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TOPIC HIGHLIGHT

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Treatment of chronic viral hepatitis with nitazoxanide and second generation thiazolides

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Abstract

Nitazoxanide, the first thiazolide, was originally developed for the treatment of *Cryptosporidium parvum*. More recently, antiviral activity of nitazoxanide against hepatitis B virus (HBV) and hepatitis C virus was recognized in *in vitro* systems. These basic studies led to phase Ⅱ clinical trials that demonstrated the safety and efficacy of nitazoxanide in combination with peginterferon, with or without ribavirin, in the treatment of chronic hepatitis C genotype 4. The sustained virologic response rate was 79% and 80% in two studies, which was higher than the response rate of 50% with the standard of care with peginterferon plus ribavirin. In very preliminary studies of patients with chronic hepatitis B, nitazoxanide suppressed serum HBV DNA and led to loss of hepatitis B e antigen in the majority of patients and hepatitis B surface antigen in approximately a quarter of patients. Randomized controlled studies of naive and nonresponder patients with chronic hepatitis C genotype 1 are underway, new second generation and controlled release thiazolides are being developed, and future studies of patients with chronic hepatitis B are planned.

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INTRODUCTION

Nitazoxanide (Alinia®, Romark Laboratories, L.C., Tampa, Fl, USA), the first thiazolide, was licensed in the USA for the treatment of *Cryptosporidium parvum* and *Giardia lamblia* in immunocompetent adults and children in 2002[1]. A number of emerging basic and clinical studies support an additional role of nitazoxanide in the treatment of chronic hepatitis C virus (HCV) and chronic hepatitis B virus (HBV) infection. This brief review summarizes current data from emerging phase Ⅱ studies related to this new potential use of nitazoxanide combined with peginterferon and ribavirin for the treatment of chronic hepatitis C and very preliminary experiences with nitazoxanide for the treatment of chronic hepatitis B.

ANTIVIRAL MECHANISM OF ACTION OF NITAZOXANIDE

The antiviral mechanism of action of nitazoxanide is different from the mechanism of action in protozoa and anaerobic bacteria *via* direct inhibition against the pyruvate-ferrodoxin oxidorectase reaction[1], and appears in recent studies to involve activation of the protein kinase activated by double-stranded RNA (PKR), an interferon-induced mediator of the cellular antiviral response[2]. The activation of PKR results in phosphorylation of its substrate, eukaryotic initiation factor 2 alpha (eIF2α). Nitazoxanide, thus, represents a new class of small molecules that modulate host antiviral pathways. By targeting a host function, the barrier to development of

antiviral resistance is significantly higher than for drugs directly targeting a viral function.

IN VITRO ACTIVITY OF NITAZOXANIDE AGAINST HBV AND HCV

After nitazoxanide was serendipitously suspected as active against viral hepatitis in patients with acquired immune deficiency syndrome (AIDS) treated with nitazoxanide for *Cryptosporidium*, the antiviral activities of nitazoxanide and its metabolite, tizoxanide, were confirmed in standard antiviral assays *in vitro*[3,4]. Both nitazoxanide and tizoxanide are potent inhibitors of HBV, and in combination with other antiviral agents such as lamivudine or adefovir, show synergistic effects. Nitazoxanide and tizoxanide are also effective against HBV-resistant mutants to lamivudine and adefovir. Additionally, both nitazoxanide and tizoxanide are potent inhibitors of HCV in genotype 1a- and 1b-derived replicon cells and genotype 2a cell culture models, and synergistic effects are observed when tizoxanide is combined with interferon[3]. Three days of pretreatment of the HCV replicon model with nitazoxanide sensitizes the virus to the effects of subsequent treatment with interferon, providing support to the clinical studies underway using a nitazoxanide lead-in phase prior to combination therapy.

LACK OF DIRECT ANTIVIRAL RESISTANCE TO NITAZOXANIDE

Studies were carried out in HCV replicons exposed to increasing concentrations of nitazoxanide or tizoxanide over 24 wk in an attempt to produce resistance to nitazoxanide and tizoxanide[5]. This serial passage did not reduce the susceptibility of HCV replicons to interferon, ribavirin, or 2'-C-methyl-cytidine, indicating that nitazoxanide and tizoxanide do not induce resistance to these agents.

TREATMENT OF CHRONIC HEPATITIS C

Completed studies

Romark made the decision to initially focus on the potential treatment of chronic hepatitis C with nitazoxanide. Three phase Ⅱ studies of nitazoxanide for the treatment of chronic hepatitis C have been completed and communicated in publications or presentations at national and international meetings[6-8]. The first study was a randomized, double-blind, placebo-controlled study of the treatment of chronic hepatitis C with nitazoxanide 500 mg twice daily in 50 adult patients with chronic hepatitis C infected with genotype 4[6]. Seven of 23 patients (30%) had a virologic end-of-treatment response (ETR) with undetectable virus, and a sustained virologic response (SVR) with undetectable virus 24 wk after the completion of treatment was observed in 4 of 23 patients (17%). All responders had low serum HCV RNA levels less than 400 000 IU/mL. This study was the first use of nitazoxanide in patients with chronic hepatitis C and for a longer time period than for its use for cryptosporidiosis and giardiasis, and the drug was well tolerated with the same number of mild gastrointestinal adverse events in the treated and placebo groups.

A second randomized, double-blind, placebo-controlled study (STEALTH C-1) evaluated the effects of nitazoxanide plus peginterferon alfa-2a (dual regimen) or nitazoxanide plus peginterferon alfa-2a and ribavirin (triple regimen) *versus* the standard of care (SOC) with peginterferon alfa-2a and ribavirin in 96 treatment-naive patients with chronic hepatitis C infected with genotype 4[7]. Nitazoxanide 500 mg twice daily was administered over a 12-wk lead-in followed by an additional 36 wk in combination with peginterferon alfa-2a 180 μg weekly with or without ribavirin 1000-1200 mg daily. The SOC group received the same doses of peginterferon alfa-2a and ribavirin for 48 wk. A rapid virologic response (RVR; undetectable serum HCV RNA after 4 wk of combination therapy) occurred in 38%, 54%, and 64% of patients receiving the SOC, dual regimen and triple regimen, respectively ($P = 0.048$, SOC *vs* triple regimen). A complete early virologic response (cEVR; undetectable serum HCV RNA at week 12 of combination therapy) occurred in 70, 68 and 86% of the SOC, dual regimen and triple regimen, respectively. The SVR rates at 24 wk post-treatment were 50%, 61% and 79%, respectively, demonstrating a 29% difference between the SOC and the triple regimen with nitazoxanide ($P = 0.023$).

A third open label study was carried out in 44 patients; 40 were infected with genotype 4, and 3 were infected with genotype 1, and 1 infected with genotype 2[8]. This study evaluated a shorter 4-wk lead-in phase with nitazoxanide 500 mg twice daily followed by 36 wk of treatment with a combination of nitazoxanide with peginterferon alfa-2a 180 μg weekly without ribavirin, and the results were compared with the historical results from the STEALTH C-1 study. The RVR, cEVR, and SVR rates were 59%, 82% and 80%, respectively, and the SVR rate of 80% in this study was significantly higher than the historical SVR rate of 50% in the SOC group ($P = 0.006$). The 3 patients infected with genotype 1 and the single patient infected with genotype 2 all had an SVR. The SVR rate of 80% raises the possibility using nitazoxanide in place of ribavirin, which requires further study.

In both this study and the STEALTH C-1 study, the administration of nitazoxanide in combination with peginterferon, with or without ribavirin, was associated with a low relapse rates (3 of 38 in the current study; and 3 of 20 patients receiving dual therapy and 1 of 23 patients receiving triple therapy, compared with 10 of 30 patients in the SOC arm in the STEALTH C-1 study). These low relapse rates, with or without ribavirin, suggest the possibility that nitazoxanide might play a similar role as ribavirin in reducing the relapse rate and be a possible substitute for ribavirin in combination with peginterferon for the treatment of chronic hepatitis C.

The results of these preliminary studies in Egypt in patients predominantly infected with genotype 4 were met with considerable interest in the hepatology community; but, studies conducted in the United States and the

response to nitazoxanide in combination with the SOC in patients with genotype 1 were recognized as the next steps required to confirm these initial interesting findings.

Ongoing studies

A phase Ⅱ randomized, double-blind, placebo-controlled study with of nitazoxanide or placebo monotherapy (2:1 randomization) over a 4-wk lead-in phase followed by nitazoxanide or placebo in combination with peginterferon alfa-2a plus ribavirin is currently underway in 112 naive patients infected with genotype 1 at 13 USA study sites. The preliminary results of the early virologic responses will be communicated in early 2009.

A second phase Ⅱ, randomized, double-blind, placebo controlled study of nitazoxanide with peginterferon and ribavirin is being conducted in 10 USA sites in 64 patients who are prior nonresponders to peginterferon and ribavirin. Preliminary results from this study will also be communicated in 2009.

CONTROLLED RELEASE TABLET

Studies have recently been initiated to study the pharmacokinetics, viral kinetics, and tolerability of a controlled release tablet of nitazoxanide in adult healthy volunteers as well as a phase Ⅱ study in patients with chronic hepatitis C[9]. The new nitazoxanide controlled release tablet contains 675 mg of the drug, and kinetics has been evaluated using either 675 mg or 1350 mg twice daily for 7 d in a phase I study. The pharmacokinetics profile is substantially improved compared to the standard tablet, with higher blood levels and an increased area under the curve of approximately 70%.

In a subsequent randomized, controlled trial, 40 treatment-naive patients with chronic hepatitis C genotype 4 have been allocated to receive either 675 mg or 1350 mg or placebo twice daily for 4 wk followed by the same regimen plus the addition of peginterferon alfa-2a 180 μg weekly and ribavirin 1000 or 1200 mg daily based on body weight. An early interim analysis has shown that the mean reduction in serum HCV RNA from baseline to week 8 (after 4 wk of combination therapy) were 5.45, 5.25, and 2.75 in the high-dose, low-dose and placebo groups, respectively[9]. Nitazoxanide was well tolerated without gastrointestinal toxicity.

TREATMENT OF CHRONIC HEPATITIS B

Nitazoxanide alone has shown preliminary evidence of efficacy in the treatment of chronic hepatitis B over a one year course of therapy[10]. Nitazoxanide 500 mg twice daily resulted in a decrease in serum HBV DNA in all of 4 HBeAg-positive patients, with undetectable HBV DNA in 2 of 4 patients, loss of HBeAg in 3 patients, and loss of HBsAg in one patient. Seven of 8 HBeAg-negative patients treated with nitazoxanide 500 mg twice daily had undetectable HBV DNA and 2 had loss of HBsAg. Additionally, nitazoxanide monotherapy in one case and nitazoxanide plus adefovir in another case resulted in undetectable HBV DNA, loss of HBeAg and loss of HBsAg[11]. These preliminary studies showed a higher rate of HBsAg loss than any currently licensed therapy for chronic hepatitis B. The similar mechanism of action of interferon and nitazoxanide suggest that stand-alone nitazoxanide therapy or nitazoxanide in concert with nucleos(t)ide analogs have the potential to increase loss of HBsAg, which is the ultimate end-point of therapy. A formal phase Ⅱ study is being planned for 2009.

SECOND GENERATION THIAZOLIDES

Newer thiazolide analogs have been identified with higher specific activity against HBV and HCV. The objective of this search for alternative thiazolides is to identify compounds that do not have antiparasitic or antibacterial activity *via* direct inhibition against the pyruvate-ferredoxin oxidoreductase reaction and increased antiviral activity. There are several lead compounds that have undergone preliminary study in experimental replicon models and have the potential to move into clinical trial.

CONCLUSION

Nitazoxanide shows significant activity against HBV and HCV, both in cell culture models as well as in patients with chronic hepatitis B and C. The HCV antiviral mechanism of action has been elucidated to involve up-regulation of PKR and eIF2α phosphorylation, which enhances natural cellular antiviral mechanisms without induction of antiviral resistance. Second generation molecules in controlled release formulations are in development. Interim results of the American phase Ⅱ trial involving treatment of naive and nonresponder patients with chronic hepatitis C infected with genotype 1 will be reported in early 2009. The thiazolides have a very favorable toxicity profile with a very low incidence of mild gastrointestinal side effects.

There are many future potential uses of nitazoxanide in treatment regimens for chronic hepatitis C. Nitazoxanide might allow reduction in the duration of a standard peginterferon-based regimen, as the results in the phase Ⅱ studies of patients with genotype 4 were achieved with 36 wk of combination therapy versus the usual 48 wk used in the current SOC. Further studies using even shorter duration of combination therapy, i.e. 24 wk, are warranted. It is also possible that the interferon-like mechanism of action of nitazoxanide will allow use of a reduced dose of peginterferon in combination treatment regimens. The findings of high SVR rates with nitazoxanide in combination with peginterferon without ribavirin require confirmation, but raise the possibility that nitazoxanide might be used in the place of ribavirin and avoid the substantial hematologic and other side effects of this drug. Finally, nitazoxanide might be used with a reduced dose of peginterferon or even without peginterferon in combination with a protease inhibitor and/or polymerase inhibitor as part of an all-oral cocktail for treatment of chronic hepatitis C, avoiding the need for injectable interferon with all of its side effects.

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