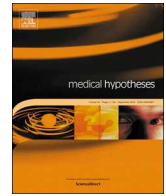




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5-Fluorouracil in combination with deoxyribonucleosides and deoxyribose as possible therapeutic options for the Coronavirus, COVID-19 infection

Shamim I. Ahmad

Department of Genetics and Genome Biology, University of Leicester, Leicester LE1 7RH, United Kingdom

ABSTRACT

The recent global pandemic created by the Coronavirus SARS-CoV-2, started in Wuhan, China in December 2019, has generated panic, both in term of human death (4–5% of infected patients identified through testing) and the global economy. Human sufferings seem to be continuing, and it is not clear how long this will continue and how much more destruction it is going to cause until complete control is achieved.

One of the most disturbing issues is Covid-19 treatment; although a large number of medications, previously used successfully with other viruses (including Chinese herbal medicines and anti-malaria drugs), are under consideration, there remain questions as to whether they can play a satisfactory role for this disease.

Global attempts are ongoing to find the drugs for the treatment of this virus but none of the antiviral drugs used for treatment of other human viral infection is working and hence attempts to find new drugs are continuing. Here the author is proposing that 5-Fluorouracil (5-FU) which when used on its own is failing as an antiviral agent due to the removal of this compound by proof reading ability exceptionally found in Coronaviruses. The author here is proposing to test 5-FU in combination with a number of deoxynucleosides on animal models infected with this Covid-19. Should encouraging results ensue, therapies could then be tried on patients.

Introduction

The Coronavirus known as SARS-CoV-2 and 2019-nCoV emerged in the city of Wuhan, China in December 2019. Soon after it started spreading quickly and reached other cities in China, Japan, and South Korea. As this paper goes to press there are more than 190 countries affected, and according to the World Health Organization it has reached pandemic stage. The previous epidemics of Coronavirus were Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) in 2002 and the Middle East Respiratory Syndrome Virus (MERS-CoV) in 2012.

Attempts are continuing to treat SARS-CoV-2 infections using certain specific and broad spectrum antiviral drugs used to treat other human viral infections. According to the media and recent research publications, no specific drug nor any vaccine are available to treat or even control the global spread of infection in humans. As a result, WHO has recently declared this to be a pandemic. The news is alarming.

Drugs in experimental phase

As SARS-Cov and SARS-CoV-2 have both emerged from China, this country has carried out more than a hundred clinical studies of this new Coronavirus infection including trials of most known antiviral drugs, antimalarial drugs, glucocorticoids, plasma therapy, virus vaccine, and certain Western drugs. Although the final results of these studies may take a long time to be completed (may be up to a year) the interim

research data may be helpful for the urgent demand arising from the pandemic [1]. Also, Chinese herbal medicines, which are commonly used in treating viral respiratory infections, are being tested for their abilities to directly inhibit the SARS-CoV-2. The results show that of the large number of natural compounds that were screened, 13 were present in Chinese medicines. These were found to have potential for anti-2019-nCoV (SARS-CoV-2) activity [2].

A number of antiviral drugs are either proposed or are in the clinical experimental phase, to test their safety and efficacies, by administering one or more of these drugs to patients suffering from Covid-19. These are Chloroquine phosphate and hydroxychloroquine sulphate (also known as anti-malarial drugs), arbidol, Remdesivir (GS-5734), Favipitavir and ribavirin (a complex structure but best viewed as ribosyl purine analogues). So far, some promising results have been seen [3–5] yet none of these antiviral drugs for managing coronavirus infection in humans has yet been approved by the relevant Drug Regulatory Authorities.

Remdesivir remains the most promising drug to treat the Ebola virus [6] infection and has the ability to inhibit replication of SARS-CoV and MERS-CoV in tissue culture and in non-human animal models. According to a recent press release, the last patient suffering from Ebola virus in Democratic Republic of Congo has been released from the hospital. Lopinavir/Ritonavir and interferon β (LPV/RTV-INF β) form another combination drug also available, but clinical trials on a humanized mice model have shown that Remdesivir is a better drug to

E-mail address: Shamim.ahmad@ntu.ac.uk.

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treat MERS-CoV [6,7]. In another study in South Korea, Lopinavir/Ritonavir (Kaletra, Abbvie) was administered to patients suffering from Covid-19. Following treatment, viral loads were found to be significantly decreased, and no or little Coronavirus titres were observed [8].

Chloroquine phosphate has been used in a multicenter clinical trial in China. Being an antimalarial, commonly used drug, the National Health Commission of the People's Republic of China has recommended these drugs to be included in the next version of the Guidelines for the prevention, diagnosis and treatment of pneumonia induced by Covid-19 virus [5]. It is furthermore requested that the scientific community should consider this drug in the field of antiviral research [9].

SARS-CoV-2, infection and inflammation

Martinez [6], based on the high mortality rates induced by the two Coronaviruses, SARS and MERS, questioned whether the induced pro-inflammatory response plays a role in pathogenesis, and, if so, should inflammation induced by the infection be targeted to control the progression of the disease. It remains debatable whether drugs targeted to treat Coronaviruses infections may be enough to reverse the highly pathogenic infection, especially when certain anti-inflammatory drugs may have the ability to decrease the most important defence machinery against viruses, the immune system.

Treatment proposed in this publication

My search of PubMed (25th March 2020) to find any research publications showing "5-Fluorouracil and Covid-19" has failed. However, a search "Coronavirus and 5-fluorouracil" has identified 10 publications. The most recent in 2019 showed that 5-Fluorouracil (5-FU) has remained ineffective to treat any coronavirus infection. None of the other 9 papers (during 2016–1983) address the application of FU in the treatment with any concrete data.

The reason proposed for the failure of 5-FU may be that Coronaviruses have RNA proofreading activities involving a 3'→5' exoribonuclease within non-structural protein 14 (nsp1-ExonN), and these remove 5-FU from their RNA during replication and metabolism hence making 5-FU ineffective when used on its own [10,11]. On the other hand the CoVs lacking exoN activity (ExonN2) were up to three hundred fold more sensitive to 5-FU [12,13].

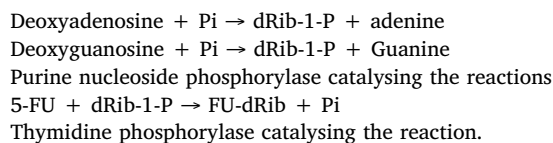
Before I propose the use of 5-FU plus compounds which may be used to treat viral infection through their insertion into RNA and probably escape identification by the proof reading activities, I consider it useful to refer to our work with these agents.

This work involved genetic, biochemical and physiological studies of a number of enzymes involved in the catabolism of nucleosides and deoxynucleosides in *Escherichia coli*. Two analogues, azauracil (Azu) and 5-FU were used, and preliminary tests showed that *E. coli* was significantly more sensitive to 5-FU (sensitive to 0.25 $\mu\text{g ml}^{-1}$) than Azu (requiring 100 $\mu\text{g ml}^{-1}$) to kill equivalent concentration of the bacteria.

Subsequently, we isolated bacterial colonies resistant to 2.5 $\mu\text{g ml}^{-1}$ of 5-FU and assumed that they had mutations of uracil phosphoribosyltransferase (EC 2.4.29). Next we exposed this mutant to 10 $\mu\text{g ml}^{-1}$ of 5-FU and from their sensitivity to this concentration we concluded that they retained purine nucleoside phosphorylase and thymidine phosphorylase activities. These enzymes are found in human cells too, and a deficiency of purine nucleoside phosphorylase can cause lymphospecific toxicity [14], a severe defect in T-cell immunity [15], and disorder of the immune system leading to neurologic symptoms and autoimmune disorders [16]. Thymidine phosphorylase is also present in humans and its deficiency can lead to mitochondrial neurogastrointestinal encephalomyopathy [17,18].

Our next step was to expose the mutant *E. coli* produced above to a combination of 5-FU (2.5 $\mu\text{g ml}^{-1}$) plus deoxyadenosine (100 $\mu\text{g ml}^{-1}$).

The culture was sensitive to this combination and we were able to isolate resistant colonies, likely to be mutants of either purine nucleoside phosphorylase or thymidine phosphorylase according to the reactions presented below. Analysis of resistant colonies showed that both class of mutants were present [19,20]. Our attempts to isolate mutants of uridine phosphorylase were based on the same principle, except that instead of deoxyadenosine we used adenosine in the selective medium [21].



As SARS-CoV-2 contains a single stranded positive sense RNA genome, I propose that laboratory experiments may be carried out on the ability of 5-FU to treat mice infected with SARS-CoV-2; even though 5-FU on its own has not acted as curing agents due to possible proof-reading of the viral genome by exoribonuclease (ExoN) hampering the activities of nucleoside-based therapeutics [22], it may be possible that when 5-fluorodeoxyuridine (5-FU-dRib) is inserted in the RNA and it may escape proof-reading and lead to lethality and/or lethal mutagenesis in the virus. In addition to 5-FU + deoxyadenosine, research could also be carried out on the efficacy of 5-FU + deoxyguanosine, 5-FU + deoxyuridine, 5-FU + deoxyribose and 5-fluorodeoxyuridine. My PubMed search could not find any publication showing the deoxyuridine and or combination drugs have been tried on any RNA viruses. As for my knowledge goes there is no deoxyuridine phosphorylase present in any RNA viruses, hence when tested in animal studies infected with SARS-CoV-2, can enter in RNA leading to lethality/lethal mutagenesis.

Finally it should be noted that 5-FU has been in use for a long time to treat certain cancers such as colorectal cancer [23,24], in pancreatic cancer [25], head and neck cancer [26], lymphoblastic leukaemia [27] and acute myeloid leukaemia [28]. According to results of a recent search of PubMed no less than 50,340 research papers have been published on this compound and hence this agent may be considered relatively safe when applied to SARS-CoV-2 infected patients.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mehy.2020.109754>.

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