STIMULATING HAIR GROWTH BY HELIUM-NEON LASER IN RATS

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ABSTRACT

We performed a study to determine whether 632.8 nm helium-neon (He-Ne) laser radiation would affect hair growth of the skin after the hairs had been pull out in 18 female rats, 8 weeks of aged. The four skin areas, 2 cm in diameter each, the hairs were pull out at the back of all rats after ketamine hydrochloride anesthesia. To determine the rate of hair growth and optimum daily energy density irradiance, energy density of 1.35, 2.70 and 4.05 J/cm^2/day of He-Ne laser were delivered for two weeks to area I (15 mins every 24 hrs), area II, two repeated exposures at 12 hrs interval and area III, three repeated exposures at 8 hrs interval, respectively. The control site (area IV) was not irradiated but received placebo light. Additional to the observation of the reaction at all areas where the hairs were pull out, length of hairs on them were measured daily by capillary tube and magnifying lens. The results shown that normal rate of hair growth was 0.27 mm/day while the growth rate at the irradiated areas with 1.35, 2.70 and 4.05 J/cm^2/day were 0.42, 0.62 and 0.36 mm/day, respectively. 2 repeated daily exposures were the optimum energy density for stimulation of hair growth. Hair growth in the control site was the same rate as 1 exposure site and 3 repeated exposures site. Hair follicles needed some energy density of He-Ne laser radiation to initiate hair growth in rats.

INTRODUCTION

Low-energy laser irradiation has been shown to have some bioeffects. Activation of local cellular and humoral level, such as increased formation of ATP,[1,2] fibroblast, mast cells,[3,4] change in prostaglandin level, increased angiogenesis,[5] increased epithelial activity and microcirculation, prevent post traumatic degeneration of nerves [6] were described as the local effect. Systematic effect of low-energy laser were to promote wound healing,[3,7,8] antiinflammation,[2,5] relief pain,[8-12] increased vascularization, increased blood flow and lymphatic drainage.[13] Some authors demonstrated that interaction at tissue level was photochemical in nature, dependent on absorption in a tissue chromophore. Each of these potential chromophores absorbed radiation of some wavelength or wavelengths.[3,4,8,14] The absorption probably increased the energy of this chromophore and its activity was thus altered in relationship to its environment and consequently the metabolism of the cell was changed affecting tissues and organs.[14]

Alopecia is a psychological problem especially in women that play a role in the leading position. Male pattern baldness can affect not only

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in men, but also in women. In patients with early male pattern baldness, the subcutaneous blood flow was 2.6 times lower than the values found in the normal individuals.[15] Aging results in a reduction of the maximal conductance of the cutaneous vasculature.[16] Stimulating hair growth by many substances, some microorganisms, immunosuppressive drugs, vasodilator drug have been reported.[17-27] Minoxidil is a potent vasodilator that has been used to stimulate cutaneous blood flow in human balding scalps.[28] If this hypothesis is true, it means drugs or substances that causes cutaneous vasodilation, will promote hair growth. He-Ne laser is a non-ionizing radiation and low -energy laser that shows vasodilation effect and angiogenesis.[5,29] It is very interesting thing whether He-Ne laser irradiation would affect hair growth. We proposed an in vivo study in rats in order to measure the rate of hair growth and to decide an optimum laser energy density per day.

MATERIALS AND METHODS

18 white female Wistar rats aged 8 weeks from National Laboratory Animal Center, Mahidol University were introduced in the same environment, same food supplement for 4 weeks before starting the experiment. Theirs mean weights were 324 ± 17.8 g ranging from 300-400 g. These rats were grouped by means of simple random sampling into 3 groups that consisted of 6 rats in each group. All rats were anesthetized with ketamine hydrochloride (25 mg/kg) intramuscularly 5 mins before pulling the hair off from the back. Each of the 4 area was 2 cm in diameter as shown in Fig 1.

Placing these rats in a control environment and food supplement for 24 days, thus the hair follicles grow into telogen stage.[30] The hairs at the same area were pull off again to induced anagen stage of hair follicles. Each skin without hair was irradiated by He-Ne laser (Professional laser: power 30 mW) of monochromatic 632.8 nm. Daily energy density was delivered to various sites according to the following programs:

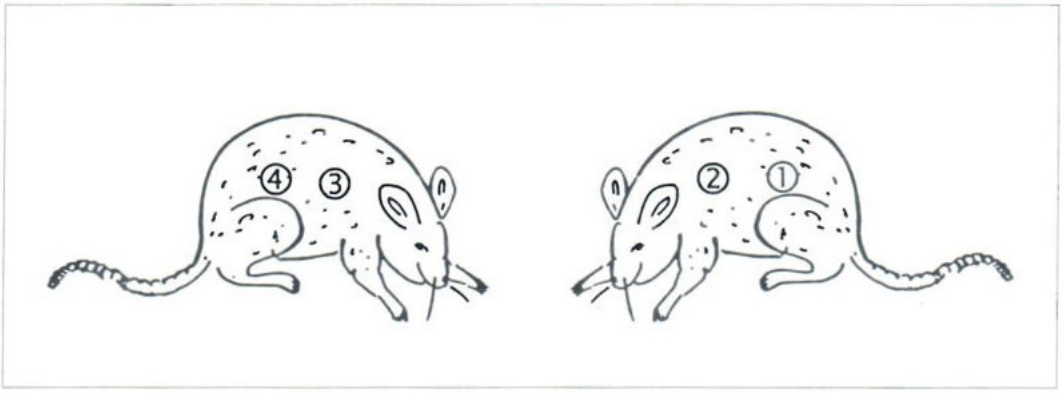


Fig. 1 4 areas of skins at the back of rat, after the hairs were pull off

Table 1. Number of fractions per day and energy density delivered to various sites

site	number of fractions (15 mins/fraction)	energy density/day (J/cm^2/day)
1	0	0.00
2	1	1.35
3	2	2.70
4	3	4.05

Area at site 1 was control, the site 2, 3 and 4 were daily treated with 1, 2 and 3 fractions of He-Ne irradiation for two weeks, respectively. Apart from the observation of the reaction at the 4 areas where the hairs were pull off, length of hairs were also measured by capillary tube and magnifying lens.

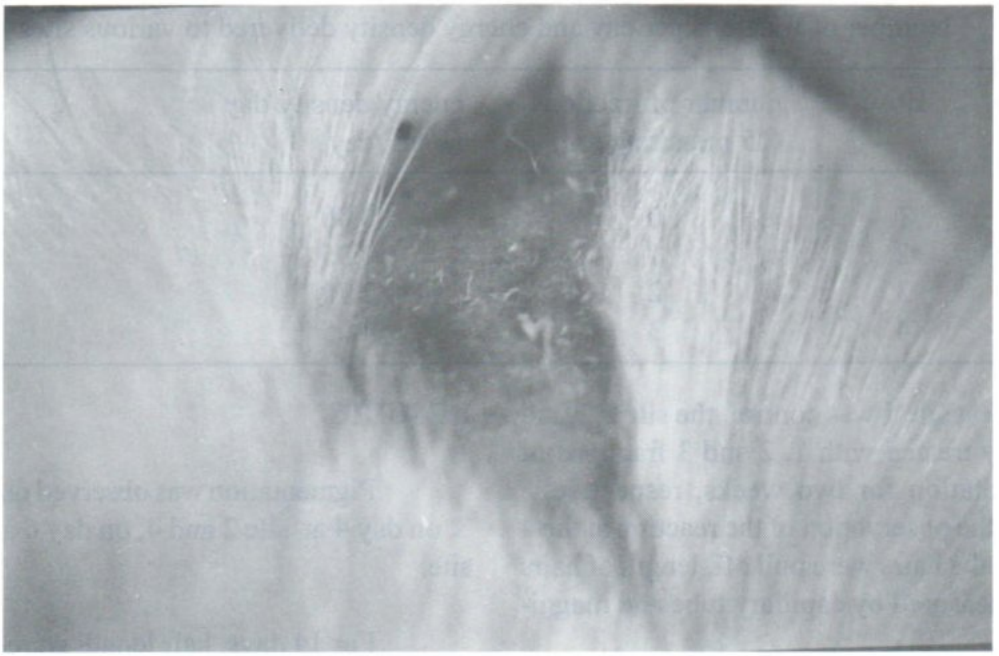
RESULTS

Pigmentation was observed on day 3 at site 3, on day 4 at site 2 and 4, on day 6 at the control site.

For 14 days, hair length were 3.9, 5.9, 8.8 and 5.1 mm on site 1, 2, 3 and 4, respectively, as shown in table 2. Daily hair growth in rats group 1, 2 and 3 were plotted versus various sites in figure 3, 4 and 5, respectively.

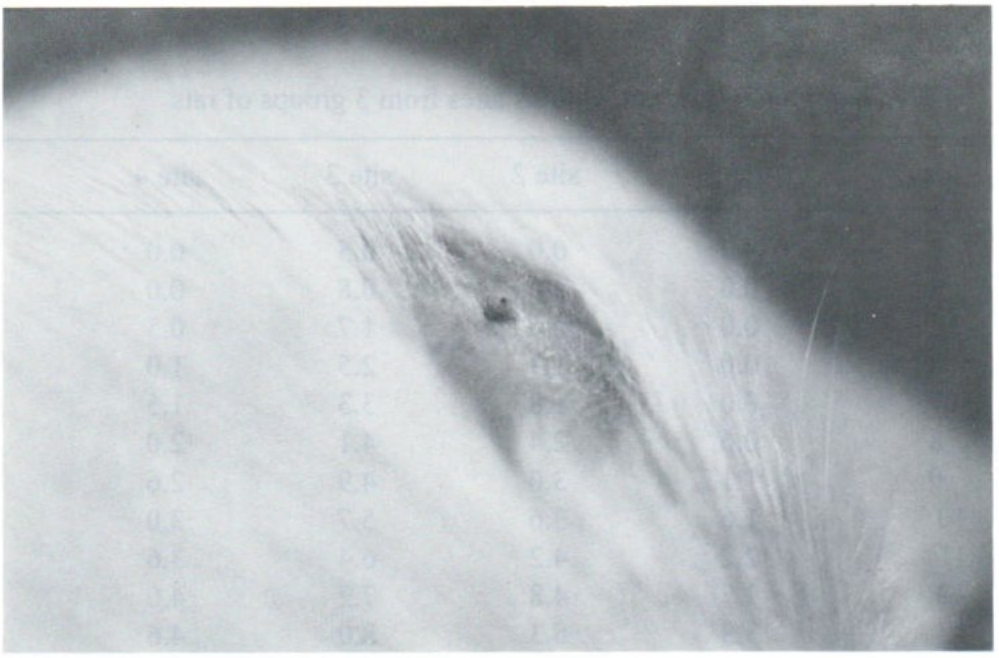
Table 2. Mean hair length (mm) on various sites from 3 groups of rats

day	site 1	site 2	site 3	site 4
3	0.0	0.0	0.5	0.0
4	0.0	0.0	0.8	0.0
5	0.0	0.6	1.7	0.5
6	0.0	1.3	2.5	1.0
7	0.0	1.8	3.3	1.5
8	0.6	2.4	4.1	2.0
9	1.1	3.0	4.9	2.6
10	1.6	3.6	5.7	3.0
11	2.2	4.2	6.4	3.6
12	2.7	4.8	7.2	4.0
13	3.3	5.3	8.0	4.6
14	3.9	5.9	8.8	5.1
mean /day	0.27	0.42	0.62	0.36



A.

Fig. 2 Observation on day 3 A. no pigmentation at control site



B.

Fig. 2 Observation on day 3 B. pigmentation was observed on site 3

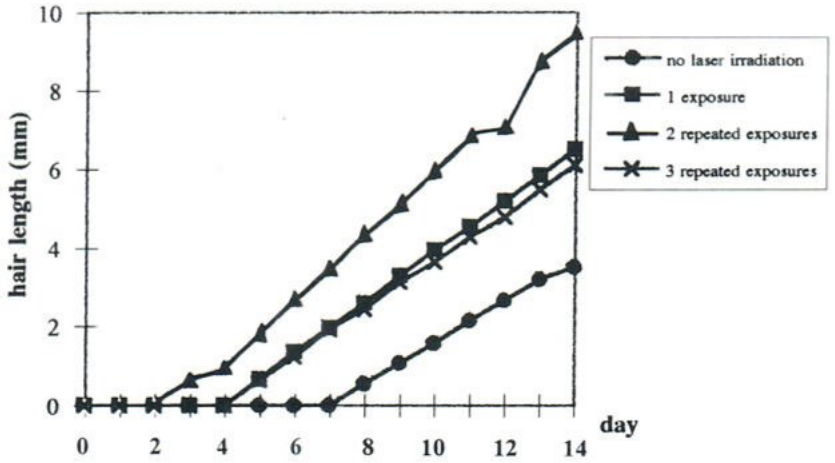


Fig.3 Mean daily hair length on various sites in rats group 1

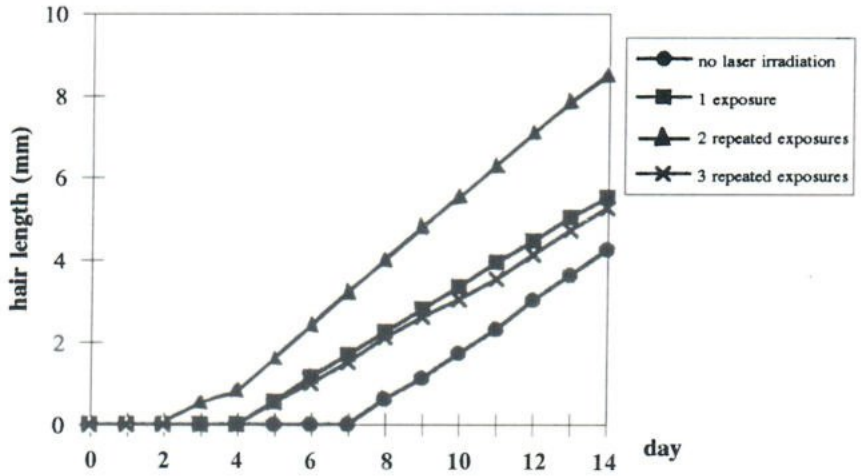


Fig.4 Mean daily hair length on various sites in rats group 2

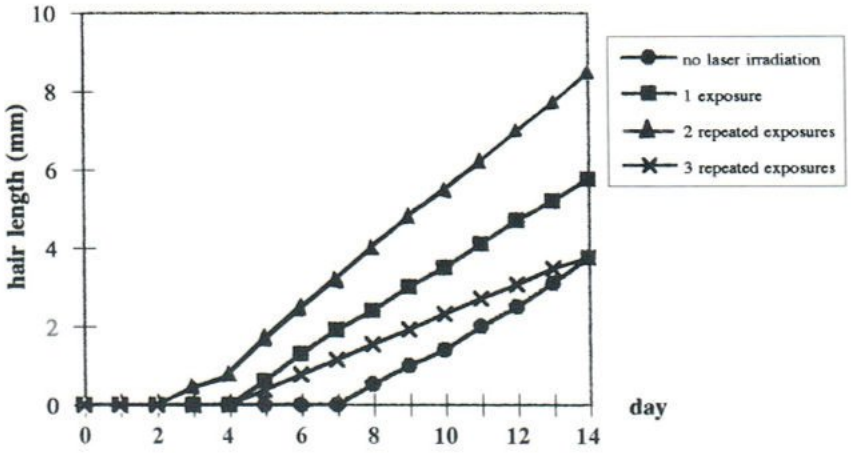


Fig.5 Mean daily hair length on various sites in rats group 3

Analysis of variance was used to analyze the differences between mean of hair length on 4 sites. From 3 groups of rats, the mean at site 1 (μ_0), 2 (μ_1), 3 (μ_2) and 4 (μ_3) were significantly different (p-value = 0.027) as shown in table 3 and hair length on site 3 that was irradiated by He-Ne laser 2 times a day was longer than site 1, 2 and 4 (p-value = 0.005, 0.04 and 0.03), respectively. Hair growth on the control site was the same rate as site 2 and 4 (p-value = 0.190 and 0.239)

Table 3. ANOVA Table

source	d.f.	sum of square	mean of square	F
treatment	3	72.58	24.19	4.54*
error	40	195.90	4.89	
total	43	268.48		

* p-value = 0.027, significant at $\alpha = 0.05$

Table 4. Comparison of means of hair length in each site

treatment	$t = \frac{\lvert (\bar{X}_i - \bar{X}_j) \rvert}{\sqrt{MSE\{(1/n_i) + (1/n_j)\}}}$	p - value
μ_0 , μ_1	0.94	0.190
μ_0 , μ_2	2.70	0.005*
μ_0 , μ_3	0.71	0.239
μ_1 , μ_2	1.76	0.040*
μ_1 , μ_3	0.22	> 0.250
μ_2 , μ_3	1.98	0.030*

* significant at $\alpha = 0.05$

DISCUSSION

The rate of hair growth is supposed to be influenced by such factors as spicies, race, sex, age, season of year, nutrition and hormones.[31,32] Observations have been made mainly on animal hair by the reason of the short cycle of its hair growth. The capillary method was used to measured the rate of hair growth. This method could be used daily with an error rate of 2.82% at 25-30°C.[30] The results showed that normal rate of hair growth was 0.27 mm/day, but the growth rate in the irradiated site with 2.7 J/cm^2/day laser energy was 0.62 mm/day (Table 2). The linearity of hair growth of the 4 areas of the skin were found in all groups (Fig. 3, 4 and 5).

Many chemicals, microorganisms, drugs, immunosuppressive agents, monochromatic light were conducted to stimulate hair growth.[17-27] Daily repeated exposures to high doses of monochromatic 260 nm radiation results in the initiation of active hair growth in telogen phase of hair follicles by occurrence of pigmentation in day 5, with hair protruding above the surface approximately two weeks after the first exposure.[17,18] The 632.8 nm monochromatic light of laser in various power energy were delivered to the various sites of rat-skin. The pigmentation were observed at day 3-4, hairs were protuded at day 4-5 with higher rate than the control site (no laser irradiation). Pigmentation can be observed at the control site on the skin at day 6 with hair protruding above the skin at day 8 after the first exposure (Table 2).

2 repeated exposures doses per day (2.7 J/cm^2) was the optimum energy density for stimulation of hair growth in all 3 groups of rats with mean length 8.8 ± 0.5 mm. This value was significantly longer than hair length at the 1 exposure site (1.35 J/cm^2) and 3 repeated exposures site (4.05 J/cm^2) (p-value = 0.04 and 0.03, respectively). With single exposure dose of 632.8 nm radiation, neither the radiation nor the concentration of protease at the level of the hair germ would be sufficient to initiate hair growth. The highest rate of hair growth was occured at 2 repeated exposures site, It is possible that a second exposure at about 12 hours after the first would release newly formed protease in the recovering cells of the epidermis and that it is this, in combination with the earlier released enzyme or acting on primed target, which caused the initiation of hair growth. The hair germ at 3 repeated exposures site will be destroyed when the third exposure was given. The thymine dimer in DNA of nuclei separate from the matrix cell and telogen hair follicle was dead.[32] It indicated that hair follicles need some energy density of monochromatic light to initiate hair growth.

Excessive dose of He-Ne laser may be harmful to the cell. In rabbit, giving an irradiance of 4.42 W/cm^2 for daily 3-30 min, the mitotic rate of corneal epithelium was reduced and finally damaged. Severe epithelial damage after 30 seconds (0.13 J/cm^2) daily irradiation and 20 min irradiation destroyed most of the epithelium. Endothelial damage occured after irradiation periods of 1 min (0.26 J/cm^2). In cat, corneal thickness increased but endothelial cell density decreased by 21% and 12%, respectively.[33] The bioeffects of low-energy laser irradiation on wound healing have been shown to be ineffective (0.37 and 0.45 J/cm^2/day)[34] or stimulating (3.8 J/cm^2/day) depending on doses.[3] All these experiments revealed that low-energy lasers do have specific bioeffects which seem to be changed from stimulation to damaging with increasing doses.

In radiotherapeutic patients, the distress events may affect from the nature of diseases and the radiation-tissue interaction.[35] Radiation induced alopecia in these patients usually appeared because of the decreasing of newly forming hair cortex.[36] He-Ne may be the alternative way to

stimulate hair growth in male pattern alopecia and radiation induced alopecia by photochemical mechanism. However, the mechanism and pathogenesis of alopecia are the important things that affect the success of the treatment.

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