Letter to the Editor

Antiviral properties of blue laser in an *in vitro* model of HSV-1 infection

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Herpes simplex virus type-1 (HSV-1) is known to cause lifelong infections in humans. First infection is characterized by gingiva-stomatitis and pharyngitis, while virus reactivation causes recurrent herpes labialis with ulcerations on intraoral mucosa, mouth or external facial skin [1].

Laser therapy (LT), set at red and infrared wavelengths, has been reported as able to reduce HSV-1 recurrence and duration of herpetic sores [2]. Despite the blue wavelength already showed its efficacy in killing different strains of bacteria, it has never been tested on viruses [3].

We explored the antiviral properties of blue light in an *in vitro* model obtained infecting human keratinocytes cell line (HaCaT) with HSV-1.

The experimental settings employed are reported in Fig.1a.

Blue LT successfully tackles HSV-1: we measured an increment in cells vitality and a decrease of HSV-1 viral load in supernatant of infected cells, previously irradiated when compared to cells infected not irradiated ($p=0.0002$ and $p=0.0007$ respectively, setting-1, Fig.1a,b,c).

Cytotoxicity test to detect the most effective LT protocol tolerated was performed, selecting the following parameters: 0.15 W/cm^2, 30 J/cm^2, 5 Hz. LT was able to decrease the viral load without affecting cells' vitality ($p=0.02$, setting-2, Fig.1a,d,e).

Instead, settings-3 and -4 (irradiation of cells prior to infection and irradiation of cells already infected for 24 hours, Fig.1a) showed no difference in viral load or cells vitality (data not shown). Finally, in cells infected for 1 hour prior to irradiation, LT reduced viral load, but statistical significance was not achieved (setting-5, Fig.1a,f,g). The results were confirmed by plaque forming assay (Fig.1h).

Our findings show the antiviral impact of blue laser on HSV-1; its influence is more effective on virus irradiated alone, while only a slightly trend on infected cultures was observed, suggesting the effect of LT in inactivating the virus prior to cell entry. On the contrary, when the virus is already inside the cells, LT effect is less evident; finally, LT does not increase cells' resistance to infection.

Different reports described the ability of LT in both reducing HSV-1 recurrence and helping the healing of oral vesicles. Only one study observed that HaCaT cells, HSV-1 infected and irradiated with infrared laser light, presented reduction in viral replication and increase of pro-inflammatory molecules, speculating that LT activates immune anti-viral response and inhibits virus spreading [4].

Unfortunately, the exact mechanism at the basis of LT anti-viral effect is still unknown; researchers hypothesized an immuno-modulatory effect rather than a direct anti-viral effect.

Our results showed a direct inhibitory effect of blue LT on the virus itself. So, further studies are warranted to understand in which way the blue LT exerts its anti-viral effect, in order to shift our in vitro results in a clinical setting, thus promoting its use on HSV-1 infected patients.

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Conflicts of Interest

Ottaviani G. has a part-time employment in K-Laser d.o.o. (Sežana, Slovenia). The other authors none.

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Figure 1. Effects of blue laser treatment on HSV-1 infection in HaCaT cell culture.

a) Schematic representation of experimental settings employed. HSV-1 (a clinical isolate of HSV-1 showing a 99% identity with the TG3 strain (sequence ID: HQ686001) in a 300 bp region of the thymidine kinase gene) was quantified using Taq-man assay on Real Time PCR platform (4). The experiments were performed in four replicates executed in two independent experimental days. Cells vitality was assessed with MTT Cell proliferation assay (Trevigen, Gaithersburg, Maryland, U.S.A.) in eight replicates in two independent experimental days. Thermal monitoring during irradiation was performed with a FLUKE Ti20 thermal imager infrared camera (Everett, Washington, U.S.A., supplementary figure 1).

b) HSV-1 were diluted at a concentration of 540000 viral copies/ul and irradiated using a gallium arsenide (GaAs) + indium gallium aluminium arsenide phosphide (InGaAlAsP) diode laser device (class IV, K-Laser Cube series, K-laser d.o.o., Sežana, Slovenia) with the following protocol: 445 nm, 0.3 W/cm^2, 60J/ cm^2, CW.

After 30' the irradiated virus was transferred to HaCaT cells culture and then, after other 24 hours HSV-1 quantification was performed in cells supernatant (setting 1). The results are reported as percentage respect to cells infected with not treated virus (Mann-Withney test, two tails).

c) Cells vitality after 24 hours of infection with irradiated virus (setting 1). The results are reported as percentage respect to untreated cells (Mann-Withney test, two tails).

d) HSV-1 quantification after 24 hours from laser irradiation (445 nm, 0.15 W/cm^2, 30 J/cm^2, 5 Hz) of virus alone (setting 2). The results are reported as percentage respect to cells infected with untreated virus (Mann-Withney test, two tails).

e) Cells vitality after 24 hours of infection with irradiated virus (setting 2). The results are reported as percentage respect to untreated cells

f) HSV-1 quantification after 24 hours from irradiation (445 nm, 0.15 W/cm^2, 30 J/cm^2, 5 Hz) of cells infected for 1 hour with HSV-1 before irradiation (setting 5). The results are reported as percentage respect to cells infected with untreated virus.

g) Cells vitality after 24 hours from irradiation: cells infected for 1 hour with HSV-1 before irradiation (setting 5). The results are reported as percentage respect to untreated cells.

h) Representative images of plaque forming unit (PFU) assay, performed using the 5 settings. At the end of the time scheduled, 200 µl of the supernatant at different dilution were used to infect Vero cells in 24 multi-wells plate for 2 days, after which the plates were fixed with paraformaldehyde 4% in PBS for 30' and colored with crystal violet (Merck KGaA, Darmstadt, Germany).

***$p<0.001$

*$p<0.05$

Supplementary figure 1. Thermal images acquired during irradiation using the following protocols: left 445 nm, 0.3 W/cm^2, 60J/ cm^2, CW; right 445 nm, 0.15 W/cm^2, 30 J/cm^2, 5 Hz.

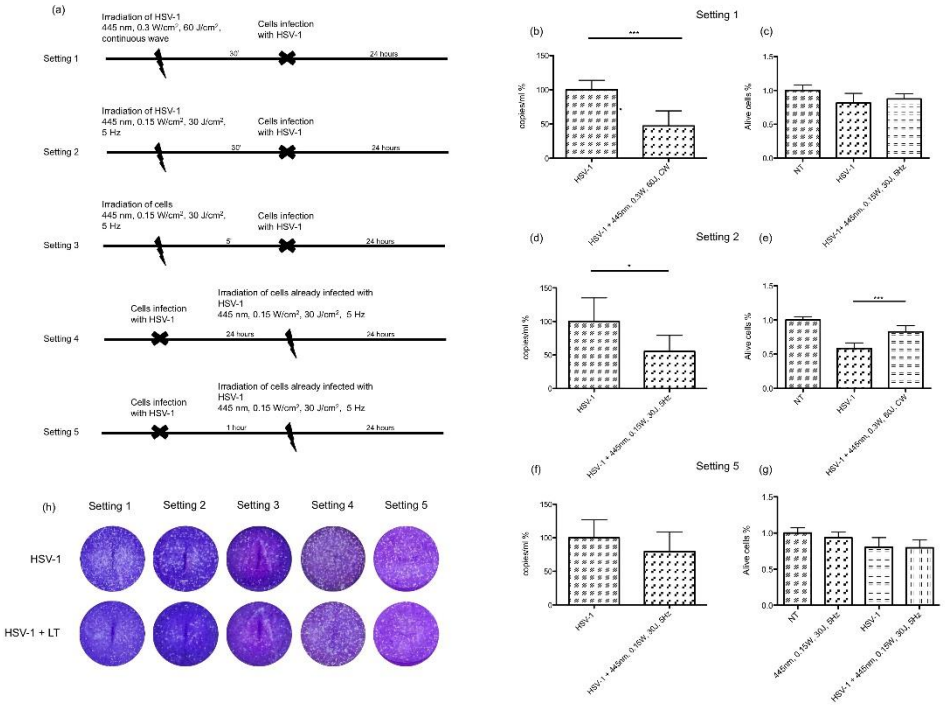

Figure. 1