Research Article

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The Repair Process of the Uterine Cervix Subjected to CO$_2$ Laser Vaporization: A Pilot Study

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Abstract

Objective: Cervical intraepithelial neoplasia (CIN) frequently affects young women in childbearing age. Present literature does not provide scientific consensus on the real incidence of obstetric complications after the various conservative techniques performed. Most of all literature data are still lacking a pathophysiological basis to explain the different impact of various treatment techniques on the functional integrity of the uterine cervix. The gold standard conservative treatment is the excisional therapy. This study tried to analyze the evolution of tissue repair of cervix subjected to CO$_2$ laser conization for high grade CIN with the aim of identifying eventual differences between the repaired tissue and the original cervical one.

Study Design: The analysis was conducted on histological seriated samples of uterine cervix obtained at standardized times after conization treatments and then analyzed using histological examination and immunohistochemical staining.

Results: This study showed that the tissue concentration of TGFβ1 was significantly low in patients treated with CO$_2$ laser, confirming the reduced inflammatory reaction after laser treatment. Another very interesting result was the high ratio of type III and type I collagen: the prevalence of type III collagen suggested a regeneration of tissue without scarring after laser therapy.

Conclusion: Tissue repair process after CO$_2$ laser cervical treatment is able to compensate the loss of substance in damaged area and at the same time to limit the deposition of disorganized connective matrix, typical of scars, leading to a morphologic and functional restitutio ad integrum of the treated cervical tissue, which tends to be similar to the original one.

Keywords: Laser CO$_2$; Cervical Intraepithelial Neoplasia; Repair Process

Condensation

Tissue repair process after CO$_2$ laser cervical treatment is able to compensate the loss of substance in damaged area and at the same time to limit the deposition of disorganized connective matrix, typical of scars, leading to a restitutio ad integrum of the treated cervical tissue, which tends to be similar to the original one.

Introduction

Patients with cervical intraepithelial disease are mostly in childbearing age. Preventive treatment of non-invasive lesions in young patients requires minimally invasive procedures with few adverse effects on female fertility and on future obstetric outcome [1-2]. The gold standard treatment for high grade cervical intraepithelial neoplasia (CIN II/III) is excisional therapy, such as LEEP (loop electrosurgical excision procedure), cold knife conization and CO2 laser conization [1]. Observational studies described excisional methods as at risk of dysfunctional repair of the cervix with a high risk of obstetric complications, such as preterm delivery caused by cervical incontinence. However, conclusions

are often not unique. Recent meta-analysis showed only a modest increased obstetric risk in women undergoing CO$_2$ laser conization. This suggests an influence of the treatment technique on the regeneration and functionality of the treated cervical tissue [2-4]. Studies on repair process of different tissues treated with CO$_2$ laser showed that this procedure is associated with less bleeding and post-operative pain than other techniques, due to the hemostatic effect of the laser and to the reduced tissue edema [5].

Furthermore, histological analysis of tissue samples underlined a less capillary neoangiogenesis, a re-epithelialization of the wound and a reduced early deposition of collagen. These elements would be at the base of a reduced rate of scarring and of a better healing process with an organic and functional restitutio ad integrum [6-7]. To authors' knowledge, no studies on the repair process of the uterine cervix affected by high grade CIN and treated with CO$_2$ laser excision are present in literature [8]. The aim of this study is to describe the characteristics of the cervical tissue damage produced by CO$_2$ laser on women of childbearing age treated for pre-invasive

cervical lesions and to follow the time evolution of reparative process.

Materials and Methods

The study was conducted at the Laser Therapy Center of the Maternal and Child Department, Careggi University Hospital, Florence. The inclusion criteria were: CIN II/III [9]; age under 40 years; no previous cervical treatment; no chronic disease; no smoking. Patients had to accept to undergo cervical biopsy on the treated area at standardized times after conization. All patients signed informed consent to the study. The conization treatments were performed in an outpatient setting under local anesthesia using CO_2 laser model SmartXide 50HS (DEKA MELA SRL, Italy), connected to a colposcope ZEISS OPMI (Carl Zeiss,Oberkochen, Germany).The beam spot diameter ranged from 0.5 to 1 mm with an irradiance ranging from 2500 to 3500W/cm, guided by a micromanipulator. After delineating the area of abnormality with Lugol's iodine, local anesthesia was administered performing at the 3, 6, 9 and 12 o'clock site of the cervix injections of 3.6-5.4mL of 2% lidocaine mixed with epinephrine 1:100,000. The first step of procedure was to direct the laser beam perpendicularly to the cervical surface achieving an initial 0.5-1cm deep circular section and then to guide it obliquely by manipulating the on-going excised specimen using a small steel hook. To assure the complete clearance of the lesion, after the excision the crater base and the walls were vaporized with defocused laser beam.

With these settings, we ensured an emission with high peak power and high energy density to induce the phenomenon of photoablation with rapid destruction of the tissue and reduced propagation of thermal damage to surrounding tissue. At the end of the treatment, we executed a sample of tissue at 12 o'clock in the treated cervical area using punch biopsy forceps. Subsequent specimens were performed 2, 7 and 14 days after surgery at 3, 6 and 9 o'clock of the treated area, respectively, in order to take samples of tissue from areas not affected by previous biopsies. A further specimen was then collected at the first follow-up visit 3 months after treatment. All specimens were sent to the laboratory and prepared for histological evaluation with light microscopy. In order to describe the characteristics of the cervical tissue damage, standard histological analysis and immunohistochemical research of inflammatory markers, neoangiogenesis and tissue regeneration were conducted. Samples providing a good histological readability in terms of stratification of the tissue and representation of its various components were considered eligible to immunohistochemical analysis.

Immuno Histochemical Evaluation on Tissue Samples Focused on

a)   IL-1β - Inflammatory cytokine (No. Catalogue: MAB 201, Clone: 8516. R & D Systems. Minneapolis, USA);

b)   TGFß1 - Inflammatory cytokine with chemotactic action for fibroblasts and angiogenesis (No. Catalogue: MAB 240; Clone: 9016. R & D Systems. Minneapolis, USA);

c)   Factor VIII Related - A marker for endothelial cells (No. Catalogue: CP 039. Bio Care Medical. Concord, USA); Type I

collagen - a protein with a structural function (No. Catalogue: 2150-0030. Abd Sero tec. Kidlington, UK);

d)   Type III collagen - A protein with a structural function (No. Catalogue: nbpi-67528. Novus Biologicals. Littleton, USA).

Tissue samples were collected and fixed in 4% buffered formalin; specimens were oriented, reduced and embedded in paraffin. Three microns thick sections were obtained by microtome. For each specimen, some sections were subjected to standard staining with hematoxylin-eosin for histological evaluation, while others were used for immunohistochemistry. Immunohistochemistry was prepared by immune enzymatic technique with indirect method with immune complexes using peroxidase as tracer, the Avidin-Biotin complex (ABC) as immune complex and the DAB (3,3'-diamino- benzidine) as chromogen. All sections were affixed on polarized glass slides, deparaffinized in xylene and then rehydrated through a descending scale of alcohol until deionized water.

Slides were passed to the microwave in citrate solution for unmasking antigens, then black incubated in hydrogen peroxide for inhibition of the endogenous peroxidases. After this first phase, goat serum was used to eliminate any effect of non-specific binding (background) antibodies, before being incubated with the primary antibody. A similar passage was performed again with the normal serum, before proceeding to incubation with the secondary antibody. In the last phase of the procedure the immune complex (ABC) and subsequently the solution DAB was added. After adding chromogen, the sections were observed under a microscope and then fixed by soaking in deionized water at the onset of the color reaction. To complete the preparation, the sections were counterstained with hematoxylin, dehydrated, passed in xylol and finally covered with a coverslip mounted. The observation of samples was performed using an optical microscope Nikon Eclipse 80th (Nikon, Shinjuku, Japan).

Results

During three months, seven patients were recruited, aged 24-37 years, who met the inclusion criteria and accepted to undergo the study schedule. The indications for treatment were CIN II (six), and CIN III (one). Four patients were able to complete all 5 specimens according to the study protocol, two patients refused to continue the study. One patient was excluded because follow-up colposcopy showed a CIN I confirmed by biopsy and underwent laser ablation. Cyto-colposcopic monitoring at three months after treatment was negative in four subjects providing a cure rate of 80%. A total of 27 specimens were collected, 7 of which at t0 (time of treatment), 6 at t1 (48 hours after treatment), 5 at t2 (one week after treatment), 5 at t3 (two weeks postoperatively) and 4 at t4 (three months after treatment) (Table 1). All specimens were evaluated by light microscopy; immunohistochemistry analysis was performed, and 15 samples were processed: 4 at t0, 3 at t1, 3 at t2, 3 at t3 and 2 at t4 (Table 1). The histological evaluation was performed on a descriptive basis caring of the presence or absence of carbonized areas, vacuolar degeneration of superficial epithelium, grading of cell maturation and stratification, inflammatory infiltration, neoangiogenesis and new gland formation.

Table 1: Enrolled patients.

	DIAGNOSIS	t_0	t_1	t_2	t_3	t_4	FOLLOW-UP
Case 1	CIN II	X	X	X	X	X	Negative
Case 2	CIN II	X	X	X	X	X	Negative
Case 3	CIN II	X	NP	NP	NP	NP	
Case 4	CIN II	X	X	X	X	NP	CIN I
Case 5	CIN II	X	X	X	X	X	Negative
Case 6	CIN II	X	X	NP	NP	NP	
Case 7	CIN III	X	X	X	X	X	Negative

t_0- Specimens taken at the time of treatment

t_1 - Specimens taken 2 days after treatment

t_2 - Specimens taken 7 days after treatment

t_3 - Specimens taken 14 days after treatment

t_4- Specimens taken during follow-up visit 3 months after treatment

X - Specimens analyzed in IHC

NP - Sampling not performed

The immunohistochemical evaluation took into consideration TGFβ1, Factor VIII Related (FVIIIR) and Collagen type I and III. All 15 samples analyzed both histologically and immunohistochemically displayed homogeneous aspects within each group (t0, t1, t2, t3, t4) for the considered markers (Table 1). At t0, the histological analysis showed only traces of carbonization on luminal side with adjacent area of vacuolar degeneration of cells, phenomena which are produced by laser-treatment. No structural alterations of the underlying tissue were found. At t0 the immunostaining was negative for TGFß1 and FVIIIR. Only a weak positivity of type I and type III collagen was seen on remaining native basal membranes. At t1 the thermal damage became evident; the carbonized area was mostly substituted by homogeneous coagulated and poorly cellular tissue with peripherical inflammatory infiltration. The limit between the two layers resulted positive to FVIIIR immunostaining, while TGFß1 was still absent. Type I collagen was completely absent while type III collagen resulted positive in the coagulated area linking denatured fibers.

At t2 a newly formed reparative tissue substituted the coagulated area; this tissue was characterized by a weak positivity to TGFß1 and by neoangiogenesis, glandular genesis and epithelial regeneration. An intense positivity to type I collagen was evident at the intermediate layer of epithelium and to type III at the basal one. FVIIIR was only present in the stromal vessels. Reduced inflammatory infiltration was noticed. At t3 no angiogenesis and inflammatory infiltration were noticed; neoangiogenesis and gland regeneration were completed, the epithelia had an increased stratification and maturation, although large and nucleated cells still remained. Presence of collagen types I and III was similar to t2. Positivity to TGFß1 and FVIIIR was no longer visible. At t4 the reparative stage was almost entirely completed even if small areas of high cellularity persisted. TGFß1 and FVIIIR were negative. The analysis of type I collagen showed a fibrillar organization at the polarizer inspection, while type III collagen antibodies were widely bound to connective tissue, highlighting a fibrillar structure.

Comment

When a tissue damage is caused for therapeutic purposes, especially in anatomical regions in which the functional integrity is important, such as uterine cervix, the clinical interest is to shift the equilibrium of reparative process to the regeneration of a tissue with mechanical and functional characteristics similar to the original one. It is known that, in the prenatal period, the tissues are able to repair without scarring, therefore able to regenerate [10]. Fetal wounds heal rapidly and without the scarring and inflammation that characterize adult wounds. The prenatal wound healing process is faster and more efficient than adult repair and produces new tissue rather than scar. However, the difference between fetal and adult repair isn't completely explained [10-12]. An important difference between embryonic and adult reparation process is highlighted by the concentration of TGFβ and its isoforms: in embryo low levels of TGFβ1 and 2 and very high levels of TGFβ3 are present.

TGFβ3 stimulates fibroblast migration and directs their orientation as to allow the correct position of connective matrix deposited by fibroblasts, ensuring a regeneration of tissue similar to the original one. Conversely, in adult damaged tissue, the degranulation of platelets, monocytes and macrophages releases large amounts of TGFβ1 and 2, while the liberation of TGFβ3 by keratinocytes and fibroblasts is poor. In the presence of a tissue damage, the adult tissue responds with a high inflammatory response, promoted by pro-inflammatory cytokines, including TGFβ1 and 2. They lead to the deposition of granulation tissue, which fills the loss of substance, but prevents the proper migration of fibroblasts from the edges of damaged areas. The low concentration of TGFβ3 does not support the interweaving of proper collagen fibers, which are arranged predominantly in parallel with each other, without ensuring adequate mechanical strength. Trying to compensate the reduced tensile strength, fibroblasts accumulate fibers giving rise to a fibrous scar that becomes prominent in time [13].

Another important element in repair tissue is type III collagen. It's responsible for maintaining the structural integrity of the fabric and it's also considered able to adjust the diameter of collagen fibers during reparative phase and to modulate the transformation of fibroblasts into myofibroblasts, responsible for contraction of wounds. The important role of this element in tissue regeneration without scarring is supported by the observation of increased expression of type III collagen in fetal tissue repair, with high ratio of type III/I collagen. From these considerations, it's evident that the method to generate a tissue damage for therapeutic purposes should consider functional needs of the tissue itself. Treatment modalities should try to stimulate a regenerative process in the damaged tissue characterized by high growth and tissue differentiation, poor inflammatory response and poor angiogenesis, in order to reconstitute a tissue as similar as possible to the original one. According to some studies, even if conducted on tissues other than the uterine cervix, the laser would be capable of inducing a repair process that tends to have these characteristics [14-15].

The carbonized area which represents the loss of tissue induced by the laser on the margins of the treated area is as small as to be fulfilled in term of a few days (t0-t1). The low positive response to TGFβ1 in all samples suggests a reduced pattern of inflammatory cytokines, that would be desirable in the context of a tissue regeneration with poor scarring. The low release of TGFβ1 after the lesion induced by CO_2 laser, compared to other techniques, has been described in literature by Manolis et al. [16]. This trial showed that the tissue concentration of TGFβ1 was significantly lower in patients treated with CO_2 laser compared to traditional surgery, confirming the reduced inflammatory reaction after laser treatment. The FVIIIR was used as an indirect element for the evaluation of inflammatory stimulus.

In fact, in our study angiogenesis, marked by FVIIIR, is stimulated both by initial vascular damage and by inflammatory cytokines including the TGFβ. In our samples, the FVIIIR presence is mainly evident 48 hours after treatment: it's localized diffusely throughout the tissue and decreases progressively to be localized exclusively in regenerated vessels at t3 and t4. This allows us to deduce that the poor neoangiogenic stimulus present in laser CO_2 damaged cervix is supported by TGFβ1. In regard to the search of type I and type III collagen, there is a trend similar to that proposed by other trials, with a progressive increase of their positivity in 7-10 days after treatment [6]. A very interesting result in our case, however, is the high ratio of type III and type I collagen observed at t4. Thus, the prevalence of type III collagen suggests a regeneration of tissue without scarring after laser therapy.

Conclusion

These initial data represent an interesting basis for the comprehension of the reparative process after CO_2 laser damage on the uterine cervix performed for therapeutic purposes. This is of utmost importance to understand how to tailor excisional therapy in order to prevent functional damage. On the basis of our results, in agreement with some studies conducted on tissues other than cervix, tissue repair after CO_2 laser seems to be more similar to tissue regeneration typical of prenatal period compared with other kinds of treatment. The immunohistochemical analysis on the collected samples showed a low concentration of TGFβ1, a pro-inflammatory cytokine which is responsible for a high inflammatory response leading to the deposition of granulation tissue and the developing of scar tissue. Another important characteristic was the high rate of collagen type III/I; type III collagen is responsible for maintaining the structural integrity of the tissue and is able to adjust the diameter of collagen fibers during reparative phase, promoting a regeneration of tissue without scarring. These features show that tissue repair process after CO_2 laser treatment is able to compensate the loss of substance in damaged area and at the same time to limit the deposition of disorganized connective matrix, typical of scars, leading to a restitutio ad integrum of the treated cervical tissue, which tends to be similar to the original one.

References

1. Martin-Hirsch PP, Paraskevaidis E, Bryant A, Dickinson HO, Keep SL (2010) Surgery for cervical intraepithelial neoplasia. Cochrane Database Syst Rev p. 6.

2. Kyrgiou M, Koliopoulos G, Martin-Hirsch P, Arbyn M, Prendiville W, et al. (2006) Obstetric outcomes after conservative treatment for intraepithelial or early invasive cervical lesions: systematic review and meta-analysis. Lancet 367(9509): 489-498.

3. Arbyn M, Kyrgiou M, Simoens C, Raifu AO, Koliopoulos G, et al. (2008) Perinatal mortality and other severe adverse pregnancy outcomes associated with treatment of cervical intraepithelial neoplasia: meta-analysis. BMJ 337: a1284.

4. Nøhr B, Tabor A, Frederiksen K, Kjaer SK (2007) Loop electrosurgical excision of the cervix and the subsequent risk of preterm delivery. Acta Obstet Gynecol Scand 86(5): 596-603.

5. Liboon J, Funkhouser W, Terris DJ (1997) Acomparison of mucosal incisions made by scalpel, CO_2 laser, electrocautery, and constant-voltage electrocautery. Otolaryngol Head Neck Surg 116(3): 379-385.

6. Orringer JS, Kang S, Johnson TM, Karimipour DJ, Hamilton T, et al. (2004) Connective tissue remodeling induced by carbon dioxide laser resurfacing of photodamaged human skin. Arch Dermatol 140(11): 1326-1332.

7. Cercadillo-Ibarguren I, España-Tost A, Arnabat-Domínguez J, Valmaseda-Castellón E, et al. (2010) Histologic evaluation of thermal damage produced on soft tissues by CO_2, Er, Cr: YSGG and diode lasers. Med Oral Patol Oral Cir Bucal 15(6): e912-918.

8. Paraskevaidis E, Kitchener HC, Malamou-Mitsi V, Agnanti N, Lolis D (1994) Thermal tissue damage following laser and large loop conization of the cervix. Obstet Gynecol 84(5): 752-754.

9. Massad LS, Einstein MH, Huh WK, Katki HA, Kinney WK, et al. (2013) 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. Obstet Gynecol; 121(4): 829-846.

10. Adzick NS, Lorenz HP (1994) Cells, matrix, growth factors, and the surgeon. The biology of scarless fetal wound repair. Ann Surg 220(1): 10-18.

11. Ferguson MW, O Kane S (2004) Scar-freehealing: from embryonic mechanisms to adult therapeutic intervention. Philos Trans R Soc Lond B Biol Sci 359(1445): 839-850.

12. Occleston NL, Metcalfe AD, Boanas A, Burgoyne NJ, et al. (2010) Therapeutic improvement of scarring: mechanisms of scarless and scar-forming healing and approaches to the discovery of new treatments. Dermatol Res Pract.

13. Volk SW, Wang Y, Mauldin EA, Liechty KW, Adams SL (2011) Diminished type III collagen promotes myofibroblast differentiation and increases scar deposition in cutaneous wound healing. Cells Tissues Organs 194(1): 25-37.

14. Grbavac RA, Veeck EB, Bernard JP, Ramalho LM, Pinheiro AL (2006) Effects of laser therapy in CO_2 laser wounds in rats. Photomed Laser Surg 24(3): 389-396.

15. Capon A, Mordon S (2003) Can thermal lasers promote skin wound healing? Am J Clin Dermatol 4(1): 1-12.

16. Manolis EN, Kaklamanos IG, Spanakis N, Filippou DK, Panagiotaropoulos T, et al. (2007) Tissue concentration of transforming growth factor beta1 and basic fibroblast growth factor in skin wounds created with a CO_2 laser and scalpel: a comparative experimental study, using an animal model of skin resurfacing. Wound RepaiRegen 15(2): 252-257.

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