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High-Voltage Low-Frequency Electric Field Exposure as an Antiviral Strategy: Effects on Viral Infectivity and Host Cell Viability

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Abstract

This study explores the antiviral properties of high-voltage low-frequency electric field exposure on the replication of human viruses, including Herpes Simplex Virus type 1 (HSV-1), Human Coronavirus OC43 (HCoV OC43), and Influenza A virus (A H1N1). Using the Healectrics™ device (model S02), which operates by applying high-voltage direct current (30 - 50 kV) with a polarity change frequency of ~0.2 Hz, we investigated the impact on viral infectivity and host cell viability. Virus cultures were exposed to electric fields during different stages: virion adsorption (0 - 1 hour), intracellular replication (1 - 8 hours), and both stages. Viral infectivity was assessed through titration, and cytotoxic effects were evaluated using MTT assays. Electric field exposure significantly reduced viral infectivity, particularly during the combined sorption and replication stages, with up to a 90% decrease in viral activity. Among the viruses tested, HCoV OC43 showed the least sensitivity, with a reduction in viral activity by a factor of 5. Comparisons revealed statistically significant reductions for influenza and herpes viruses, and a trend towards significance for HCoV OC43. The electric field treatment did not significantly affect the viability of Vero and MDCK cells, indicating the method's safety. Our findings suggest that high-voltage low-frequency electric fields can effectively reduce viral infectivity and may serve as a potential therapeutic and preventive measure against a wide range of membrane-bound viruses, including SARS-CoV-2.

Keywords

High-Voltage Therapy, Cell Viability, Viral Infectivity, Herpes Simplex Virus Type 1 (HSV-1), Human Coronavirus OC43 (HCoV OC43), Influenza A Virus (A H1N1)

1. Introduction

Standard treatments for viral infections today involve the use of antiviral drugs such as antiretrovirals for HIV infection that hinder the virus's ability to transcribe its genetic material within human immune cells, or Tamiflu for influenza, which suppresses the ability of an infected cell to release new virus copies, among others. These antiviral drugs demonstrate considerable effectiveness at certain stages of their use, but over time the virus mutates, and the drugs cease to work, or at least their efficacy significantly decreases. Alternative approaches to treating viruses are based on studies in the relatively new field of physical virology, which seeks to identify the physical mechanisms controlling virus development. This knowledge can provide the information needed for the rational design of new antiviral strategies that are less specific to mutations.

One area of physical virology is studying the sensitivity of viruses to electric fields. Various in vitro experiments confirm the effectiveness of certain types of electric fields on different viruses. The effectiveness of electric field exposure on the infectivity of lentivirus was studied in [1], infectious bronchitis virus in [2], human immunodeficiency virus type 1 (HIV-1) in [3], and herpes simplex virus type 1 (HSV-1) and adenovirus type 5 (AdV-5) in [4].

The problem is that in all these experiments, electric field exposure was applied directly to the extracellular virus solution before incubation with target cells. Moreover, electrodes were directly introduced into the solution [1] [2], and a low potential high-frequency alternative current field was applied. In [3] and [4], viruses were adsorbed onto special electrodes to which a low potential constant electric field was applied. Such exposures are local and do not allow the creation of a model of electric field exposure on a patient where the field exposure through electrodes attached to the skin would influence virus development in deep tissue cells during infection and viral replication within cells.

This paper presents an in vitro model of high-voltage DC field exposure on body cells during infection and viral replication without direct contact of electrodes with the viral solution.

The viruses chosen for this study—Herpes Simplex Virus type 1 (HSV-1), Human Coronavirus OC43 (HCoV OC43), and Influenza A virus (H1N1)—represent membrane-bound viruses commonly encountered in virology studies. They were selected based on their accessibility and relevance to various fields of viral study, providing an opportunity to test the broad applicability of high-voltage electrotherapy across different viral families.

2. Methods and Experimental Design

2.1. Technology of High-Voltage Low-Frequency Electrotherapy

This study utilized high-voltage low-frequency therapy using the stationary electrotherapy devices Healectrics^{TM} device (model S02) [5]. The therapy operates based on the application of high-voltage direct current (30 - 50 kV), with a polarity change frequency of up to 1 Hz. In this experiment, a frequency of ~0.2 Hz was used. To ensure effective therapy and safety, the subject undergoing therapy must be electrically isolated from other large conductive objects. For instance, when treating humans, the subject sits on a chair made of dielectric materials. Similarly, when treating animals, they are placed in a plastic cage on a dielectric stand at least 5 cm above a conductive surface, and other conductive objects should also be maintained at a distance of at least 5 cm. This method differs from previously considered electrotherapy techniques by inducing polarization effects throughout the subject's body, thereby increasing the body potential to a level of 30 - 50 kV. This saturation of the body with ions begins to dissipate into the air, and microcurrents flow through the body, exerting biological effects that were further studied for their antiviral properties.

2.2. Experimental Design

Abbreviations and Notations

- CPE: Cytopathic effect.
- HCoV OC43: Human Coronavirus OC43.
- MEM: Minimal Essential Medium.
- MOI: Multiplicity of infection.
- HSV-1: Herpes Simplex Virus type 1.
- TCID50: 50% Tissue Culture Infecting Dose.

The aim of this study was to characterize the antiviral properties of low-frequency, high-voltage exposure on the infectious activity of human viruses such as Herpes Simplex Virus type 1, Human Coronavirus OC43, and Influenza A virus (A/Puerto Rico/8/34 H1N1). The viruses were cultured in permissive Vero cell lines (ATCC CCL-81) while the influenza virus was cultured in MDCK cell lines (ATCC CCL-34). The experimental material used was cell-free cultural fluid obtained after virus cultivation.

Viral Infection and Treatment Application

Viral cells corresponding to the permissive cell lines were seeded in 24-well plates at 10^5 cells per well and incubated overnight at 36˚C in a 5% CO_2 atmosphere until a monolayer was formed. Cells were washed with MEM and inoculated with the respective viruses in a volume of 0.3 ml (infectious dose of 0.1 TCID50 per cell), marking this as the zero time point. Virion adsorption, the process by which viruses attach to host cell surfaces, was performed over 1 hour at room temperature. Following this period, unbound virions were washed off with MEM, and the plates were transferred to a CO_2 incubator for 7 hours to allow further cycles of intracellular viral replication. An equal volume of cell culture medium was added to control wells instead of virus-containing material. These samples served as controls for assessing the cytotoxicity of the effect.

Sample Groups

1) Group 1: High-voltage low-frequency exposure during virion adsorption phase (0 - 1 hour).

2) Group 2: Exposure during the intracellular replication phase of the virus (1 - 8 hours), for 20 minutes every 2 hours.

3) Group 3: Exposure during both the adsorption and replication phases, for 20 minutes every 2 hours.

4) Group 4: Control samples, under the same conditions but without exposure to high-voltage low-frequency electric field.

The electric field effect was implemented by placing a conductive plate (electrode) under the plates, which delivered a constant current potential of about 30-50 kV, changing polarity every 5 seconds (~0.2 Hz) using the Healectrics™ device (model S02). The intensity of the electric field in the exposure zone was approximately 30 - 50 kV/cm or ~10^5 V/m.

Virus Titration and Cytotoxicity Assessment

After 8 hours post-infection, the infectious activity of the viral progeny was assessed as described below. Cells from respective permissive lines were seeded in 96-well plates at 10^4 cells/well in 100 µl of complete MEM and incubated for 24 hours at 36˚C in a 5% CO_2 atmosphere to form a monolayer. Viral titers were determined by calculating the highest dilution of samples that showed viral CPE, using the Reed-Muench method [6], and expressed in logarithms of the number of 50% infectious doses (lg TCID50). The cytotoxic effects of the electric field were evaluated by assessing the viability of cells post-exposure using the MTT assay, which reflects cell viability through the reduction of the tetrazolium dye by mitochondrial and partly cytoplasmic dehydrogenases [7].

Cells from the control plate without field exposure (group 4) and the plate subjected to maximum field exposure (group 3) were washed with MEM, and 100 µl of a solution (0.5 mg/ml) of 3-(4,5-dimethylthiazol-2)-2,5-diphenyltetrazolium bromide in cell medium was added to the wells. Cells were incubated at 36˚C in a 5% CO_2 atmosphere for 2 hours and washed with physiological solution for 5 minutes. The purple formazan precipitate was dissolved in DMSO, and the optical density was measured using a Multiscan FC plate analyzer (Thermo Scientific) at a wavelength of 540 nm. Cell viability was assessed by the optical density in the wells compared to the control cells without treatment.

3. Results

The effects of high-voltage low-frequency electric field exposure on the infectivity of various human viruses were thoroughly investigated in this study. The results, which summarize the infectivity rates under different treatment conditions, are displayed in **Tables 1-4**.

Tables 1-3: Reproduction Rates of Human Viruses Under Electric Field Exposure

- S: Exposure during the sorption phase.
- R: Exposure during the intracellular replication phase.
- S + R: Exposure during both phases.
- KV: Control, no field exposure.

Table 1. HSV-1.

Group	Infectivity (lg TCID50/0.2 mL)
Control (KV)	3.75 ± 0.50
Sorption (S)	3.00 ± 0.00
Replication (R)	3.50 ± 0.50
Sorption + Replication (S + R)	2.75 ± 0.50

Table 2. Influenza H1N1.

Group	Infectivity (lg TCID50/0.2 mL)
Control (KV)	4.25 ± 0.50
Sorption (S)	3.50 ± 0.50
Replication (R)	4.00 ± 0.00
Sorption + Replication (S + R)	3.25 ± 0.50

Table 3. HCoV OC43.

Group	Infectivity (lg TCID50/0.2 mL)
Control (KV)	3.00 ± 0.00
Sorption (S)	2.50 ± 0.50
Replication (R)	3.00 ± 0.00
Sorption + Replication (S + R)	2.25 ± 0.50

Table 4. T-Test Results for Viral Infectivity.

Comparison	HSV-1 P-value	Influenza H1N1 P-value	HCoV OC43 P-value
Sorption (S) vs Control (KV)	0.058**	0.098	0.182
Replication (R) vs Control (KV)	0.391	0.537	0.391
Sorption + Replication (S + R) vs Control (KV)	0.030*	0.030*	0.058**

The following observations for each type of virus were recorded.

HSV-1: Exposure during the combined sorption and replication phases (S + R) resulted in a significant reduction in infectivity, achieving up to a 90% decrease in viral activity.

Influenza H1N1: Similarly, a significant reduction in infectivity was observed with the combined phase exposure, confirmed by statistically significant p-values.

HCoV OC43: While the electric field exposure led to a reduction in infectivity by a factor of 5, this virus was comparatively less sensitive to the treatment. The p-value was close to the significance threshold (0.05), indicating a potential trend toward sensitivity that may warrant further investigation.

Table 5 demonstrates that electric field exposure did not significantly affect cell viability, as measured by optical density (OD 540). Even under the longest exposure conditions, cell viability remained comparable to controls, suggesting minimal cytotoxicity within the parameters tested.

Table 5. Optical density (OD 540) in cell culture.

	Vero (OD 540)		MDCK (OD 540)	
	Without exposure	After treatment	Without exposure	After treatment
Mean ± SD	1.1328 ± 0.0076	1.1244 ± 0.0050	1.1837 ± 0.0302	1.2142 ± 0.0658
P-value		P = 0.1220		P = 0.4455

4. Detailed Descriptions and Interpretations

4.1. Viral Infectivity

Comparing the control group with the group subjected to exposure at both stages for influenza and herpes viruses yielded a statistically significant p-value. The comparison of the control group and the group with exposure at both stages for the coronavirus HCoV OC43 showed a p-value close to the significance level (0.05), which may also indicate a trend towards statistical significance.

Among the viruses used, HCoV OC43 was the least sensitive to the electric field exposure. Sequential exposure at the sorption and replication stages for this virus led to an average reduction in viral activity by 5 times. For the influenza virus and HSV1, the infectivity of the viral progeny decreased by an average of 10 times (inhibition degree of 90%).

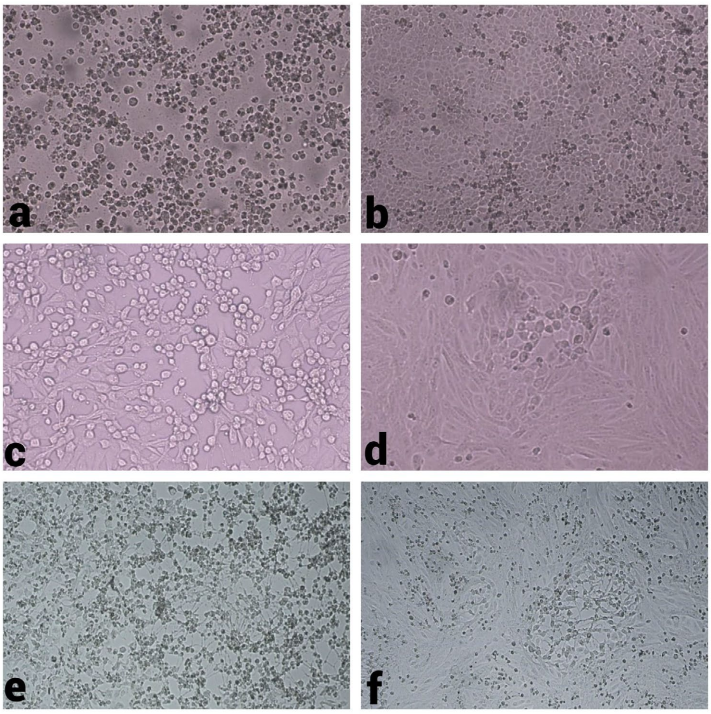

Figure 1. Cytopathic effect of the influenza virus in MDCK cells (a, b), Herpes Simplex Virus type 1 in Vero cells (c, d), and Human Coronavirus OC43 in Vero cells (e, f) under the influence of high-voltage low-frequency electric field. (a, c, e) control samples; (b, d, f) cells infected with viruses cultured under the influence of the electric field.

These results were confirmed by morphological analysis of virus-specific CPE development 48 hours after cell infection during the titration of viral infectivity. Virus-induced cell damage caused by viruses cultured under the influence of the electric field was less pronounced than with control virus infection. The foci of viral reproduction and the overall area of damage were smaller compared to the control samples (**Figure 1**).

As shown by the presented results, the effect of the electric field on viruses replicating in cells led to a reduction in viral activity. This effect was most pronounced during the sequential exposure at the sorption and replication stages and to a lesser extent during the sorption stage alone. The impact on infected cells at the replication stage had the least influence on the infectivity of the viral progeny. In this case, the virus titers in the culture fluid were statistically identical to the control values. However, this impact during the replication stage had a synergistic effect, enhancing the impact during the sorption stage.

The findings align with the atomistic modeling results by [8], showing that external electric fields (~10^5 V/m) can destabilize viral membrane proteins such as the spike (S) protein of SARS-CoV-2. These changes include the transformation of critical structural elements, significantly reducing the virus's ability to bind to cell receptors. This mechanism explains the observed reduction in viral activity and supports the potential application of high-voltage low-frequency electric fields as an antiviral strategy.

4.2. Cell Viability

As can be seen from the obtained results (**Table 5**), even the longest exposure to the electric field on the cell culture did not lead to a decrease in cell viability. This was also confirmed visually (**Figure 2**). In the morphological analysis of the cell

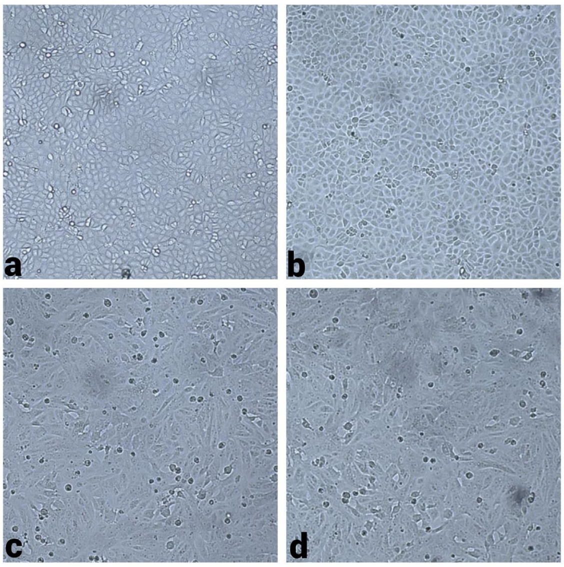

Figure 2. Morphology of MDCK cells (a, b) and Vero cells (c, d) under the influence of high-voltage low-frequency electric field. (a, c) control cells; (b, d) cells after exposure.

monolayer, no differences were observed between the control cells without field exposure and the cells exposed to the field during the sorption and replication stages. The lack of observable cytotoxic effects during the experiment supports the safety of this approach in short-term exposure. However, additional long-term studies are essential to confirm that repeated or prolonged exposure does not result in delayed cytotoxic effects, ensuring a comprehensive safety profile for potential therapeutic applications.

5. Conclusions

The study demonstrated the effects of low-frequency high-voltage electric field exposure on the replication of Influenza, HSV-1, and HCoV OC43 viruses in permissive cell cultures. The key findings are:

1) Reduction in Viral Activity: The electric field exposure led to a significant reduction in viral infectivity, particularly during the sorption and combined sorption-replication stages, with a decrease in viral activity by up to 90% in certain conditions.

2) Least Sensitivity: Among the viruses tested, HCoV OC43 showed the least sensitivity to the electric field exposure, with a reduction in viral activity by a factor of 5 in the combined group. This can be explained by the relatively slow replication rate of the virus.

3) Statistical Significance: Comparing the control group with the group subjected to exposure at both stages for influenza and herpes viruses yielded a statistically significant p-value. For the coronavirus HCoV OC43, the comparison showed a p-value close to the significance level (0.05), indicating a potential trend towards statistical significance.

4) Potential Applicability to SARS-CoV-2: The human coronavirus OC43 used in this study belongs to the same phylogenetic group (family Coronaviridae) as the epidemiologically significant SARS-CoV-2 virus, which caused the COVID-19 pandemic in 2019. Both viruses are large enveloped viruses with a single-stranded (+)-RNA genome of approximately 30 kb. The high degree of similarity between these viruses, along with the observed effectiveness of the electric field treatment on other enveloped viruses, including influenza virus (family Orthomyxoviridae) and herpes virus (family Herpesviridae), suggests that SARS-CoV-2 might also be sensitive to high-voltage, low-frequency electric field exposure.

5) No Significant Cytotoxicity: The electric field treatment did not significantly affect the viability of Vero and MDCK cells, as confirmed by both MTT assays and morphological analysis, indicating the safety of the electric field exposure for host cells. However, additional long-term studies are needed to confirm the absence of delayed cytotoxic effects, ensuring comprehensive safety for therapeutic application.

6) Indirect Application Potential: During the experiment, there was no direct contact between the viral solution and the electrode to which the electric field was

applied, suggesting the possibility of affecting viral activity through electrodes applied to the skin of the patient.

7) Supporting Findings from Literature: Our study corroborates the findings of [8], demonstrating that high-voltage electric fields have the greatest effect at the sorption stage. In their study, it was shown through atomistic modeling that external electric fields (~10^5 V/m) can destabilize the spike (S) protein, causing long-term structural damage. These changes include the transformation of two parallel beta-sheets, responsible for high affinity to the ACE2 receptor, into an unstructured tangle that significantly reduces binding capability. This effect was observed across various mutant RBDs, including VOCs B.1.1.7 (UK), B.1.351 (South Africa), and P.1 (Brazil). The structural flexibility of the S-protein, while enhancing the virus's ability to penetrate the cell, also makes it highly vulnerable to high-voltage electric fields. The conclusions of their study suggest that a purely physical method can weaken SARS-CoV-2 without further biochemical treatment. Based on the fact that the sorption stage contributes the most to reducing viral activity, we believe that, as in the study [8], the applied high-voltage low frequency field destabilizes the S-protein or other membrane proteins of the viruses, thereby hindering their ability to bind to cell receptors such as ACE2. Thus, low frequency high voltage electric field exposure can be beneficial not only as a therapeutic intervention but also primarily as a preventive measure against a wide range of membrane viruses, including SARS-CoV-2. Further research is necessary to explore the full potential and optimize the parameters of this promising antiviral strategy.

8) This destabilization complicates the process of virus-cell receptor binding, making the application of electric fields potentially useful for preventing infections by various types of membrane-bound viruses.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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