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Low-power laser irradiation effects on cell proliferation and viability of cultured Jurkat E6.1 T-lymphocyte leukemia cell line

Layla M.H. Al-Ameri[1], Amel M. Maki[2], Ali H. Ad'hiah[1]*, Qiuyu Wang[3] and Mayada H. ALQaisi[3]

[1]Tropical-Biological Research Unit, College of Science, [2]Institute of Laser for Postgraduate Studies, University of Baghdad, Baghdad, Iraq and [3]School of Healthcare Science, Manchester Metropolitan University, Manchester, UK.
*Corresponding author: adhiah1756@yahoo.com

Abstract

The effects of low-power laser irradiation (LPLI) on cell proliferation and viability of cultured Jurkat E6.1 T-lymphocyte leukemia (JETL) cells were assessed at 635nm (visible) and 780nm (near infrared) wavelengths. For each wavelength, the cells were exposed to three energy densities (6.116, 9.174 and 12.232 J/cm^2), and then the cells were examined post-irradiation at three incubation time periods, which were 24, 48 and 72 hours. The results showed significant variations between irradiated and unirradiated cells, and the effects were dose-, wavelength- and incubation time period-dependent. The lowest cell count (1.987 x 10^6 cell/ml) was observed 72 hours post-irradiation at energy density 9.174 J/cm^2 of 780nm wavelength, while the lowest cell viability (59.3%) was also observed at 9.174 J/cm^2 energy density, but for 635nm wavelength. It was concluded that LPLI can exert a significant impact on the viability and proliferative activity of the cultured cell line.

Keywords: Low-power laser irradiation, Jurkat E6.1 T-lymphocyte leukemia cell line, Proliferation, Viability.

Introduction

In the late 1960s, low-power laser irradiation (LPLI) was introduced to modulate biological processes, especially in the field of medicine, and since then medical treatment with LPLI has made pronounced advancements, and currently there is no doubt that such type of irradiation acts directly on the organism at the cellular level, in which molecular changes have been observed (Abrahamse, 2012). In this regard, it has been demonstrated that LPLI can alter metabolic processes in mammalian cells, and can accelerate or inhibit cellular metabolism, depending on wavelength, dose and treatment protocol (Prindeze *et al*., 2012). As a consequence, *in vitro* studies have suggested several potentials of LPLI; for instance, changes in growth-factor production, ATP synthesis and cell biological activities, and further observations also indicated that the photobiological effects of LPLI may involve changes in cell cycle progression, and growing body of experimental and clinical studies have demonstrated that LPLI regulates cell survival, proliferation, and differentiation (Pires-Oliveira *et al*., 2010; AlGhamdi *et al*., 2012).

LPLI effects on cell proliferation have been under intensive investigations, and it has been suggested that cell proliferation is a very important physiological target for LPLI. Increased proliferation after LPLI has been shown in many cell types *in vitro*, including fibroblasts, keratinocytes, osteoblasts, lymphocytes, stem cells, smooth muscle cell and endothelial cells (Frigo *et al*., 2010; Feng *et al*., 2012; Gomes Henriques *et al*., 2014). In contrast to these stimulatory effects, other investigations have found a damaging or even a destructive action of LPLI, and an observation made by Ocaña Quero *et al*. (1997a) described a degenerative effect of He-Ne laser irradiation on bovine oocytes. The same group of investigators demonstrated that Helium-Neon laser radiation induced sister chromatid exchanges in sheep peripheral blood mononuclear cells (Ocaña Quero *et al*., 1997b). A further investigation demonstrated that stimulation by 458nm and 467nm mercury lamps inhibited pig kidney embryo cell division in the metaphase (Rocha *et al*., 2002). In addition, Ohara *et al*. (2002) found that blue light inhibited the growth of B16 melanoma cells *in vitro*. Recently, Lev-Tov *et al*. (2013) studied the effect of red light-emitting diode-generated low-level light therapy on

fibroblast proliferation and viability *in vitro*, and demonstrated that such irradiation can effectively inhibit fibroblast proliferation.

Accordingly, the effects of LPLI on cell proliferation has been debatable because studies have found both an increase and a decrease in proliferation frequency of irradiated cultured cells; therefore there is still controversy concerning the effect of LPLI on cell proliferation, in which both stimulatory and inhibitory actions of visible laser light on cell cultures have been reported. Such inconsistency might be related to laser irradiation parameters (for instance; wavelength, power density and fluence), as well as, cell type may also account for such differences (Prindeze *et al*., 2012).

In the present investigation, cell proliferation and viability were evaluated in cultured human Jurkat E6.1 T-lymphocyte (JETL) cell line after LPLI at two wavelengths (635nm and 780nm) that each corresponded to three energy densities (6.116, 9.174 and 12.232 J/cm^2) and for three incubation time periods (24, 48 and 72 hours).

Materials and Methods

Cell line: Jurkat Clone E6.1 is a human T lymphoblastoid cell line, which was established from the peripheral blood of a 14-year-old boy with acute T cell leukemia by Schneider and co-workers in 1977 (Schneider et al., 1977). The cell line was purchased from Sigma-Aldrich (UK), and it was maintained at 37°C under humidified air supplemented with 5% CO_2 in RPMI- 1640 medium containing 10% fetal bovine serum, 2 mM L-glutamine, 100 µg/ml streptomycin, and 100 units/ml penicillin (GIBCO, UK).

Seeding of JETL cells: After assessing viability, the JETL cell suspension (1 ml) was made-up to 15 ml with RPMI- 1640 medium and transferred to a tissue culture flask (25 cm^2) and incubated at 37°C, 5% CO_2 and 80% relative humidity for three days. After that, the flask contents was mixed gently and transferred to two 10ml centrifuge tubes. The tubes were centrifuged (1200 rpm for 5 minutes) to pellet cells, and first, each cell pellet was suspended in 1 ml of culture medium to assess cell viability, and then, the cell suspension of each tube was made-up to 15 ml with culture medium and transferred to a tissue culture flask (25 cm^2) and incubated at 37°C, 5% CO^2 and 80% relative humidity for three days to sub-culture cells. After incubation, the cells were cryopreserved in liquid nitrogen for a later use.

Setting-up cultures for LPLI: The cryotubes were obtained from the liquid nitrogen, and cell suspension was thawed and washed. The cell viability was assessed by a dye-exclusion (trypan blue) test, and cell count was adjusted to 4 × 10^5 cell/ml with culture medium. The cultures were set-up in 12-well flat-bottomed tissue culture plate (Sigma-Aldrich), and in each well, 2 ml of cell suspension were dispensed. The plate was then incubated overnight, and after incubation, the cells were ready for LPLI. For each treatment, there were hexa-replicates (6 wells).

LPLI of cultures: A continuous wave portable GaAlAs (Gallium, Aluminium, Arsenide) laser (Scientific Ltd., UK) with wavelengths of 635nm and 780nm were utilized for all experiments. Before starting the experiments, the GaAlAs laser equipment was calibrated in a laser power energy monitor (Scientific Ltd., UK). The laser parameters were: spot size, 5 mm; output power, 20, 30 and 40 mW; exposure time, 60 seconds; energy density, 6.116, 9.174 and 12.232 J/cm^2 and power density of 0.1019, 0.1529 and 0.2038 W/cm^2.

Each well in the tissue culture plate was irradiated with LPLI at a 635nm wave length that had energy density 6.116, 9.174 or 12.232 J/cm^2. Further plates were similarly irradiated but at a wavelength of 780nm. After irradiation, the plate was incubated (37°C, 5% CO_2 and 80% relative humidity) for 24 hours, and after incubation, the cultured cells were assessed for cell proliferation and cell viability. Further plates were incubated for 48 hours and 72 hours; therefore the laboratory assessments were carried out at the end of three incubation time periods (ITPs). Each type of irradiation and ITP was paralleled by a control culture plate, in which the cells were not exposed to LPLI.

Cell proliferation and cell viability: After the end of an ITP, 0.01 ml of cell suspension was diluted up to 0.20 ml of trypan blue stain (1:19), and after mixing, 10 µl of cell suspension in trypan blue was dispensed at the edge of the of cover-slip until the chamber of Neubauer-improved haemocytometer was filled by capillary action. The haemocytometer was left three minutes to settle the cells before examination by phase contrast inverted microscope using 10X objective lens. The total number of cells was counted and then percentage of viable cells was recorded.

Statistical analysis: Data were given as mean ± standard deviation (SD), and differences between means were assessed by ANOVA (Analysis of Variance), followed by the least significant difference (LSD), in which the probability (P) was considered significant when it was ≤ 0.05. The analyses were carried out using the statistical package SPSS version 13.0.

Results and Discussion

Cell proliferation at 635nm wavelength: In unirradiated cell cultures (controls), the cell count was 3.182 ± 0.088 × 10^6 cell/ml after 24 hours ITP, which was progressed further to 5.258 ± 0.114 and 6.433 ± 0.112 × 10^6 cell/ml, respectively at 48 and 72 hours ITP, and such difference was significant ($P \leq 0.001$). When cell cultures were laser irradiated, the cell count mean in general was decreased in the three ITPs compared to controls. The cell counts were 2.773 ± 0.071, 2.822 ± 0.097 and 2.648 ± 0.146 × 10^6 cell/ml for the three ITPs (24, 48 and 72 hours, respectively) at energy density 6.116 J/cm^2. The corresponding counts for 9.174 J/cm^2 were 3.067 ± 0.326, 3.334 ± 0.142 and 2.552 ± 0.121 × 10^6 cell/ml, respectively, and for 12.232 J/cm^2, they were 3.287 ± 0.083, 2.307 ± 0.153 and 3.463 ± 0.192 × 10^6 cell/ml, respectively (Figure 1).

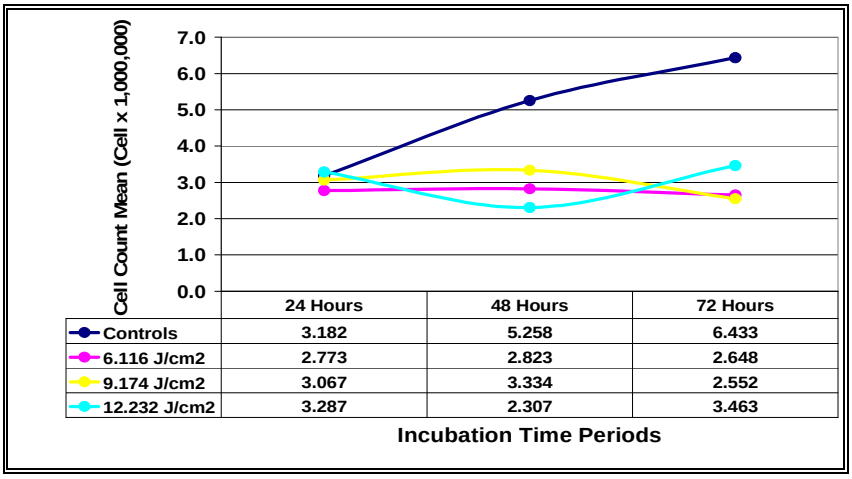
Cell proliferation at 780nm wavelength: Control cell cultures revealed a progressive cell count as the ITP was progressing. They started with a cell count of 4.330 ± 0.050 × 10^6 cell/ml after 24 hours ITP; 5.730 ± 0.088 × 10^6 cell/ml after 48 hours ITP; and 7.283 ± 0.096 × 10^6 cell/ml after 72 hours ITP. The difference between these means was significant ($P \leq 0.001$). Cultures treated with LPLI were in general showed a less cell count than in controls with a different manner of progression. At the first energy density (6.116 J/cm^2), the count was 3.060 ± 0.203 × 10^6 cell/ml after 24 hours ITP, and then, it was increased to 3.668 ± 0.317 × 10^6 cell/ml after 48 hours ITP, and finally, it was decreased to 2.340 ± 0.154 × 10^6 cell/ml after 72 hours ITP. A similar pattern of cell count distribution was observed at energy densities 9.174 J/cm^2 (2.690 ± 0.180, 3.713 ± 0.082 and 1.9867 ± 0.080 × 10^6 cell/ml, respectively) and 12.232 J/cm^2 (3.510 ± 0.151, 4.366 ± 0.071 and 3.154 ± 0.125 × 10^6 cell/ml, respectively). In the three cases, the count after 48 hours ITP was significantly ($P \leq 0.01$) higher than the count of 24 and 72 hours ITPs (Figure 2).

Cell viability at 635nm wavelength: The four cell cultures (Controls and energy densities 6.116, 9.174 and 12.232 J/cm^2) showed a similar pattern of cell viability distribution in the three investigated ITPs (24, 48 and 72 hours). The cell viability demonstrated a gradual decrease as ITP was progressing. It was 86.2 ± 0.3, 78.9 ± 0.7 and 68.5 ± 0.4%, respectively in controls; 80.7 ± 0.9, 76.2 ± 0.4 and 63.7 ± 1.4%, respectively at 6.116 J/cm^2; 80.8 ± 2.6, 71.7 ± 0.9 and 59.3 ± 1.4%, respectively at 9.174 J/cm^2; and 84.5 ± 3.1, 70.5 ± 3.4 and 69.7 ± 1.2%, respectively at 12.232 J/cm^2. In the four cell cultures, the difference between the means of the three ITPs was significant ($P \leq 0.001$) (Figure 3).

Cell viability at 780nm wavelength: The manner of cell viability distribution in the four investigated cell cultures was almost similar with some fluctuation in the percentage of cell viability, which was correspondent to the type of culture (controls or LPLI cultures with the energy densities 6.116, 9.174 and 12.232 J/cm^2) and the ITP (24, 48 or 72 hours). In unirradiated cultures (controls), the cell viability percentage was 96.2 ± 1.8% after 24 hours ITP, and then it was decreased to 77.3 ± 1.0% after 48 hours ITP, and a further decrease was observed after 72 hours ITP (59.5 ± 0.9%). The corresponding percentages in cell line cultures irradiated with LPLI at 6.116 J/cm^2 were 84.0 ± 2.8, 73.0 ± 1.5 and 59.7 ± 2.4%, respectively; at 9.174 J/cm^2 were 87.2 ± 2.4, 73.2 ± 1.9 and 64.5 ± 0.9%, respectively; and at 12.232 J/cm^2 were 86.2 ± 1.4, 76.0 ± 0.7 and 67.7 ± 0.9%, respectively. The difference between the means of the three ITPs in each type of the four cell cultures was significant (Figure 4).

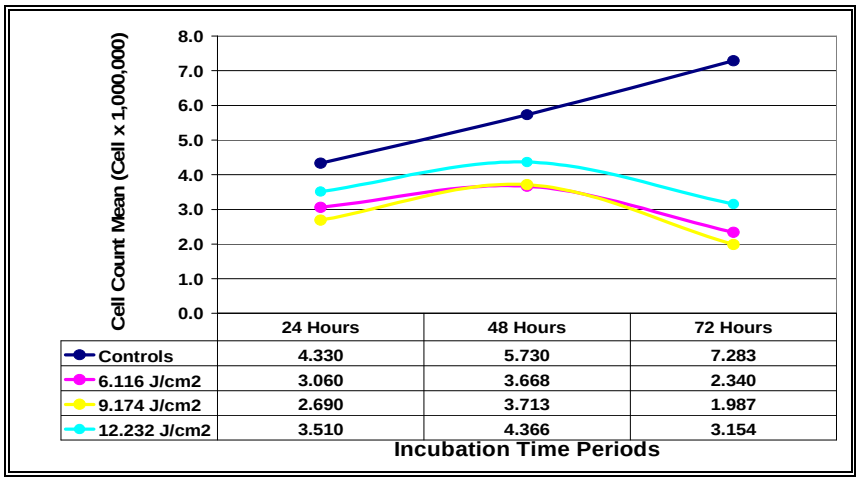
The present results demonstrated that the proliferation activity of the investigated cell line (JETL) showed different responses to LPLI in a manner that was dependent on wavelength, energy density and ITP. The lowest cell count (1.987 x 10^6 cell/ml) was observed 72 hours post-irradiation at energy density 9.174 J/cm^2 of 780nm wavelength. Such findings may contrast the suggested bio-stimulatory effects of LPLI by different investigations, in which LPLI has been demonstrated to enhance cell proliferation (Frigo *et al*., 2010; Feng *et al*., 2012; Gomes Henriques *et al*., 2014). However, other studies were in favor of that LPLI showed inhibitory effects on cell proliferation. Liu *et al.* (2004) demonstrated that proliferation of HepG2 and J-5 cells was inhibited by LPLI, and maximal effect was achieved with 90 and 120 seconds of exposure time (of energy density 5.85 and 7.8 J/cm^2, respectively) for HepG2 and J-5, respectively.

The decreased ratio of cell number by this dose of irradiation was 72% and 66% in HepG2 and J-5 cells, respectively. Besides that, they found that the architecture of intermediate filaments in these cells was disorganized by laser irradiation, and the expression of intermediate filament-associated protein, synemin, was also reduced. The authors suggested that the mechanism of inhibition might be due to down-regulation of synemin expression and alteration of cytokeratin organization that was caused by laser irradiation.



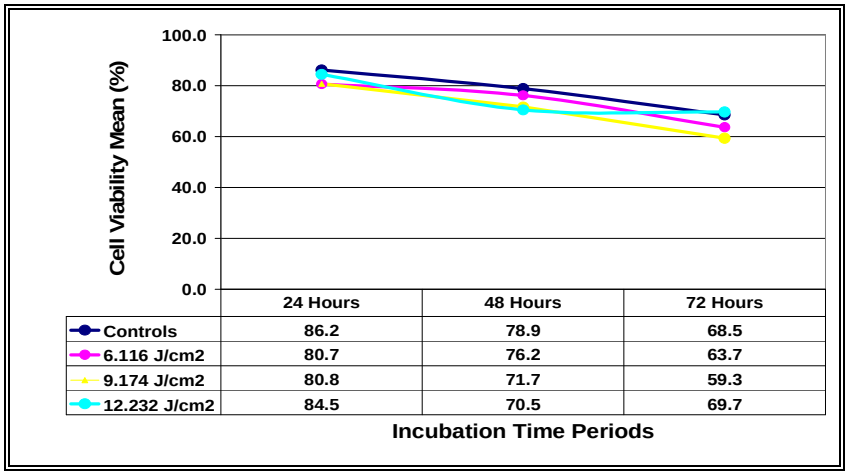
	24 Hours	48 Hours	72 Hours
Controls	3.182	5.258	6.433
6.116 J/cm2	2.773	2.823	2.648
9.174 J/cm2	3.067	3.334	2.552
12.232 J/cm2	3.287	2.307	3.463

Figure (1): Cell count mean of cultured Jurkat E6.1 T-lymphocyte cell line after low-power laser irradiation at 635nm wavelength (energy densities: 6.116, 9.174 and 12.232 J/cm^2) for three incubation time periods (24, 48 and 72 hours).



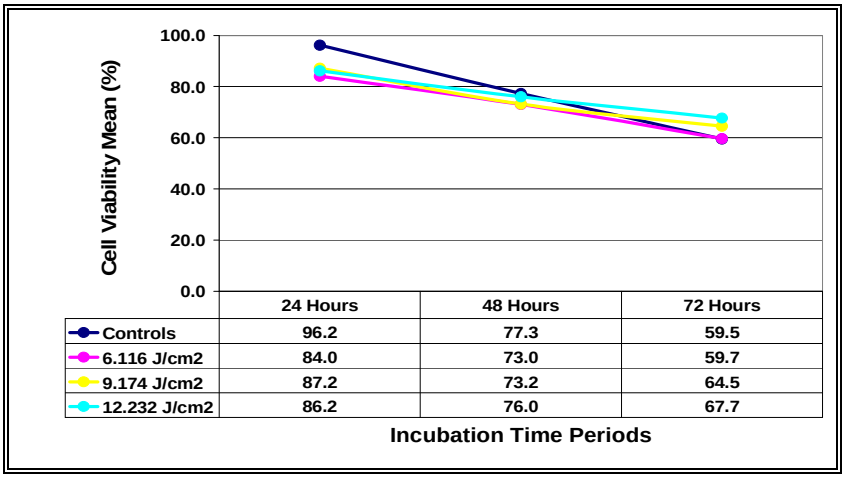
	24 Hours	48 Hours	72 Hours
Controls	4.330	5.730	7.283
6.116 J/cm2	3.060	3.668	2.340
9.174 J/cm2	2.690	3.713	1.987
12.232 J/cm2	3.510	4.366	3.154

Figure (2): Cell count mean of cultured Jurkat E6.1 T-lymphocyte cell line after low-power laser irradiation at 780nm wavelength (energy densities: 6.116, 9.174 and 12.232 J/cm^2) for three incubation time periods (24, 48 and 72 hours).



	24 Hours	48 Hours	72 Hours
Controls	86.2	78.9	68.5
6.116 J/cm2	80.7	76.2	63.7
9.174 J/cm2	80.8	71.7	59.3
12.232 J/cm2	84.5	70.5	69.7

Figure (3): Cell viability mean of cultured Jurkat E6.1 T-lymphocyte cell line after low-power laser irradiation at 635nm wavelength (energy densities: 6.116, 9.174 and 12.232 J/cm^2) for three incubation time periods (24, 48 and 72 hours).



	24 Hours	48 Hours	72 Hours
Controls	96.2	77.3	59.5
6.116 J/cm2	84.0	73.0	59.7
9.174 J/cm2	87.2	73.2	64.5
12.232 J/cm2	86.2	76.0	67.7

Figure (4): Cell viability mean of cultured Jurkat E6.1 T-lymphocyte cell line after low-power laser irradiation at 780nm wavelength (energy densities: 6.116, 9.174 and 12.232 J/cm^2) for three incubation time periods (24, 48 and 72 hours).

The same group of investigators confirmed their findings further by using Western blot and immunofluorescent staining to examine the expression and distribution of histone and cytoskeletal proteins after LPLI, and presented evidence that histone synthesis was reduced, and such effect of LPLI may further reduce the proliferation rate of human hepatoma cell lines HepG2 and J-5 (Liu *et al*., 2006). In addition, cell viability, alkaline phosphatase activity and the expression of osteopontin and collagen type I mRNA were investigated in human osteoblast-like cells after LPLI (Stein *et al*., 2008). Their results revealed that the three investigated parameters were slightly enhanced in cells irradiated with 1 J/cm^2 compared with untreated control cells, but increasing the laser dose to 2 J/cm^2 reduced cell viability during the first 48 hours and resulted in persistently lower alkaline phosphatase activity. More recently, it has been demonstrated that LPLI markedly inhibited LPS-induced, pro-inflammatory cytokine expression at an optimal dose of 8 J/cm^2. The inhibitory effect was suggested to be triggered by LPLI and might occur through an increase in the intracellular level of cyclic AMP (cAMP), which acts to down-regulate nuclear factor kappa B (NF-κB) transcriptional activity (Wu *et al*., 2013).

The cell viability of cultured cells was also affected by LPLI, and it was also subjected to the effect of LPLI parameter, as well, ITP, and the lowest cell viability (59.3%) was also observed at 9.174 J/cm^2 energy density, but for 635nm wavelength. However, approximated cell viability (59.7%) was recorded for 780nm wavelength, but at lower power intensity (6.116 J/cm^2). Such findings may augment the view that LPLI can exert a cytotoxic effect on cultured cells. Although, Liu *et al*. (2006) presented a similar finding in human hepatoma cell lines *in vitro*, it is difficult to explain such effect. However, two previous investigations demonstrated that the irradiation with a He-Ne laser caused an increase in frequency of chromosome aberrations in diploid cells of human fibroblasts (Stepanov *et al*., 1977) and irradiation with a semiconductor laser at 660 nm increased output of single-strand breaks of DNA in dose-dependent manner (McKelevey *et al*., 1992). Furthermore, Ocaña Quero *et al*. (1997b) demonstrated that Helium-Neon laser radiation induced sister chromatid exchanges in sheep peripheral blood mononuclear cells. These findings suggest that LPLI may have mutagenic effects, and accordingly, the cell viability is affected. However, Karu (2010) argued that such effects cannot be explained by a direct action of visible light on DNA, and one has to suppose indirect effects, especially if we consider that DNA is sensitive to oxidative damage and reactive oxygen species (ROS) can cause mutations particularly in mitochondrial DNA. In addition, *in vitro* studies confirmed that LLLT was found to alter gene expression, inter-cellular pH balance, mitochondrial membrane potential, generation of transient ROS and calcium ion level in irradiated cells (Pal *et al*., 2007). Such indirect effects of LPLI may impact the genetic make-up of the cell and risk its viability.

However, it might be too early to understand the molecular mechanism(s) behind the decreased proliferation and viability rates induced by LPLI, and further determinations are certainly required; for instance, cell cycle progression, apoptosis and

expression of genes involved in controlling cell cycle.

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