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Effects on DNA

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Low level laser therapy (LLLT) - Does it damage DNA?

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Abstract

Low level laser therapy (LLLT) has been found beneficial in a wide variety of therapeutic applications (see for example 1). However, some concern has arisen on possible DNA damage. May it be possible that it benefits the patient only at a first glance but damages DNA and therefore increases the risk of therapy induced disease up to an increased cancer risk ?

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What are the facts ? LLLT is usually performed with red (630 nm) or near infrared (830nm) laser light. Typical accumulated doses per area are of the order of a few Joules per square centimeter. What an effect may such irradiation may have on DNA ? Unfortunately, most studies on the effects of radiation on DNA are performed with ionizing radiation (alpha, beta , gamma rays) or with UV light. There, DNA damage may be dramatic, although such studies have revealed a surprisingly strong DNA repair capacity of otherwise healthy human cells. Even when the overall integrity of a cell's genome is seriously degraded, the damaged DNA can be repaired without directly detectable consequences (although long term mutational damage cannot be completely excluded).

One efficient and comparably simple technique, requiring basically only a fluorescence microscope and a gel electrophoresis device, to study DNA damage and repair is "Single Cell Gel Electrophoresis" (SCGE). Cells are embedded in an electrophoresis gel, their cell nuclei are perforated chemically and subsequently an electric field is applied (2-5). Since under suitable physicochemical conditions DNA is negatively charged, it migrates towards the electrically positive side of the gel. In a given time, small DNA fragments migrate a long distance (10-20 micrometers), large molecules migrate a correspondingly shorter distance. Very large DNA molecules cannot leave the cell nucleus. When the DNA of a cell is undamaged, it remains in the nucleus, which appears in a microscope, after staining with a fluorescence dye, as a sphere, or two dimensionally as a circle. When

part of the DNA is damaged, the latter migrates out of the nucleus.After staining, such a cell has the appearance of a comet with bright head and a tail whose length (or a more quantitative parameter called tail moment) is a measure for the degree of damage. Therefore, SCGE is also called the COMET assay.

Using this COMET assay, light induced DNA damage has been studied in the wavelength range from 308 nm (UV) to approx. 450 nm (blue) (6) . While at 308 nm (UV) 0.0001 Joules per square centimeter were sufficient to induce detectable DNA damage, 1 Joule per square centimeter was required at 450 nm. The damage declined exponentially with wavelength. When one extrapolates this to the wavelengths which are used for LLLT, one can estimate that at least a thousandfold dose for 630 nm and a millionfold dose for 830 nm would be required to induce DNA damage detectable by the COMET assay. Probably the effects are even smaller, since in ref.6 a pulsed laser source was used, which often generates more damage than a corresponding continuous laser.

There is still the possibility that the COMET assay is just not sensitive enough to detect minor, but harmful DNA damages. However, one can compare the amount of radiation with that of sunlight. Bright sunlight has a power per area (=intensity) of 0.1345 Watts per square centimeter, which. after irradiation for only ten seconds, results in a dose per area of 1.345 Joule per square centimeter, comparable to what is used in LLLT, integrated over the whole spectral range. When one filters out a wavelength band of +/- 10 nm, Sun radiation for a few minutes is required to accumulate a few Joules per square centimeter. Such an irradiation is not generally supposed to cause disease. Since we are on the red side of the optical spectrum which, as mentioned above, is less DNA damaging than the average sunlight, we are on the safe side when we assume that the doses per area as they are used in LLLT correspond to the DNA damaging effects of a few minutes sunbath. If any DNA breaks are induced by such irradiation, they will be repaired immediately, otherwise even a short sunbath would cause mutations and finally cancer.
May that mean that LLLT does produce no effect at all, that everything is placebo effect ? Again, COMET assay experiments give a hint: when one first irradiates cells of the bacterium Escherichia coli (7) or human lymphocytes (8) with red (He-Ne) laser light (0.054 - 0.27 Joules per square centimeter) and then tries to damage DNA by UV irradiation, DNA fragmentation is much lower than without pre- irradiation with red light. An interpretation of this effect is that the pre-irradiation activates enzymes of the DNA repair machinery which immediately repairs possible UV damages. Since the effect is similar for cells as different as bacterial and mammalian cells one may conclude that it is evolutionarily conserved. In addition, these experiments indicate that low level laser illumination indeed can cause beneficial effects.

In conclusion, COMET assay experiments reveal possible therapeutic effects of LLLT but do not indicate a risk of DNA damage.

Reference

1. Z.Simunovic editor Lasers in Medicine and Dentistry : LLLT 2000 Eur.Med.Laser Association. Access via www.lasermedico.ch

2. O.Östling, K.J. Johanson Microelectrophoretic study of radiation induced DNA damages in individual mammalian cells 1984 Bioch.Bioph.Res.Comm.123,291-2982
3. N.Singh, M.Mc Coy, R.Tice, E.Schneider A simple technique for quantification of low levels of DNA damage in individual cells 1988 Exp.Cell Res. 175,184-191
4. P.L.Olive,D.Wlodek,J.P.Banath DNA double strand breaks measured in individual cells subjected to gel electrophoresis 1991 Cancer Res.51, 4671-4676
5. A.Rapp, C.Bock, A.Rapp, H.Dittmar, K.O.Greulich 2000 J.Photochem. Photobiol. in press UV-A breakage sensitivity of human chromosomes measured by COMET-FISH depends on gene density and is not dependent on chromosome size,
6. A.de With, K.O.Greulich Wavelength dependence of laser induced DNA damages in lymphocytes observed by single cell gel electrophoresis 1995 J.Photochem.Photobiol.B,30,71-76
7. R.Kohli, P.K.Gupta, A.Dube He-Ne laser pre-irradiation induces protection against UV C radiation in wild type E coli strain K12B1157 2000 Rad.Res.153, 181-185
8. A.Dube, C.Bock, E.Bauer, R.Kohli, P.K.Gupta, K.O.Greulich He-Ne laser protects B-lymphoblasts from UV induced DNA damage 2000 Rad.Env. Bioph.submitted

Radiat Environ Biophys. 2001 Mar;40(1):77-82.

He-Ne laser irradiation protects B-lymphoblasts from UVA-induced DNA damage.

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The effect of He-Ne laser (632.8 nm) pre-irradiation on UVA (343 nm)-induced DNA damage in the human B-lymphoblast cell line NC37 was investigated using the comet assay. He-Ne laser pre-irradiation was observed to result in a dose-dependent decrease in UVA-induced DNA damage. This effect was also found to be dependent on the incubation period between He-Ne laser pre-irradiation and the UVA exposure. Whereas the control cells with a higher DNA damage point to an initial ability of faster repair, both the control and the He-Ne laser pre-irradiated cells subsequently show the same rate of DNA repair. The results suggest that He-Ne laser irradiation protect the cells from UVA-induced DNA damage primarily through an influence on processes that prevent an initial DNA damage.

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Exact action spectra for cellular responses relevant to phototherapy.

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OBJECTIVE: The aim of the present work is to analyze available action spectra for various biological responses of HeLa cells irradiated with monochromatic light of 580-860 nm. BACKGROUND DATA: Phototherapy (low-level laser therapy or photobiomodulation) is characterized by its ability to induce photobiological processes in cells. Exact action spectra are needed for determination of photoacceptors as well as for further investigations into cellular mechanisms of phototherapy. METHODS: Seven action spectra for the stimulation of DNA and RNA synthesis rate and cell adhesion to glass matrix are analyzed by curve fitting, followed by deconvolusion with Lorentzian fitting. Exact parameters of peak positions and bandwidths are presented. RESULTS: The peak positions are between 613.5 and 623.5 nm (in one spectrum, at 606 nm), in the red maximum. The far-red maximum has exact peak positions between 667.5 and 683.7 nm in different spectra. Two near infrared maxima have peak positions in the range 750.7-772.3 nm and 812.5-846.0 nm, respectively. CONCLUSIONS: In the wavelength range important for phototherapy (600-860 nm), there are four "active" regions, but peak positions are not exactly the same for all spectra.