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RESEARCH ARTICLE

NITAZOXANIDE: ANTIVIRAL PROPERTIES RELEVANT TO CURRENT GLOBAL SITUATION

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ABSTRACT

Nitazoxanide is a broad-spectrum, orally active anti-infective initially developed for the treatment of protozoal disease. However, nitazoxanide and its active metabolite, tizoxanide, have demonstrated antiviral activity against a variety of viruses in preclinical and clinical settings. This review of the literature aims to present the pharmacological profile of nitazoxanide, mechanisms of antiviral action, and its demonstrated and potential antiviral applicability.

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INTRODUCTION

Nitazoxanide is a broad-spectrum, orally active nitrothiazolysalicylamide anti-infective drug targeting extracellular and intracellular classes of pathogens including protozoa, helminths, anaerobic and microaerophilic bacteria, and viruses. It is approved by the US Food and Drug Administration and other health agencies worldwide as a first-line, standard treatment of *Cryptosporidium parvum* or *Giardia lamblia* infection in healthy subjects (Anderson & Curran, 2007). Nitazoxanide is also used in the treatment of patients with HIV coinfected with intestinal classes of parasites such as protozoa or helminths, in addition to enteroviruses (Shakya *et al*., 2017).

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Indications and Applications: Although originally developed and commercialized for treatment of protozoal disease, the antiviral properties of nitazoxanide and its metabolite, tizoxanide, have generated increasing interest in recent years due to its demonstrated ability to act as a broad-spectrum antiviral agent, through multiple mechanisms of action that appear to vary by virus type but that in general interfere with host-regulated pathways – including interferon or mTORC1 signalling pathways – involved in viral replication (Rossignol, 2014; Rossignol, 2016). These antiviral mechanisms include actions triggering inhibition of viral RNA and DNA replication, direct inhibition of viral protein expression, interference in host cellular metabolism, as well as circumvention of viral immune evasion mechanisms (Rossignol, 2014; Hickson *et al*., 2018). Importantly, the antiviral effect of nitazoxanide in triggering host immune functions rather than on the virus directly may represent a

barrier to development of drug resistance (Keefe & Rossignol, 2009). Nitazoxanide appears to modulate the innate immune response after a pathogen has entered a susceptible cellular target, a time at which amplification of the host immune control of viral proliferation is critical, especially for viruses that employ immune avoidance mechanisms to avoid triggering the innate immune response (Rossignol, 2016). Use of nitazoxanide has also been suggested in strategies aimed at preventing viral infection or eradication of viral reservoirs, which can potentially persist for varying periods of time (Jasenosky *et al*., 2019).

Safety Profile: The safety profile of nitazoxanide is well-known, having been evaluated in extensive pharmacological testing in animals and humans. It is considered to be a generally well-tolerated drug, especially in comparison to other drugs used in the treatment of intestinal parasitic infections, such as metronidazole (Rosenthal, 2017). Post-marketing surveillance studies estimate that more than 75 million adults and children have been exposed to nitazoxanide since its registration in the United States, and no significant drug-related safety concerns have emerged (Rossignol, 2014). Of particular note, Taubel *et al*. (2014) noted no effect on cardiac repolarization in a clinical study evaluating cardiac safety of nitazoxanide. Known side effects include systemic effects (asthenia, fever, pain, allergic reactions), central nervous system effects (dizziness, somnolence, insomnia), gastrointestinal effects (vomiting, dyspepsia, anorexia), urogenital effects (urine discoloration, dysuria, amenorrhea), metabolic side effects (increased SGPT), hematological effects (anemia, leukocytosis), dermatologic (rash, pruritis), respiratory, and cardiovascular effects, among others (Wishart *et al*., 2017). These side effects occur at a rate of <1%.

Absorption, Distribution, Metabolism, Elimination: Following oral administration and gastrointestinal absorption, nitazoxanide is rapidly hydrolyzed by plasma esterases to form tizoxanide, its active plasma protein-bound circulating metabolite, which then is glucurono-conjugated in the liver (Rossignol, 2014) and eliminated in the urine and bile. Nitazoxanide absorption is significantly improved when administered with food and this factor, together with the relatively short half-life of tizoxanide in plasma (1.3h) and the potential impairment of oral drug absorption in seriously ill or hospitalized patients, must be taken into consideration when the drug is used in clinical trials of conditions other than intestinal infections (Rossignol, 2014, Gamiño-Arroyo *et al*., 2019).

Antiviral Mechanisms of Nitazoxanide: In contrast to the direct mode of action in the setting of its initial indication an antiparasitic drug, nitazoxanide antiviral mechanisms act selectively at the post-translational level and interfere with viral intracellular trafficking and insertion into the host plasma membrane, rather than exerting a direct effect on the virus (Pizzorno *et al*., 2019). Several mechanisms have been proposed for the observed action of nitazoxanide against specific viruses, and most of them are likely derived from inhibition of mitochondrial oxidative phosphorylation (Rossignol & Bréchot, 2019). Proposed antiviral mechanisms based on the results of cell-based assays include enhancement or induction of IFN-stimulated gene (ISG), depletion of ATP-sensitive intracellular Ca(2+) stores, and studies point to a weak association between nitazoxanide and PKR activation (Ashiru *et al*., 2014; Elazar *et al*., 2009; LaFrazia *et al*., 2013; Li *et al*., 2017; Piacentini *et al*., 2018; Rossignol *et al*., 2009b; Sekiba *et al*., 2018). Nitazoxanide also appears to decrease pro-inflammatory cytokine levels in peripheral blood mononuclear cells (McKimm-Breschkin *et al*., 2018).

Various authors have reported viral inhibition in tissue culture and small animal models by nitazoxanide and its circulating metabolite tizoxanide, at concentrations ranging between 0.2-1.5µg/ml (Rossignol *et al*., 2009a, Ashton *et al*., 2010, Belardo *et al*., 2011, Sleeman *et al*., 2014, Gubareva *et al*., 2014). These include respiratory viruses such as influenza A and B (Influenza A subtypes H1N1, H3N2, H3N2v, H3N8, H5N9, H7N1, H1N1-PR8, and H5N9; Influenza B subtype Parma/3/04), hepatitis B, hepatitis C, ebola, and viral infections of the gastrointestinal tract. In the case of flaviviruses, nitazoxanide has been shown in cell cultures to inhibit the protease activity of the MBP-NS3 and His-NS2B heterocomplex in zika virus and Japanese encephalitis virus (Li *et al*., 2017; Shi *et al*., 2014). Antiviral action of nitazoxanide against paramyxoviruses has been attributed to the targeting of F protein folding by ERp57 inhibition. ERp57 is a member of the protein disulfide isomerase family located in the endoplasmic reticulum; silencing of this protein's expression in cell cultures has been reported to decrease viral replication (Piacentini *et al*., 2016; McKimm-Breschkin *et al*., 2018). In vitro tests evaluating the action of nitazoxanide on chikungunya virus indicated its ability to suppress cell-to-cell viral transmission, block the cytopathogenic effects induced by the virus, and limit viral entry, genome synthesis, and viral release (Wang *et al*., 2016). Importantly, the antiviral IC_{50} concentrations observed in preclinical settings can be achieved in humans following oral administration of nitazoxanide, with respective peak and trough plasma concentrations of 4.6 and 0.8µg/ml (Rossignol, 2016)

Viral Respiratory Tract Infections

Influenza: Nitazoxanide has been evaluated in the treatment of influenza viruses, which remain a public health problem despite the development of new antivirals. The emergence of new circulating strains, in addition to the emergence of resistant strains to classic antivirals, necessitates the development of new treatments or the identification of existing treatments that may be effective (Pizzorno *et al*., 2019). It is here that nitazoxanide is one candidate drug that showed promise among candidates for drug repurposing. Advantages include long-time market use and availability of post-marketing surveillance data together with an acceptable safety profile. In the case of influenza, the mechanism of action of the tizoxanide metabolite is proposed to result from selective blockade of influenza viral hemagglutinin maturation, thus impairing viral hemagglutinin intracellular trafficking and insertion of the viral protein into the host membrane (Rossignol *et al*., 2009). Tilmanis *et al*. (2017) demonstrated the in vitro susceptibility of influenza virus strains to tizoxanide, reporting on the results of 210 circulating influenza A and B strains. Nitazoxanide has also demonstrated synergistic effects in combination with neuraminidase inhibitors oseltamivir and zanamivir in cell culture assays of influenza A, including amantadine-resistant and oseltamivir-resistant strains (Belardo *et al*., 2015). Early results from placebo-controlled phase II studies showed promise in time-to-resolution outcomes of symptomatic treatment of viral respiratory infection in children using twice-daily oral doses of 100-200mg (Lopez-Chegne *et al*., 2011a); similar results were

reported among adolescents and adults at oral dose of 500mg (Lopez-Chegne *et al*., 2011a). A 2014 study comparing the 5-day use of 600mg and 300mg dosing regimens with placebo in the treatment of acute uncomplicated influenza infection reported reduction of symptom duration in the 600mg treatment group with minor adverse events (Haffizulla *et al*.,2014). While these studies showed promising results, further confirmatory studies are warranted, especially in light of a recent study that reported duration of hospitalization with nitazoxanide 600mg twice daily vs. placebo was similar among children and adults infected with influenza A and B strains and other respiratory viruses (Gamiño-Arroyo *et al*., 2019).

Other respiratory viruses: Tizoxanide has been shown in vitro to inhibit replication of canine coronavirus (S-378), in addition to murine, bovine and human strains of coronavirus, specifically by inhibition of viral N protein expression (Cao *et al*., 2015). Both nitazoxanide and tizoxanide also inhibit MERS-CoV in vitro, with respective IC50 of 0.92 and 0.83µg/ml (Rossignol, 2016). Preclinical data demonstrating inhibition of pro-inflammatory cytokine production (including TNF-α, IL-2, IL-4, I-5, IL-6, IL-8 and IL-10) suggest that nitazoxanide could improve clinical outcomes of MERS-CoV infections in humans by suppression of overproduction of these pro-inflammatory cytokines, particularly IL-6 (Rossignol, 2016).

Gastrointestinal Tract Viral Infections

Nitazoxanide has shown effectiveness and has been increasingly used empirically in the treatment of acute pediatric gastroenteritis of varying etiologies, including Giardia, Entamoeba, Bacteroides, Clostridium, Cryptosporidium, Rotavirus *and* Norovirus (Rossignol *et al*., 2012). It is especially used in remote and low-income settings, given the advantages of oral administration, thermal stability, and known safety and tolerability in children (Waddington *et al*., 2018).

Rotavirus: A cell culture assay on antiviral activity of nitazoxanide and tizoxanide against two different rotaviruses, simian SA11-G3P[2] and human Wa-G1P[8], resulted in viral inhibition with EC_{50} between 0.3-2µg/ml and CC_{50}s above 50µg/ml (La Frazia *et al*., 2013). In the case of noroviruses, nitazoxanide and other thiazolides interfere with viral replication by disrupting the interaction between the non-structural proteins NSP5 and NSP2, thus reducing the size and altering viroplasmal architecture and decreasing rotavirus dsRNA formation (La Frazia *et al*., 2013). In the treatment of pediatric rotavirus diarrhea, Rossignol *et al*. (2006) reported results of a 3-day treatment utilizing 7·5 mg/kg nitazoxanide as an oral suspension, which significantly reduced the duration of rotavirus disease in hospitalized pediatric patients. A clinical trial is currently underway evaluating outcomes of oral nitazoxanide for the empiric treatment of acute gastroenteritis among Australian Aboriginal children (Waddington *et al*., 2018).

Norovirus: Dang *et al*. (2018) reported on the mechanism by which nitazoxanide and its metabolite tizoxanide inhibited human norovirus replication in vitro: tizoxanide-activated cellular antiviral response and stimulated the expression of a subset of interferon-stimulated genes (ISGs), particularly interferon regulatory factor 1 (IRF-1). Overexpression of exogenous IRF-1 inhibited noroviral replication, whereas knockdown of IRF-1 largely attenuated the antiviral activity of nitazoxanide, suggesting that IRF-1 mediates nitazoxanide induced inhibition of human norovirus. Nitazoxanide induced antiviral response independently of the classical JAK-signal transducers and activators of transcription (JAK-STAT) pathway. Furthermore, TZD and ribavirin synergized to inhibit HuNV replication and completely depleted the replicons from host cells after long-term treatment. In the clinical setting, nitazoxanide has been successfully employed in the treatment of norovirus diarrhea among immunocompromised individuals, and is suggested as a safe therapeutic alternative, for cases in which a reduction in immunosuppression is not a viable option (Morris *et al*., 2013; Siddiq *et al*., 2011; Woodward *et al*., 2017).

Adenovirus: Nitazoxanide has been suggested as a treatment alternative for adenovirus infection, presenting a more favorable tolerability profile in comparison to antivirals such as cidofovir (Esquer Garrigos *et al*., 2018). Further investigation into this indication is needed with well controlled studies, because although data in the literature shows promise, to date they are limited to case reports (Esquer Garrigos *et al*., 2018).

Hepatitis

Hepatitis B: In cell cultures, nitazoxanide and tizoxanide inhibited viral replication of Hepatitis B virus (HBV), reducing the levels of extracellular HBV surface and e antigens (HBsAg, HBeAg), as well as the levels of intracellular HBV nucleocapsid core antigen (HBcAg) in a dose-dependent manner (Korba *et al*., 2008). Nitazoxanide also displayed synergistic anti-HBV activity when combined with lamivudine or adefovir dipovoxil (Korba *et al*., 2008). Nitazoxanide was reported to inhibit expression of HBV cccDNA and HBV RNA transcription by interference with hepatitis B X protein (HBx)–damage-specific DNA-binding protein 1(DDB1) interaction (Sekiba *et al*., 2019). A recently published proof-of-concept clinical trial evaluating use of nitazoxanide in the treatment of chronic hepatitis B reported rapid decrease of serum HBV DNA following twice-daily oral treatment with 500 mg nitazoxanide (Rossignol & Bréchot, 2019). Clinical trials are currently underway evaluating nitazoxanide in combination with oral nucleos(t)ide analogues (NUCs) in the treatment of chronic hepatitis B.

Hepatitis C: In cell cultures, both nitazoxanide and tizoxanide selectively reduced intracellular Hepatitis C virus (HCV) replication in AVA5 cells. Nitazoxanide exhibited synergistic inhibitory activity in combination with interferon (IFN) or 2 CmeC (2 -C-methyl cytidine) against HCV, and was an effective inhibitor of an NS5B and two NS3 drug-resistant mutants in Huh7 cells.

Pre-treatment of HCV replicon cells with nitazoxanide potentiated the effect of subsequent treatment with combinations containing IFN, but not 2 CmeC, suggesting an interesting complementary activity with IFN (Korba *et al*., 2008). Clinical studies of hepatitis C employing nitazoxanide as monotherapy or as add-on have been carried out on hepatitis C genotype 1 or 4, with varying results, and further well-designed, robust, randomized trials are needed to better evaluate the potential benefit of nitazoxanide in the treatment of chronic hepatitis C (Nikolova *et al*., 2014).

Negative-Strand RNA Viruses

Nitazoxanide has been reported to counteract virus-specific immune evasion mechanisms, through enhancement of RNA sensing and the interferon axis that is triggered by exposure to foreign cytoplasmic RNA (Jasenosky *et al*., 2019). Although this premise has yet to be tested in the clinical setting, Ebola virus replication is inhibited by nitazoxanide in vitro, in addition to exerting a host innate immune response amplification that improves viral detection. The mechanisms by which nitazoxanide has demonstrated efficacy in Ebola virus infection inhibition include enhancement of retinoic-acid-inducible protein I (RIG-I)-like-receptor, mitochondrial antiviral signaling protein, interferon regulatory factor 3, interferon activities and transcription induction of the antiviral phosphatase GADD34. In human cells, nitazoxanide inhibits Ebola virus replication through enhancement of RIG_I and PKR, and consequently enhancement of viral detection and circumvention of the virus's ability to block these specific sensing mechanisms. In the case of vesicular stomatitis virus (VSV), nitaxozanide appears to act in a manner similar to that of Ebola virus in circumventing viral immune evasion mechanisms, through RIG-I and GADD34 (but not PKR).

Potential for Sars-Cov-2:

Monotherapy and/or Combination Therapy: To date, a number of authors have suggested that use of nitazoxanide at dose regimens previously employed in randomized controlled clinical trials of SARS CoV-2 whether as monotherapy or in combination with other drugs, including azithromycin and hydroxychloroquine. These propositions take into account previous studies of nitazoxanide monotherapy in the treatment of influenza and aim to assess the potential of nitazoxanide alone or in combination to decrease SARS CoV-2 morbidity and mortality (Kelleni *et al*., 2020; Padmanabhan, 2020). We support these suggestions based on the urgent need for an easily accessible, safe, and effective treatment in light of the current pandemic.

CONCLUSION

This review highlights the potential therapeutic role of nitazoxanide as an antiviral, having shown inhibitory activity in a variety of viral species and strains through a number of mechanisms. In the current scenario, we await the results of clinical trials using nitazoxanide in the treatment of SARS-Cov-2. Given the promising preclinical results reported previously, it may be that potential positive results will offer a greatly needed therapeutic option in the global response to the coronavirus pandemic.

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