Effect of He-Ne Laser Radiation on the Amount of ATF in HeLa Cells

T. I. Karu, L. V. Pyatibrat, G. S. Kalendo, and N. G. Serebryakov

UDC 577.391.621.375.8

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 6, pp. 617–618, June, 1993
Original article submitted January 20, 1993.

Key Words: *laser radiation; He-Ne laser; ATP; cell proliferation*

The stimulatory action of red light from a He-Ne laser (λ=632.8 nm) on cell proliferation has been described by a number of scientists (see [5] for a review), and a possible molecular mechanism relating the absorption of light by primary photoacceptors and by respiratory chain components to the final macroeffect and to changes in the cell proliferation rate has been proposed [4]. In this paper, a scheme is presented in which extrasynthesis of ATP is suggested as one link in the chain of photosignal transmission and amplification. Several authorities observed intensified ATP synthesis when isolated rat liver mitochondria were exposed to red light radiation [1,3,7,10]. In the available literature, however, we could not find any data on the measurement of ATP in irradiated cells cultured *in vitro*.

The purpose of the present study was to measure ATP in He-Ne laser-irradiated HeLa cells. It should be mentioned that this model has been used previously to examine how proliferation is affected in cells so irradiated [2,6].

MATERIALS AND METHODS

HeLa cells grown in penicillin vials containing 1 ml of medium 199 supplemented with 10% bovine serum and antibiotics (penicillin and streptomycin, 100 U/ml each) were used. The cells were irradiated with a He-Ne laser through a light guide (d=4 mm) so that the diameter of the beam corresponded to that of the vial bottom covered with the cell monolayer. The light intensity at the level of the vial bottom was 10 W/m^2, the time of irradiation was 10 sec, and the radiation dose was 100 J/m^2. These He-Ne laser parameters had been found optimal for altering the proliferation rate of HeLa cells [1,6]. The irradiation was carried out through the bottom of the vial in darkness. During irradiation the vial was fixed in a stand.

The amount of ATP in the cells was determined by a bioluminescence technique [8] based on a quantitative measurement of the intensity of the light flux

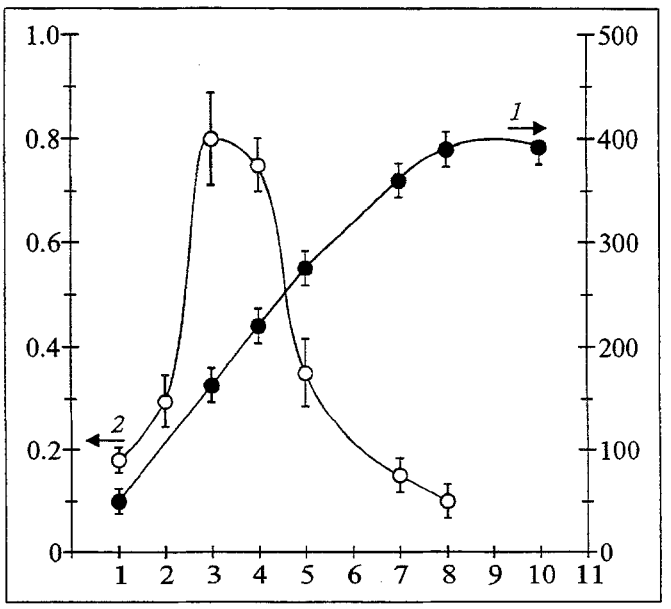

Fig. 1. Numbers of cells in monolayer (1) and ATP levels in individual cells during their growth in culture (2). 1) ATP concentration in cells×10^{-15} moles; 2) number of cells×10^3.

Research Center for Technological Lasers, Russian Academy of Medical Sciences, Troitsk, Moscow Region; and Cancer Research Center, Moscow. (Presented by N. N. Trapeznikov, Member of the Russian Academy of Medical Sciences)

0007-4888/93/0006-0688$12.50

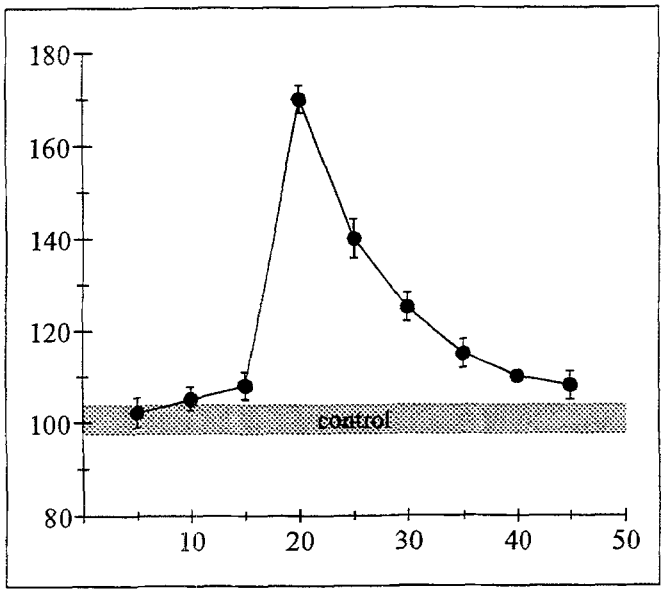

Fig. 2. Amounts of ATP in HeLa cells in the exponential growth phase after irradiation of their monolayer with He–Ne laser at 100 J/m² for 10 sec (the control level, taken as 100%, is 8×10^{-16} moles/cell).

induced in the reaction ATP + luciferin + O_2 $\xrightarrow{\text{luciferase}}$ oxyluciferin + AMP + phosphate + CO + light using an appropriate reagent kit (LKB Wallac, Finland). The absolute amount of ATP was determined by use of a calibration graph constructed through measurement of reference ATP solutions (LKB Wallac, Finland). The luminescence of the samples was measured with a Biolumat LB 9500 instrument (Berthold, Germany).

At specified times postirradiation, the medium was decanted from the vial, the cell monolayer was rinsed with cold Hanks balanced salt solution, and 0.5 ml of 0.7% trichloroacetic acid was added to the monolayer, which was then kept on an ice bath for 5 min without shaking to release ATP from the cells. Next, the sample was transferred to a clean test tube and appropriate dilutions were prepared in HEPES buffer (pH 7.75), after which 200 µl of the sample were taken into the cuvette of the measuring instrument and 20 µl of the luciferin-luciferase solution were added to the cuvette. The maximum luminescence of the sample was determined for 10 sec after the start of the reaction. The number of cells was determined in each vial. The results were expressed in units of ATP per cell. The values obtained are means of three replicate measurements.

RESULTS

Figure 1 shows the ATP concentrations in the cultured HeLa cells during their growth. The amount of ATP remained low in the lag period and in the early exponential growth phase 1 to 2 days after seeding. After 3-4 days, i.e. in the midexponential growth phase, the ATP concentration rose to its highest value of 8×10^{-16} mol/cell and then declined by the start of the early stationary growth phase (on days 7-8 after seeding). This pattern of variation in ATP agrees with the data reported in the literature [9].

Figure 2 shows the ATP concentrations in the exponential growth phase cells (72 h after seeding) in relation to the time after irradiation. It can be seen that the concentration of ATP in the cell remained virtually at the control level within the first 15 min postirradiation (100 J/m², 10 sec, 10 W/m²). Some increase in its concentration occurred 15 min after irradiation, followed by a sharp rise in the next few minutes. After the ATP concentration reached its peak (at 20 minutes), it slowly declined to the control level.

The dependence of ATP levels 20 min postirradiation of HeLa cells in the exponential growth phase (day 3 after seeding) on the radiation dose is shown in Table 1. As seen in this table, the effect was virtually nil at the dose rate of 10 J/m² while at 1000 J/m² it was much greater than at 100 J/m².

Since the amount of ATP in the cell depends on the growth phase of cultured cells (Fig. 1), it was of interest to explore how ATP levels were altered by irradiation according to the growth phase or, in other words, to find out whether the sensitivity of the cultured cells to red light radiation depends on their growth phase. To this end, similar tests were run at various times after seeding (1 to 8 days), irradiating the monolayer at 100 J/m² and measuring ATP 20 min postirradiation, i.e., when the amount of ATP was at its maximum (see Fig. 1).

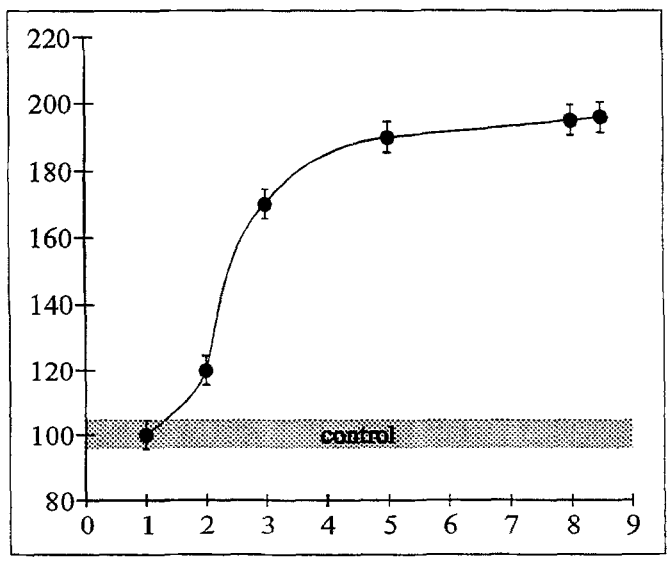

Fig. 3. Sensitivity of cultured HeLa cells to He–Ne laser radiation (100 J/m², 10 sec, 10 W/m²) during their growth (in each test the amount of ATP was determined 20 min after irradiation).

TABLE 1. Amounts of ATP in HeLa Cells in the Exponential Growth Phase 20 min after their Irradiation with He–Ne Laser in Various Doses (mean±SEM)

Dose, J/m²	Amount of ATP, % of control level
–	100±2.0
10	100.5±3.5
10^2	170.8±2.3
10^3	238.3±5.5

Irradiating the cells with the He-Ne laser on day 1 or 2 after seeding was found not to have increased the amount of ATP as compared to intact cells (Fig. 3), whereas irradiating them on day 3, 5, or 8 increased its level; their sensitivity to the radiation remained virtually the same and peaked on day 5 (190%) under the experimental conditions used.

The results of this study indicate that He-Ne laser radiation-induced ATP extrasynthesis does not occur just only in isolated rat liver mitochondria [2,3,7,10]. After He-Ne laser irradiation, ATP also increases in cells cultured *in vitro*.

REFERENCES

1. T. I. Karu, G. S. Kalendo, V. S. Letokhov, and V. V. Lobko, *Kvant. Elektronika*, **10**, № 9, 1771-1776 (1983).
2. S. M. Zubkova and O. A. Krylov, in: *Experimental and Clinical Aspects of Health Resort Treatment and Physical Therapy* [in Russian], Moscow (1976), pp. 18-20.
3. S. A. Gordon and K. Surrey, *Radiat. Res.*, **12**, 325-329 (1960).
4. T. Karu, *Lasers Life Sci.*, **2**, № 1, 53-74 (1988).
5. T. Karu, *Photochem. Photobiol.*, **52**, № 6, 1089-1099 (1990).
6. T. Karu, L. V. Pyatibrat, and G. S. Kalendo, *Nuovo Cimento D.*, **9**, № 12, 1485-1494 (1987).
7. M. Kato, S. Shinzawa, and S. Yoshikawa, *Photochem. Photobiophys.*, **2**, № 2, 263-271 (1981).
8. G. Kimmich, J. Randes, and J. S. Brand, *Analyt. Biochem.*, **69**, № 1, 187-206 (1975).
9. F. Kramb, D. Kummerand, and K. Schmitt, *Analyt. Biochem.*, **71**, 500-506 (1976).
10. S. Passarella, F. Casamassina, S. Molinari, *et al.*, *FEBS Lett.*, **175**, № 1, 95-99 (1984).

Epidermal Ornithine Decarboxylase Activity in Psoriasis: a Biochemical Indicator of a Hyperproliferative Process

A. T. Kagramanova, L. D. Tischenko, and T. T. Berezov

UDC 616.517–07:616.591–008.931–074

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 6, pp. 618–620, June, 1993
Original article submitted December 29, 1992

Key Words: *polyamines; ornithine decarboxylase; epidermis; psoriasis*

There has been an upsurge of interest in the synthesis and degradation of polyamines in different biological objects in the last decade in connection with the vital role played by polyamines in the regulation of cell metabolism and in DNA, RNA, and protein synthesis, particularly in the course of malignant growth [5, 7, 11, 14, 15]. Convincing evidence has been obtained of a direct relationship between polyamine concentration of eukaryotic cell differentiation rate. A specific level of polyamines regulated by the ratio of enzymes catalyzing polyamine synthesis and degradation has been found to be characteristic of different stages of cell differentiation and growth [13, 16]. An increased activity of enzymes catalysing polyamine synthesis and, conversely, a decreased activity of enzymes of polyamine catabolism are characteristic biochemical features of some rapidly growing tissues (embryonic and malignant, regenerating liver, etc.) [4, 12]. We should like to mention here our previous

Department of Skin and Venereal Diseases and Department of Biochemistry, Russian Peoples Friendship University, Moscow

0007-4888/93/0006-0690$12.50