in patients with hypercholesterolemia, including familial hypercholesterolemia (20-25). In May, Isis presented results for 3 patients in an ongoing phase II study in patients with homozygous FH. This study was designed mainly to assess the safety of ISIS-301012 in combination with high-dose lipid-lowering agents. However, due to the good safety profile at 5 weeks at a dose of 200 mg/week, the trial was amended to include a cohort of patients treated at 300 mg/week for 3 months. At 12 weeks, these patients achieved additional reductions in LDL cholesterol of 45-51% over and above those seen on lipid-lowering therapy, and additional reductions of 43-54% in apoB. No serious adverse events were reported (26).

Drug Interactions

Potential pharmacokinetic interactions of ISIS-301012 with oral hypolipidemic agents were studied as part of a phase I trial conducted in healthy volunteers (n=10 in each group). In the study, healthy individuals first received an oral hypolipidemic agent, either simvastatin (40 mg) or ezetimibe (10 mg), and were then given four 2-h i.v. infusions of ISIS-301012 (200 mg) over an 8-day period, with the last dose of ISIS-301012 co-administered with another dose of the oral hypolipidemic agent. Administration of ISIS-301012 alone or together with oral hypolipidemic agents yielded similar pharmacokinetic parameters, including $AUC_{0\text{-}24h}$, C_{max} and $t_{1/2}$. In addition, neither administration of ISIS-301012 alone nor co-administration with simvastatin led to significant plasma protein displacement. The results indicated a lack of pharmacokinetic interactions between ISIS-301012 and oral hypolipidemic agents (27).

Source

Isis Pharmaceuticals, Inc. (US).

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