

Tetrandrine as a Therapeutic Agent for COVID-19

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April 28, 2020

Abstract

More than one million patients worldwide have been diagnosed with coronavirus disease 19 (COVID-19) to date (WHO situation report, 8th April 2020). There is neither a vaccine to prevent infection with the causative organism, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), nor a cure. In the struggle to devise potentially useful therapeutics in record time, the repurposing of existing drugs is a key route of action. In this review we argue that the bisbenzylisoquinoline and calcium channel blocker tetrandrine, originally extracted from the plant *Stephania tetrandra* and utilised in traditional Chinese medicine, could be repurposed to treat COVID-19. We collate and review evidence for tetrandrine's putative mechanism of action in viral infection, specifically its recently discovered antagonism of the two-pore channel 2 (TPC2). Consideration of its pharmacodynamics and pharmacokinetics suggests that oral tetrandrine at doses currently used in clinical practice could be an effective agent for the treatment of SARS-CoV-2 infection in humans.

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Key words: coronavirus, SARS-CoV-2, two-pore channel 2 (TPC2), endolysosomal pathway, calcium channel blocker, drug repurposing, pharmacodynamics, pharmacokinetics, clinical trial, traditional Chinese medicine.

Word count: 1861

Figure count: 1

Introduction

We wish to draw attention to the possibility of repurposing the existing calcium channel blocking drug tetrandrine for the treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

Tetrandrine is a bisbenzylisoquinoline that can be extracted from the perennial vine plant *Stephania tetrandra* *S. Moore* (Chinese patent WO2004009106A1, 2002), which has been used in traditional Chinese medicine ([1], [2] and references therein). It can also be synthesised chemically (US Patent 10,023,584 B2, 2018, [3]) .

There is extensive literature on the anti-inflammatory, immunosuppressive, oncological and cardiovascular effects of tetrandrine [2;4-6]. It is licensed in China for the treatment of silicosis, but to our knowledge has not been licensed for viral illnesses in any country to date.

Mechanism of Action

The putative mechanism of action of tetrandrine that underlies its potential use as a coronavirus disease 2019 (COVID-19) treatment is its ability to block the two-pore channel 2 (TPC2) in host cells and thus inhibit virus replication at low micromolar concentrations [7]. However, it has been reported also to block other targets, including L- and T-type calcium channels ([2], [8], and references therein).

Two-pore channels (TPCs) are intracellular calcium/cation channels located in the membranes of host endolysosomal compartments which SARS-CoV-2, the virus causing COVID-19 (and several other viruses) depend upon for egress from these organelles and replication. TPC2 has already been implicated in Middle East Respiratory Syndrome-Coronavirus (MERS-CoV) [9], Ebola virus (EBOV) [10], and Merkel cell polyoma virus (MCPyV) [11] infection as a key drug target.

There are specific TPC blocking agents (such as Ned-19, [12]), which have been shown to inhibit EBOV replication *in vitro* [10], but are currently only chemical tools, though they may engender drug candidates in the future. The different stereoisomers of Ned-19 can have different effects *in vitro* and their respective effects may differ according to tissue type [12;13].

However, many existing calcium channel antagonists block TPCs at relatively high concentrations (for example nifedipine, verapamil, etc. [14;15], and references therein), and there is evidence that such calcium channel blockers (CCBs) could be repurposed in viral illnesses [16;17]. While these are attractive candidates for repurposing due to their widespread use; their efficacy with respect to SARS-CoV-2 infection needs further investigation in the first instance.

Tetrandrine, while also not specific to TPC2 [2], shows greater efficacy *in vitro* against MERS-CoV than other CCBs [9]. It also decreased human coronavirus strain OC43 (HCoV-OC43) infection of MRC-5 human lung cells *in vitro*, with an inhibitory concentration (CC_{50}) of 0.33 μ M, lower than that of related alkaloids [18]. Critically, tetrandrine was found recently to be effective against SARS-CoV-2 *in vitro* [7].

Administration and Dosage

It has been suggested that a 90 – 99 % reduction of viral replication is required for effective therapy of quickly progressing acute viral infections; the general standard for chronic viral infections being 50 % [19]. The dose for a ~ 90 % reduction of SARS-CoV-2 pseudovirus replication *in vitro* was shown to be approximately 3 μ g/ml [7].

Tetrandrine has been reported to be poorly soluble (saturation is 0.015 mg/ml in phosphate buffered saline at pH 7.4), and to have low and variable oral bioavailability (cited in [20], and references therein). Pharmaceutical methods have thus been investigated to improve the bioavailability; including lipid nanocapsules, nanoparticles, ethosomes, and microspheres [20]. Tetrandrine has also been administered by inhalation in humans with a metered dose inhaler for the treatment of asthma (reviewed in [21], original not available).

Administration in mice of 30 mg/kg tetrandrine intraperitoneally, a frequently-used dose in animal studies, was shown to give a peak plasma concentration of 2 μ M (1.2 μ g/ml) [22], whilst 10 mg/kg of oral tetrandrine resulted in a peak plasma concentration of 500 ng/ml in rats [20]. If the drug was emulsified, the same dose gave a peak concentration of 1.2 μ g/ml [20]. Pharmacodynamic studies in human subjects [23] showed that a single oral dose of 100 mg tetrandrine produced an average maximal serum concentration of 67.26 ng/ml ($n = 6$). However, these data require confirmation, as there seems to be a discrepancy between this value and a graph in the same publication.

In rats, the average maximal serum concentration obtained after inhalation of 8 mg/kg of tetrandrine was 140 ng/ml. While this serum concentration was moderate, average maximal wet lung tissue concentration obtained at post mortem examination following inhalation of the same dose of tetrandrine was over 90

$\mu\text{g/g}$. Lung concentrations following intravenous administration of 7.5 mg/kg were also relatively high with an average maximal concentration of $\sim 20 \mu\text{g/g}$ [24]. In another study investigating the spatiotemporal distribution of tetrandrine in rats using mass spectrometry imaging, 30 mg/kg intravenous administration also resulted in high drug concentrations specifically in the lungs with a peak of $\sim 120 \mu\text{g/g}$ [25].

The above data suggest that tetrandrine may reach therapeutic concentrations in lung tissue even when serum concentrations remain relatively low. This is in keeping with its two compartment kinetic profile and high apparent volume of distribution (though up to 90 % of the drug can be plasma protein bound; [2], and references therein).

Toxic doses have been reported in an MRC-5 human lung cell line study with cytotoxic concentration (CC_{50}) $> 10 \mu\text{M}$ [18]. Similar results were reported in WI-38 human lung fibroblast and NL-20 human bronchial epithelial cell lines [26]. In animal experiments, the reported median lethal dose (LD_{50}) for oral administration was 3700 mg/kg in mice and 2230 mg/kg in rats (cited in [6]). Acute lung injury was demonstrated in mice at a dose of 150 mg/kg intraperitoneally [26]. Toxic effects of tetrandrine in humans have been reported with 10 mg/kg intravenous administration (cited in [2] and [6]).

According to the above-mentioned Chinese patent (WO2004009106A1, 2002), dosage in humans is usually in the range of 0.02 - 1.5 g/day depending on the weight and symptoms of the patient. Tetrandrine has been used at an oral dose of 60 – 100 mg three times a day in recent studies on silicosis ([27], [28]), and at 60 mg four times a day in an ongoing clinical trial (see below). In intravenous administration in humans, doses between 240 mg and 300 mg have been described as a ‘safe range’ (cited in [6], original not available). The maximum utilised doses in human studies that could be found were 200 mg orally three times a day (cited in [2]), and 400 mg three times a day with the route of administration not stated (cited in [6]).

It is difficult to conduct relevant animal to human dose conversions given the lack of *in vivo* studies of tetrandrine in SARS-CoV-2 infection and the lack of lung concentration data for oral administration of tetrandrine. The following human equivalent dose (HED) calculation serves only to illustrate that established clinical doses of tetrandrine in humans are likely to lead to potentially effective doses for viral inhibition in lung, as based on animal/cell line studies. The HED of a 7.5 mg/kg intravenous dose in rat (shown to give rise to lung concentrations of tetrandrine many times that required for a ~ 90 % reduction of SARS-CoV-2 pseudovirus replication *in vitro*) is $\sim 1.21 \text{ mg/kg}$ [29], i.e. $\sim 72.6 \text{ mg}$ for a standard 60 kg human. This is well below reported toxic doses in humans, and even with low bioavailability should be achievable with oral equivalent doses in the range of the above mentioned clinical trial (240 mg/day in divided doses).

Potential for the Use of Tetrandrine in the Treatment of SARS-CoV-2 Infection

It is probable from the available evidence that oral tetrandrine could be an effective agent for the treatment of SARS-CoV-2 infection in humans.

The level of inhibition of viral replication in lung attainable with standard oral dosing of tetrandrine could be of value for the treatment, or potentially the prophylaxis (pending caveats about long-term use and toxicity) of SARS-CoV-2 infection. Prophylaxis is suggested because early administration of tetrandrine relative to infection has been shown to be more effective *in vitro* in the context of HCoV-OC43 [18] and this may also apply to SARS-CoV-2.

As tetrandrine is already licensed and used in China as an oral tablet at the doses cited above, it is probable that these doses are safe to be administered more widely. However, evidence of conclusive pharmacokinetic and toxicological studies, as well as clinical trials are required.

Additionally, as tetrandrine is likely to operate in a different manner to antiviral agents currently being considered for the treatment of SARS-CoV-2 infection, it might make for a suitable synergistic adjunct. The administration of tetrandrine using inhalation by aerosol also stands a possibility of being effective and would have the advantage of direct delivery to the key site of infection. However, this requires further research.

Ongoing Research and Availability

There are currently no CCBs on the WHO list of drug candidates for SARS-CoV-2 infection:

https://www.who.int/blueprint/priority-diseases/key-action/Table_of_therapeutics_Appendix_17022020.pdf?ua=1.

To date, there is at least one clinical trial registered that employs CCBs in the context of COVID-19 (NCT04330300, Galway, Ireland). It is based around the observation that SARS-CoV-2 uses angiotensin converting enzyme 2 (ACE2) as a cell entry point, and that commonly prescribed antihypertensives, angiotensin converting enzyme inhibitors (ACE inhibitors) might have a detrimental effect on patients who contract COVID-19. The study thus compares patients who remain on ACE inhibitors throughout their illness with patients who are switched to alternative antihypertensives, including CCBs. While this work is not concerned with the mechanism of action discussed above, it will provide useful information on the potential therapeutic role of these drugs in SARS-CoV-2 infection.

No patents specifically relating to tetrandrine and the therapy of SARS-CoV-2 infection were found online (but see Chinese and US patents, cited above). However, a phase 4 clinical trial of tetrandrine for the treatment of SARS-CoV-2 was set up in China in March 2020, <https://clinicaltrials.gov/ct2/show/NCT04308317>, of one year's duration. It is geared towards preventing pulmonary fibrosis, and does not mention a pharmacological basis for the mechanism of action of tetrandrine. However, primary and secondary outcome measures are stated as survival rate and body temperature, respectively, suggesting that the trial will provide valuable insights into the role of tetrandrine as an acute therapeutic agent.

Suggested Actions

The following is a non-exhaustive list of suggested studies that could be considered at this point:

1. Confirmatory pharmacokinetic and toxicological studies of oral tetrandrine
2. A clinical trial to investigate tetrandrine's potential, using a standard oral dose, as an acute therapeutic or prophylactic agent against SARS-CoV-2 infection, provided safety can be confirmed for the particular length of use
3. *In vitro* investigations to assess the potential of other calcium channel blockers known to block TPCs, such as nifedipine or verapamil, to inhibit SARS-CoV-2 pseudovirus replication
4. If indicated by (3), a clinical trial to investigate the potential role of clinically established calcium channel blockers such as nifedipine or verapamil as therapeutic agents in SARS-CoV-2 infection
5. A retrospective analysis of the relative outcomes of COVID-19 patients already on CCBs
6. *In vitro* investigations to assess the potential of analogues of Ned-19 and other chemicals with expected specificity as TPC inhibitors to inhibit SARS-CoV-2 pseudovirus replication
7. An autopsy toxicology study to assess tetrandrine (or other calcium channel blocker) concentration in human lung tissue in patients prescribed these drugs during life

Acknowledgements

The authors have no competing financial interests.

We would like to thank Professor Antony Galione, Department of Pharmacology, University of Oxford, for helpful discussions; and Kiera Jamison, for the co-production of Fig. 1.

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