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Repurposing of Adamantanes for the Potential Prevention or Treatment of COVID-19

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Repurposing of Adamantanes for the Treatment of COVID-19: Rationale and Perspectives

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Abstract

Intensive efforts are underway in the search for novel antiviral agents and in the repurposing of existing antivirals with the potential to mitigate the effects of COVID-19. Amantadines represent a large family of tricyclic agents some of which are known to manifest efficacy against a range of viruses including influenza A and several human and animal coronaviruses including SARS-CoV and HCoV-OC43 with neuroinvasive characteristics. The adamantane derivative memantine improves clinical scores and motor disabilities while reducing HCoV-OC43 viral replication in a dose-dependent manner. Anti-viral actions of memantine against HCoV are independent of the agent's action as a non-competitive NMDA receptor antagonist. Amantadine and the novel spiroadamantane amine possess significant activity against coronavirus 229E. Mechanisms proposed to date to account for the anti-SARS CoV-2 actions of adamantanes include blocking of the viroporin channel of the virus E protein preventing release of viral nucleus into the host-cell cytoplasm and down-regulation of the host protease CTSL and lysosomal disruption leading to decreased viral replication. Further investigations are now required including the assessment of other adamantanes as antivirals in the experimental setting and controlled clinical trials to assess their safety and efficacy for the prevention and treatment of COVID-19.

Introduction

In view of the time required for the discovery, safety/efficacy testing and eventual large-scale production of vaccines for COVID-19, large numbers of commercial laboratories and clinical research institutions in various world centers are now actively concentrating their efforts on the discovery of novel antiviral agents. In many cases, the repurposing of existing antiviral agents with the potential to mitigate the effects of COVID-19 is well under way. At least 15 potential COVID-19 treatments are currently under evaluation in clinical trials and they include not only established antivirals but also anti-malarial and newly-synthesized compounds shown to exhibit antiviral potential in animal studies. Antibody-rich plasma preparations from patients recovering from COVID-19 are also under evaluation. As part of the race to find effective treatments for COVID-19, WHO launched a major trial to test repurposed drugs and experimental drug candidates that included the experimental antiviral remdesivir, the antimalarial drugs [chloroquine and hydroxychloroquine], a combination of the HIV drugs lopinavir and ritonavir alone or together with interferon-beta, an immune system messenger [1].

Repurposing of the adamantanes for COVID-19

The antiviral activity of the adamantanes has a long and chequered history. To quote from an article published in Science in 1964,

"1-Adamantanamine [amantadine] causes a selective, reproducible, dose-related inhibition of influenza infections in tissue culture, chick embryos and mice. The compound is not viricidal and appears to act by interfering with the penetration of the host cell by the virus" [2]. This avenue of research remains active today.

From a molecular structural standpoint, the adamantane molecule consists of three condensed cyclohexane rings fused in an armchair configuration, chemical formula $C6 H16$ with a functional group characteristic of each individual adamantane family member substituted at one of the four methyne positions. Names and structures of currently-available adamantanes are depicted in (Figure 1).

The best characterized adamantane, amantadine, is widely prescribed for the treatment of Parkinson's Disease [PD] where it has proven efficacy for the treatment of motor disturbances as well as for the dyskinesias resulting from long-term treatment of PD patients with L-Dopa [2]. Amantadine is increasingly providing benefit for the management of disorders of cognition and consciousness in patients with traumatic brain injury [3].

Several members of the adamantane family have established antiviral properties. The M2 proton channel of the influenza A virus is the target for the anti-influenza drugs amantadine and rimantadine that inhibit the endosomal uncoating of the virus [4]. However, the efficacy of these agents became limited by the advent of drug-resistant mutations in the pore of the channel. Consequently, a search is underway for the discovery of additional agents. Making use of a high throughput yeast growth restoration assay, several other adamantanes were shown to possess inhibitory potential at the M2 channel of influenza A virus [5]. Moreover, a novel adamantane derivative spiroadamantane amine was found to be a highly potent inhibitor of the amantadine-resistant V27A mutant but was also a 3-fold more potent inhibitor of the WT M2 channel compared to amantadine or rimantadine making it a potential candidate for antiviral drug development. This novel agent had previously been reported to possess significant activity against the coronavirus strain 229E [6] (Figure 2 and Table 1).

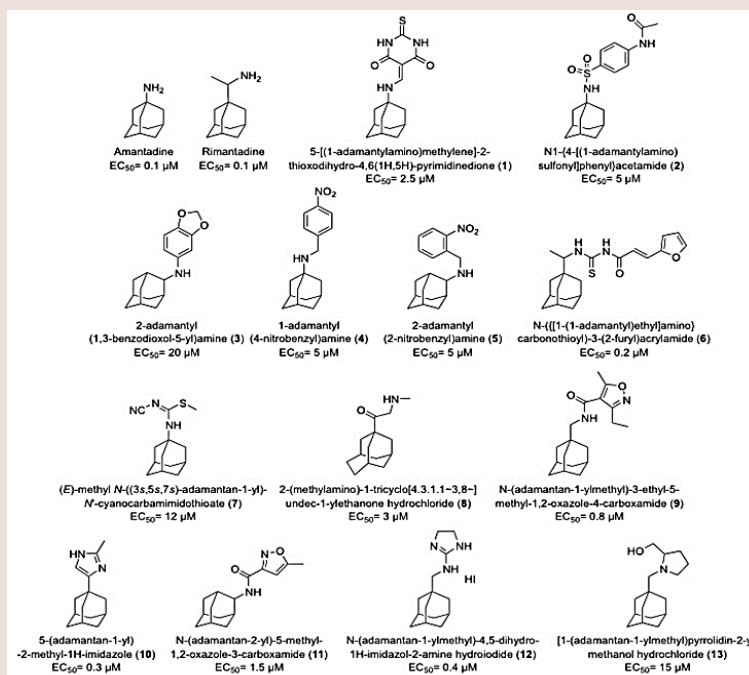Tromantadine is an effective inhibitor of both early and later

Figure 1: Structures, names and EC50 values of active adamantanes with inhibitory action against the influenza A virus M2 proton channel by high throughput yeast growth restoration assay [5].
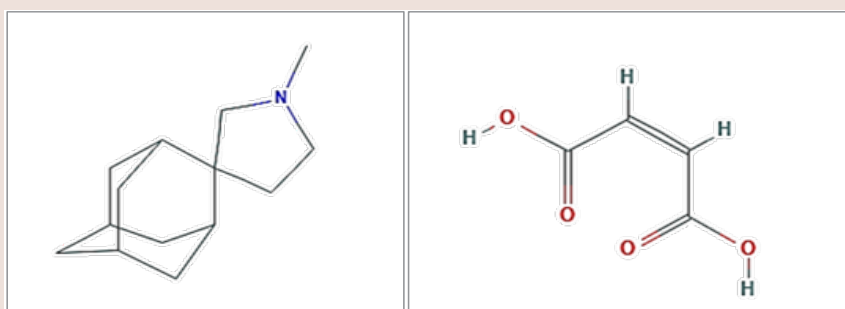

Figure 2: Structure of 1'-Methylspiro (adamantane-2,3'-pyrrolidine) maleate. **Source:**https://pubchem.ncbi.nlm.nih.gov/compound/1_-Methylspiro_adamantane-2_3_-pyrrolidine_maleate

events in Herpes Simplex virus type 1 replication [7]. More recently, in a case of severe AH1N1 viral pneumonia causing CNS disorder and multi-organ failure, amantadine was found to be neuroprotective while also appearing to result in cessation of viral shedding that contributed to positive outcome and discharge from the ICU [8]. Importantly, a series of novel 2-aminoadamantanes have been synthesized and shown to manifest persistent *in vitro* efficacy against H1N1 [2009] Influenza A where addition of as little as one CH2 group to the methyl adduct of the amantadine/rimantadine analogue 2-methyl-2-aminoadamantane resulted in activity of a range of H1N1 viral strains [9].

For coronaviruses, modest antiviral effects have been reported for amantadine, rimantadine and the structurally-related memantine and the adamantine-derived bananins* for bovine coronavirus, human coronavirus HCoV-OC43 and SARS-CoV-1 although amantadine had no inhibitory effect on replication of the coronavirus Feline Infectious Peritonitis [FIPV] virus [10-13]. Screening against

clinical isolates by neutralization tests with confirmation by plaque reduction assay revealed that rimantadine showed antiviral activity against SARS-CoV-1 [14]. Human coronaviruses [HCoVs] are well-recognized respiratory pathogens that manifest neurotropic and neuroinvasive actions. The OC43 strain [HCov-OC43] activates mechanisms implicated in neuroinflammatory and neurodegenerative processes in susceptible animals [12]. Moreover, treatment with the adamantane derivative memantine led to improvements in both clinical scores and motor disabilities in [HCoV-OC43]-infected mice. Memantine treatment also resulted in attenuation of body weight loss and mortality rates while reducing HCoV-OC43 replication in a dose-dependent manner. It was concluded that the antiviral action of memantine was independent of its NMDA receptor antagonist properties. Rather, it was proposed that memantine could act by inhibition of viral replication following viral attachment to the host cell receptor or by inhibition of the ATPase activity of HCoV-OC43 helicase as was previously shown for other adamantane derivatives

Table 1: ᵃDeterminations of coronavirus 229E titrations in L132 cells to the maintenance medium to which spiroadamantane drug was added 0.5 (-0.5 h) or 18 h (-18 h) before the virus [6].

Drug concn	Virus titer ($\log_{10}$ TCID$_{50}$/0.1 ml)	
µg/ml	-0.5h	-18 h
0	4.2	4.2
8	3.7	2.2
16	1.4	1.7
32	1.4	1.7

[12].

*Bananins are a class of antiviral compounds constituted of a trioxa-adamantane moiety covalently bound to a pyridoxal derivative. At least one family member is an effective inhibitor of the SARS-CoV-1 virus [11].

Mechanisms of anti-viral action of adamantanes repurposed for COVID-19

Three independent mechanisms of action have been proposed to explain the anti-SARS-CoV-2 actions of adamantanes in general and of amantadine and memantine, in particular. These mechanisms, simply stated, are:

i] Blockage of the viroporin channel of the E protein of SARS-CoV-2 preventing the release of the viral nucleus into the host cell cytoplasm [15].

ii] Down regulation of expression of the host cell protease Cathepsin L and lysosomal dysfunction leading to protection against viral entry and, ultimately, its replication [16].

iii] Mechanisms related to non-competitive antagonism of NMDA receptors.

According to hypothesis [i], amantadine enters the E-channel of the coronavirus where it prevents release of the viral nucleus into the cell. Docking studies suggest that amantadine interacts with amino acids ALA 22 and PHE 26 and, in so doing, blocks the proton channel [17].

Hypothesis [ii] is predicated on the observation that SARS CoV-2 entry into the host cell depends upon binding of the viral spike protein to cellular receptor and upon its subsequent cleavage by host cell proteases such as Cathepsin [CTSL] located in the lysosomes. Amantadine, in addition to causing down-regulation of CTSL also has the capacity to further disrupt the lysosome pathway resulting in decreased viral replication with the potential to decrease viral load and improve clinical outcome.

The expression of NMDA receptors in the lungs and airways provides a solid basis for the notion that signalling via these receptors is implicated in the pathogenesis of the acute respiratory distress syndrome [18]. In order to evaluate Hypothesis [iii], experiments were conducted in mouse primary CNS cell cultures known to express NMDA receptors compared to those of a human epithelial cell line commonly employed to amplify HCoV-OC43 that does not express NMDA receptors. The observation that memantine affected viral replication in both cell types suggested that the antiviral action of memantine was independent of its NMDA receptor antagonist properties. Alternative mechanisms were proposed namely that memantine may act by inhibition of viral replication following viral attachment to the host cell receptor or alternatively by inhibition of ATPase activity of HCoV-OC43 helicase as was shown for other adamantane derivatives [12].

Conclusions and Prospects

Whilst awaiting the availability of an effective vaccine against SARS-CoV-2, intense efforts have been underway towards the discovery of novel antiviral agents or the repurposing of existing compounds known to manifest antiviral properties to be used as potential novel approaches to the prevention and treatment of COVID-19. Based upon recent literature citations, calls for the repurposing of adamantanes have repeatedly been made [19-21]. Members of the adamantine family of agents including amantadine, rimantadine, tromantadine and bananins and their derivatives memantine, spiroamantadine manifest antiviral activities against a range of viruses from Herpes Simplex and influenza A to the coronaviruses 229E, HCoV-OC43, SARS-CoV-1 and SARS-CoV-2].

Evidence in support of multiple mechanisms of action has been presented whereby the adamantanes manifest their antiviral effects that include inhibition of the viroporin channel of the E protein, down-regulation of expression of the host cell protease Cathepsin L and lysosomal dysfunction leading to impaired replication and, thirdly, non-competitive antagonism of brain glutamate [NMDA] receptors. An appeal is made for further assessment of the potential of these and other members of the adamantine family of agents for their capacity to act against SARS-CoV-2.

Alternatively, combination therapies involving the use of adamantanes could be envisaged. For example, it has been suggested that the combined use of serine protease inhibitors and CTSL inhibitors could offer a safer and effective therapy compared to other available therapeutics to block coronavirus host cell entry and intracellular replication [22]. Combination therapy with amantadine [shown to manifest potential benefit against some coronaviruses with low-dose dexamethasone might offer one such combination for COVID-19.

Given the relatively short period of time [barely 6 months] since the arrival of SARS-CoV-2 and its associated disease COVID-19, clinical assessments of efficacy of adamantanes with established antiviral properties have been restricted to a handful of Case Reports. Some suggest the potential for the prevention of COVID-19 as summarised in the current report. The time has now come for the creation of an evidence base for these suggested benefits. Randomised controlled clinical trials for the assessment of efficacy and safety of these agents for the prevention and/or treatment of COVID-19 are now required.

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