Lack of Efficacy of an Inhibitor of PDE4 in Phase 1 and 2 Trials of Patients With Nonalcoholic Steatohepatitis

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BACKGROUND & AIMS: **ASP9831 is a phosphodiesterase-4 inhibitor developed to treat nonalcoholic steatohepatitis (NASH); it showed potent anti-inflammatory and antifibrotic effects in preclinical studies. We evaluated the efficacy and safety of ASP9831 in patients with NASH.**

METHODS: **In a phase 1 trial, we determined the optimal therapeutic window of ASP9831 in healthy volunteers and evaluated 2 doses (50 and 100 mg) in patients with NASH. Based on the positive outcomes of the phase 1 study, we performed a phase 2 trial to compare the biochemical effects of ASP9831 vs placebo. Patients with NASH were assigned randomly to groups given either 50 mg (n = 33) or 100 mg (n = 33) ASP9831 twice daily, or placebo (n = 30), for 12 weeks. The primary end point was the mean percentage change, from baseline to the end of ASP9831 administration, in serum level of alanine aminotransferase (ALT); secondary outcomes included changes in aspartate aminotransferase (AST) levels, ratio of AST:ALT, and various biomarkers of NASH.**

RESULTS: **After 12 weeks of administration, there was no significant change in mean serum levels of ALT ($P = .42$) or AST ($P = .20$) or other biomarkers in any group, and no significant differences were observed among groups. Most adverse events were mild; gastrointestinal disorders occurred more frequently in the ASP9831 groups than the placebo group.**

CONCLUSIONS: **Despite a relevant mechanism of action, ASP9831 did not significantly alter the biochemical markers of NASH, compared with placebo, in a clinical trial. This highlights the difficulties of developing therapeutics for NASH and the need for more extensive preclinical testing of mechanisms of potential drug candidates. Clinicaltrialsregister.eu: 2005-001687-31; EudraCT numbers: 2007-002114-19.**

Keywords: Fatty Liver Disease; Therapeutic Targets; Treatment; PDE4.

Abbreviations used in this paper: **AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under the plasma drug concentration time curve; AUC_{tau}, area under the plasma drug concentration time curve over dosing interval tau; cAMP, adenosine 3′,5′-cyclic monophosphate; C_{max}, maximum drug concentration; LPS, lipopolysaccharide; MCD, methionine-choline deficient; NASH, nonalcoholic steatohepatitis; PDE, phosphodiesterase; t_{max}, time to attain maximum drug concentration; TNF, tumor necrosis factor; TEAE, treatment-emergent adverse event.**

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Nonalcoholic steatohepatitis (NASH) is an increasingly prevalent liver disease worldwide. Patients at risk for NASH include those sharing clusters of comorbidities intimately related to insulin resistance (eg, obesity/overweight, type 2 diabetes, dyslipidemia).[1] Although nonalcoholic fatty liver (ie, steatosis alone or with mild lobular inflammation) usually is considered a benign, nonevolving condition, NASH is a cause of cirrhosis, end-stage liver disease, and hepatocellular carcinoma, which increases liver-related mortality 10-fold and significantly reduces survival.[2] No drug therapy currently is available for NASH; however, in some trials, thiazolidinediones or vitamin E have shown modest therapeutic efficacy on histologic parameters.[3]

NASH differs from steatosis alone by the presence of hepatic inflammation and cell injury in response to lipotoxic intermediates that accumulate or are metabolized in the steatotic liver.[4] Several experimental studies in animal models of steatohepatitis have shown the activation of intrahepatic inflammatory pathways leading to the activation of inflammatory mediators and recruitment of inflammatory cells.[5] However, selective pharmacologic blockades of these different pathways did not result in unambiguous improvement in NASH models, and they have not been proven applicable to human beings with this disease.

Adenosine 3′,5′-cyclic monophosphate (cAMP), a second messenger, mediates numerous signaling responses that result in suppression or inhibition of inflammatory-mediator release, as well as inhibition of fibrosis and smooth muscle relaxation. Intracellular levels of cAMP are modulated by phosphodiesterases (PDEs) that degrade cAMP into 5′-adenosine monophosphate.[6] The superfamily of PDE enzymes comprises 11 or more members differing in structure, substrate specificity, inhibitor selectivity, tissue and cell distribution, and regulation by kinases.[7] Inflammatory cells crucial to the pathogenesis of inflammatory diseases preferentially express PDE4, which has led to the development of highly potent and selective PDE inhibitors. These compounds increase the intracellular levels of cAMP and reduce the activation of a wide range of inflammatory cells.[8] Some of these compounds have been approved (ie, roflumilast) or are in development for inflammatory diseases (eg, asthma, chronic obstructive pulmonary disease, arthritis, inflammatory bowel disease, and skin inflammatory disorders). Pentoxifylline, a nonselective inhibitor of PDEs, reduces some lethal complications of end-stage cirrhosis (eg, encephalopathy, bacterial infection) and increases complication-free survival.[9] It is not known if these effects resulted from the inhibition of PDEs or tumor necrosis factor (TNF)-α synthesis.

ASP9831 is a new PDE4 inhibitor with anti-inflammatory properties mainly directed at activated macrophages and Kupffer cells. Upon stimulation by lipopolysaccharide (LPS), ASP9831 inhibits TNF-α production, in the nanomolar range, from both human and rat peripheral blood mononuclear cells (data on file; Astellas Pharma Europe BV, Leiden, The Netherlands). Moreover, ASP9831 significantly reduced alanine aminotransferase (ALT) activity with a dose effect in 3 distinct acute hepatitis models: D-galactosamine and D-galactosamine plus LPS in rats, and concanavalin A in mice (data on file; Astellas Pharma). When administered concurrently with a methionine-choline deficient (MCD) diet for 16 weeks, ASP9831 achieved 40% to 50% reduction in aminotransferase levels and improved histologic necroinflammation at doses of 1 and 3 mg/kg. There was a significant reduction in hydroxyproline hepatic content and an improvement in fibrosis score, suggesting that ASP9831 also might have antifibrotic effects as a result of its anti-inflammatory activity (data on file; Astellas Pharma). These preclinical data strongly support an anti-inflammatory and antifibrotic potential for ASP9831 in chronic models of steatohepatitis and prompted the initiation of phase I and IIa clinical trials in healthy volunteers and patients with NASH.

Methods

The Supplementary Materials and Methods section describes in detail the methodology and patient eligibility criteria.

In brief, the phase I study first evaluated single- and multiple-ascending doses of ASP9831 in healthy volunteers. Outcome variables were frequency and severity of adverse events (AEs) and pharmacokinetic parameters for plasma and urine. Ex vivo TNF-α production was assessed as a biomarker for inflammation, and safety data were collected throughout the study. ASP9831 then was evaluated in patients with NASH to assess whether fibrotic liver injury in NASH significantly modifies the pharmacokinetic parameters. Based on the results obtained in healthy volunteers, 2 doses of ASP9831 (50 and 100 mg) were administered sequentially to patients with NASH and advanced fibrosis for 8 days (Supplementary Figure 1*A*). Outcome variables were similar to those in healthy volunteers (Supplementary Materials and Methods).

The phase II study was a randomized, double-blind, parallel-group, placebo-controlled study investigating the effect of 12 weeks of administration of 50 and 100 mg ASP9831 twice daily vs placebo on serum level of ALT, various liver injury biomarkers in NASH, and safety (Supplementary Figure 1*B*). Adult outpatients (age, $\geq$18 y) with biopsy-confirmed NASH and increased serum ALT level ($\geq$1.5 $\times$ upper limit of normal: 41 U/L for men and 31 U/L for women, but $\leq$300 U/L) at screening were included and randomized to one of the treatment groups in a 1:1:1 ratio (Supplementary Materials and Methods). Each subject signed an institutional review board–approved written informed consent form.

The primary efficacy variable was the percentage change in serum ALT level after 12 weeks of administration vs baseline. Because this was a proof-of-concept study to evaluate the efficacy of ASP9831 in NASH, a biochemical