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Effect of the Faradarmani Consciousness Field on Mutant Strain of SARS-CoV-2

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Abstract

The COVID-19 pandemic is still a threat to global health. In late 2020, the rapid emergence of SARS-CoV-2 mutant strains was reported, raising concerns in the prevention and treatment of COVID-19. SARS-CoV-2 carrying the D614G mutation in the viral spike (S) protein predominated globally, and this change increased virus transmission. Furthermore, false-negative results in respiratory specimens are a problem in COVID-19 detection. Taheri Consciousness Fields (TCFs) introduced by Mohammad Ali Taheri are novel fields that neither matter nor energy. Therefore, they are not quantifiable and cannot be directly observed or measured. However, it is possible to demonstrate and measure the effects of these fields through standard scientific experiments. The present study aimed to evaluate the effect of Faradarmani CF, one of the TCFs, on the titer and RNA copy number of the mutant D614G virus and on the quality of amplification of coronavirus genome fragments. The results of this study showed that Faradarmani CF increased the replication of both non-mutated and mutated viruses compared with the control, thereby improving their replication fitness. The Faradarmani CF increased the rate of replication of the mutant strain in cells to a level close to that of the non-mutant strain, and, in fact, reached a biological equilibrium of compatibility similar to that of the Wuhan strain with the cells. The study suggests that Faradarmani CF acted as a modulator, equalizing replication rates between mutant and non-mutant strains, thereby achieving a biological equilibrium akin to the Wuhan strain. Scientifically, this is provocative; it challenges conventional molecular biology and demands rigorous studies. In addition, the PCR products of coronavirus genome fragments under the influence of the Faradarmani CF showed sharper bands on gel electrophoresis and higher-quality amplification compared with the control. This result suggests that Faradarmani CF can be used in diagnostic assays to reduce false-negative results and improve test sensitivity. Based on the results, it is recommended that the effects of TCFs on other mutants be investigated.

Keywords: COVID-19, Faradarmani, Taheri Consciousness Fields, T-Consciousness, Mutant, RT-PCR

Introduction

SARS-CoV-2 is a type of RNA virus. The process of viral replication is characterized by a high mutation rate due to the absence of a mismatch repair mechanism. Hence, coronavirus mutations are quite predictable and reasonable. The virus may become more transmissible and difficult to eliminate due to mutations (Domingo et al., 1997). Korber *et al*. identified the D614G variant (the amino acid in the 614 position was mutated from aspartic acid to glycine), which is more contagious and has been dominant worldwide (Korber et al., 2020; Hou et al., 2020). Currently, other new strains are spreading rapidly worldwide, raising concerns about the prevention and treatment of COVID-19. Recent research has shown that only mutations with important biological functions showed high transmissibility, suggesting that these key mutations may affect COVID-19 severity, virus spread, and escape from natural or vaccine-induced immunity (Zhou et al., 2021). SARS-CoV-2 infects human cells by binding to angiotensin-converting enzyme (ACE2) through the receptor-binding domain (RBD) of the Spike protein. These key mutations appear to affect the ability to bind to ACE2 (Starr et al., 2020).

Vaccination is carried out worldwide as a fundamental strategy to combat COVID-19. However, with the emergence of several SARS-CoV-2 variants, vaccine effectiveness has become a major global debate. Current studies show that SARS-CoV-2 variants significantly affect vaccine effectiveness (Zhou et al., 2021).

Detection of COVID-19 is performed by SARS-CoV-2 RNA detection via real-time RT PCR. Studies on false-negative results in respiratory specimens for SARS-CoV-2 are varied and report false-negative rates of 1-30% (Long et al., 2020; Arevalo-Rodriguez et al., 2020). The false-negative results can happen for several reasons, such as the non-optimal collection of samples, low analytical sensitivity, early testing of disease, unsuitable type of specimen, variability in the shedding of virus, or low viral load (Kinloch et al., 2020; Kucirka et al., 2020; Kanji et al., 2021; Pan et al., 2020). Given the urgency of controlling COVID-19, it is necessary to find a novel way to curb SARS-CoV-2 mutation and transmission and reduce false-negative SARS-CoV-2 detection results.

This study investigates the effects of Taheri Consciousness Fields (TCFs). According to Taheri's theory, TCFs are non-frequency in nature and are a subset of the Cosmic Consciousness Network (CCN), and it is possible to investigate their effects on a variety of subjects, including living beings and materials (Taheri, 2013).

In previous research, the effects of the TCFs on the MCF7 cancer cell line (Taheri et al., 2020a), spatial memory and avoidance behavior of a rat model of Alzheimer's disease (Taheri et al., 2021a), wheat plant (Torabi et al., 2020), bacterial population growth (Taheri et al., 2021b), and the electrical activity of the brain during the application of Faradarmani in the *Faradarmangars* population (Taheri et al., 2020b) have been investigated. The present study aimed to evaluate the effect of Faradarmani CF as one of the TCFs on mutant D614G virus titer, RNA copy number, and quality of amplification of coronavirus genome fragments.

Material and Methods

Application of the Faradarmani Consciousness Field

The study samples were influenced by Faradarmani Consciousness Field according to the protocols mentioned on the website of research management in the TCFs (www.COSMOintel.com). More details are provided in the Common Considerations section of this issue. In the present study, Faradarmani CF was announced exactly at the same time as the virus inoculation in cell culture flasks in the treatment groups of the examination.

Preparation of mutant and non-mutant strains

For the current study, non-mutant strains similar to the original Wuhan strain, as well as mutant strains with the D614G mutation in the virus spike protein, were isolated from the nasopharyngeal and oropharyngeal hospital specimens of patients admitted to the intensive care unit in the acute phase of the disease with positive real-time RT-PCR results and Ct values of 13 and 12.

Cell culture and virus titration

The T-25 flasks were seeded with 5×10^6 Vero cells in culture media composed of high glucose DMEM (Gibco) with 10% fetal bovine serum (Gibco) and incubated in 5% CO2 at 37°C until 80% confluency. Then they were divided into five groups, each with six flasks: mutant virus and non-mutant virus with and without the Faradarmani CF treatment, and a negative control (without virus). Cell cultures were prepared for virus inoculation after reaching 70% confluency. A biosafety level 3 (BSL-3) laboratory was used for all surveys on the virus (WHO, 2020). The virus culture with TCID50/ml ~ log 6 was selected for inoculations into the flasks. TCID50/ml was assessed in 96-well plates, and all the plates were monitored every 24 h for Cytopathic effect (CPE). After 4 days, the results were reported, and the virus titer was calculated using the Reed & Munch method (Reed et al., 1938).

Real-time RT-PCR for evaluation of SARS-CoV-2 virus RNA copy number

The LabPrep™ Viral DNA/RNA Mini Kit was used to extract RNA according to the manufacturer's protocol. SinaClone cDNA synthesis kit was used for cDNA synthesis. All steps were performed with RNase-free tools and solutions. Also, to make cDNAs with the same concentration in each sample, based on the RNA concentration, the volume was determined to contain 1000 ng of RNA, and the final volume was adjusted to 20 microliters with water and Master Mix. The Biotechrabbit GmbH kit was used for real-time PCR testing. RNA copy numbers were evaluated using Nucleocapsid (N) gene primers according to the protocol and materials. A Rotor-Gene Q 6000 thermocycler (Corbett, Australia) was used for all real-time RT-PCR reactions.

RT-PCR and electrophoresis of PCR products of S protein fragments of mutated SARS-CoV-2

For six selected and positive real-time PCR samples with a Ct value of 13 for which RNA was extracted, and cDNA was previously fabricated, and with three pairs of primers called B26, B27, and B28 for different parts of the S1 spike protein, PCR was performed (Table 1).

Table 1: Sequences of applied primers for PCR of S1 spike protein.

Primer number	Sequence of primer (5' to 3')	Length	Annealing temp (°C)	Target protein	Ref
B26	F: TATCTTGGCAAACCACGCGA R: ACCAGCTGTCCAACCTGAAG	1057	58	Spike	(20)
B27	F: CCCTCAGGGTTTTTCGGCTT R: CTGTGGATCACGGACAGCAT	1093	60	Spike	(20)
B28	F: CCAGCAACTGTTTGTGGACC R: GTGGCAAAACAGTAAGGCCG	1027	60	Spike	(20)

PCR products were run on a 1% agarose gel in TBE buffer containing Tris base, boric acid, EDTA, and ethidium bromide, at pH 8. For electrophoresis, the products were run at 90 V for 55 minutes, using a 100 bp Plus DNA Ladder (Germany, Fermentas) as a marker.

Statistical analysis

Statistical significance was set at $p < 0.05$ (95% confidence level). Virus titer and real-time RT-PCR data were analyzed using two-way ANOVA to assess the effects of treatment and virus strain.

Results

Faradarmani CF significantly increased the replication of both non-mutated and mutated viruses compared with control samples ($p< 0.05$). Moreover, Faradarmani CF elevated the replication rate of the mutant strain to a level approaching that of the non-mutant strain in cell culture; however, this difference in replication was not statistically significant ($p> 0.05$) (Table 2). The $TCID_{50}$/mL increased from 3×10^7 in the control of the mutated virus to $2 \times 10^{7.2}$ (approximately 3.17×10^7) in the Faradarmani CF-treated mutated virus group, with no statistically significant difference observed ($p > 0.05$). Also, Faradarmani CF increased the time required for cell destruction and mortality.

Table 2: The titer of mutant and non-mutant SARS-CoV-2 under the influence of Faradarmani CF (FCF) and control groups. All conditions, except virus strains, were the same for both Faradarmani CF groups.

	Inoculum (Seed)	Non-mutated control	Mutated control	Non-mutated virus+ FCF	Mutated Virus+ FCF	Cell culture (Cell control)
TCID50/mL	1×10^6	$2 \times 10^{6.5}$	3×10^7	$2 \times 10^{7.2}$	$2 \times 10^{7.2}$	-
Ct Value in real-time RT-PCR	14	11	9	8	8	-

In real-time PCR results, the RNA copy number of the non-mutant control group was changed compared to the non-mutant group affected by Faradarmani CF, with a difference of 3 C_t value ($p<0.05$). Also, in the mutant group, this difference between the control mutant and the mutant affected with Faradarmani CF was changed by one C_t value, which was not statistically significant ($p>0.05$).

To investigate the effect of the Faradarmani CF on the RT-PCR of the mutated coronavirus genome and the amplification rate of coronavirus genome fragments, PCR was performed on three important fragments of the coronavirus glycoprotein spike gene responsible for cell binding. Then gel electrophoresis was done on the PCR products. The sharper detected bands in the samples treated with Faradarmani (data not shown) might signify the effect of Faradarmani on the quality of amplification in the PCR, but proving this assumption requires doing complementary experiments.

Discussion

The D614G substitution in the spike glycoprotein of the Wuhan primary strain increased viral replication (Plante et al., 2021). Plante *et al.* highlighted the importance of the G614 variant in virus spread and its implications for vaccine and antibody therapy efficacy. The G614 virus replicated more than the original D614 virus in primary human upper airway tissues and the human airway epithelial cell line (Calu-3). The increased replication fitness was associated with

increases in specific stability and infectivity of the G614 virus (Plante et al., 2021). Higher levels of viral RNA were detected in patients infected with the D614G mutant, which is more infectious than the original Wuhan strain (Korber et al., 2020). Ozono *et al*. showed that the D614G mutation confers increased entry efficiency by enhancing binding affinity for ACE2, with no influence on the antigenicity of the S protein (Ozono et al., 2021).

This study showed that Faradarmani CF increased replication of non-mutated and mutated viruses compared to the group without Faradarmani CF treatment, increased replication fitness, and enabled the virus to gain greater control over the cellular replication machinery. It seems that the Faradarmani CF increases the rate of replication of the mutant strain in cells to a level close to that of the non-mutant strain and reaches a biological equilibrium of compatibility similar to that of the Wuhan strain with the cells. In order to elucidate the effects of this treatment, we recommend that the influence of the TCFs on animal models with mutated and non-mutated virus infection be evaluated.

False-negative results have vital implications for the isolation and transmission risk and for the management of COVID-19 (West et al., 2020). According to the results of the Faradarmani CF effect on the RT-PCR of the mutated coronavirus genome, Faradarmani CF may improve different steps of PCR, such as primer binding, amplification, and electrophoresis motion. According to the results, the Faradarmani CF may play an effective role in PCR for virus fragments and can help increase laboratory test accuracy and reduce false-negative results.

As mentioned, TCFs are not directly measurable, but their effects can be investigated indirectly through various experiments. We suggest investigating other mutations of the COVID-19 virus to assess the effect of the Faradarmani CF on the efficacy of immunization and vaccination *in vivo*.

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Conflicts of Interest

The authors declare no conflict of interest.

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