Effect of Blended Carbon Dioxide and Erbium:YAG Laser Energy on Preauricular and Ear Lobule Keloid Fibroblast Secretion of Growth Factors

A Serum-Free Study

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Background: A serum-free in vitro model was used to determine the effect of combined carbon dioxide (CO₂) and erbium (Er):YAG laser (Derma K; ESC/Sharplan Medical Systems, Yokneam, Israel) irradiation on keloid-producing fibroblasts (KFs) from 2 distinct facial sites. Transforming growth factor-β1 (TGF-β1) and basic fibroblast growth factor (bFGF) play an integral part in wound healing and were assayed using this model. It has always been a clinical impression that fibroblasts from different regions of the face behave differently. This is exemplified by patients prone to lobule keloid formation after ear piercing, who heal normally after a facial incision.

Design: Laboratory-based wound healing.

Methods: Human KF cell lines were established from operative specimens using standard explant techniques. At 48 hours after seeding, 20% of each well was exposed to 1.7 J/pulse of Er:YAG laser energy and CO₂ delivered at 3 or 5 W and at a duty cycle of 25%, 50%, or 100%. Using a quantitative enzyme-linked immunosorbent assay, TGF-β1 and bFGF were assayed from collected supernatants.

Results: Laser-treated ear lobule KFs demonstrated decreased TGF-β1 production when compared with preauricular KFs. Statistical significance (P<.005) was seen in the 3-W CO₂ 25% duty cycle; a trend was seen in the 3-W CO₂ 50% duty cycle (P<.08). Preauricular KFs secreted increased bFGF when compared with lobule KFs. Significance was seen in the 3-W CO₂ 25% and 50% duty cycles (P<.05). Laser-treated preauricular KFs had increased bFGF secretion when compared with non–laser-treated preauricular KFs in the 3-W CO₂ 25%, 50%, and 100% duty cycles.

Conclusions: Combined CO₂ and Er:YAG laser treatment decreases the production of TGF-β1 in preauricular and ear lobule KFs. This laser may have clinical promise in the treatment of keloids. Finally, the different growth factor profiles obtained suggest that KFs from the ear lobule and preauricular regions are different.

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Regional variability exists in the body in its response to wound healing. Clinical experience has shown a predilection for keloid or hypertrophic scar formation after trauma in certain regions of the body. As physicians, we commonly see ear lobule keloids, but we uncommonly see keloids in the midportion of the face. Beauty and vanity have led people to induce trauma to the face in search of a youthful effect. Since ancient times, exfoliation of the skin has been used to improve its quality and texture. Ancient Egyptians used salts and animal oils, whereas Turks used fire to wound the skin with the goal of aesthetic improvement. In the 20th century, chemical peels, dermabrasion, and laser treatments have allowed the surgeon specializing in facial aesthetics a controlled means of ablating skin. A fine line exists between inducing normal wound healing vs wound healing gone awry.

Keloids remain a perplexing problem for the surgeon. Differing from a hypertrophic scar that grows within the scar borders, the keloid extends beyond like a crab claw and produces an excess of collagen. Despite being described as far back as 3000 BC in the Smith papyrus, the treatments for keloids have been moderately effective at best. These have ranged from simple excision and closure, injection with intralesional corticosteroids, and closure with local flaps, to cryotherapy, systemic chemotherapy, ionizing radiation, and pressure. The fact that no single treatment is universally accepted is a testament to the lack of a successful treatment modality for this benign tumor.

Lasers have been increasingly used in the field of facial aesthetic surgery. Since the mid to late 1980s, the indications for...
the release of transforming growth factor-β1 (TGF-β1) and basic fibroblast growth factor (bFGF) has shown promising results in increasing the secretion of growth factors by fibroblasts during wound healing. This suggests that the use of growth factors may enhance tissue regeneration and minimize scarring.

In our laboratory, a superpulsed CO2 laser has been used to ablate thin layers of tissue with minimal thermal damage. This instrument has been shown to be effective in treating keloid, rhinophyma, and actinic cheilitis by precisely ablating thin layers of tissue. The laser energy is delivered at a wavelength of 10,600 nm, which is absorbed by water, allowing for selective coagulation of target tissues.

Combined CO2 and Er:YAG Laser Treatment

Fifty-eight hours after seeding (time, 0 hours), cells were prepared for laser treatments. Each cell well was washed 3 times with PBS, then aspirated before laser treatments. Control wells received no irradiation. The scanning system (Dermacan; ESC/Sharplan Medical Systems) was set to the smallest parallelogram (6.0 × 4.8 mm). This pattern irradiated 18% of each cell well. All trays were treated in duplicate. After irradiation, 1 mL of serum-free medium was added to each well and placed in the incubator for the appropriate time. The supernatant from each well was collected at the appropriate time intervals for later growth factor assays. Cell counts and viability were obtained from each well using a hemacytometer, phase-contrast microscope, and the trypan-blue dye exclusion method.

Enzyme-Linked Immunosorbent Assay

Human growth factors TGF-β1 and bFGF were assayed from each well using commercially available enzyme-linked immunosorbent assay kits (Quantikine and Quantikine High Sensitivity, respectively; R&D Systems, Minneapolis, Minn). Optical densities were analyzed using commercially available software (KC4; Bio-Tek Instruments, Inc, Winooski, Vt). Assays were read using an automated plate reader (Elex800; Bio-Tek Instruments, Inc). The TGF-β1 and bFGF enzyme-linked immunosorbent assay sensitivities are 7 pg/mL and 0.50 pg/mL, respectively.

Statistical Analysis

Each data point represents duplicate cell counts with assays performed in duplicate. Cell population-doubling times (PDT) were calculated from logarithmic best-fit curves. Data analysis and statistics were performed using commercially available software (Microsoft Excel for Windows 97, Version 7.0; Microsoft Corp, Redmond, Wash). Statistical differences between groups were assessed using the paired t test. Differences at the 5% level were considered statistically significant.
The fibroblast growth factor family consists of 2 closely related isoforms, basic (bFGF) and acidic (aFGF). In general, most cells are much more sensitive to bFGF than aFGF. Basic FGF has been found to be 10- to 30-fold more active than aFGF and can maximally stimulate proliferation of some cells at 1 ng/mL.\(^7,8\) Basic FGF is stored as an inactive peptide in the extracellular matrix, integrated within the basement membrane. It functions locally and is released when injury has occurred.\(^3,6,8\)

Basic FGF is a potent modulator of collagen production by keloid-producing fibroblasts (KFs). Tan et al\(^3\) have shown that this cytokine inhibits and down-regulates type I collagen gene expression by KFs. Others have shown that the altered collagen metabolism results from stimulating the expression of collagenase in these cells.\(^9\)

Transforming growth factor \(\beta 1\) is a homodimeric structure with 2 disulfide-linked polypeptide chains of 12.5 kd. It is a member of a family of pleiotropic growth factors and is produced by platelets, macrophages, fibroblasts, and smooth muscle cells.\(^10\) One of 2 transforming growth factors (the other being TGF-\(\alpha\)), TGF-\(\beta 1\) was originally characterized by its ability to reversibly induce the transformed phenotype in certain nontumori-
genic cells.\(^11\) Now known to elicit a variety of biological activities, TGF-\(\beta 1\) has been shown to dramatically increase the expression of several collagen types in cultured fibroblasts.\(^10\) This growth factor is now postulated to play an active role in keloid formation.

The purpose of this study was to examine the effects of blended Er:YAG and CO\(_2\) laser energy on KFs from 2 different facial regions in a serum-free in vitro model. Our laboratory has shown in previous studies the importance of a serum-free medium for analysis of growth factor production by fibroblasts.\(^2,12\) This laser is believed to produce less intense erythema of a shorter duration resulting from facial laser resurfacing as well as excellent clinical results.\(^13\) The laser's effect on KP proliferation and production of bFGF and TGF-\(\beta 1\) may provide insights into the following: (1) this laser's ability to prevent aberrant wound healing during facial laser resurfacing; (2) its efficacy in treating facial keloids; and (3) different behavior of preauricular KFs compared with ear lobule KFs.

**RESULTS**

All populations of cells exhibited exponential growth in the serum-free medium (Figure 1 and Figure 2). Viability at 0 hours for all populations of cells ranged from 85% to 98%. The PDT was calculated for each population of cells. The mean PDT of preauricular KFs (44.3 hours) was faster than that of ear lobule KFs (51.2 hours). This difference was not statistically significant. The control groups, which received no laser irradiation, had shorter PDTs than all other groups that received laser treatment (Figure 3).

Laser-treated preauricular KFs secreted more bFGF than did laser-treated ear lobule KFs, except when comparing the non–laser-treated groups (Figure 4). The preauricular KFs treated with 3 W of CO\(_2\) at a duty cycle of 25%, 50%, and 100% all secreted more bFGF than did the non–laser-treated preauricular KF group. Statistical significance was not reached.

Secretion of TGF-\(\beta 1\) increased progressively up to 96 and 120 hours for preauricular and ear lobule KFs, respectively. Preauricular KFs secreted more TGF-\(\beta 1\) than did ear lobule KFs in all groups. Statistical significance
(P<.005) was seen in the 3-W CO2 25% duty cycle, whereas a trend was seen in the 3-W CO2 50% duty cycle (P<.08). Laser-treated preauricular and ear lobule KFs demonstrated decreased TGF-β1 secretion when compared with non–laser-treated KFs (Figure 5). In the preauricular KFs, statistical significance was seen in the 3-W CO2 25% and 50% duty cycle groups when compared with the non–laser-treated preauricular KFs (P=.04 and P=.05, respectively). In the ear lobule KFs, statistical significance was also seen in the 3-W CO2 25% duty cycle group (P=.04), whereas a trend was observed in the 3-W CO2 100% duty cycle group (P=.09).

The incidence of keloid formation is equal between men and women, and occurs in anywhere from 5% to 15% of wounds in high-risk populations, ie, dark-skinned individuals and those aged 2 to 40 years.1 Keloids appear to run in families,14 and despite some assumptions that inheritance is autosomal dominant, the form has not been clearly determined.13 There are numerous theories as to the cause of keloids, but none has been proven. A few of these are ischemia-related proliferation of perivascular myofibroblasts and endothelial cells; tension-induced excess collagen production by fibroblasts; inflammatory-, immunity-, and autoimmunity-induced antigen and antibody responses; and induction of fibroblasts by estrogen-, androgen-, and melanocyte-stimulating hormones and other hormonally related processes.

Keloids seem to occur with different frequencies in different regions of the body. They almost never appear on eyelids, central portion of the face, palms of the hand, soles of the feet, or genitalia.10 On the other hand, they are commonly seen on earlobes, especially after ear piercing; presternal and deltoid regions; wounds that cross skin-tension lines; wounds that are closed under tension; and wounds that develop in thicker skin.1

Normal fibroblasts and KFs appear to have no difference in cellular morphology but have vastly different cellular function.11 Keloids are histologically characterized by an abundance of extracellular matrix of connective tissue. Increased production of elastin, chondroitin sulfates proteoglycans, fibronectin, and especially collagen occurs in KFs compared with their normal counterparts.3,10,11,18 Collagen is the predominant extracellular matrix component of keloids with an excess of type I collagen production.19 Keloids contain randomly organized sheets of thick collagen fibers, compared with discrete, organized collagen bundles in normal fibroblasts.

Light amplification by the stimulated emission of electrons (or LASER) is a tool invented in the 1960s. The ability to increase precision and uniformity of tissue ablation was very attractive, and the search for other treatment modalities for keloids and hypertrophic scars was begun. Castro et al10 discovered that the Nd:YAG laser selectively suppresses collagen production without affecting cell proliferation; they concluded that this laser treatment could be used to reduce collagen deposition in conditions such as keloids and hypertrophic scars. The work of Abergel et al21 continued that of Castro et al. They confirmed that the Nd:YAG laser selectively suppresses collagen production in fibroblast cultures and in healthy skin in vivo irrespective of a thermal effect.21 Thus, both conclusions were similar, ie, that this laser may be useful for the treatment of fibrotic conditions, such as keloids and hypertrophic scars. Abergel et al24 were also able to show that 2 low-energy lasers, helium-neon and gallium-arsenide, stimulated collagen production in human skin fibroblast cultures, suggesting that these lasers could be used for enhancement of wound-healing processes. Apfelberg et al15 worked with the argon and CO2 lasers on trunk and earlobe keloids. Only 1 patient of 13 responded to their multiple bore-hole argon technique, followed by total excision with the CO2 laser. Alster and Williams23 discovered that the 585-nm flashlamp-pumped pulsed-dye laser improved erythema, scar height, skin surface texture, and pruritus on hypertrophic or keloidlike median sternotomy scars, with effects lasting 6 months. Nowak et al20 have shown that the superpulsed CO2 laser stimulates the release of bFGF and inhibits the release of TGF-β1 in keloids. These profiles of growth...
factors imply that this laser may have beneficial effects in the treatment of keloids. Recently, we have also shown that simultaneous Er:YAG and CO₂ laser irradiation also produces a favorable profile of growth factors when delivered to facial keloids (E. T. C. and R. J. K., unpublished data, October 1999).

We used combined Er:YAG and CO₂ irradiation to the same tissue area simultaneously by means of a collimated hand piece with a 3-mm spot diameter and a scanning system manufactured to use the described laser system. The laser system consists of a 0.1–to 1.7-J/pulse Er:YAG laser that delivers a wavelength of 2.94 µm and a pulse duration of 350 microseconds combined with a 0- to 10-W CO₂ laser that delivers a wavelength of 10.6 µm and a pulse duration of up to a 100% duty cycle. Since the system delivers both laser energies simultaneously, the CO₂ pulsing is dependent on the rate of the Er delivery. The high energy of the Er:YAG laser allows precise ablation, whereas the subablative CO₂ heats the tissue and provides laser energy for hemostasis and tissue tightening. The laser system combines a pulsed (350-microsecond) Er:YAG system, operable at a fluence of 5 to 25 J/cm², and a continuous-wave low-power CO₂ system. The CO₂ laser component delivers pulses of variable duration, operating mostly at 30 to 50 milliseconds. The laser has a spot size of 3 mm, and with an integrated scanning pattern generator has the ability to irradiate an area as large as 20 mm. The early results using this laser system appear to be positive, with shorter healing times secondary to less thermal damage and consistent results. Healing generally occurs within 5 days, and the resulting erythema completely disappears within 4 to 6 weeks. At present, there are no published reports in the literature on the efficacy of this laser on regional keloid differences.

In vitro studies have shown the cellular function of fibroblasts and keloids to be altered by growth-inhibitory and growth-stimulatory factors, such as TGF-β1 and bFGF. Basic FGF alters cellular function in keloids by stabilizing cell morphology, increasing cell migration and proliferation, and cell survival. Our results showed that laser-treated preauricular KFs secreted more bFGF than did ear lobule KFs. This difference in growth factor profile may be our first bit of data showing a regional difference between the 2 keloid groups. The increased rate of proliferation of preauricular compared with ear lobule KFs and the mitogenic role of this growth factor may explain the increased bFGF secretion.

Laser-irradiated preauricular KFs produced more bFGF when exposed to 3 W of laser energy compared with the control group. The ability of bFGF to repress the synthesis of type I collagen in fibroblasts has been known since the early 1980s. The work of Tan et al confirmed that bFGF down-regulates type I collagen production by KFs. The laser’s ability to increase the release of bFGF at 3 W in the preauricular KFs implies a possible protective and preventative role in the development of keloids during facial laser resurfacing. It also implies a role for the treatment of preauricular keloids. The concentration of TGF-β1 was decreased in laser-treated preauricular KFs when compared with that of non–laser-treated preauricular KFs. Prolonged or excessive TGF-β1 secretion in fibroblast cell culture has been postulated to contribute to the development of keloids. Transforming growth factor β1 selectively increases collagen production in keloid cell culture as opposed to normal fibroblasts in culture. Statistical significance was reached when preauricular KFs underwent laser irradiation with 3 W of CO₂ at a duty cycle of 25% and 50%.

The concentration of TGF-β1 was also decreased in ear lobule KFs when compared with that of non–laser-treated ear lobule KFs. Statistical significance was reached when ear lobule KFs underwent laser irradiation with 3 W of CO₂ at a duty cycle of 25%, and a definite trend was seen with 3 W of CO₂ at a duty cycle of 100%.

Skin resurfacing by means of the CO₂ laser for the treatment of facial elastosis has become a standard modality of treatment in clinical practice. The beneficial role of the CO₂ laser has been thought to result from thermal contraction of collagen leading to tightening of the dermal layer. The combined Er:YAG and CO₂ laser is a new-generation laser that has the capability of combining 2 laser modalities in precisely ablating thin layers of tissue with minimal thermal damage under a hemostatically controlled environment.

To our knowledge, this is the first study in the literature examining the effects of this laser on regional keloid variability in a serum-free in vitro model. The ability of this laser to treat facial elastosis safely without excessive scar formation is very significant. Our study demonstrates that this laser increases the release of bFGF, which has been shown to promote tightly organized collagen bundles, and decreases the concentration of TGF-β1, which has also been shown to promote fibrosis formation, in preauricular KFs.

This study demonstrates the following important key points: (1) The Er:YAG and CO₂ laser set at 3 W at a duty cycle of 25% or 50% may help prevent excessive scar formation during facial laser resurfacing. (2) Through its increase in bFGF and decrease in TGF-β1 secretion, the Er:YAG and CO₂ laser may provide a viable treatment modality for preauricular keloids. (3) Regional differences exist between preauricular and ear lobule keloids and are based on the growth factor profiles.

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REFERENCES


Correction

Misspelling of an Author's Surname. In the byline of the article titled “Reconstruction of Nasal Alar Defects” published in the April-June issue of the ARCHIVES (2001;3:91-99) the first author’s name should have read Brian P. Driscoll, MD. The journal regrets the error.