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Nitazoxanide, a new drug candidate for the treatment of Middle East respiratory syndrome coronavirus

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Summary Nitazoxanide is a broad-spectrum antiviral agent undergoing clinical development for treatment of influenza and other viral respiratory infections. Nitazoxanide exhibits *in vitro* activity against Middle East respiratory syndrome coronavirus (MERS-CoV) and other coronaviruses, inhibiting expression of the viral N protein. Nitazoxanide also suppresses production of pro-inflammatory cytokines in peripheral blood mononuclear cells and suppresses interleukin 6 production in mice. Having been used extensively in clinical trials and in post-marketing experience, nitazoxanide is an attractive drug candidate for treatment of Middle East respiratory syndrome. Future research should include *in vitro* mechanism studies, animal models of MERS-CoV infection, clinical trials, including dose-ranging trials, and evaluation of combination therapy with other potential MERS-CoV antivirals.

Introduction

Middle East respiratory syndrome coronavirus (MERS-CoV) is an emerging viral disease of global concern. More than three years after the first discovery of MERS-CoV in 2012, fundamental questions related to its epidemiology, pathogenesis, immune responses and optimal treatment remain unanswered. Nevertheless, it is associated with a high rate of mortality and there is no approved antiviral treatment. Host-directed therapies and the repurposing of existing drugs have been proposed as promising strategies for the development of MERS-CoV-specific antiviral therapy [1].

Nitazoxanide is a broad-spectrum antiviral agent undergoing development for the treatment of influenza and other viral respiratory infections. Originally developed as an antiprotozoal agent, immediate-release dosage formulations of nitazoxanide are licensed in the United States for the

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treatment of intestinal infections caused by *Cryptosporidium parvum* and throughout Latin America, India, Bangladesh and Egypt as a broad-spectrum antiparasitic agent. A new extended-release oral tablet has been developed to deliver the drug systemically, and nitazoxanide is being repurposed for use in treating viral respiratory infections. It is presently undergoing Phase 3 clinical development for treating acute uncomplicated influenza [2].

In vitro, tizoxanide, the active circulating metabolite of nitazoxanide, inhibits the replication of a broad range of influenza A and B strains including influenza A subtypes H1N1, H3N2, H3N2v, H3N8, H5N9, H7N1 and oseltamivir- and amantadine-resistant strains. The concentrations required to inhibit viral replication by 50% (IC_{50}s) are between 0.2 and 1.5 μg/ml in multiple human and canine cell lines using single-step virus growth with high multiplicity of infection (5 PFU/cell) and multi-step growth with low multiplicity of infection (0.001 PFU/cell). These IC_{50} concentrations are easily achieved in humans following administration of nitazoxanide extended-release tablets, as peak and trough plasma concentrations during repeated twice daily dosing have been reported to be 4.6 and 0.8 μg/ml, respectively. Tizoxanide acts synergistically with oseltamivir and zanamivir in inhibiting *in vitro* replication of influenza viruses, and it exhibits a high barrier to resistance with no decrease in sensitivity to influenza A viruses after passage for 30 days in increasingly sub-inhibitory concentrations of drug [2—4].

In addition to influenza viruses, tizoxanide inhibits replication of a broad range of other RNA and DNA viruses in cell culture assays, including respiratory syncytial virus, parainfluenza, coronavirus, rotavirus, norovirus, hepatitis B, hepatitis C, dengue, yellow fever, Japanese encephalitis virus and human immunodeficiency virus [2]. The broad-spectrum antiviral activity of tizoxanide is attributed to interference with host-regulated pathways involved in viral replication, rather than a virus-targeted mechanism. These pathways may include interferon or mTORC1 signaling pathways [2,5]. In the case of influenza, tizoxanide ultimately blocks the maturation of viral hemagglutinin at the post-translational stage. It does not affect the neuraminidase glycoprotein (the target of oseltamivir, zanamivir and peramivir) or the M2 protein (the target of amantadine), and it has no effect on viral infectivity, adsorption or entry into target cells [3].

Importantly, nitazoxanide has been studied extensively in humans and has undergone preclinical and clinical testing required for licensure in the United States as a treatment of diarrhea caused by *Cryptosporidium parvum* and *Giardia lamblia*. Exposure in clinical trials has included specific experience in patients with influenza and influenza-like illnesses and extended courses of treatment (up to 48 weeks) in patients with chronic hepatitis C. Worldwide, it is estimated that more than 150 million people have been treated with nitazoxanide for the treatment of intestinal parasitic infections [2].

In vitro activity against MERS-CoV and other coronaviruses

In vitro studies have shown that tizoxanide inhibits replication of canine coronavirus S-378 grown in A72 cells with an IC_{50} of 1 μg/ml [2]. Other studies have shown that nitazoxanide inhibits murine coronavirus, mouse hepatitis virus strain A59 (MHV-A59), bovine coronavirus strain L9 (BCoV-L9) and human enteric coronavirus 4408 (HECoV-4408) grown in mouse astrocytoma DBT and fibroblast 17Cl-1 cells with IC_{50}s of approximately 0.3 μg/ml. In these studies, nitazoxanide inhibited expression of the viral N protein [7].

The parent compound, nitazoxanide, and the metabolite, tizoxanide, generally show similar inhibitory activity against viruses *in vitro*. Both compounds have been shown to inhibit MERS-CoV cultured in LLC-MK2 cells with IC_{50}s of 0.92 and 0.83 μg/ml for nitazoxanide and tizoxanide, respectively. Notably, these *in vitro* IC_{50}s are similar to those observed for influenza and other viruses.

Effect on production of pro-inflammatory cytokines, including interleukin 6 (IL-6)

In addition to its antiviral activity, nitazoxanide inhibits the production of pro-inflammatory cytokines TNF-α, IL-2, IL-4, I-5, IL-6, IL-8 and IL-10 in peripheral blood mononuclear cells (PBMCs) (Romark Laboratories, personal communication [6]). *In vivo*, oral administration of nitazoxanide in mice at a dose of 100 mg/kg given two hours before a 1-mL intraperitoneal injection of 4% thioglycollate (TG) reduced plasma IL-6 levels six hours after TG injection by 90% compared with vehicle-treated mice [8]. The relevance of these data to humans has not been studied, but these data suggest that nitazoxanide could improve outcomes in patients infected with MERS-CoV by suppressing overproduction of pro-inflammatory cytokines, including IL-6.

Human clinical trials in viral respiratory infections

Nitazoxanide extended-release tablets are currently undergoing clinical development for the treatment of acute uncomplicated influenza.

A phase 2b/3 randomized, double-blind, placebo controlled trial was conducted in 74 outpatient clinics in the United States between December 2010 and May 2011. Subjects who were 12—65 years of age with fever $\geq$38 °C and at least one respiratory symptom and one constitutional symptom of influenza were enrolled within 48 h of symptom onset while flu was circulating in the community. Subjects who had been vaccinated for seasonal influenza and those at high-risk for influenza-related complications were excluded. The subjects received either 600 mg of nitazoxanide, 300 mg of nitazoxanide or placebo orally twice daily for five days and were followed for 28 days. Each subject maintained a diary and graded each of nine symptoms as absent, mild, moderate or severe twice daily for seven days, or longer if all symptoms had not returned to absent or mild by day 7. The primary endpoint was the time from the first dose until the alleviation of symptoms (all symptoms mild or moderate for at least 24 h). The primary analysis was by intention-to-treat for subjects with influenza infection confirmed by RT-PCR or culture at baseline [9].

A total of 624 subjects were enrolled, 257 of whom had influenza identified from nasopharyngeal swabs collected at baseline. Subjects who received nitazoxanide experienced shorter times to alleviation of symptoms compared with subjects who received placebo. The median times from first dose to symptom alleviation were 95.5 h (95% CI 84.0—108.0; $p = 0.0084$) for subjects who received 600 mg of nitazoxanide and 109.1 h (95% CI 96.1—129.5; $p = 0.521$) for subjects who received 300 mg of nitazoxanide, compared with 116.7 h (95% CI 108.1—122.1) for subjects who received placebo. The lack of statistical significance in the low dose group may have been due to a lack of statistical power. Adverse events were similar for the three groups, the most common being headache reported by 24 subjects (11%) in the placebo group, 12 subjects (6%) in the low-dose group and 17 subjects (8%) in the high-dose group, and diarrhea reported by 7 subjects (3%) in the placebo group, 4 subjects (2%) in the low-dose group and 17 subjects (8%) in the high-dose group [9].

Based upon data from the Phase 2b/3 trial, nitazoxanide extended-release tablets (600 mg) administered twice daily for five days are undergoing Phase 3 clinical development for treatment of acute uncomplicated influenza. Nitazoxanide is being studied as a monotherapy and in combination with oseltamivir [3].

Recommendations for future research

Existing data that suggest a potential role for nitazoxanide in treating MERS-CoV include (i) *in vitro* activity of nitazoxanide against MERS-CoV and other coronaviruses, (ii) inhibition of pro-inflammatory cytokines in PBMCs and inhibition of IL-6 production in mice, (iii) activity in reducing the duration of symptoms of influenza in humans, and (iv) a favorable safety profile demonstrated in clinical trials and in widespread post-marketing use. Future research should include:

- *In vitro* studies of the effect of nitazoxanide on viral proteins to further elucidate the mechanism of action against coronaviruses.
- Animal models of MERS-CoV infection.
- Clinical trials that evaluate different doses of nitazoxanide.
- Combination therapies of nitazoxanide with other proposed antivirals for MERS-CoV should be studied *in vitro* and then potentially in clinical trials.

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Competing interests

None declared.

Ethical approval

Not required.

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