Iranian Journal of Medical Physics

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Effect of Helium-Neon Laser and Sodium Hypochlorite on Calf Thymus Double-Stranded Deoxyribonucleic Acid Molecule: An in Vitro Experimental Study

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ARTICLE INFO

Article type:
Original Article

Article history:
Received: Jul 02, 2017
Accepted: Sep 13, 2017

Keywords:
Hene Laser
DNA Double Stranded
Sodium Hypochlorite
Hypochromic
Hyperchromic

ABSTRACT

Introduction: Low-energy helium-neon (He-Ne) laser beam light is used in combination with sodium hypochlorite (Na_2HOCl_3) for clinical purposes. Regarding this, the present study aimed to investigate the effect of He-Ne laser (632.8 nm) and sodium hypochlorite on the calf thymus double-stranded deoxyribonucleic acid (ctdsDNA) molecule.

Materials and Methods: For the purpose of the study, ctdsDNA solutions (30µg/ml) were exposed to He-Ne laser (632.8 nm) light in the absence and presence of different concentrations of sodium hypochlorite for up to 60 sec. The levels of nucleic acids released as uncontaminated and contaminated proteins were considered as the markers of DNA damage in terms of hypochromasia (i.e., DNA strand breakage) and hyperchromasia (i.e., of DNA strands separation).

Results: The mean concentration of nucleic acids insignificantly ($P > 0.05$) decreased after exposure to laser light irradiation (hypochromic effect). Furthermore, laser irradiation insignificantly and inconsistency protected the ctdsDNA molecules from the effect of sodium hypochlorite. Sodium hypochlorite at concentrations of 1 and 3 mmol reduced the levels of the nucleic acids released from contaminated protein by 29.2% and 78.3% of the pre-incubated levels (hyporchromasia effect). The He-Ne laser (632.8 nm) irradiation induced hypochromic effect on the uncontaminated and contaminated proteins, while sodium hypochlorite induced a remarkable hyperchromic effect at higher concentrations.

Conclusion: As the finding indicated, a short time He-Ne laser light (632.8 nm) irradiation exerted minor significant effect on the ctdsDNA molecule. This laser light did not interact with sodium hypochlorite as a synergistic combination against the ctdsDNA molecule.

► *Please cite this article as:*
Al-Nimer SM, Al-Deen M, Ibrahim T. Effect of Helium-Neon Laser and Sodium Hypochlorite on Calf Thymus Double-Stranded Deoxyribonucleic Acid Molecule: An in Vitro Experimental Study. Iran J Med Phys 2018; 15: 6-11.10.22038/ijmp.2017.24314.1240.

Introduction

Low-energy helium-neon laser beam light (He-Ne, 632.8 nm) is a therapeutic option to treat the skin depigmentation disorders such as vitiligo. Laser light disturbs the function of the mitochondria, therefore, it exerts a little effect on the DNA molecule [1, 2]. The effect of He-Ne laser depends on the duration of the exposure. In this regards, a single exposure of fibroblast cells to the laser light induces damage to the DNA molecule [3, 4].

When the fibroblast cells were irradiated for a long time up to 30 min with an interval the cell viability increases, and the damage to DNA molecule decreases [3, 4]. The higher doses of He-Ne laser irradiation (up to 10-16 J/cm^2) significantly reduces the viability of the human fibroblast and induces a significant damage to the cell membrane as well as the DNA molecule compared to a small dose of 5 J/cm^2 [5, 6]. On the other hand, He-Ne laser irradiation protects the cell from DNA damage induced by ultraviolet radiation. Kohli and Gupta [7] demonstrated that He-Ne laser irradiation protected the DNA damage of the bacterial cells induced by ultraviolet-C, and that its effect was extended to induce DNA repair at a dose of 2 W/m^2. With respect to the interaction between reactive oxygen species and He-Ne laser irradiation, Zaichkina et al. [8] found that the levels of the reactive oxygen species released from the cells of the mice irradiated with ionizing radiation or non-ionizing radiation (represented by laser irradiation) were insignificantly different. In a study, a group of mice were irradiated at the thymus zone with a single dose of He-Ne laser (632.8 nm, 0.2 mW/cm^2) after induction of stress by lipopolysaccharide injection (250 mg/100 g of animal

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body weight). The results of the mentioned study revealed a significant increase in the levels of heat shock protein. However, in the mentioned study, the He-Ne laser had no significant effect on the nitrosative free radicals represented by nitric oxide levels [9].

Laser light irradiation not only produces significant impacts on the cellular structure, but also affects the chemical structures particularly when the free radicals are available. The importance of performing the present was to clarify the simultaneous effect of laser light and sodium hypochlorite (as a source of free radicals) on the DNA molecule looking for their synergistic, additive, or antagonistic (protective) interaction.

In this study, we applied an in vitro experimental model to avoid the effects of laser light or sodium hypochlorite on the cellular components other than the nucleus and DNA molecule. Sodium hypochlorite spontaneously released free radicals in aqueous solution, which affect the DNA molecule.

With this background in mind, the present study aimed to investigate the effect of low energy irradiation with a He-Ne laser (632.8) light on the calf thymus double-stranded deoxyribonucleic acid (ctdsDNA) molecule incubated with and without sodium hypochlorite as a source of free radicals using in vitro experimental model.

Materials and Methods

Sample Preparation

This in vitro study was reviewed by the Scientific Committee in the Department of the Physiology and approved by the Council of the College of Medicine at Diyala University in Diyala, Iraq. This study was conducted in the Department of Physiology from 1st January to 30th June 2016. A ctdsDNA was purchased from BDH Chemicals (UK).

The specifications of ctdsDNA were the calculated molecular weight of 3560 g/Mol from Sv20, white threads like appearance, and optical density of 16 at 260 nm ultraviolet wavelength. A stock solution of ctdsDNA was prepared by dissolving a known weight of ctdsDNA in isotonic phosphate buffer solution (consisting of 0.0015M sodium chloride and 0.00015M trisodium citrate) to get a final concentration of 30 µg/ml. The following series of experiments were carried [10].

Effect of Helium-Neon Laser (632.8 Nm) Light on the Aqueous Solution of Ctdsdna

In the first series of the experiments, known volume of ctdsDNA solutions in a glass test tube were irradiated with He-Ne laser (632.8 nm) beam light (Model JCQ-250 He-Ne Laser, minimum output power of 1.6 nm, China). The light beam was at 1 cm vertical distance from the upper opening of the test tube, and the irradiation was maintained for the following periods of time: 0 (the data representing before irradiation), and 15, 30, 45, and 60 sec (the data representing after irradiation).

The UV-spectra (200-900 nm) of ctdsDNA solutions at each time was scanned by UV-Visible spectrophotometer (UV-1800, SHIMADZU, Japan). The optic densities (absorbance) were recorded at the wavelengths of 205, 260, and 280 nm. Each experiment was repeated four times.

Effect of Sodium Hypochlorite at Different Concentrations on the Aqueous Solution of Ctdsdna

In the second series of the experiments, the ctdsDNA solutions were treated with different concentrations (1, 3, 7, and 10 mmol) of sodium hypochlorite as a source of chlorinated free radicals. Subsequently they were incubated for 0, 15, 30, 45, and 60 sec. Then the optic densities (absorbance) of ctdsDNA were recorded at the wavelengths of 205, 260 and 280 nm [10]. Each experiment was repeated four times.

Simultaneous Effects of Sodium Hypochlorite and Helium-Neon Laser (632.8 Nm) Light on the Aqueous Solution of Ctdsdna

In the third series of the experiments, the ctdsDNA solutions were treated with sodium hypochloritre as in the second series. Subsequently, the solutions were irradiated with laser beam light as in the first series in order to investigate the simultaneous effect of the free radicals and laser irradiation after 0, 15, 30, 45, and 60 sec of exposure. Each experiment was repeated four times.

From the UV-spectra scan record (including the absorbance value of each test at 205, 260, and 280 nm wavelengths), the following measurements were calculated using the given equations [11-13]:

Uncontaminated protein concentration (µg/ml) representing the nucleic acids concentration = 31 × absorbance at λ 205.

Contaminated protein representing the nucleic acids concentration (µg/ml) = (1.55 × absorbance at λ 280 nm) – (0.75 × absorbance at λ 260 nm).

The enhancement of the optical density, (i.e., the absorbance at each wavelength) indicated the separation of the double strands of DNA molecules. On the other hand, the reduction of the optical density signified the breakage of the double-stranded DNA molecule termed hyperchromic effect [10].

The extinction coefficient (e) of uncontaminated protein (Mol, cm^{-1}) was as follows:

$$e = (27 \times 120) + \frac{\text{absorbance at } \lambda\ 280\ nm}{\text{absorbance at } \lambda\ 260\ nm} \quad [14] \qquad (1)$$

The extinction coefficient (e) of contaminated protein (Mol, cm^{-1}) was calculated according to [14] using the following equation:

$$e = (27 \times 120) + \frac{\text{concentration of contaminated protein}}{\text{absorbance at } \lambda\ 280\ nm} \qquad (2)$$

Statistical Analysis

The data were presented as percentage, mean, and standard error. The results were analyzed by using paired and unpaired student t-tests. P-value less than 0.05 was considered statistically significant. The statistical analysis was performed in Excel software (2007).

Results

Effects of Sodium Hypochlorite Solution and Helium-Neon Laser (632.8 Nm) Light on the Concentrations of Nucleic Acids Released From Uncontaminated Protein of Ctdsdna Molecule

Figure 1 presents the effect of laser irradiation and sodium hypochlorite on the DNA molecule in terms of the nucleic acids released from uncontaminated protein. The mean baseline concentration of nucleic acids (pre-exposure to the laser light or pre-incubation with sodium hypchloriteo was 65.77±5.24 µg/ml.

Laser light irradiation insignificantly (P>0.05) reduced the mean level nucleic acid to 52.89 ± 10.0 µg/ml after 15 sec (80.4% of the baseline value). This mean even decreased to a lesser lower level after 30-60 sec of exposure to laser irradiation, representing the hypochromic effect (Figure 1A).

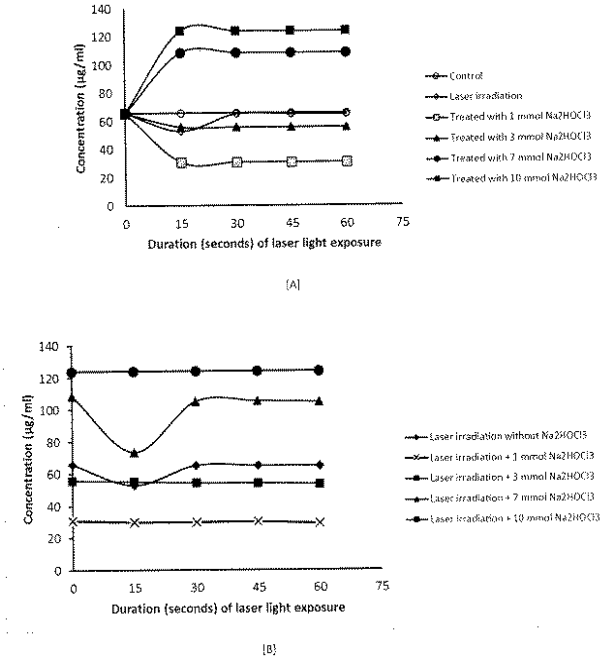

Figure 1. Effects of laser irradiation or sodium hypochlorite [A] solution separately and in combination [B] on the nucleic acids that released from uncontaminated protein of ctds DNA molecule

Sodium hypochlorite solution at concentrations of 1 and 3 mmol reduced the levels of the nucleic acid to 80.4% (P >0.05) and 46.9% (P<0.02) of the pre-incubated levels (i.e., hypochromasic effect), respectively. However, this solution enhanced the release of the nucleic acids to 65% (P<0.01) and 189% (P<0.01) of the baseline value at concentrations of 7 and 10 mmol (Figure 1A) respectively.

Figure 1B illustrates the simultaneous effects of laser light exposure and sodium hypochlorite at different concentrations. Laser irradiation insignificantly counteracts the hyperchromasia that was induced by sodium hypochlorite solution at 7mmol concentration. This was indicated by reducing of the nucleic acid levels from the mean value 108.53 to the 73.38 µg/ml (P<0.05) after 15 sec of exposure (Figure 1B).

Effects of Sodium Hypochlorite Solution and Helium-Neon Laser (632.8 Nm) Light on the Extinction Coefficient of Nucleic Acids Released From Uncontaminated Protein of Ctdsdna Molecule

According to Table 1 neither the effect of laser irradiation nor sodium hypochlorite treatment resulted in a significant change in the determination of the extinction coefficient of the uncontaminated DNA molecule.

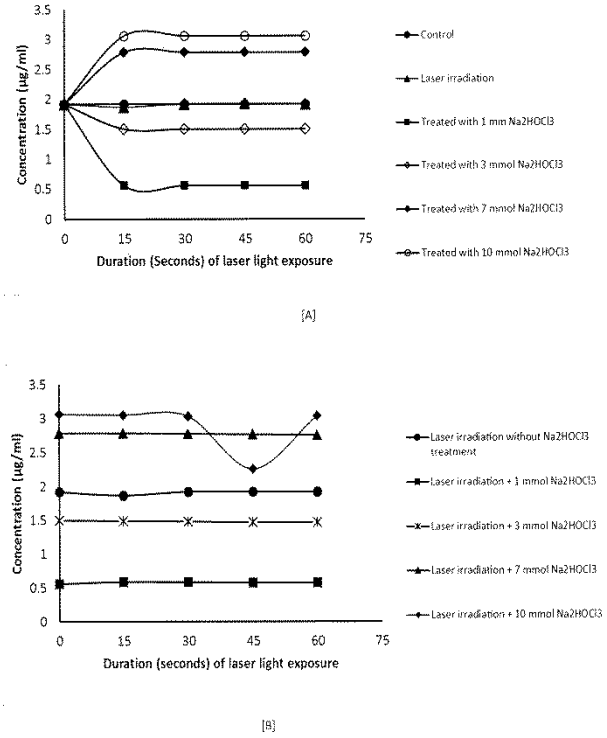

Figure 2. Effects of laser irradiation or sodium hypochlorite [A] solution separately and in combination [B] on the nucleic acids that released from uncontaminated protein of ctds DNA molecule

Table 1. Effects of He-Ne laser (632.8 nm) light irradiation and/or sodium hypochlorite (Na_2HOCl_3) at different concentrations (as a source of free radicals on the extinction coefficient of uncontaminated protein (nucleic acids)

Characteristics of exposure	Duration (seconds)				
		15	30	45	60
Pre-exposure	3240.44				
Laser irradiation		3240.45	3240.44	3240.45	3240.44
Treatment with sodium hypochlorite					
1 mmol	3240.76				
3 mmol	3240.57				
7 mmol	3240.53				
10 mmol	3240.66				
Treatment with sodium hypochlorite and laser irradiation					
1 mmol		3240.75	3240.75	3240.74	3240.73
3 mmol		3240.56	3240.55	3240.55	3240.54
7 mmol		3240.52	3240.52	3240.52	3240.52
10 mmol		3240.65	3240.66	3240.79	3240.65

The results are expressed as mean of *n* =4.

Table 2. Effects of l He-Ne laser (632.8 nm) light irradiation and/or sodium hypochlorite (Na_2HOCl_3) at different concentrations (as a source of free radicals on the extinction coefficient of contaminated protein (nucleic acids)

Characteristics of exposure	Duration (seconds)				
		15	30	45	60
Pre-exposure	1.218				
Laser irradiation		1.215	1.220	1.215	1.220
Treatment with sodium hypochlorite					
1 mmol	0.979	0.989	0.991	0.999	1.029
3 mmol	1.126	1.127	1.133	1.14	1.148
7 mmol	1.153	1.158	1.160	1.163	1.164
10 mmol	1.057	1.060	1.058	0.960	1.066
Treatment with sodium hypochlorite and laser irradiation					
1 mmol		1.257	1.258	1.256	1.258
3 mmol		1.280	1.282	1.228	1.118
7 mmol		1.212	1.228	1.228	1.228
10 mmol		1.108	1.113	1.095	1.112

The results are expressed as mean of *n* =4.

Effects of sodium hypochlorite solution and helium-neon laser (632.8 nm) light on the concentrations of nucleic acids released from contaminated protein of ctdsDNA molecule

As displayed in Figure 2A, the mean level of nucleic acids released from contaminated protein was 1.918±0.5 µg/ml. Laser irradiation did not induce any changes in the levels of nucleic acids. The levels of the nucleic acids reduced to 29.2% ($P<0.01$) and 78.3% ($P>0.05$) of the baseline value (hyporchromasia effect) when the aqueous solution of ctDNA molecule were incubated with sodium hypochlorite the concentrations of 1 and 3 mmol, respectively (Figure 2A).

Based on Figure 2B, the laser irradiation produced inconsistent effect on the ctdsDNA molecule that was treated simultaneously with sodium hypochlorite. Laser irradiation for 45 sec significantly ($P<0.02$) reduced the levels of nucleic acids treated with sodium hypochlorite at a concentration of 10 mmol (Figure 2B).

Effects of Sodium Hypochlorite Solution and Helium-Neon Laser (632.8 Nm) Light on the Extinction Coefficient of Nucleic Acids Released From Contaminated Protein of Ctdsdna Molecule

As indicated in Table 2 the laser irradiation and sodium hypochlorite treatment caused a remarkable change in the determination of the extinction coefficient of the contaminated DNA molecule.

Discussion

As the findings indicated, laser irradiation induced minor effect on the DNA molecules compared to the free radicals generated from sodium hypochlorite solution. Moreover, He-Ne laser irradiation inconsistently counteracted the effect of sodium hypochlorite. Furthermore, the calculated extinction coefficients of contaminated protein were changed by laser irradiation and treatment with sodium hypochlorite solution. Therefore, according to the results of the present study, the effect of laser light on the uncontaminated or contaminated proteins was determined in terms of DNA damage (hyporchromasia) which was observed after 15 sec. This finding is in agreement with those reported in

other studies, demonstrating that laser irradiation immediately induces DNA damage, and that this effect depends on the intensity of irradiation [15]. Nonetheless, our results inconsistent with the findings of a similar study reporting that the amount of the DNA damage is related to the duration of irradiation [15]. This discrepancy may be due to the use of different research methods in the two studies. Other studies demonstrated that a combination of He-Ne laser irradiation with nitrative free radicals produced a favorable effect against the stress induced by ultraviolet B. This finding is indicative of the important role of the free radical source in the net results of laser irradiation [16].

In the present study, we used the solution of sodium hypochlorite as a source of chlorinated free radicals producing different effects. Our results are in agreement with those of other studies demonstrating that a single exposure of He-Ne irradiation (632.8 nm) at a frequency of 5 J/cm^2 for 30 min leads to cellular damage, while frequent irradiation is less likely to induce DNA molecule damage [3].

Sodium hypochlorite solution resulted in DNA damage in terms of uncontaminated and contaminated proteins in a concentration-independent manner. This solution induced hyperchromasia (i.e., separation of DNA double strands) at concentrations of 7 and 10 mmol; in addition, it resulted in hypochromasia (i.e., breakage of DNA double strands) at concentrations of 1 and 3 mmol.

In the living tissue, the sodium hypochlorite solution chlorinates the cellular DNA and RNA nucleobases, and thereby directly induces DNA double-strand breakage (hypochromasic effect) [17, 18]. This explains our results that sodium hypochlorite at low concentrations (i.e., 1 and 3 mmol) induces damage to the ctdsDNA [19].

There is evidence that sodium hypochlorite produces a wide spectrum of DNA damage, including single- and double-strand breaks via chloramine-mediated reactions. Accordingly, this evidence explicates the hyperchromic effect observed in this study [20]. Sodium hypochlorite induces double-strand DNA damage in the living cells; moreover, it accelerates the lipid peroxidation process via releasing a chlorinated free radicals [21].

Therefore, the results of this study confirmed those of the previous studies revealing that sodium hypochlorite induces double-strand DNA damage. The interesting finding in this study was that sodium hypochlorite solution induced a similar damage in both uncontaminated and contaminated proteins of the DNA molecule. This finding indicates that the effect of sodium hypochlorite is extended to different protein molecules.

It is well-known that sodium hypochlorite at a high concentration acts as a deproteinizing agent even at the extracellular levels, unlike hydrogen peroxide which only affects the nuclear portion [22, 23]. The hypochlorite ion released from sodium hypochlorite has the ability to induce oxidative cleavage of chemical molecule. Therefore, this ion is responsible for the inconsistent effects of laser irradiation on the DNA molecule treated with sodium hypochlorite [24].

Regarding the living tissue, there is evidence revealing that the cell exposure to the simultaneous effect of short-term laser irradiation (the output power 100 mW and emission of 660 nm) and sodium hypochlorite results in the cell death [25]. However, this finding was not observed in the present study due to use of no cellular elements and the differences in the duration of irradiation.

Finally, the extinction coefficient of the contaminated protein showed variations in respect to the treatment. Nevertheless, the uncontaminated protein demonstrated no variation in its extinction coefficient. This observation is acceptable since the purified protein has a specific extinction coefficient. Accordingly, the variability in extinction coefficients is usually observed whenever there is unpurified protein and there is a change in the amino acid sequences.

Conclusion

As the findings of the present study revealed, a short-time He-Ne laser light (632.8 nm) irradiation exerted minor significant effect on the DNA molecule. Furthermore, this irradiation showed no remarkable synergistic effect with sodium hypochlorite in inducing ctdsDNA molecule damage in vitro experimental study.

Acknowledgment

The authors thank and appreciate the Faculty of the Diyala university for their kind support and encouragement in performing this study.

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