ORGINAL SCIENTIFIC PRESENTATION

Vaginal Fractional CO_2 Laser: A Minimally Invasive Option for Vaginal Rejuvenation

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Introduction: *To provide evidence that the use of a fractional CO_2 laser in combination with platelet-rich plasma (PRP) locally in the vagina mucous with minimal to moderate atrophy and pelvic floor exercise with perineometer would have effects of greater impact in the 3 layers of vaginal walls, including an important decrease of discomfort during sex.*

Materials and Methods: *The study was composed of 2 groups: a study group and a control group. The main consultation purposes were vaginal dryness, dyspareunia, and local irritation. The study group underwent PRP, CO_2 laser, and pelvic exercise, whereas only PRP and pelvic exercise were applied to the control group. We used a special vaginal scanner, which was able to fractionize the laser and could be inserted in the vagina. Both groups were evaluated with a sexual questionnaire and vaginal biopsies.*

Results: *An important improvement of vaginal mucous histology and a decrease of discomfort during sex were observed in most patients in the study group compared with the control group.*

Conclusion: *Through the local use of vaginal fractional CO_2 laser, PRP, and pelvic floor exercises in women with symptoms of vaginal atrophy, beneficial effects are exerted in the 3 layers of the vagina rather than only the epithelium, as achieved with estrogens. We also observed a significant decrease of discomfort during sex. More data will be needed to better address the use of this new procedure.*

The progressive decline in circulating estrogen levels in peripheral blood in women in their menopausal transition can cause different degrees of vaginal atrophy. This often leads to vaginal dryness, often causing painful or uncomfortable sexual intercourse.[1] A wide range of patients (15% to 45%) will show signs or symptoms associated with atrophic vulvovaginitis such as burning, pruritus, vaginal bleeding, or leucorrhoea, and approximately 25% of these patients will seek medical help.[2] While the use of estrogen in the treatment of atrophic vulvovaginitis is the treatment of choice,[3] we consider that the search for a nonhormonal option for this condition represents an important alternative to the known limitations of hormone replacement therapy.

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Objective

Our objective was to provide evidence that the use of a fractional CO_2 laser in combination with the local application of platelet-rich plasma (PRP) in the hypotrophic or atrophic vaginal mucosa, accompanied by pelvic floor exercise with a perineometer, would have beneficial effects of great impact in the layers of the vagina, including the epithelium, lamina propria, and muscularis. We noticed a marked decrease of discomfort during sex, unlike what happens with the use of local estrogen, which produces effects only on the epithelium of the mucous layer.

Materials and Methods

Between December 2009 and December 2010, a total of 92 patients with signs and/or symptoms of vaginal hypotrophy or atrophy (mild to moderate) were recruited. Patients with severe vaginal atrophy, as well as patients with stress urinary incontinence and damage in the rectovaginal fascia and/or fascia of Halban, were excluded from this protocol. The patients were evaluated and divided into 2 groups. One was called the study group (or "group of cases") and the other the control group. The study group consisted of 40 patients (12 in the premenopausal period and 28 in the postmenopausal period). These patients underwent

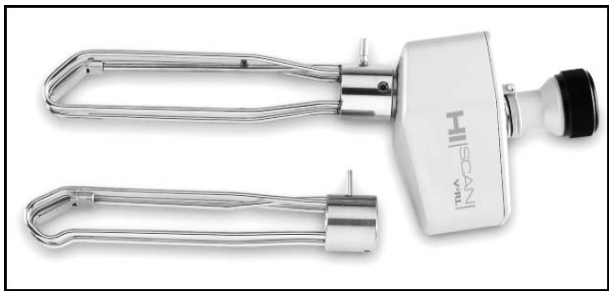

Figure 1. *Vaginal scanner for CO_2 laser.*

a vaginal fractional CO_2 laser application in each session, together with the application of PRP and pelvic floor exercise with a perineometer. In the control group, 52 patients were included (14 in the premenopausal period and 38 in the postmenopausal period), and only PRP and pelvic floor exercise with a perineometer were prescribed to them. In both groups, the main consultation purposes were vaginal dryness, dyspareunia, burning sensation, or local irritation. Before starting the protocol, all patients underwent a vaginal biopsy so as to determine the degree of vaginal hypotrophy or atrophy. In the study group, PRP was applied 14 days before the session with an intravaginal fractional CO_2 laser. After 30 days of the laser application, a vaginal biopsy was performed. This protocol was repeated a total of 3 times, with an interval of 14 days between each session. In the control group, PRP was applied every 60 days, on 3 occasions. For the application of PRP, 20 mL of peripheral venous blood was used, and it was placed in 8 citrated tubes of 2.5 mL. A Gelec G-142D centrifuge was used. To obtain the PRP, the sample was centrifuged at 1900 rpm for 9 minutes, with an average of 4 mL of PRP. The average platelet concentration of the samples was 1 100 000/mm^3, with a minimum of 700 000/mm^3 and a maximum of 1 500 000/mm^3. This count was done by a biochemical laboratory. The PRP was activated previous to its vaginal application, with 10% calcium chloride, using 0.05 mL of calcium chloride for every 1 mL of PRP. Once activated, the PRP was applied at the level of the vaginal walls as mesotherapy.

Tischler biopsy forceps were used for taking the vaginal biopsy, getting a sample of the union of the middle-third to lower-third of the anterior wall of the vagina, which was fixed in 10% buffered formol. For processing, the sample was paraffin embedded, cut into 5-μm sections, and stained with hematoxylin-eosin, Gomori, and Mason trichromic.

In both groups, patients were prescribed perinean exercise using perineometry technique for the strengthening of the pelvic floor (levator ani muscle and endopelvic fascia). We used a perineometer ExTT-101, which consists of a program with 3 increasing phases of training. The exercises of the pelvic floor muscles were indicated in the first month, 4 times a week; in the second month, 3 times a week; in the third month, only 2 sessions per week; and, finally, in the fourth and last month, only 1 session per week. Each session lasted about 25 minutes.

For the vaginal application of the CO_2 laser, we used equipment from the Italian company DEKA with a special intravaginal scanner (Figure 1) able to fractionize the laser light and to reduce the initial pulse (D-Pulse), minimizing the epithelial vaporization while maintaining the thermal effect (Figure 2) that stimulates collagenesis, angiogenesis, and cellularity of the mucous layer of the vagina (Figure 3).

The parameters within which the laser was applied varied from 20 to 25 W. The difference in values in the application (dwell time and spacing) depended mainly on the condition of the mucous layer prior to the use of the laser, the woman's hormonal state (premenopause or menopause), and whether it was the first or subsequent session.

To evaluate the result of the protocol, we used a sexual health questionnaire at the beginning and end of the treatment. Each sign or symptom was rated as mild, moderate, or severe. Clinical improvement was considered to be a change from severe to moderate, moderate to mild, or mild to normal.

Results

We evaluated the results, not only through the sexual health questionnaire but also through a vaginal biopsy performed 30 days after the last laser session in the study group and 30 days after the last session of PRP in the control group. A significant decrease of discomfort during sex was observed in the study group compared with the control group. This improvement was mainly due to a decrease in vaginal dryness during sexual intercourse and therefore a reduction in pain during the activity. Based on a sexual health questionnaire, after 3 treatment protocols in the study group, we observed the following improvements: 67.5% (27/40) in vaginal dryness, 62.5% (25/40) in dyspareunia, and 50% (20/40) in irritation sensation or local burning. In the control group, we could observe an improvement of 23% (12/52) in vaginal dryness, 15.4% (8/52) in dyspareunia, and 19.2% (10/52) in the sense of local irritation (Figure 4).

The treatment abandonment rate in the study group was 5% (2/40) due to an absence of clinical results after

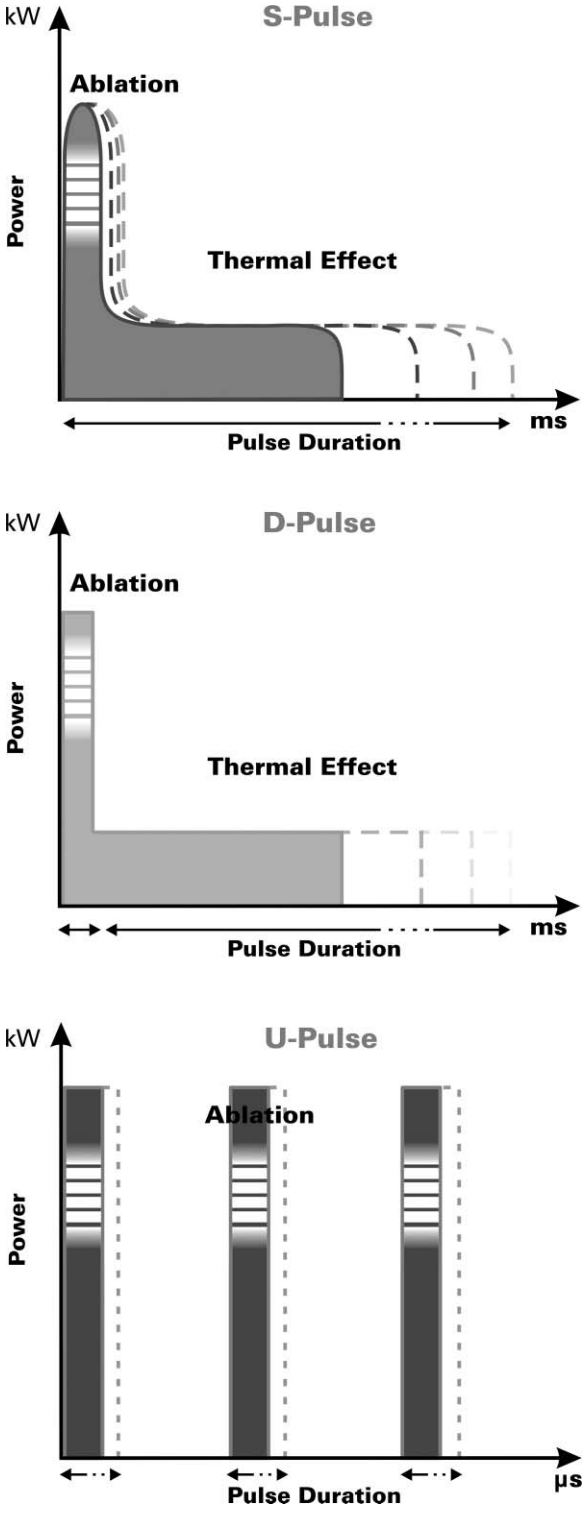

Figure 2. *S-Pulse is the skin pulse used for skin rejuvenation where we need more initial ablation to properly reach the connective tissue below the epithelium, because the skin has more layers than the vagina; lasers for skin rejuvenation have more initial ablation but the same thermal effect. D-Pulse is a diminished initial pulse having less initial ablation but keeps the thermal effect that will produce the rejuvenation of the target (connective tissue bellow in the vaginal mucosa). U-Pulse is the ultra-pulse used for surgical insitions without thermal effect, meaning without rejuvenation.*

performing 2 treatment protocols. In the control group, the abandonment rate was 16% (8/50) because of the absence of clinical results after the patients underwent 1 protocol (3/8) or 2 protocols (5/8), respectively.

Regarding the complications observed with the use of the described technique, 6 cases of minimal vaginal bleeding after the local application of PRP, due to the Nappage technique, were observed. After the use of the vaginal scanner, almost 30% of patients reported mild discomfort, such as pain or burning sensation, at the time of the application, even within 72 hours. This discomfort was alleviated with the local application of diclofenac gel once a day, for up to 5 days. None of the complications, such as bleeding, pain, or burning, required the administration of oral medication.

Concerning the histological changes in the study group, in a comparison between the biopsy performed at the beginning and a biopsy at the end of the treatment (time period between 160 and 180 days), a significant increase was observed in the fibrillar component of the extracellular matrix and fibroblast activity bins while stressing the marked neoangiogenesis observed. Similarly, the thickness of the vaginal epithelium and its glycogenic load increased significantly after laser treatment (Figure 5), in contrast to the discrete changes obtained in the control group (Figure 6).

We note that these histological findings, obtained in both the laser and the control group, kept a clinical correlation, with a more marked clinical improvement in the laser group (Figure 5).

Discussion

The aging of the vagina includes changes that are widely studied, affecting not only the epithelium but also all the layers of the vaginal wall. The thickness of the vaginal epithelium and its glycogenic charge are significantly reduced after menopause.[4] At the level of the lamina propria, there is a decrease in the number and activity of blasts and all the extracellular matrix components, with a marked decrease in vascularization and water retention capacity.[5] These changes at the epithelial and connective tissue level are similar to those seen in the epidermis and dermis, but they are more noticeable in the vagina because this tissue is

Figure 3. *The use of the new vaginal scanner in the anterior and posterior vaginal walls.*

much more sensitive to estrogen.[6] The functions of this mucosa (absorption, secretion, protection, and response to stimuli) depend on the function and integrity of its 3 layers: mucosa (including epithelium and lamina propria), muscularis, and adventitia. There is also a progressive relaxation of the pelvic floor in variable degrees, with a weakening of the vaginal walls and endopelvic fascia.[7]

Role of Fractional CO_2 Laser in Vaginal Rejuvenation

In recent years, enough evidence has been provided about what the significant beneficial effects the CO_2 laser has at the level of the connective tissue. We rely on tissue remodeling by cytokine in response to the injury caused by the laser thermal effect.[8] We used this technique to improve tissue conditions at the level of vaginal lamina propria caused by aging. As this is an epithelium lacking stratum corneum, we used a scan with lower initial pulse to reduce epithelial vaporization caused by each column of the laser light (D-Pulse), keeping the thermal effect at the level of the underlying connective tissue in the mucous layer. This is known as the bottle effect. After the application of the fractional CO_2 laser on the surface of the vaginal walls, the process of repairing the injury starts, and it is mediated by the local increase of basic

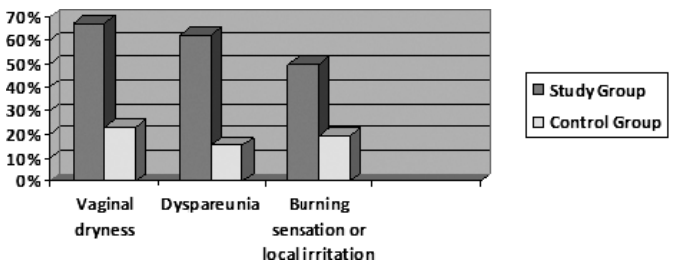

Figure 4. *Improvements in consultation purposes after completing the protocols in the study and control groups.*

fibroblast growth factor and the decrease of transforming growth factor beta 1. Based on studies of animal samples, the outcome of this process will be an increase in the amount of collagen, blasts, and lymphocytes in the connective tissue as well as neoangiogenesis (Figure 7).

It has been hypothesized that during wound healing of normal tissues, the increase and activation of the basic fibroblast growth factor is responsible for the neoangiogenesis effect. The mechanisms by which it does this are poorly defined. Nevertheless, it has been demonstrated to induce fibroblast activation with increased production of collagen, elastin, glycosaminoglycans, and adhesive glycoproteins.[9] In the same way and mediated by cytokines, there is a cell recruitment and activation of neutrophils and lymphocytes.

Transforming growth factor beta 1 is a cytokine that, among other functions, is a mediator of inflammation and wound healing. It is produced by lymphocytes, monocytes, macrophages, and dendritic cells. It participates in the chemotactic attraction of inflammatory cells and the activation and suppression of them, depending on the local concentration of this cytokine and the degree of differentiation from target cells.[10] Transforming growth factor beta 1 is also a cytokine apoptosis-inducing factor and regulates the synthesis of extracellular matrix components. Finally, the fractional CO_2 laser induces cell replication, and, at the same time, it regulates collagenesis, preventing the appearance of any excessive fibrosis.[11]

Platelet-Rich Plasma

As water is the CO_2 laser chromophore, and as we are dealing with a target (vaginal mucous layer) in a variable state of dehydration, we use the PRP to improve the local conditions of the stratified vaginal

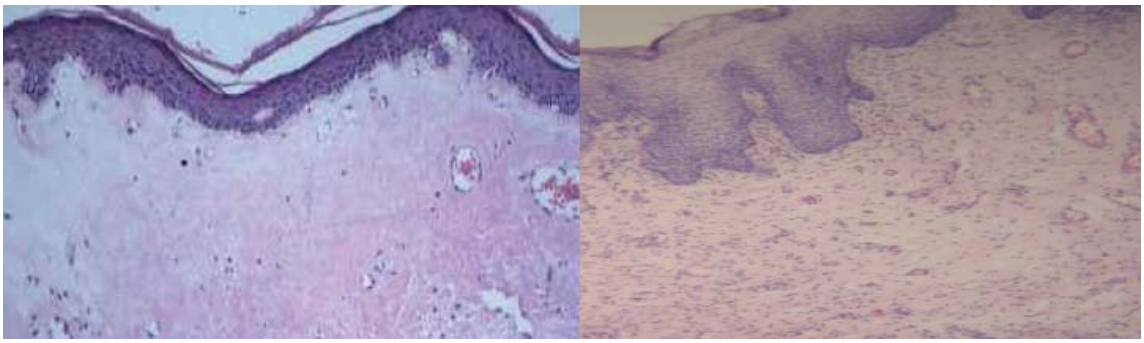

Figure 5. *Vaginal atrophy. Before and after the laser/platelet-rich plasma treatment (study group).*

epithelium. The platelets are activated and applied in the vaginal wall, releasing platelet alpha granules, which are responsible for epithelial cell replication. In addition, growth factors derived from platelets are platelet-derived growth factor with mitogenic fibroblast action and insulin-like growth factor, responsible for the synthesis of proteoglycans and collagen while inducing fibroblast proliferation. Finally, basic fibroblast growth factor exhibits a significant angiogenic and inductor effect of fibroblast proliferation,[12] as discussed earlier.

Perineometry

Considering that most reports show a vaginal prolapse rate of 11%; that there is also a postoperative recurrence rate after 4 years of approximately 30%[13]; that with aging the perineal muscle tone decreases, especially after the onset of menopause; and the importance of the strength of the vaginal walls in a young vagina, a training program for pelvic floor muscle toning (levator ani muscle) was registered through the use of perineometric software. The Kegel exercise strengthens the pelvic floor muscles.[14] These exercises are proven to ultimately build up and increase vaginal contraction force. Thus, this contributes to sexual enhancement and the facilitation of orgasms.[15,16] As vaginal tightness improves, erectile strength for males can improve as well. In general, Kegel exercises are performed alone by oneself, so in many cases, the exercises are not properly performed.[17] Therefore, success rates can be low, and on many occasions, people stop the exercise altogether and give up. To counteract this problem, we used the ExTT-101 Perineometer Biofeedback Kegel Exerciser, which allows a woman to diagnostically measure her own vaginal contractions and pressure. Furthermore, the device allows her to monitor her own progress and success rates. There was an improvement that showed an increase in resting muscle tone, fast maximum contraction, maximal contraction maintained, and maximal contraction after a series of 10 contractions. In our study, we observed at the beginning of the research that nearly 20% (19.2%) of patients had values measured in mm Hg below normal.

Conclusion

Through the use of local vaginal fractional CO_2 laser and PRP (applied every 60 days in 3 sessions), in association with pelvic floor exercises in perimenopausal women with minimal to moderate symptoms of vaginal atrophy, beneficial effects were observed in

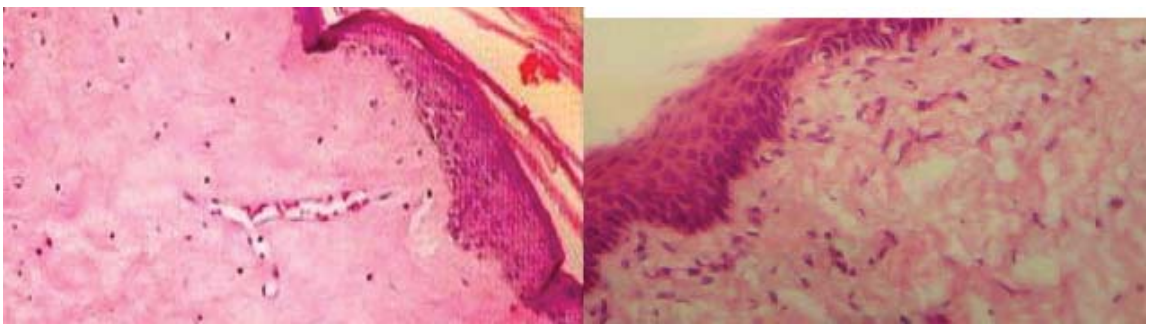

Figure 6. *Vaginal atrophy. Before and after the platelet-rich plasma treatment (control group).*

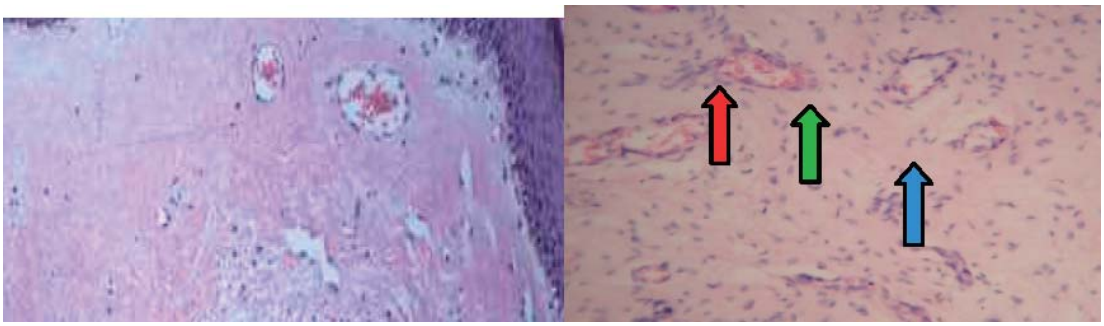

Figure 7. *The vaginal lamina propria. Before and after CO_2 laser. Increase of collagen (blue arrow), blasts (red arrow), and neovessels (green arrow).*

the 3 layers of the vagina in addition to a marked decrease of discomfort during sex. Although these findings suggest the possibility of using a new nonhormonal treatment for the prevention of urovaginal atrophy and for achieving benefits in the quality of life, more data will be needed to better address the use of this new procedure.

Acknowledgments

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