CYTOGENETIC EFFECTS OF LOW LEVEL LASER IRRADIATION OF HUMAN LEUKOCYTES

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Lasers have been applied in many aspects of medicine. In this report, the cytogenetic effects of low power laser irradiation on the cultured human peripheral lymphocytes were studied. The lasers used are argon, He-Ne and GaAlAs diode lasers in low power density. The mitotic index, chromosomal aberrations (including chromosome or chromatid breaks and gaps) and scores for sister chromatid exchange of lymphocytes were observed. Our experiments showed that irradiation with argon laser at the dose of 30–300 J/cm² has dose-related cytostatic effects. Mitosis was inhibited completely when dose was 240 J/cm² or over. There was a significant increase in the frequency of chromosome and chromatid breaks and gaps ($p < 0.05$ with paired *t*-test) and also an increase of sister chromatid exchange after 180 J/cm² ($p < 0.05$ with paired *t*-test). As to the other lasers, there were no significant increase in the frequency of chromosome aberrations. There was an increase of mitosis after GaAlAs diode (0.5 to 1.5 J/cm²) and He-Ne laser (1 to 4 J/cm²) irradiation, however, it was not significant statistically.

KEY WORDS Chromosome aberrations Sister chromatid exchange

Introduction

Lasers have been used for the therapeutic purposes in recent years. However, laser energy may be potentially dangerous because of its photothermal effect, photochemical effect or photoacoustic effect. Therefore, the study of its unwanted biological effects is very important. Although there were no proved evidence for the mutagenic or carcinogenic effects of conventional medical lasers, it had been reported that lasers could induce significant increase in chromosome aberrations in Chinese hamster cells and rat bone marrow cells.¹ Since there are reports of increased chromosomal aberrations of lymphocytes in patients exposed to ionizing radiation, it is logical to use chromosomal aberration frequency as a 'biological dosimeter' in evaluation of the adverse effect of radiation. In this study, we investigate the

cytogenetic effects of argon, He-Ne and GaAlAs diode laser irradiation in low energy range on human lymphocytes. The dose-response relationship and the possible potentiation effects of clastogens such as caffeine were also studied.

Materials and Methods

Laser Irradiation

Argon laser system (Innova 60/70, Coherent Co.) was used. The emission spectrum was most intense at 514 and 488 nm. Cells were irradiated with a defocused laser beam which gave almost even irradiation of a circular area of 1 cm diameter. The energy was calibrated with a laser power meter. The energy density calculated was 1.12 W/cm². Irradiation doses of 30 to 300 J/cm² were given with fixed output and various duration. To avoid thermal effect caused by prolonged irradiation, the dishes were kept cool with ice and irradiated intermittently.

He-Ne laser system (105-LHR-141, Coherent Co.) with peak wave length of 632.8 nm was used. The output power was 4 mW and energy density

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calculated was 0.27 mW/cm². Irradiation doses were ranged from 1 to 4 J/cm².

GaAlAs diode laser (Model JQ 304, Nippon Infrared Co.) with wavelength 904 nm and output power 2 mW was used. The energy density calculated was 0.635 mW/cm² and the doses were from 0.5 to 1.5 J/cm².

Lymphocyte Culture

Peripheral blood was obtained from healthy donors. In each 5 ml RPMI 1640 medium, 0.3 ml of cell containing buffy coat was inoculated. The medium contained phytohemagglutinin, L-glutamine, streptomycin and 20% fetal calf serum. The lymphocytes were cultured for a total of 72 h. These cells were irradiated for 48 h before harvesting for chromosome preparation.

In each experiment, samples were divided into control and treated groups and then each was subdivided into four subgroups: (1) culture medium only, (2) Caffeine with a concentration of 1 mM was added in culture medium, (3) 5-Bromodeoxyuridine with concentration of 1 µM was added in medium, (4) Caffeine and 5-bromodeoxyuridine were both added.

Chromosome Preparation

Chromosome preparation was performed with routine method and stained with GTG banding technique. For each group, we scored for mitotic index (metaphase cells among interphase cells) and observed chromosome aberrations including chromatid gaps, chromatid breaks, chromosomal

breaks and dicentric chromosomes among 100 metaphase cells in each sample.

Sister Chromatid Exchange (SCE)

In cultured medium 1 µM of 5-bromodeoxyuridine was added to the medium immediately after irradiation. These irradiated cells were cultured in the dark for 48 h. Chromosome samples were prepared and stained with fluorescence-plus-Giemsa (FPG) technique.² Analysis was carried out under light microscope. Terminal SCEs were recorded as a single SCE and interstitial SCEs as two exchanges. Centromeric exchanges were included in the analysis unless there was uncertainty whether the chromatids might be twisted. In each sample at least 20 complete metaphases suitable for SCE counting are analysed.

Results

The results of mitotic index, chromosome changes and induction of sister chromatid exchange (SCE) after argon, He-Ne and GaAlAs diode laser irradiation are summarized in Tables 1 to 6. After multiple regression analysis, the mitotic index after argon irradiation showed a dose-dependent decrease (Figure 1) and chromosome breakage was also noticed to have a dose-dependent increase (Figure 2). The mitotic index decreased when the medium contained bromodeoxyuridine, which was probably due to the inhibition of cell growth by bromodeoxyuridine itself. And as shown in Figures 3 and 4, there were no synergic effects of caffeine with bromodeoxyuridine or argon laser. There was sig-

Table 1. Mitosis and chromosome changes after argon laser irradiation

Laser irradiation dose (J/cm ²)		Mitotic index (mitoses/200 cells)	Chromosome or chromatid gap or break	
			Gaps/100 cells	Breaks/100 cells
a.	30	3.38	6/100	2/100
	Control	3.16	3/100	1/100
b.	60	3.34	5/100	1/100
	Control	3.50	2/100	0/100
c.	90	3.72	6/100	2/100
	Control	3.70	2/100	0/100
d.	120	3.04	7/100	2/100
	Control	3.02	2/100	0/100
e.	150	2.48	6/100	3/100
	Control	2.80	2/100	0/100
f.	180	1.06	8/100	2/100
	Control	4.02	0/100	0/100
g.	210	0.38	9/100	3/100
	Control	4.18	1/100	0/100
h.	240	0	—	—
	Control	4.14	—	—
i.	300	0	—	—
	Control	2.68	—	—

Table 2. Induction of SCE by argon laser irradiation

Laser irradiation dose (J/cm ²)	Without caffeine SCE/cell (mean $\pm$ SD)	With caffeine (10 ⁻³ M)
Control	9.1 $\pm$ 1.0	13.7 $\pm$ 1.3
60	9.0 $\pm$ 0.9	13.0 $\pm$ 1.1
120	8.8 $\pm$ 1.6	13.8 $\pm$ 0.9
180	10.8 $\pm$ 1.9*	15.2 $\pm$ 2.1*
210	13.5 $\pm$ 2.6*	20.4 $\pm$ 2.3*

*Significantly different from control with *t*-test ($p < 0.05$).

Table 3. Effects of bromodeoxyuridine and caffeine after argon irradiation

A. control group				
Mitotic index*	Control 3.68	c BrdU 2.18	c Caffeine 3.73	BrdU+Caffeine 1.94
Chromosome gaps	2	2	4	3
breaks**	0	0	1	1
B. Argon irradiation				
	Argon	c BrdU	c Caffeine	BrdU+Caffeine
60 J/cm ²				
Mitotic index	3.34	2.13	3.10	1.60
Chromosome gaps	5	7	14	16
breaks	1	1	4	2
120 J/cm ²				
Mitotic index	3.04	2.11	2.92	1.87
Chromosome gaps	7	8	10	14
breaks	2	2	3	3
180 J/cm ²				
Mitotic index	1.06	0.86	1.10	0.78
Chromosome gaps	8	5	9	10
breaks	2	3	2	2
210 J/cm ²				
Mitotic index	0.38	0.36	0.40	0.31
Chromosome gaps	9	6	9	12
breaks	3	1	3	4

Figure 1. Regression of mitotic index with argon irradiation doses

Table 4. Mitosis and chromosome changes after He-Ne laser irradiation

A. Control group				
	Control	c BrdU	c Caffeine	c BrdU + Caffeine
Mitotic index	3.68	2.18	3.73	1.94
Chromosome gaps	2	2	4	3
breaks	0	0	1	1
B. He-Ne laser irradiation				
	Control	c BrdU	c Caffeine	c BrdU + Caffeine
1.0 J/cm ²				
Mitotic index	4.25	3.45	4.67	1.74
Chromosome gaps	0	1	2	1
breaks	0	0	1	1
2.0 J/cm ²				
Mitotic index	4.04	2.55	4.80	1.59
Chromosome gaps	1	1	2	3
breaks	0	0	0	0
3.0 J/cm ²				
Mitotic index	4.50	2.56	3.95	1.48
Chromosome gaps	0	1	3	1
breaks	0	0	1	1
4.0 J/cm ²				
Mitotic index	3.55	2.35	2.96	1.58
Chromosome gaps	1	0	1	1
breaks	0	0	1	0

Table 5. Induction of SCE by He-Ne laser irradiation

Irradiation dose (J/cm ²)	Without caffeine SCE/cells (mean ± SD)	With caffeine (10 ⁻³ M)
Control	9.1 ± 1.0*	13.7 ± 1.3
1	8.9 ± 1.2*	13.2 ± 0.8
2	9.0 ± 1.4*	13.8 ± 1.0
3	8.6 ± 1.3*	12.9 ± 0.9
4	9.3 ± 1.0*	12.5 ± 1.3

*Difference not significant ($p > 0.05$ paired t -test)

Table 6. Mitosis and chromosome changes after GaAlAs low energy laser irradiation

Laser doses (Joules/cm ²)	Mitotic index (metaphase/200 cells)	Chromosomal	
		Gaps	Breaks/100 cells
Control	3.68*	2	0
0.5	4.52*	2	0
1.0	3.84*	1	0
1.5	4.33*	3	0

*Difference not significant ($p > 0.05$ paired t -test)

nificantly more SCEs when cells received argon laser irradiation up to 180 J/cm² (Table 2). There were more SCEs in the caffeine-treated group, however, this seemed to be the effect of caffeine itself. As for He-Ne and GaAlAs laser irradiation, there was no difference either in mitotic index or chromosome breakage (Tables 4 and 6). There was no difference in SCEs after He-Ne irradiation (Table 5).

Discussion

In this study, we demonstrated that irradiation from argon laser could suppress cell proliferation and cause increased chromosome breakage. Sister chromatid exchange is considered to be an expression of chromosome damage and repair process, therefore, we examined SCEs as an indicator of possible genotoxicity of laser irradiation. Our study also showed increases in SCEs after argon irradiation. Comparing with the previous report by Nakajima *et al.*,¹ they demonstrated a twofold increase of SCEs at doses of 100 J/cm² and a fivefold increase of SCEs at doses of 200 J/cm². In our study, there were no differences at 120 J/cm² and at a dose of

Figure 2. Regression of chromosome breakage with irradiation dose

Figure 3. Effects of bromodeoxyuridine and caffeine on mitotic index after argon laser irradiation

210 J/cm² there was only 1.5 time increase in SCEs. This difference probably can be explained by different sensitivity between human cells and hamster cells.

The mechanism of the cytogenetic effects of argon laser irradiation was discussed in Nakajima's report, however, the possible effects due to pressure recoil, elastic shock or electromagnetic force were difficult to prove experimentally. In X-ray or UV irradiation, photochemical reactions such as thymidine dimer formation may result in breakage of DNA strands.³ From several studies of other lasers, Green⁴ reported 193 nm and 248 nm UV-excimer laser would increase unscheduled synthesis. Seiler *et al.*⁵ and Chilbert *et al.*⁶ reported increase strand break of

DNA after excimer laser. Therefore, investigating the effects of argon laser on DNA strand break may give some light on this cytogenetic effect.

As for He-Ne and GaAlAs low energy lasers, although there were studies demonstrating low-watt lasers would stimulate lymphocyte proliferation, altering cell motility and respiration,⁷⁻⁹ we did not observe any statistical difference in mitosis or chromosome changes after He-Ne or GaAlAs laser irradiation. Possibly these parameters were more suitable in evaluating damaging effects rather than stimulating effects.

For He-Ne and GaAlAs lasers, we did not drive the irradiation dose up to 30 J/cm² to compare with the effects of argon laser. Because to achieve this level, the long duration of cultured cells outside of CO₂ incubator would reduce cell survival and proliferative activity. Other devices will be needed to obtain higher doses from He-Ne and GaAlAs lasers. In this study, because of the disparate irradiation dosage, we therefore do not attempt to compare the effects of He-Ne and GaAlAs lasers from argon laser. However, this must be done in a future study.

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Figure 4. Effects of bromodeoxyuridine and caffeine on chromosome breakage after argon laser irradiation

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