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Commentary Potential Enhancement by 3-Deazauridine of the Antiviral Activity of Molnupiravir in Patients with COVID-19

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Abstract

Background: Molnupiravir, N4-hydroxycytidine-5'-isopropyl ester, is an oral prodrug of N4-deoxycytidine (NHC), a nucleoside analog, which has *in vitro* activity against SARS-CoV-2. NHC is phosphorylated in cells to NHC triphosphate (NHC-TP), which is incorporated into viral RNA, leading to epigenetic catastrophe of the viral genome and inhibition of viral replication. The antiviral activity against SARS-CoV-2 is dependent on the number of molecules of NHC-TP incorporated into viral RNA. Clinical studies in patients with COVID-19 showed that treatment with molnupiravir for 5 days decreases the risk of hospitalization and death as compared with placebo. **Objective:** It should be possible to enhance the antiviral activity of NHC-TP against SARS-CoV-2 by the use of the biochemical modulator, 3-deazauridine (3DU). 3DU is an inhibitor of CTP synthetase. Inhibition of this enzyme results in a reduction in the intracellular pool size of CTP. Since NHC-TP competes with CTP for incorporation into viral RNA in the reaction catalyzed by the SARS-CoV-2 viral RNA-dependent RNA polymerase, the reduction in the level of CTP should result in a significant enhancement of the incorporation of NHC-TP into viral RNA and an enhancement of its antiviral activity. **Methods:** Analysis of the publications of 3DU and cytosine nucleoside analogues support the hypothesis that 3DU enhances the pharmacological action of the analogues. **Results:** 3-DU increased the incorporation of 5-azacytidine into RNA and 5-aza-deoxycytidine into DNA of leukemic cells with an enhancement of their antineoplastic action. 3-DU potentiated the antiviral activity against HIV-1 activity by the cytosine nucleoside analogues: 2'-deoxy-3'-thiacytidine (3TC; lamivudine) and 2',3'-dideoxycytidine (ddC). This anti-HIV-1 activity of 3DU was associated with a reduction in the intracellular pool size of dCTP and increased incorporation of triphosphates of

3TC and ddC into DNA by the HIV-1 reverse transcriptase. The reduction of CTP levels in cells by 3-DU also leads to a reduction in dCTP since CTP is its precursor. **Conclusion:** The preclinical studies on 3-DU indicate that it can enhance the pharmacological activity of both ribo- and deoxyribonucleoside analogues against neoplastic cells and viral infected cells. These observations suggest that 3-DU also has the potential to enhance the antiviral activity of molnupiravir and arrest the progression of the disease in patients with COVID-19.

Keywords

Molnupiravir, N4-Hydroxycytidine-5'-Triphosphate, 3-Deazauridine, COVID-16, Antiviral Therapy

1. Introduction

The human pandemic coronavirus Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) has produced substantial worldwide morbidity and mortality [1]. What is urgently needed is effective drug therapy to treat patients with advanced COVID-19 to arrest progression prevent death due to the viral infection. An interesting agent to test for therapeutic activity against COVID-19 is molnupiravir, a prodrug of the cytosine ribonucleoside analog, beta-D-N4-hydroxycytidine (NHC) that contains isopropylester at its 5' end [2]. After removal of the isopropylester by esterases, NHC is phosphorylated by host kinases to its 5'-triphosphate form (NHC-TP) and incorporated into RNA by the viral RNA-dependent RNA polymerase. The presence of NHC in the viral RNA results in mutation ns during viral replication that leads to a non-infective virus [3] [4]. NHC exhibits *in vitro* antiviral activity against all coronaviruses tested, including SARS-CoV-2 [5]. Treatment of animals with NHC significantly inhibited SARS-CoV-2 [6]. Therapeutic treatment of infected animals with NHC significantly reduced upper respiratory tract SARS-CoV-2 load and completely suppressed spread to untreated animals [6]. These interesting preclinical results on the antiviral activity of NHC lead to the design of a clinical trial where patients diagnosed with advanced COVID-19 were treated with oral NHC to determine if this antiviral therapy can prevent progression to severe illness, and block transmission of severe acute respiratory syndrome [7]. The formulation of NHC for oral administration was accomplished by the insertion of isopropylester to the 5'end of NHC [8]. This chemical modification facilitated the oral administration of molnupiravir to patients with COVID-19.

The preliminary results of the oral treatment with molnupiravir exhibited positive responses in many COVID-19 patients treated with NHC [7]. This novel therapy in patients with COVID-19 was well tolerated and associated with potent antiviral efficacy as shown by reduced infectious virus isolation, reduction in the time to elimination of SARS-CoV-2 RNA and a greater reduction in

SARS-CoV-2 viral RNA from baseline compared to placebo treatment [7] [8]. These remarkable preliminary results suggest that NHC has the potential to prevent the progression of patients with advanced COVID-19 to a fatal outcome.

An interesting question is whether this promising antiviral therapy with NHC to arrest and prevent COVID-19 progression can be enhanced by the addition of another drug. Studies on the pharmacology of cytosine nucleoside analogues suggest that it may be possible to enhance the antiviral action of NHC by its use in combination with an agent that modulates its metabolism.

3-Deazauridine (3DU) is a cytosine ribonucleoside analogue that after its phosphorylation to its triphosphate and acts as a competitive inhibitor of CTP synthetase [9] (**Figure 1**). Inhibition of this enzyme reduces the level of CTP in the cells by the reduction of its competition with cytosine nucleoside analogues for incorporation into RNA. A good example of this interaction is shown by the remarkable enhancement by 3DU of the antileukemic activity of the cytosine ribonucleoside analogue, 5-azacytidine [10]. Treatment of leukemic cells with 3DU increased the incorporation of 5-azacytidine into RNA by 80%. By this similar mechanism, 3DU has the potential to enhance the incorporation of NHC-TP into viral RNA, which should increase significantly its antiviral action against SARS-CoV-2 (**Figure 2**). The antiviral activity of NHC is most likely dependent on the number of molecules of NHC-TP incorporated into the viral RNA. If the number of incorporated molecules of NHC-TP falls below the threshold for complete viral inactivation, some viruses may survive the treatment. Treatment with 3DU in combination with HMC can prevent these possibilities.

The reduction in the level of CTP in cells by 3DU also leads to the reduction

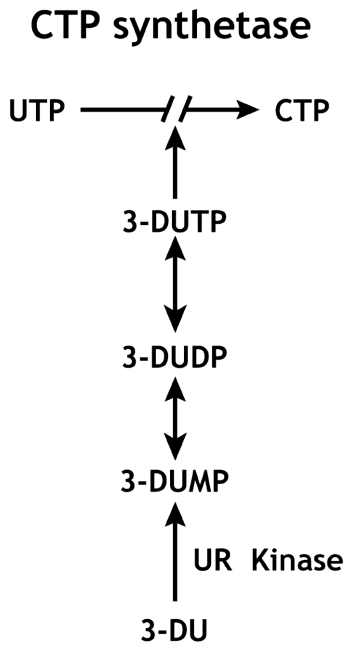

Figure 1. Molecular mechanism of action of 3-deazauridine (3-DU). The phosphorylation of 3-DU is catalyzed by uridine-cytidine (UC) kinase to its monophosphate (3-DUMP) and by cellular kinases to its diphosphate (3-DUDP) and triphosphate (3-DUTP).

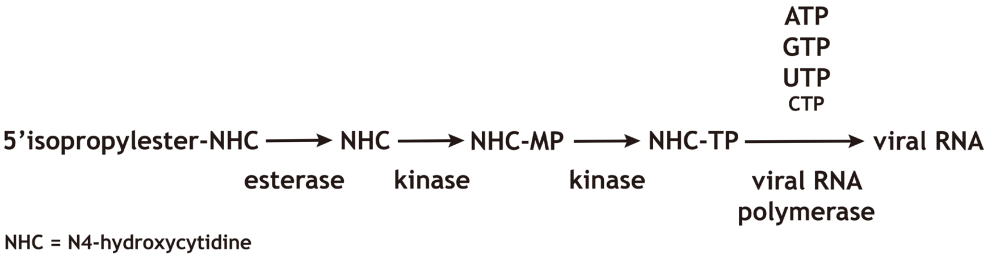

Figure 2. The metabolism of molnupiravir (5'-isopropylester-NHC). After removal of the 5'-isopropylester, NHC is phosphorylated by cellular kinases to its monophosphate (NHC-MP), diphosphate (NHC-DP) and triphosphate (NHC-TP). The viral RNA-dependent RNA polymerase incorporates NHC-TP into viral RNA. The reduced level of CTP (shown by smaller size) permits a greater incorporation of NHC-TP Into viral RNA by the viral RNA polymerase.

in dCTP, since CTP is its precursor. This is the mechanism by which 3DU enhanced the anti-HIV activity of the cytosine deoxyribonucleoside analogues, zalcitabine and lamivudine, which have to compete with dCTP for incorporation into the viral DNA in the reverse transcriptase reaction [11]. The reduction of dCTP by 3DU is also responsible for the enhancement of the antileukemic action of 5-aza-2'-deoxycytiine due to its increased incorporation into DNA [12] [13]. In these latter studies, 3DU exhibited remarkable reproducible inhibitory activity against leukemic cells in cell cultures, animal models and in patients. These observations indicate that the antineoplastic action of 3DU is very reproducible both *in vitro* and *in vivo*. The clinical studies on 3DU in patients with leukemia indicate that it exhibits minimal side effects and would be safe to use in combination with molnupiravir [13]. The preclinical and clinical studies on 3DU show that the effective plasma concentration of 3DU to modulate the metabolism of cytosine nucleoside analogues is in the range of 50 μM [13].

3DU in combination with NHC merits preclinical investigation to confirm its enhancement of the *in vitro* antiviral activity of NHC against SARS-CoV-2 in cell culture and its *in vivo* antiviral activity in animal models. Positive results in these preclinical models will provide a very good rationale for the investigation of 3DU plus NHC in patients with advanced COVID-19. Initial studies on patients with advanced COVID-19 can start with an oral dose of molnupiravir of 200 mg twice per day for 2 days [8]. After oral administration of molnupiravir, NHC achieves its maximum serum concentration with a median time of 1.00 to 1.75 h [8]. The serum concentration of NHC declines slowly with a half-life of approximately 1 h. Therapeutic concentrations of NHC against SARS-CoV-2 after oral administration are estimated to have a duration of about 6 h. Therefore, after the oral dose of molnupiravir, the duration of the infusion of 3DU should be about 6 h to obtain maximum incorporation of NHC into viral RNA. In patients with leukemia, 3DU was infused at a rate of 5 mg/kg/h for up to 72 h without any sign of adverse events [13]. Pharmacokinetic studies in patients administered an i.v. infusion of 3DU the estimated plasma half-life of 3DU was 4 h [14].

The objective is to find the optimal dose schedule of molnupiravir and 3DU

that will arrest the progression of viral disease. The rapid development of SARS-CoV-2 variants that are resistant to standard COVID vaccines provides a very good rationale to improve the effectiveness of antiviral drug therapy to rescue patients with progressive disease due to the infection with vaccine-resistant variants.

2. Conclusion

Molnupiravir after its metabolic conversion to the active inhibitor, NHC-TP, and incorporation into viral RNA, exhibits remarkable antiviral against COVID-19 [15] [16]. The pharmacology of 3-DU predicts that it has the potential to enhance the incorporation of NHC-TP into the SARS-CoV-2 RNA leading to a non-functional viral RNA. This action predicts that 3-DU will increase the antiviral potency of molnupiravir in patients with COVID-19. This novel antiviral therapy merits preclinical and clinical investigation.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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