Enhancement of monoclonal antibody production in CHO cells by exposure to He–Ne laser radiation

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Abstract This study tested the effectiveness of laser biostimulation in small-scale cultures *in vitro*. We investigated the response of recombinant CHO cells, which are used for the production of monoclonal antibody, to low level laser radiation. The cells were irradiated using a 632.8 nm He–Ne laser in a continuous wave mode at different energy doses. We incubated the irradiated cells in small batch cultures and assessed their proliferation and productivity at various time intervals. Compared to untreated cells, the irradiated cells showed a significant increase in antibody production. Moreover, the results showed that laser irradiation did not affect viability and slightly enhanced proliferation rate.

Keywords CHO cells · Laser irradiation · ATP · Monoclonal antibodies

Introduction

Chinese hamster ovary cells (CHO) are the most popular host cell line for the production of therapeutic proteins (Wurm 2004, 2005). Efforts to optimize the production process by improving culture systems, media, and culture conditions have resulted in substantial increase in yields (Kuystermans and Al-Rubeai 2011). Another approach to cell culture development and optimization could be achieved through metabolic engineering of cell lines to improve their survivability, growth and productivity (Fengi et al. 2005; Birch et al. 2006; Kuystermans et al. 2007; Ma et al. 2007; Li et al. 2010; Gorfien and Jayne 2011). On the basis of this cellular improvement novel strategies for optimization of cell culture should be possible.

Further improvements may be achieved by applying some of the advances in our understanding of the biological effects of low energy radiation. It should be possible to manipulate cellular responses to the external environment, inducing cells to grow faster or expressing higher amounts of recombinant product. This approach of metabolic alteration by radiation exposure has been highlighted by the reports that low dose radiation could stimulate most immune system parameters in animals, including antibody formation, natural killer activity, secretion of interferon as well as other cellular changes (Shu-Zheng 2007).

Several other studies have also shown that low-power laser irradiation has a positive therapeutic effect

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in a number of clinical situations (Havel et al. 2012)—e.g., pain relief (Fonseca et al. 2012), wound healing (Kirkby et al. 2012), and sports medicine (Wheeland 2012). This effect occurs through a mechanism involving acceleration of cell division, increased enzyme activity, collagen production (Calatrava et al. 1997), regulation of mitochondrial processes (Karu 2008; Eells et al. 2004; Prusa et al. 2012), increased expression of certain proteins (Ozog et al. 2012), induced synthesis of cell cycle regulatory proteins (Ocanã-Quero et al. 1998a) and increase ATP production (Kirkby et al. 2012; Karu 2010; Gavish et al. 2004).

Further, increasing exposure to laser light affects the mitochondrial respiratory chain and their selective permeability for sodium, potassium, and calcium ions (Moore et al. 2005). More laser exposure also increases DNA synthesis and may increase cell proliferation or stimulate the release of growth factors (Ocanã-Quero et al. 1998b). In contrast, some investigators have found a destructive and inhibitory action of laser radiation (Ito et al. 2000; Koutna et al. 2003; Lapotko et al. 2006). These studies seem to indicate that laser biomodulation speeds up healing by improving inflammation, lymphocytes, fibroblast proliferation, and tissue regeneration (Karu et al. 2001; Gavish et al. 2004; Al-Watban and Andres 2012).

This study was designed to investigate the impact of low-level laser (LLL) radiation (at the 632.8 nm wavelength) on the growth, energy metabolism and production of monoclonal antibody (MAb) in CHO cell culture. We found that exposure of cells up to seven min of LLL radiation enhances MAb concentration without affecting viability and this enhancement was related to the stimulation of ATP production.

Materials and methods

Cell-line maintenance

The recombinant Chinese hamster ovary cell line CHO 22H11, was kindly supplied by LONZA Biologics (Slough, UK) and had previously been transfected with the glutamine synthetase (GS) expression system carrying a gene for a mouse-human chimeric antibody (cB72.3). We determined the viable cell number and percentage viability before and after centrifuging the cells at 1,000 rpm for five min and

resuspending them in fresh growth medium at a cell density of 2×10^5 cells per ml into a final volume of 15 ml in vented 50 ml Erlenmeyer flasks (Corning, Corning, NY, USA). The culture was agitated at a rate of 125 rpm at 37 °C in the presence of 5 % CO₂.

Cell count and viability

We used a Cell Lab Quanta SC flow cytometer (Beckman Coulter, Brea, CA, USA) to monitor cell number and viability. We analysed cell viability immediately after adding 10 µl of propidium iodide (PI) to a 490 µl cell suspension to make a 1 µg/ml PI solution. Analysis was undertaken by loading an appropriate protocol for the acquired parameters: FS, log SS, and PI integral.

Determination of glucose and lactate concentrations

Glucose measurements were carried out in triplicate using a blood glucose meter (Bayer HealthCare LLC, Berkeley, CA, USA). Lactate concentrations in supernatant were determined from triplicate cultures using either a lactate meter (Accutrend, Roche Diagnostic, Burgess Hill, UK).

Determination of monoclonal antibody concentrations

We determined the concentration of human-mouse B72.3 IgG4 chimeric MAb using enzyme-linked immunosorbent assay (ELISA). Chimeric MAb produced by cells was sandwiched by monoclonal antihuman IgG (Fc specific; Sigma-Aldrich, Dublin, Ireland) and peroxidase-conjugated anti-human kappa light chain antibody (Sigma-Aldrich). The amount of chimeric MAb was determined by measuring absorbance using o-phenylenediamine dihydrochloride (OPD, Sigma-Aldrich) as a substrate for peroxidase.

He–Ne laser stimulatory dose determination

After plating the CHO cells, we exposed them to irradiation using the He–Ne laser at a wavelength of 632.8 nm with a beam profile in continuous wave TEM00 mode at 13 mW power (Fig. 1). Different doses of energy were applied during cultivation in order to detect the existence of a dose response and its

Fig. 1 Laser irradiation system setup in operating mode. The numbers indicated on the figure are: 1 He–Ne laser source; 2 and 6 holder; 3 power supply; 4 test sample; 5 focus lens

effects. The irradiation with the 632.8 nm laser was made at doses of 0.821, 5.7, and 11.424 J/cm², representing a low, medium, and high dose. We divided the plated cells into four groups: group 1 as a control (not irradiated); group 2 (0.821 J/cm², 60 s); group 3 (5.7 J/cm², 420 s); and group 4 (11.424 J/cm², 840 s). We irradiated cells immediately after plating them and changed medium for each condition. The laser was positioned vertically above each well at a distance of 10 cm from the bottom of the plate, and the irradiated area was 1.1 cm². We performed a calibration for laser power by using a power meter (Thorlabs, Newton, NJ, USA). In order to avoid any accidental influence, we covered all wells that we did not intend to irradiate during each application. The non-irradiated control cells were exposed to room light for the same periods of time and maintained outside the incubator under the same conditions as the laser-irradiated cells.

Determination of ATP by HPLC

We prepared a 1 mM working concentration of ATP and ADP standard from 16.5 mM stock solution by adding 60.6–939.3 µl of deionized water. We then

diluted the 1 mM working solution to 0.5, 0.25 and 0.125 mM to generate the standard curve. Mobile phase A consisted of 20 mM ammonia acetate (pH 4.5), while mobile phase B consisted of 100 % acetonitrile. Air bubbles from both solutions were removed by sonication. We diluted cells to a concentration of 10⁶ cells/ml; we then centrifuged the samples at 9,000 × g for 10 min and carefully discarded the supernatant. We resuspended the pellets in 100 µL of lysis buffer and incubated for 2 min at 100 °C. Samples were centrifuged at 13,000 rpm for 5 min, and supernatant was transferred to fresh tubes and kept at −80 °C until analysis. The gradient elution was performed on a Kinetex XB-C18 column (4.6 × 75 mm Phenomenex, Cheshire, UK) with two buffers at a rate of 1 ml/min. Buffer A contained 20 mM ammonia acetate (pH 4.5), while organic mobile phase B consisted of 100 % acetonitrile. We auto injected 5 µl of prepared sample or standard and monitored UV at 260 nm for between zero and 10 min. Peaks were identified by their retention times and by using chromatography with standards.

Statistical analysis

Means and standard deviations (SD) were calculated for descriptive statistical documentation. The student *T* test was applied for analytical statistics. We considered values of *p* < 0.05 and *p* < 0.01 to be significant.

Results and discussion

Effect of LLL irradiation on proliferation, metabolism and ATP level of CHO cells

We measured the proliferation of CHO cells at the same time every day from day 1 to day 8 (Fig. 2a). The results showed that seven min of daily laser irradiation significantly increased the number of viable cells (*p* < 0.001) compared to the control group on days 5 and 7. When we used fourteen min of irradiation, we observed significant increases (*p* < 0.05) in cell numbers by day 7. However, we detected no significant increase in the viable cells of 1 min exposure by day 7. These findings could not be attributed to thermal changes since culture temperature did not change during irradiation. The non-significant effects on cell viability found in this study are in accordance

Fig. 2 Effects of various laser doses of 632.8 nm He-Ne laser radiation on viable cell number (a) and % viability (b) in CHO cell culture. Cells were cultured in 50 ml Erlenmeyer flasks. The culture was agitated at a rate of 125 rpm at 37 °C in the presence of 5 % CO₂. Viability measurements are presented for day 7 of batch cultures. The error bars represent the standard deviations calculated from the data obtained from experimental replicates

with the findings of other authors (Marchesini et al. 1989; Chan et al. 2003) suggesting that alterations in cell cycle and mitosis could be responsible. It was reported that high energy laser irradiation resulted in an increase in G0/G1 phase of the cell cycle in melanoma cell lines (Chan et al. 2003).

The percentage of viability in control and irradiated cells was higher than 95 % and did not show any difference up to day 7 of the culture (Fig. 2b). No significant change in glucose consumption rate among cells that had different irradiation times was observed. However, the lactate production rate decreased significantly after irradiation (Fig. 3a) of seven ($p < 0.05$) and fourteen ($p < 0.01$) min by day 7 (Fig. 3b). Typically lactate is produced during growth phase of culture which at high concentration can have

Fig. 3 Effect of various laser exposure times on glucose consumption rate (a) and lactate production (b) in CHO cell culture. Measurements are taken at day 5, 6 and 7 of batch cultures. The error bars represent the standard deviations calculated from the data obtained from experimental replicates

detrimental effects on cell metabolism and viability. Glacken et al. (1986) found that a high concentration of lactate decreases the growth rate and the specific production rate of antibody in hybridoma cells even though pH was maintained constant. Thus, the reduction in lactate accumulation in the culture environment is desirable and may lead to improvement in cell growth and increase in MAb production. It is worthy to note that several processes have been developed for the reduction of lactate in cell culture (Chen et al. 2001; Xie and Wang 1994).

We observed changes in the mitochondrial activity of laser irradiated cells. ATP levels increased significantly ($p < 0.05$) by days 5 and 7 in cells that had been irradiated for one and 7 min compared to their respective controls that had not been irradiated (Fig. 4). Several studies reported a significant increase in ATP

Fig. 4 Effect of various laser exposure times on ATP level in CHO cell culture. ATP measurements are taken at day 5, 6 and 7 of batch cultures by HPLC. The error bars represent the standard deviations calculated from the data obtained from experimental replicates

production in living cells after laser irradiation (Benedicenti et al. 2008). Nevertheless, no explanation for the mechanisms associated with such an increase in ATP production was given. We postulate that this increase in ATP levels could be due to the absorption of laser energy by photoreceptor molecules and hence energy conversion from electronic to metabolic energy.

Effect of LLL irradiation on MAb production by CHO cells

Exposure to laser for 1, 7, and 14 min increased MAb concentrations significantly ($P < 0.01$)—by 17, 50, and 33 %, respectively, on day 7 (Fig. 5). Part of this increase in total productivity can be explained by the increased cell number of irradiated cultures but it also resulted from an increase in cell specific productivity. Specific productivity of cells at day 7 of 0, 1, 7 and 14 min exposure were 1.23, 1.56, 1.60 and 1.72 mg/10⁹ cell/h, respectively.

The stimulatory effect of laser irradiation on MAb cellular production rate might have been a consequence of increased ATP production (Fig. 4) and protein synthesis. Studies have shown that LLL irradiation promotes proliferation of many cell types and induces the synthesis of many molecules, like growth factors, interleukins and inflammatory cytokines, as well as can alter cellular homeostasis parameters, such as pHi and redox potential (Gao and Xing 2009), which can lead to a protein production increase (Khoo and Al-Rubeai 2009).

Fig. 5 Effect of various laser exposure times on monoclonal antibody (MAb) concentrations in the supernatant of CHO cell culture. ELISA measurements are presented for day 7 of batch cultures. The error bars represent the standard deviations calculated from the data obtained from experimental replicates

It would be useful for further analysis of laser irradiation on CHO cells to explore the biochemical, bioelectric and bioenergetics changes which lead to increased metabolism and protein synthesis. Many investigations have attempted to determine the biological effects of low-intensity lasers on tissues, especially during the repair process. However, not all of them have produced satisfactory results. Kreisler et al. (2003) studied the effect of low level of laser irradiation on human periodontal ligament fibroblasts and demonstrated the stimulation of photoreceptors in the mitochondrial respiratory chain, changes in cellular ATP levels, and release of growth factors. The mechanism of action of stimulation on cellular activities has not been clarified yet. Photoreception at the mitochondrial level may intensify the respiratory metabolism and electrophysiological properties of the membrane, thus promoting changes in cell physiology. Moreover, laser radiation increases the synthesis of ATP within mitochondria, thus accelerating the processes of cell proliferation and protein synthesis. For such reason, effort should be explored to define the laser parameters and culture conditions which support optimum productivity in CHO cells.

Conclusion

Low dose of laser irradiation exerts a biostimulative effect on CHO cells producing monoclonal antibodies. The increase in the recombinant antibody production

is associated with an enhancement in energy metabolism as expressed by the increase in ATP and glucose uptake rate. The narrow area of laser irradiation might hamper the large scale development and limits the potential industrial application of antibody production from recombinant cells.

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References

- Al-Watban FAH, Andres BL (2012) Laser biomodulation of normal and neoplastic cells. *Lasers Med Sci* 27(5):1039–1043
- Benedicenti S, Pepe IM, Angiero F, Benedicenti A (2008) Intracellular ATP level increases in lymphocytes irradiated with infrared laser light of wavelength 904 nm. *Photomed Laser Surg* 26:451–453
- Birch PR, Rehmany AP, Pritchard L, Kamoun S, Beynon JL (2006) Trafficking arms: Oomycete effectors enter host plant cells. *Trends Microbiol* 14:8–11
- Calatrava R, Valenzuela JMS, Gomez-Villamandos RJ, Redondo JJ, Gomez-Villamandos JC, Jurado IA (1997) Histological and clinical responses of articular cartilage to low-level laser therapy: experimental study. *Lasers Med Sci* 12:117–121
- Chan HHL, Xiang L, Leung JCK, Tsang KWT, Lai K (2003) In vitro study examining the effect of sub-lethal QS 755 nm lasers on the expression of p16INK4a on melanoma cell lines. *Lasers Surg Med* 32:88–93
- Chen K, Liu Q, Xie L, Sharp PA, Wang DIC (2001) Engineering of a mammalian cell line for reduction of lactate formation and high monoclonal antibody production. *Biotechnol Bioeng* 72:55–61
- Eells JT, Wong-Riley MTT, VerHoeve J, Henry M, Buchman EV, Kane MP, Gould LJ, Das R, Jett M, Hodgson BD, Margolis D, Whelan HT (2004) Mitochondrial signal transduction in accelerated wound and retinal healing by near-infrared light therapy. *Mitochondrion* 4:559–567
- Feng L, Joe XZ, Xiaoming Y, Tim T, Brian L (2005) Current therapeutic antibody production and process optimization. *Bioprocess J* :1–8
- Fonseca AS, Geller M, Filho MB, Valença SS, Paoli FD (2012) Low-level infrared laser effect on plasmid DNA. *Lasers Med Sci* 27:121–130
- Gao X, Xing D (2009) Molecular mechanisms of cell proliferation induced by low power laser irradiation. *J Biomed Sci* 16:4
- Gavish L, Asher Y, Becker Y, Kleinman Y (2004) Low level laser irradiation stimulates mitochondrial membrane potential and disperses subnuclear promyelocytic leukemia protein. *Lasers Surg Med* 35:369–376
- Glacken MW, Fleischaker RJ, Sinskey AJ (1986) Reduction of waste product excretion via nutrient control: possible strategies for maximizing product and cell yields on serum in cultures of mammalian cells. *Biotechnol Bioeng* 28:1376–1389
- Gorfien SF, Jayme DW (2011) Development and optimization of serum- and protein-free culture media. In: Davis JM (ed) *Animal Cell Culture: Essential Methods*. Wiley, Chichester, UK. pp 153–184 doi:10.1002/9780470669815.ch5
- Havel M, Sroka R, Englert E, Stelter K, Leunig A, Betz CS (2012) Intraindividual comparison of 1,470 nm diode laser versus carbon dioxide laser for tonsillotomy: a prospective, randomized, double blind, controlled feasibility trial. *Lasers Surg Med* 44:558–563
- Ito K, Senda N, Sugano N, Moriya Y, Nanba K, Hirano Y, Murai S (2000) Inhibitory effect of yellow He-Ne laser irradiation mediated by crystal violet solution on early plaque formation in human mouth. *Lasers Med Sci* 15:174–180
- Karu TI (2008) Mitochondrial signaling in mammalian cells activated by red and near IR radiation. *Photochem Photobiol* 84:1091–1099
- Karu TI (2010) multiple roles of cytochrome c oxidase in mammalian cells under action of red and IR-A radiation. *IUBMB Life* 62:607–610
- Karu TI, Pyatibra LV, Kalendo GS (2001) Cell attachment modulation by radiation from a pulsed light diode (k.820 nm) and various chemicals. *Lasers Surg Med* 28:227–236
- Khoo SHG, Al-Rubeai M (2009) Metabolic characterization of a hyper-productive state in an antibody producing NS0 myeloma cell line. *Metab Eng* 11:199–211
- Kirkby KA, Freeman DE, Morton AJ, Ellison GW, Alleman AR, Borsa PA, Reinhard MK, Robertson SA (2012) The effects of low-level laser therapy in a rat model of intestinal Ischemia–Reperfusion injury. *Lasers Surg Med* 44:580–587
- Koutna M, Janisch R, Veselska R (2003) Effects of Low-Power Laser Irradiation on Cell Proliferation. *Scripta Medica (Brno)* 76(3):163–172
- Kreisler M, Christoffers AB, Willershausen B, d’Hoedt B (2003) Effect of low-level GaAlAs laser irradiation on the proliferation rate of human periodontal ligament fibroblasts: an in vitro study. *J Clin Periodontol* 30:353–358
- Kuystermans D, Al-Rubeai M (2011) Bioreactor systems for the production of antibody from mammalian cells in: antibody expression and production. *Cell Eng* 7:25–52
- Kuystermans D, Krampe B, Swiderek H, Al-Rubeai M (2007) Using cell engineering and omic tools for the improvement of cell culture processes. *Cytotechnology* 53:3–22
- Lapotko DO, Lukianova E, Oraevsky AA (2006) Selective laser nano-thermolysis of human leukemia cells with micro-bubbles generated around clusters of gold nanoparticles. *Lasers Surg Med* 38(6):631–642
- Li Y, Han D, Hu G, Sommerfeld M, Hu Q (2010) Inhibition of starch synthesis results in overproduction of lipids in *Chlamydomonas reinhardtii*. *Biotechnol Bioeng* 107:258–268
- Ma H, Sorokin A, Mazein A, Selkov A, Selkov E, Demin O, Goryanin I (2007) The Edinburgh human metabolic network reconstruction and its functional analysis. *Mol Syst Biol* 3:135
- Marchesini R, Dasdia T, Melloni E, Rocca E (1989) Effect of low-energy laser irradiation on colony formation capability in different human tumor cells in vitro. *Lasers Surg Med* 9:59–62

- Moore P, Ridgway TD, Higbee RG, Howard EW, Lucroy MD (2005) Effect of wavelength on low-intensity laser irradiation-stimulated cell proliferation in vitro. *Lasers Surg Med* 36:8–12
- Ocaña-Quero JM, Gomez-Villamandos R, Moreno-Millan M, Santisteban-Valenzuela JM (1998a) Effect of Helium-Neon (He-Ne) laser irradiation on dog neoplasm cells in culture. *Lasers Med Sci* 13:143–147
- Ocaña-Quero JM, Perez de la Lastra J, Gomez-Villamandos R, Moreno-Millan M (1998b) Biological effect of Helium-Neon (He-Ne) laser irradiation on mouse myeloma (Sp2-Ag14) cell line in vitro. *Lasers Med Sci* 13:214–218
- Ozog D, Qu L, Liu A, Zhou L, He C, Grossman PH, Moy RL, Mi QS (2012) Clinical and molecular effects on mature burn scars after treatment with a fractional CO₂ laser. *Lasers Surg Med* 44:517–524
- Prusa AM, Plass CA, Wieselthaler GM, Podesser PK (2012) Low-Level-Laser irradiation induces photorelaxation in coronary arteries and overcomes vasospasm of internal thoracic arteries. *Lasers Surg Med* 44:705–711
- Shu-Zheng L (2007) Cancer control related to stimulation of immunity by low-dose radiation. *Dose-response* 5:39–47
- Wheeland RG (2012) Permanent hair reduction with a home-use diode laser: safety and effectiveness 1 year after eight treatments. *Lasers Surg Med* 44:550–557
- Wurm FM (2004) Production of recombinant protein therapeutics in cultivated mammalian cells. *Nat Biotechnol* 22:1393–1398
- Wurm FM (2005) The industry's workhorses-mammalian expression systems. In: Knablein J (ed) *Modern Biopharmaceuticals*, vol 3. Wiley-VCH Weinheim, pp 723–759
- Xie L, Wang DIC (1994) Fed-batch cultivation of animal cells using different medium design concepts and feeding strategies. *Biotechnol Bioeng* 43:1175–1189