Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Medical Hypotheses 142 (2020) 109754

Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

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

A B S T R A C T

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 administrating 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-INFb) 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.

https://doi.org/10.1016/j.mehy.2020.109754
Received 5 April 2020; Accepted 21 April 2020

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 μg ml^{-1}) than Azu (requiring 100 μg ml^{-1}) to kill equivalent concentration of the bacteria.

Subsequently, we isolated bacterial colonies resistant to 2.5 μg ml^{-1} of 5-FU and assumed that they had mutations of uracil phophoribosyltransferase (EC 2.4.29). Next we exposed this mutant to10μg 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 μg ml^{-1}) plus deoxyadenosine (100 μg 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].

Deoxyadenosine + Pi → dRib-1-P + adenine
Deoxyguanosine + Pi → dRib-1-P + Guanine
Purine nucleoside phosphorylase catalysing the reactions
5-FU + dRib-1-P → FU-dRib + Pi
Thymidine phosphorylase catalysing the reaction.

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 proofreading 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 SASRS-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.

Acknowledgements

Acknowledgement goes to my colleague Dr Sandra Kirk to polish this manuscript. Also to Professor Jacqui Shaw, Head of the Department of Leicester University, for allowing me to put the name of this university as my affiliation. Certain materials presented in the article are from my Ph. D. thesis which was done at the Leicester University.

Appendix A. Supplementary data

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

References

[1] Zhang Q, Wang Y, Qi C, et al. Clinical trial analysis of 2019-nCoV therapy registered in China. J Med Virol 2020. https://doi.org/10.1002/jmv.25733. [Epub ahead of print].
[2] Zhang DH, Wu KL, Zhang X, et al. In silico screening of Chinese herbal medicines with the potential to directly inhibit 2019 novel coronavirus. T Integr Med 2020;18(2):152–8. https://doi.org/10.1016/j.joim.2020.02.005 Epub 2020 Feb 20; In this issue.

[3] Al-Tawfiq JA, Al-Homoud AH, Memish ZA. Remdesivir as a possible therapeutic option for the COVID-19. Travl Med Infect Dis 2020:101615. https://doi.org/10.1016/j.tmaid.2020.101615. [Epub ahead of Print].
[4] Dong L, Hu S, Gao J. Discovering drugs to treat coronavirus disease 2019 (COVID-19). Drug Discov 2020;14(1):48–60.
[5] Gao J, Tian Z, Yang X. Breakthrough: chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. BioSci Trends 2020. https://doi.org/10.5582/bst.2020.01047. [Epub ahead of print].
[6] Martinez MA. Compounds with therapeutic potential against novel respiratory 2019 coronavirus. Antimicrobes Agents Chemother 2020. Pil:AAC.00399-20. doi: 10.1128/AAC.00399-20. [Epub ahead print}.
[7] Gordon CJ, Tchesnokov EP, Feng JY, et al. The antiviral compound Remdesivir potently inhibits RNA-dependent RNA polymerase from Middle East respiratory syndrome. J Biol Chem 2020. Pii: jbc.AC120.013056. doi: 10.1074/jbc.AC120.013056. [Epub ahead of print].
[8] Lim J, Jeon S, Shin HY, et al. Case of the index patients who caused tertiary transmission of COVID-19 infection in Korea: the application of Lopinavir/Ritonavir for the treatment of COVID-19 infected pneumonia monitored by quantitative RT-PCR. J Korean Med Sci 2020;35(6):e79. https://doi.org/10.3346/jkms.2020.35.e79.
[9] Touret F, de Lamballerie. Of chloroquine and COVID-19. Antiviral Res 2020;177:104762. https://doi.org/10.101016/j.antiviral.2020.104762. [Epub ahead of print].
[10] Smith EC, Case JB, Blanc H, et al. Mutation in Coronavirus non-structural protein 10 decreases virus replication fidelity. J Virol 2015;89(12):65418–26. https://doi.org/10.1128/JVI.00110-15. Epub 2015 Apr 8.
[11] Bassi MR, Sempere RN, Meyn P et al. Extinction of Zika Virus and Usutu Virus by lethal mutagenesis reveals different patterns of sensitivity to three mutagenic drugs. Antimicrob Agents Chemother. 2018 August 27;62(9). pii: e00380-18. doi: 10.1128/AAC.00380-18. Print 2018 Sep.
[12] Smith EC, Blanc H, Surdel MC, et al. Coronavirus lacking exoribonuclease activity are susceptible to lethal mutagenesis: evidence for proofreading and potential therapeutics. PLoS Pathog 2013;9(8):e1003565https://doi.org/10.1371/journal ppat.1003565. Epub 2013 Aug 15.
[13] Sexton NR, Smith, EC, Blanc H et al. Homology-based identification of a mutation in the coronavirus RNA-dependent RNA polymerase that confers resistance to multiple mutagenesis. J Virol, 2016 July 27;90(16):7415-7428. doi: 10.1128/JVI.00080-16. Print 2016 Aug 15.
[14] Carson DA, Kaye J, Seegmiller JE. PNAS December 1, 1977 74(12)5677-5681; doi: 10.1073/pnas.74.12.5677.
[15] Staal GE, Stoop JW, Zegers BJ et al. Erythrocyte metabolism in purine nucleoside phosphorylase deficiency after enzyme replacement therapy by infusion of erythrocytes. Free Access – 25.3.2020. doi: 10.1172/JCL109639.
[16] Purine nucleoside phosphorylase deficiency Genetic and Rare Diseases information Center25.3.2020.
[17] Hirano M, MartiR, Spinazzola A. Thymidine phosphorylase deficiency causes MNGIE: an autosomal recessive mitochondrial disorder. Nucleosides Nucleotides Nucl Acids 2004 Oct;23(8-9):1217–1225.
[18] Yadak, R, Smitt, PS, van Gisbergen et al. Mitochondrial neurogastrointestinal encephalomyopathy caused by thymidine phosphorylase enzyme deficiency: from pathogenesis to merging therapeutic options. Fron Cell Neurosci. 2017, 11:31 Feb 15. doi: 103389/fncel.2017.00031.
[19] Ahmad SI, Pritchard RH. A map of four genes specifying enzymes involved in catabolism of nucleosides and deoxynucleosides in *Escherichia coli*. Molec Gen Genet 1969;104:351–9.
[20] Ahmad SI, Pritchard RH. A regulatory mutant affecting the synthesis of enzymes involved in the catabolism of nucleosides in *Escherichia coli*. Molec Gen Genet 1971;111:77–83.
[21] Pritchard RH, Ahmad SI. Fluorouracil and the isolation of mutants lacking uridine phosphorylase in Escherichia coli: location of gene. Mol. Gen. Genet. 1971;111:84–8.
[22] Agostini ML, Pruijesser AJ, Chappell JD et al. Small molecule anti viral Beta-d-N4-hydroxycytidine inhibits a proofreading-intact coronavirus with a high genetic barrier to resistance. J Virol, 2019 Nov 26;93(24). pil: e01348-19. doi: 10.1128/JVI.01348-19. Print Dec 15.
[23] CaoY Lin Y, Wang D, et al. Enhancing 5-fluorouracil efficacy through suppression of PKM2 in colorectal cancer. Cancer Chemother Pharmacol. 2018 Dec;82(6):1081–6.
[24] Demetriadou C, Pavlou D, Mpekris F, et al. NAA40 contributes colorectal cancer growth by controlling PRMT5 expression. Cell Death Dis 2019;10(3):236. https://doi.org/10.1038/s41419-019-1487-3.
[25] Inoko K, Hiraoka K, Inagaki A et al. Therapeutic activity of retroviral replicating vector-mediated prodrug activator gene therapy for pancreatic cancer. Cancer Gene Ther. 2018 Aug;25(7-8);184-195. doi: 10.1038/s41417-018-0020-7. Epub 2018 May 8.
[26] Van den Bovenkamp K, Dorgelo B, Noordhuis MG, et al. Viable tumour in salvage neck dissertations in head and neck cancer: relation with initial treatment, change of lymph node size and human papillomavirus. Oral Oncol 2018:131–6. https://doi.org/10.1016/j.oraloncology.2017.12.017.Epub 2018 Jan 8.
[27] Wang D, Chen Y, Fang H et al. Increase of PRPP enhances chemosensitivity of PRPS1 mutatnt acute lymphoblastic leukaemia cells to 5-fluorouracil. J Cell Mol Med 2018 Dec;22(12):6202-6212. doi: 10.1111/jcmm.13907. Epub 2018 Sep 25.
[28] Maurer S, Salih HR, Smirnow I, et al. Suicide gene armed measles vaccine virus for the treatment of AML. Int J Oncol 2019;55(2):347–58. https://doi.org/10.3892/ijo:2019.4835. Epub 2019 jul2.