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BIOSTIMULATIVNI EFEKAT LASERA MALE SNAGE: IN VITRO ISTRAŽIVANJE

BIOSTIMULATORY EFFECT OF LOW-LEVEL LASER THERAPY: AN IN VITRO STUDY

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SAŽETAK

Uvod: Laseri male snage nalaze široku primenu u medicini i stomatologiji zahvaljujući svojim analgetskim, antiinflamatornim, antiedematoznim i biostimulativnim efektima. Primena lasera male snage je potpuno bezbolna, neinvazivna i bez štetnih efekata. Laseri male snage koriste zračenje niskog intenziteta koje dovodi do stimulacije ćelijskog metabolizma i bioloških procesa bez termičkog oštećenja tkiva. U stomatologiji se najčešće koristi u terapiji bola, za ubrzavanje zarastanja rana, smanjenje edema i inflamacije, kao i u pripremi i očuvanju mekih tkiva tokom protetskih, parodontoloških i implantoloških procedura.
Cilj: Cilj ovog istraživanja bio je ispitivanje efekta lasera male snage na proliferaciju HeLa ćelije u kulturi u in vitro uslovima da bi se odredili optimalni parametri zračenja koji mogu imati biostimulativni efekat na ćelije.
Meterijal i metode: HeLa ćelijska linija održavana je u hranljivom medijumu DMEM (Dulbecco's Modified Eagle's Minimal Essential Medium, PAA Laboratories GmbH) obogaćenom dodatkom l-glutamina, penicilin-streptomicina (100 IU/ml) i 10% fetalnog goveđeg seruma (FBS). Ćelijska kultura održavana je u inkubatoru (Binder, Germany), u atmosferi zasićenoj vodenom parom, sa 5% CO2, na temperaturi od 37ºC. Ćelije su sađene u sterilne ploče za kultivaciju ćelija sa 96 mesta. Ukupan broj ćelija koji je primenjen po pojedinačnom mestu je 1x104 ćelija u 200 µl DMEM-a. Nakon inkubacije od 24 h vršeno je zračenje ćelija. Ćelije su zračene laserom talasne dužine 658 nm, u kontinuiranom modu. Ćelije su zračene odozgo, kroz kultivacioni medijum, u vertikalnoj sterilnoj komori (Thermo Scientific, United States). Primenjene su tri različite doze zračenja od 4, 8 i 16 J/cm2, tri puta u razmacima od po 24 h. Efekat lasera na proliferaciju ćelija utvrđen je 24 h nakon poslednjeg zračenja MTT testom (3-(4,5-dimetiltiazolil-2)-2,5-difeniltetrazolijum bromide), gde je procenat apsorbance srazmeran rastu ćelija u kulturi.
Rezultati: Rezultati istraživanja pokazali su da doza 8 J/cm2 pokazuju blagi stimulatorni efekat, da je doza od 4 J/cm2 najpribližnija vrednostima kontrole, a doza od 16 J/cm2 pokazuje blag inhibitorni efekat na trodnevni rast HeLa ćelija.
Zaključak: Rezultati istraživanja ukazuju da laser male snage, primenjen u odgovarajućim dozama, može imati biostimulativni efekat na proliferaciju ćelija u kulturi. HeLa ćelije su metabolički najaktivnije pod tretmanom dozom zračenja od 8 J/cm2, a najmanje aktivne pod tretmanom dozom od 16 J/cm2. Ovo istraživanje doprinosi boljem razumevanju uticaja lasera male snage na biološke procese i značaja optimizacije parametara zračenja kako bi se postigli željeni biološki efekti, čime se potencijalno poboljšava mogućnost primene lasera u stomatološkoj terapiji.
Ključne reči: laser male snage, HeLa ćelije, zračenje, ćelijski metabolizam, stomatološka terapija

ABSTRACT

Introduction: Low-level lasers are widely used in medicine and dentistry due to their analgesic, anti-inflammatory, antiedematous and biostimulatory effects. The application of low-level laser therapy (LLLT) is completely painless, non-invasive and free from adverse effects. These lasers emit low-intensity radiation that stimulates cellular metabolism and biological processes without causing thermal damage to tissues. In dentistry, LLLT is most commonly used for pain management, acceleration of wound healing, reduction of edema and inflammation, as well as in the preparation and preservation of soft tissues during prosthodontic, periodontic, and implantological procedures.
Aim: The aim of this study was to investigate the effect of low-level laser irradiation on the proliferation of HeLa cells in vitro, in order to determine the optimal irradiation parameters that may exert a biostimulatory effect on cells.
Materials and Methods: The HeLa cell line was maintained in DMEM (Dulbecco's Modified Eagle's Medium, PAA Laboratories GmbH) enriched with L-glutamine, penicillin-streptomycin (100 IU/ml) and 10% fetal bovine serum (FBS). Cell cultures were incubated in a humidified atmosphere with 5% CO_2 at 37°C (Binder, Germany). Cells were seeded in sterile 96-well culture plates, with 1×10^4 cells in 200 µl of DMEM per well. After 24 hours of incubation, cells were irradiated with a continuous-wave laser at a wavelength of 658 nm. The irradiation was performed from above, through the culture medium, in a vertical sterile chamber (Thermo Scientific, United States). Three different irradiation doses were applied: 4, 8, and 16 J/cm², administered three times at 24-hour intervals. The effect of laser irradiation on cell proliferation was assessed 24 hours after the last irradiation using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), where the absorbance level is proportional to the rate of cell growth in culture.
Results: The results showed that the dose of 8 J/cm² exhibited a mild stimulatory effect, the dose of 4 J/cm² was most similar to control values, while the dose of 16 J/cm² showed a mild inhibitory effect on the three-day proliferation of HeLa cells.
Conclusion: The findings suggest that low-level laser irradiation, when applied at appropriate doses, can have a biostimulatory effect on cell proliferation in vitro. HeLa cells showed the highest metabolic activity when treated with a dose of 8 J/cm² and the lowest with 16 J/cm². This study contributes to a better understanding of the biological effects of low-level laser therapy and highlights the importance of optimizing irradiation parameters to achieve desired biological outcomes, thereby enhancing the potential for laser applications in dental therapy.
Key words: low-level laser, HeLa cells, irradiation, cell metabolism, dental therapy