Carboxytherapy and Platelet Rich Plasma: A New Therapy for Trigonitis, Abacterial and Interstitial Cystitis

Muzi Fabrizio, Delicato Giampaolo, D'Andria Daniele, Baffigo Giulio, Tartaglia Edoardo, Tati Eleonora, Corvese Francesco, Signore Stefano, Perla Alessandro, Montagna Giuseppe and Tati Gaetano

Department of Urology and Oncologic Urology, S. Eugenio Hospital, Rome, 00144, Rome, Italy

Abstract: Cystitis often appears even in absence of bacteria colonization. Trigonitis and interstitial inflammation are the most common morphological features of abacterial cystitis in young and post menopausal women. Arterial obstructive disease and bladder ischemia might play an important role in bladder dysfunction. Activated inflammatory cells produce ROS (radicals of oxygen), NF kB seems involved in ROS synthesis. Clinical studies have indicated that high CO_2 levels can impact upon peripheral tissue, reducing ischaemia, responsible of recurrent inflammation and consequently reducing oxydative phenomena. PRP (platelet-rich plasma) is a volume of fractionated plasma from the patient's own blood that contains platelet concentrate rich of alpha granules. PRP interacts tissue repair mechanisms by placing supra-physiological concentrations of autologous platelets at the site of tissue damage. This study proposes a single PRP transvaginal injection followed by 10 weekly applications of carboxytherapy, using subcutaneous injections of sterile CO2 gas. We have selected 6 Women (50-75 years), affected by recurrent abacterial cystitis with Pain and urge incontinence. All patients showed a subjective sensible reduction of symptoms. After 2 months all patients have neither inflammatory symptoms nor endoscopic evidence of trigonitis. Preliminary qualitative results could encourage the use of carboxytherapy and PRP in treatment of abacterial and interstitial cystitis.

Key words: Carboxytherapy, platelet rich plasma, cystitis, trigonitis, interstitial cystitis.

1. Introduction

UTIs (urinary tract infections) are common in females, and cystitis (bladder infection) represents the majority of these infections. Trigonitis, despite its name suggestive of inflammation, is a metaplastic process. Although the precise underlying cause is not known, squamous metaplasia in the bladder usually occurs in response to an irritative (eg, chronic indwelling catheter) or infectious process. UTIs in women are very common; approximately 25-40% of women in the United States aged 20-40 years have had a UTI. UTIs account for over 6 million patient visits to physicians per year in the United States. Approximately 20% of those visits are to EDs.

General symptoms of cystitis include urgency, frequency, dysuria, and, occasionally, hematuria,

dyspareunia, abdominal cramps, and/or bladder pain and spasms. Establishing or excluding a specific diagnosis often requires repeated cultures and various urologic procedures, including cystoscopy with bladder biopsies, various bladder tests, and immune system function examinations.

Nonbacterial cystitis is a catchall term that encompasses various medical disorders, including infectious and noninfectious cystitis, as well as PBS/IC (painful bladder syndrome/interstitial cystitis). PBS/IC describes a syndrome of pain and genitourinary symptoms (eg, frequency, urgency, pain, dysuria, nocturia) for which no etiology can be found.

Interstitial cystitis is a chronic condition most often with a variable course characterized by intermittent periods of exacerbations and remissions; however, rarely, there is chronic progression to a small-capacity bladder with resultant severe lower urinary tract symptoms and risk of upper tract deterioration.

Corresponding author: Fabrizio Muzi, M.D., specialist in general surgery and andrology, consultant, research fields: urologic, physiology and pathology. E-mail: fabrizio.muzi@yahoo.it.

Unfortunately, the disorder responds poorly to treatment in many cases.

2. Materials and Methods

We have selected 6 women (50-75 years), 4 patients affected by recurrent abacterial cystitis and 2 patients affected by interstitial cystitis. All patients have been submitted to cystoscopy before treatment, with evidence of trigonitis. All patients showed very recurrent pain and urge incontinence as symptoms.

We have submitted all patients to one single transvaginal shot injection of PRP (platelet rich plasma).

PRP has been prepared by centrifugation of 4 samples of patient's blood 2500 rpm, 780 RCF for 7-9 minutes. We have selected only PRP fraction nearly by buffy coat fraction. After having summoned up the 4 fractions, we have injected the dose in upper vestibulus of vagina with 30 gauge needle. We have not used any activator.

Consequently, all patients have been submitted to 10 applications of carboxytherapy once a week, with sovrapubic dose and time controlled injection of 900 cc of sterile medical CO_2. We have used a certified medical device for carboxytherapy, usually employed in aesthetic medicine with a velocity of 30-40 cc/min

3. Results and Discussion

After the entire treatment, all patients showed a subjective sensible reduction of pain and urgence symptoms. No patient had adverse effects. No patients referred problems neither during or after the single application of carboxytherapy nor after the PRP injection. All patients refer a subjective improvement of urinary flux. After 2 months all patients have neither inflammatory symptoms nor endoscopic evidence of trigonitis.

UTIs (urinary tract infections) are common in females, and cystitis represents the majority of these infections. Very ill patients may be referred to as having urosepsis.

A presumptive diagnosis of uncomplicated cystitis can be made on the basis of findings on the history and physical examination, along with urinalysis.

The classic symptoms of UTI (urinary tract infection) in the adult are primarily dysuria with accompanying urinary urgency and frequency. A sensation of bladder fullness or lower abdominal discomfort is often present. Bloody urine is reported in as many as 10% of cases of UTI in otherwise healthy women; this condition is called hemorrhagic cystitis. Fevers, chills, and malaise may be noted in patients with cystitis, though these findings are associated more frequently with upper UTI (ie, pyelonephritis) [1].

A history of vaginal discharge suggests that vaginitis, cervicitis, or pelvic inflammatory disease is responsible for symptoms of dysuria; therefore, a pelvic examination must be performed. Important additional information includes a history of prior STD (sexually transmitted disease) and multiple current sexual partners.

UTIs in women are very common; approximately 25-40% of women in the United States aged 20-40 years have had a UTI. UTIs account for over 6 million patient visits to physicians per year in the United States. Approximately 20% of those visits are to EDs.

UTIs have been well studied in Sweden and other parts of Europe. These studies have shown that 1 in 5 adult women experience a UTI at some point, confirming that it is an exceedingly common worldwide problem.

Nonbacterial cystitis encompasses various medical disorders, including infectious and noninfectious cystitis, as well as PBS/IC (painful bladder syndrome/interstitial cystitis). PBS/IC describes a syndrome of pain and genitourinary symptoms (eg, frequency, urgency, pain, dysuria, nocturia) for which no etiology can be found [1-2].

The pathophysiology of interstitial cystitis is poorly understood. Various etiologies have been proposed, including infectious, inflammatory, autoimmune, hypoxia-related, and neurologically related. However,

none of these adequately explains the variable presentations, clinical course, or response to therapies. This may indicate that interstitial cystitis represents a number of as yet undefined, disparate pathologic conditions that, over time, ultimately present as the clinical syndrome of urinary frequency, urgency, and pelvic pain [3].

There are many controversies regarding nonbacterial cystitis, including possible etiologic agents, methods of diagnosis, and treatment, especially for noninfectious causes. General symptoms of cystitis include urgency, frequency, dysuria, and, occasionally, hematuria, dyspareunia, abdominal cramps, and/or bladder pain and spasms. Establishing or excluding a specific diagnosis often requires repeated cultures and various urologic procedures, including cystoscopy with bladder biopsies, various bladder tests, and immune system function examinations. Some conditions, such as carcinoma in situ, bladder calculi, and urethral foreign bodies, may result in symptoms that mimic those of nonbacterial cystitis [1-3].

Trigonitis refers to the nonkeratinizing squamous metaplastic changes in the bladder trigone. The trigone is the triangular area of the bladder bound by the ureteral orifices and the internal urethral sphincter, which is normally lined by urothelium, a type of transitional epithelial tissue. This entity was first described by Heymann in 1905 as cystitis trigoni and was subsequently described by Cifuentes as a true trigonal membrane. It is also referred to in the literature as pseudomembranous trigonitis or vaginal metaplasia [3-4].

Trigonitis, despite its name suggestive of inflammation, is a metaplastic process. Although the precise underlying cause is not known, squamous metaplasia in the bladder usually occurs in response to an irritative (eg, chronic indwelling catheter) or infectious process.

Because nonkeratinizing squamous metaplasia is most commonly found in adult women of childbearing age, a hormonal influence is posited. In a study of

bladder biopsies performed in women with pseudomembranous trigonitis and women who underwent cystoscopy for staging gynecological cancer, estrogen and progesterone receptors were found in the trigone in association with squamous metaplastic changes. In a more extensive mapping of estrogen and progesterone receptors in the female lower urinary tract, both were found in squamous epithelial tissue, including the transitional cell epithelium in the trigone and proximal urethra that has undergone squamous metaplastic change [4].

However, whether hormonal influences lead to squamous metaplasia is unclear. Others have suggested that squamous cell metaplasia is not associated with increased estrogen activity. Kvist et al did not find estrogen receptors in 36 historically collected samples of squamous metaplasia of the bladder urothelium, although the authors posited that relatively few existing receptors might have been destroyed in the tissue preparation process [5].

Instead, other explanations focus on the potential role of chronic inflammation and/or a deficient urothelium. In one autopsy study of adult women, histological evidence of chronic inflammation was found significantly more often in bladders with squamous metaplasia. The authors suggest that squamous metaplasia is not a consequence of chronic inflammation but rather that its surface characteristics may predispose to chronic infection [5, 6].

Squamous metaplasia is observed over edematous or inflamed lamina propria. Electron microscopy of the keratinizing variant has demonstrated that squamous metaplastic cells lack tight junctions seen in normal transitional epithelial cells, which might allow urine to permeate the subepithelial layers and result in ongoing inflammation. The interstitial cystitis literature suggests that the surface mucus of the bladder, also known as the GAG (glycosaminoglycan) layer, plays an important role in preventing the permeability of urinary solutes into the bladder wall [7-8].

Defects in or injuries resulting from chronic irritation or recurrent infections to this protective mechanism may result in chronic inflammatory changes leading to metaplasia. However, not all individuals with squamous metaplasia are symptomatic, and not all symptomatic patients have squamous metaplasia. In absence of a clearly bacterial infection, ischaemia and inflammation seem to be common factors causing all these clinical features. It seems to be involved some specific inflammatory proteins that provoke the rising of radicals of oxygen and Nitic oxide. The principal protein involved in the genesis of ROS is NF-kB [9].

The nuclear transcription factor NF-kB is a heterodimeric, sequence-specific transcription factor found in many cell. In unstimulated cells, NF-kB is found in the cytoplasm and is bound to its inhibitor kB, which prevents it from entering nuclei. In stimulated cells, however, specific kinases phosphorylate kB, leading to rapid degradation by proteasomes and subsequent nuclear translocation and binding of NF-kB to specific sequences in the promoter regions of target genes. A number of stimuli have been shown to activate NF-kB, including cytokines, activators of protein kinase C, viruses, and oxidants [9].

Urothelium repair mechanism balances oxydative effects of inflammation. Many blood factors are involved in urothelium regeneration. PRP (platelet rich plasma), also termed autologous platelet gel, PRGF (plasma rich in growth factors), PC (platelet concentrate), is essentially an increased concentration of autologous platelets suspended in a small amount of plasma after centrifugation. Basically, patient's blood is collected and centrifuged at varying speeds until it separates into 3 layers: PPP (platelet poor plasma), PRP, and red blood cells. The efficacy of certain growth factors in healing various injuries and the concentrations of these growth factors found within PRP are the theoretical basis for the use of PRP in tissue repair.The platelets collected in PRP are activated by the addition of thrombin and calcium chloride, which induces the release of the mentioned factors from alpha granules. The growth factors and other cytokines present in PRP include: PDG factor,TGF beta, fibroblast growth factor, IGF-1 and IGF-2, vascular endothelial growth factor, EGF and IL-8 [10].

Usually 2 spins are used. The first spin ("hard spin") separates the PPP from the red fraction and PRP. The second spin ("soft spin") separates the red fraction from the PRP. The material with the highest specific gravity (PRP) will be deposited at the bottom of the tube. Immediately prior to application, a platelet activator/agonist (topical bovine thrombin and 10% calcium chloride) is added to activate the clotting cascade, producing a platelet gel. The whole process takes approximately 12 minutes and produces a platelet concentration of 3-5 x that of native plasma [11-13].

Carboxytherapy is a non-surgical cosmetic medicine treatment. Carboxytherapy employs injections to infuse gaseous carbon dioxide below the skin into the subcutaneous tissue through a needle. It originated at thermal Spas of France in 1932 with the treatment of patients afflicted by peripheral arterial occlusive disease. In South America and Europe, carbon dioxide therapy has been applied to the treatment of stretch marks, cellulite, and hypertrophic scars. Studies have demonstrated that carboxytherapy improves skin elasticity, improves circulation, encourages collagen repair, improves the appearance of fine lines and wrinkles, and destroys localized fatty deposits [14, 15].

Clinical studies have indicated that such altered CO_2 levels (controlled hypercapnia) can impact upon disease progression. CO_2 levels can be sensed by cells resulting in the initiation of physiologic and pathophysiologic responses. A role for CO_2 in the regulation of gene transcription has recently been identified with exposure of cells and model organisms to high CO_2 leading to suppression of genes involved in the regulation of innate immunity

and inflammation. This latter, transcriptional regulatory role for CO_2 has been largely attributed to altered activity of the NF-κB family of transcription factors [9].

While the effects of in vivo hypercapnia on gene expression likely occurs in part through indirect mechanisms such as altered neuronal activity or the release of stress hormones, recent evidence suggests that CO_2 may also directly regulate gene expression through the NF-kB pathway. The suppression of NF-κB activity by hypercapnia was recently provided by the demonstration of CO_2-induced nuclear localization of the IKKα subunit [16].

Therapeutic hypercapnia has been reported to be of benefit in ischemia/ reperfusion injury in the mesentery and recently in the liver. The mechanisms for this protection are not yet fully elucidated in vivo however the latter study reports attenuated IRI-mediated pro-inflammatory gene expression (TNFα), enhanced anti-inflammatory cytokine production (IL-10), decreased apoptosis and decreased immunohistochemical staining for NF-κB in the hypercapnia treated groups. These studies are consistent with the observations described above for CO_2 (independent of extracellular pH) having a suppressive effect on NF-κB signaling (Cummins et al., 2010)and of hypercapnic acidosis blunting endotoxin-stimulated NF-κB signaling, resulting in decreased ICAM-1and IL-8 expression in pulmonary endothelial cells [9].

Carboxytherapy refers to the administration of CO_2 for therapeutic purposes. It has been shown that, because of the interaction between CO_2 and regulating factors of tissue perfusion, Carboxytherapy acts on the microcirculation at the level of metarterioles, arterioles and precapillary shpincteres by increas ing tissue flow velocity and consequently, by improving lymphatic drainage. Analysis of literature data shows a wide range of today applications for this treatment involving either phlebology or non-phlebology fields. Specifically, the positive effect on the increase of

lymphatic drainage has more recently made Carboxytherapy useful for treatment of lymphatic stasis. Carboxytherapy has not serious toxicity, easier management in a free risk subministration [14-19].

4. Conclusions

Preliminary qualitative results could encourage the use of carboxytherapy and PRP in treatment of abacterial and interstitial cystitis.PRP in association with carboxytherapy use is completely safe, well tolerated, with appearently good long-lasting results after a preliminary follow up of 2 months. Even if we have considered only a very strictly number of patients, this method should be a new approach for chronic cystitis even in association with other conservative therapies.

References

[1] Gupta, K., Hooton, T. M., and Naber, K. G. 2011. "International Clinical Practice Guidelines for the Treatment of Acute Uncomplicated Cystitis and Pyelonephritis in Women: A 2010 Update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases". *Clin. Infect. Dis.* 52 (5): e103-20.

[2] Kallen, A. J., Welch, H. G., and Sirovich, B. E. 2006. "Current Antibiotic Therapy for Isolated Urinary Tract Infections in Women." *Archives of Internal Medicine* 166: 635-9.

[3] O'Connor, P. J., Solberg, L. I., Christianson, J., Amundson, G., and Mosser, G. 1996. "Mechanism of Action and Impact of a Cystitis Clinical Practice Guideline on Outcomes and Costs of Care in an HMO." *The Joint Commission Journal on Quality Improvement* 22: 673-82.

[4] Stamm, W. E. 2007. "Evaluating Guidelines." *Clin. Infect. Dis.* 44: 775-6.

[5] Kahlmeter, G. 2003. "An International Survey of the Antimicrobial Susceptibility of Pathogens from Uncomplicated Urinary Tract Infections: The ECO.SENS Project." *J. Antimicrob Chemother* 51: 69-76.

[6] Warren, J. W., Abrutyn, E., Hebel, J. R., Johnson, J. R., Schaeffer, A. J., and Stamm, W. E. 1999. "Guidelines. for Antimicrobial Treatment of Uncomplicated Acute Bacterial Cystitis and Acute Pyelonephritis in Women. Infectious Diseases Society of America (IDSA)." *Clin. Infect. Dis.* 29: 745-58.

[7] Huang, E. S., and Stafford, R. S. 2002. "National Patterns in the Treatment of Urinary Tract Infections in Women by Ambulatory Care Physicians." *Archives of Internal Medicine* 162.

[8] Kahan, N. R., Chinitz, D. P., and Kahan, E. 2004. "Longer than Recommended Empiric Antibiotic Treatment of Urinary Tract Infection in Women: An Avoidable Waste of Money." *Journal of Clinical Pharmacy and Therapeutics* 29: 59-63.

[9] Michael, K. 2011. "NF-κB in Health and Disease." *Springer, 5 Maggio* ISBN 978-3-642-16016-5.

[10] Yu, W., Wang, J., and Yin, J. 2011. "Platelet-Rich Plasma: A Promising Product for Treatment of Peripheral Nerve Regeneration after Nerve Injury." *Int. J. Neurosci.* 121 (4): 176-80.

[11] Arora, N. S., Ramanayake, T., Ren, Y. F., and Romanos, G. E. 2009. "Platelet-Rich Plasma: A Literature Review." *Implant Dent.* 18 (4): 303-10.

[12] Gonshor, A. 2002. "Technique for Producing Platelet-Rich Plasma and Platelet Concentrate: Background and Process." *The International Journal of Periodontics and Restorative Dentistry* 22 (6): 547-57.

[13] Griffin, X. L., Smith, C. M., and Costa, M. L. 2009. "The Clinical Use of Platelet-Rich Plasma in the Promotion of Bone Healing: A Systematic Review." *Injury* 40 (2): 158-62.

[14] Varlaro, V., Manzo, G., and Bisacci, R. 2007. "Carboxytherapy: Effects on Microcirculation and Its Use in the Treatment of Severe Lymphedema—A Review." *ACTA PHLEBOL* 8: 000-000.

[15] Borrione, P., Gianfrancesco, A. D., Pereira, M. T., and Pigozzi, F. 2010. "Platelet-Rich Plasma in Muscle Healing." *Am. J. Phys. Med. Rehabil.* 89 (10): 854-61.

[16] Parassoni, L., and Varlaro, V. 1997. *La Carbossiterapia: Una Metodica in Evoluzione. Riv La Medicina Estetica, Anno 21, N.L.* Roma: Gennaio-Marzo Editrice Salus Internazionale.

[17] Parassoni, L., Albergati, F., Varlaro, V., and Curri, S. B. 1997. *La Carbossiterapia in Tema Di Meccanismi D'azione. Riv. La Medicina Estetica, Anni 21, N.L.* Roma: Gennaio-Marzo Editrice Salus Internazionale.

[18] Cummins, E. 2011. "Regulation of Gene Expression by Carbon Dioxide." *Physiol.* 15: 589 (Pt 4): 797-803.

[19] Georgia, S. K. 2010. "Lee Carbon Dioxide Therapy in the Treatment of Cellulite: An Audit of Clinical Practice." *Aesthetic Plastic Surgery* 34, 2: 239-43.