

***In vitro* Immunogenetic Effects of 532 nm Short YAG Laser Pulses on Human Lymphocytes**

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ABSTRACT Incident High Energy Lasers have shown to cause damages at gross cellular and organelle levels and also at the level of DNA. In this study, the effect of high energy 532 nm short YAG Lasers at 10 pulses per second on circulating lymphocytes was performed. Apart from the damage to the genetic material of the lymphocytes as expressed as "micronuclei", the phenotypic manifestations of such a damage in terms of Immunoglobulin G production were taken into consideration. Blood samples were exposed to 532nm Nd:YAG Laser. The irradiated samples were cultured and the cells screened for the occurrence frequency of micronuclei and the culture supernatant were screened for the IgG levels by the Mancini's test or the Single Radial Immunodiffusion. The results obtained showed that 532nm Lasers induced damage to the DNA of lymphocytes which were expressed as micronucleated cells. Interestingly, at lower doses, the damages observed were more than that for the higher doses. This can be due to the lethal effects of higher doses on cells thereby, preventing the amplification of damage expression in culture. Similarly, there was no change in the IgG levels at lower doses, but showed a significant increase at higher doses.

INTRODUCTION

Advances in Laser technology has led to a surge for applications in basic research, biological sciences, medicine and surgery. As the usage of lasers is increasing rapidly, it is also essential to understand the mechanisms of harmful effects, if any. Incident High Energy Lasers have shown to cause damages at gross cellular and organelle levels and also at the level of DNA as a result of non-linear multi-photon absorption or through some other photochemical processes, (Cao et al. 1993). Such processes have also shown to damage DNA and also produce mutagenesis in mammalian cells (Boll et al. 1987). The mechanisms for laser induced effects on mammalian cells for a laser of 193-nm was studied and reported (Kochevar et al. 1990). Previous studies showed that 532nm irradiations produced mutations which are ten fold higher than that of the controls in cultured Syrian hamster fibroblasts, (Leavitt et al. 1997). It was also shown

that ultraviolet light (240-280nm) produces thymine dimers in DNA (Setlow 1966; de With et al. 1994). It was also shown that Nd:YAG 532 nm laser light exposure of DNA in solution produced cyclobutylpyrimidine dimers in DNA that are potentially mutagenic. Hitherto, the damage to the genetic material was also well demonstrated in cultured cells induced by pulsed excimer laser irradiation and also continuous UV radiation (Rasmussen et al. 1989). The present study was conducted with these background information about the possible DNA damaging effect of the green (532 nm) laser light. The cytotoxic and mutagenic effects of low intensity, 248 and 193 nm excimer laser radiations was also demonstrated in the mammalian cells.

In this study, the effect of high energy 532 nm short YAG Lasers at 10 pulses per second on circulating lymphocytes was performed. Lymphocytes, by their relative sensitivity to damage and their abundant distribution in peripheral circulation have been the choicest of cells of study to understand the mechanism of mutagenesis. Lymphocytes, apart from being useful cytogenetic subjects, have a very vital role to play for a higher organism by being instrumental for the immune responses and therefore the effective immunological status of an organism to a large measure. In the present study, apart from the damage to the genetic material of the lymphocytes as expressed as "micronuclei", the phenotypic manifestations of such damage in terms of Immunoglobulin G production were taken into consideration.

Micronuclei represent chromosome fragments, or even whole chromosomes that have not been incorporated into the main nucleus in dividing cells and are useful markers for studying damage to the genetic material of a mammalian nucleated cell type. Immunoglobulin G is an abundant protein molecule, which is responsible for the humoral immune status of an individual to a large extent, and is produced by the B-Lymphocytes. This class of Immunoglobulin molecule is also the most abundant in circulation. As the damage due to the light pulses are stud-

ied on lymphocytes, it is only natural to also look into the phenotypic expression of such a damage which will give us a deeper understanding of the health effects of mutagenesis.

MATERIALS AND METHODS

2ml blood samples were collected aseptically and were exposed to 532nm Nd:YAG Laser irradiation. Irradiation of the blood was carried out by using a SP-Quanta Ray GCR-2 (10) Nd:YAG laser using fundamental harmonic output of 1064 nm having 6.4 mm diameter with a pulse width of 9ns and energy 100 mj, whose configuration is given in the figure 1. The passive Q switch is used to control the uni-directional release of 1064 nm laser pulse. A DHS-2 dichoric harmonic seperator was used to separate the second harmonic of 532nm with pulse width of 7ns and energy 50 mj from the fundamental beam. The samples were irradiated by the laser beam of 532nm of repetition rate of 10 pulses per second with different time intervals.

The irradiated samples were cultured and the cells screened for the occurrence frequency of micronuclei and the culture supernatant was screened for the IgG levels by the Mancini's test or the Single Radial Immunodiffusion.

Micronucleus Assay: 4 ml RPMI-1640+1 ml

FCS + 200 μ l of PHA + 500 μ l blood was taken per culture and Incubated at 37°C for 44 hours. At 44th hour 150 μ l of Cytochalasin-B was added and the culture incubated for another 28 hours. The content was transferred into a centrifuge tube and centrifuged for 10 minutes (800 rpm). Further to 0.45% KCL hypotonic treatment for 2 minutes at 37°C, the mixture was centrifuged for 10 minutes (800 rpm). The supernatant was removed and the cell button was fixed using Carnoy's fixative. After three washes in Carnoy's fixative, the cells are cast on a pre-cooled glass slide. The slides were stained with 10% giemsa, and mounted with DPX and the binucleated cells were scored for micronuclei.

Single Radial Immunodiffusion: The culture supernatants were quantified for ImmunoglobulinG using the Mancini's or the SRID technique. With this technique, it is possible to make accurate quantitative determinations of the antigen samples. 10 microliters of standards of known concentrations (serially diluted) along with 10 microliters of the sample culture supernatants were loaded into wells punched in a glass plate containing Agarose gel having Anti human IgG. The gel plates thus prepared were incubated in a humid chamber overnight. The antigen-antibody complexes take a shape of

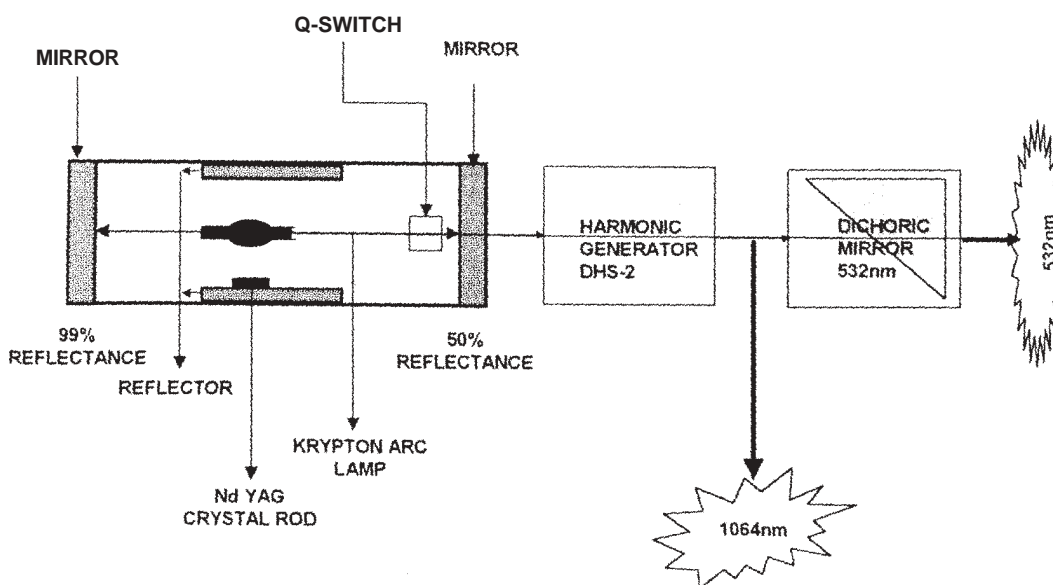


Fig. 1. Nd:YAG Laser set-up as used for irradiation of the samples

circular precipitates sharply defined as concentric rings. The diameter of the circular precipitate patterns is directly proportional to the amount of ImmunoglobulinG present in the culture supernatant and the same were measured and documented.

The immunogenetic marker levels thus obtained were analyzed to ascertain correlations if any.

Lasers induced damage to the DNA of lymphocytes which were expressed as micro nucleated cells. Interestingly, at lower doses, the damages observed were more than that for the higher doses. The levels of ImmunoglobulinG present in the culture were inversely proportional to the damage as was expected. The results obtained are given in the table 1 and figure 2.

RESULTS

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DISCUSSION

The results obtained showed that 532nm Lasers induced damage to the DNA of lympho-

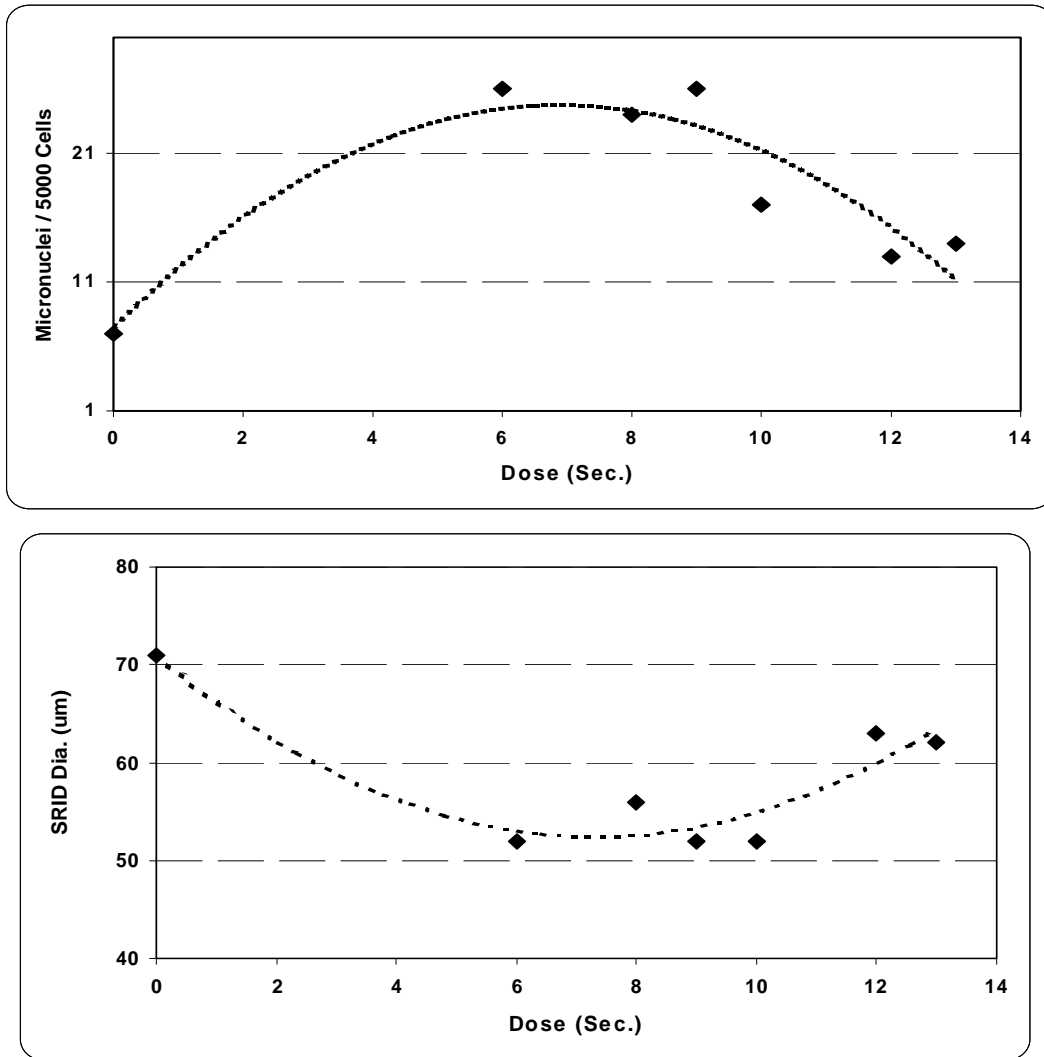


Fig. 2. The two graphs show the effects of 532nm pulsed Nd:YAG laser on the micronuclei and subsequent immunoglobulinG production *in vitro*.

Table 1: The table shows the effect of 532nm Nd:YAG laser as to the DNA damage as expressed as Micro-nuclei and the corresponding Immunoglobulin synthesis of the exposed lymphocytes.

S.No.	Sample	Dosage (Sec)	No. of Cell Scored	No. of MN	IgG (Dia) μ m
1.	Control	0	5000	7	71
2.	A1	4	5000	25	42
3.	A2	6	5000	26	52
4.	A3	8	5000	24	56
5.	A4	9	5000	26	52
6.	A5	10	5000	17	52
7.	A6	11	5000	18	46
8.	A7	12	5000	13	63
9.	A8	13	5000	14	62

cytes which were expressed as micro nucleated cells. Interestingly, at lower doses, the damages observed were more than that for the higher doses. This can be due to the lethal effects of higher doses on cells thereby, preventing the amplification of damage expression in culture. Similarly, the IgG levels were inversely proportional to the extent of damage to the cells. Lesser the damage, more the production of IgG and the opposite was noticed. This can be attributed to the fact that in cultures of lymphocytes exposed to higher doses, only the healthy cells multiplied resulting in higher levels of IgG production in culture.

At this stage it is pertinent to understand the effects of laser irradiation at sub-lethal doses at the genetic level and their long-term phenotypic manifestations of such a damage to somatic cells.

REFERENCES

Cao EH, Wang JJ, Xin SM 1993. Nonlinear biological

- effects of high-intensity visible laser radiation on DNA. *Proc SPIE Int Opt Eng*, **1882**: 309-313.
- de With A, Leitz G, Greulich KO 1994. UV-B-laser induced DNA damage in lymphocytes observed by single-cell gel electrophoresis. *J Photochem Photobiol B Biol*, **24**: 47-53.
- Green H, Boll J, Parrish J, Kochevar I, Seroff A 1987. Cytotoxicity and mutagenicity of low intensity, 248 and 193 nm excimer laser radiation in mammalian cells. *Cancer Res*, **47**: 410-413.
- John Leavitt, Michael Fatone, Charles Hestdalen, John W. Obringer, Henry S Tillinghast Jr 1997. Mutagenic Activity of High-Energy 532 nm Ultra-Short Laser Pulses. *Radiat Res*, **147**: 490-494.
- Kochevar IE, Walsh AA, Held KD, Gallo RL, Mirro J 1990. Mechanism for 193-nm laser radiation-induced effects on mammalian cells. *Radiat Res*, **122**: 142-148.
- Rasmussen RE, Hammer-Wilson M, Berns MW 1989. Mutation and sister chromatid exchange induction in Chinese hamster ovary (CHO) cells by pulsed excimer laser irradiation at 193 nm and 308 nm and continuous UV radiation at 254nm. *Photochem Photobiol*, **49**: 413-418.
- Setlow RB 1966. Cyclobutane-type pyrimidine dimmers in polynucleotides. *Science*, **153**: 3779-3786.