

Brief Report

Long-Term Control of Refractory Hemorrhagic Radiation Proctitis With Ozone Therapy

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Abstract

Context. Persistent or severe hemorrhagic radiation proctitis (HRP) has limited therapeutic options.

Objectives. To describe our experience with ozone therapy (O₃T) in the management of refractory HRP.

Methods. Patients ($n = 17$; median age 69 years [range 42–80 years]) previously irradiated for prostate or uterine cancer and suffering persistent or severe HRP without response to conventional treatment were enrolled to receive an O₃/O₂ gas mixture via rectal insufflations and topical application of ozonized oil. Most of the patients (83%) had Grade 3 or Grade 4 toxicity. Median follow-up post-O₃T was 40 months (range 3–56 months).

Results. Endoscopic treatments required were: 43 (median 1; range 0–10) pre-O₃T; 17 (median 0; range 0–8; $P = 0.063$) during O₃T; and five (median 0; range 0–2; $P = 0.008$) during follow-up. Hemoglobin levels were 10.35 g/dL (7–14 g/dL) pre-O₃T and 13 g/dL (9–15 g/dL) ($P = 0.001$) post-O₃T. Median toxicity grades were 3 (range 2–4) pre-O₃T, 1 (range 0–2; $P < 0.001$) at the end of O₃T, and 0 (range 0–1; $P < 0.001$) at the last follow-up.

Conclusion. Persistent advanced HRP was significantly improved with O₃T. The addition of O₃T can be useful as a complementary treatment in the long-term

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management of HRP and, as such, merits further evaluation. *J Pain Symptom Manage* 2013;46:106–112. © 2013 U.S. Cancer Pain Relief Committee. Published by Elsevier Inc. All rights reserved.

Key Words

Gynecologic tumors, integrative and complementary medicine, pelvic radiotherapy, prostate cancer, rectal bleeding, side effects, toxicity

Introduction

Radiation proctitis is a potential complication after radiation therapy for pelvic tumors. Higher risk can be expected after the treatment of uterine cervix or prostate tumors because higher radiation doses are delivered. There are several possible symptoms, of which rectal bleeding is the most significant. Mild symptoms of radiation proctitis may remit spontaneously without specific therapy or with medical treatments involving different endoscopy interventions. When hemorrhagic radiation proctitis (HRP) becomes persistent or severe, therapies before possible high-risk surgery are limited to hyperbaric oxygen (HBO), a treatment that is cumbersome and often of limited availability.¹

In 1917, *The Lancet* carried an article on ozone therapy (O₃T) in which local application was shown to improve radiation-induced side effects.² Internal O₃ administration via rectal insufflation was described 20 years later.³ Several effects with O₃T have been observed that are similar to those obtained with HBO.³ We do not have HBO facilities in our hospital and have successfully explored O₃T in the management of several radiation-induced toxicities.^{4–6} Over the years, O₃T has been used in our hospital for complementary and palliative treatment of pain and ischemic syndromes and administered on compassionate grounds. In this article, we report our experience in treating 17 patients with HRP who were refractory to other therapeutic interventions.

Materials and Methods

Between April 2004 and June 2009, we used O₃T to treat 17 patients (median age 69 years; range 42–80 years) suffering from HRP, who had not responded to conventional treatment. All patients provided informed written consent to the therapy, and all procedures conformed

to the Helsinki Declaration of 1975. Included in the present study were 12 males with prostate cancer and five females with gynecologic cancer. All patients had a history of pelvic radiotherapy (RT).

Diagnosis of HRP was done by endoscopy in all patients, eight of whom had additional confirmation by rectal biopsy. Before O₃T, all patients had been treated with medical therapy including diet, oral or rectal medications, and/or endoscopy procedures (argon laser, formalin, coagulation). Rectal hemorrhage grading (Fig. 1) was according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) grading system (version 4.0).⁷ In our group of patients, there were no concomitant episodes of radiation-induced hematuria before or during O₃T.

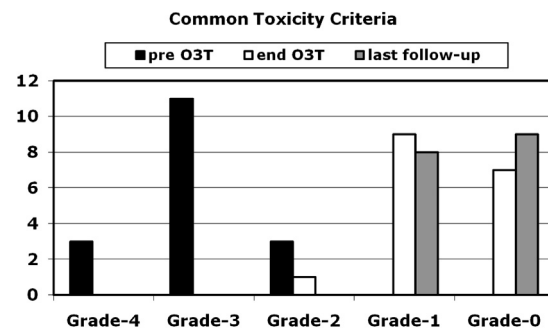


Fig. 1. Rectal hemorrhage grading according to CTCAE (see Materials and Methods section for details). Initial CTCAE toxicity grade (median 3; range 2–4) was significantly decreased at the end of O₃T (median 1; range 0–2; $P < 0.001$) and at the last follow-up assessment (median 0; range 0–1; $P < 0.001$). Grade 0: no symptoms; Grade 1: mild symptoms, intervention not indicated; Grade 2: moderate symptoms, medical intervention or minor cauterization indicated; Grade 3: severe symptoms, transfusion, radiologic, endoscopic, or elective surgical intervention indicated; Grade 4: life-threatening consequences, urgent intervention indicated. CTCAE = Common Terminology Criteria for Adverse Events; O₃T = ozone therapy.

Treatment Procedure

O₃T was by rectal insufflations of O₃/O₂ gas mixture and topical application of ozonized oil. For rectal insufflations, the ozone was obtained from clinical grade oxygen using a medical ozone generator (Ozonosan Alpha-plus®; Dr. Hänsler GmbH, Iffezheim, Germany). The O₃/O₂ gas mixture (µg/mL: µg of O₃ per mL of O₂) provided by the device was administered via a rectal cannula using standard 60 mL syringes. Typically, the gas mixture for insufflation in each treatment session ranged between 150 and 300 mL, depending on patient tolerance to the abdominal distension (or bloating) produced by the insufflation gas. Exceptionally, one patient was able to tolerate a volume of only 50 mL over the whole course of treatment. O₃ concentrations commenced at 5 µg/mL in 10 patients (total 750 µg of O₃ in the first session) and at 10 µg/mL in the rest of the patients (total 1500 µg of O₃ at the first session). The concentration was progressively increased up to 30 µg/mL in seven patients and up to 20 µg/mL in the rest of the patients. In the overall patient group, the majority of the sessions were performed at an O₃ concentration of 20 µg/mL, with an insufflated gas volume between 180 and 240 mL and with a total O₃ administration between 3600 and 4800 µg in each session. The total amount of O₃ administered over the whole period of treatment varied between 20 and 653 mg (patients with five and 107 sessions of O₃T, respectively). Initially, the O₃T sessions were scheduled for three times a week. When clinical improvement was apparent, the sessions were progressively reduced to two and one session a week, followed by two sessions a month and, finally, one session a month for two to three additional months. Because O₃ as a gas spontaneously decomposes to O₂, with a half-life of 40 minutes at 20°C,³ the production and administration needs to be performed in situ in the outpatient department. Around 15–20 minutes are required for each full session.

For topical application, commercial ozonized oil (Dr. Hänsler GmbH) was mixed in our hospital's pharmacy with liquid petroleum jelly to provide ozonized oil concentrations ranging from 14% to 25%. The 25% mixture

(5–10 mL) was administered, initially, one to two times a day. The frequency of administration and the O₃ concentration were progressively reduced depending on clinical improvement. Because the half-life of ozonized oil can range from months to years (depending on the storage temperature), the ozonized oil mixture for extended treatment was prepared individually for each patient for self-administration at home. All standard aspects of patient management, including endoscopy, were at the discretion of the attending gastroenterologist.

Statistical Analysis

The SPSS® software package v. 15 for Windows (SPSS Inc., Chicago, IL) was used for all statistical analyses. Nonparametric tests were used for all comparisons because several variables were “non-normally” distributed. Values are presented as median (range). Comparisons of paired data pre- and post-therapy were done with the two-sided Wilcoxon signed rank test and Friedman's test for comparisons of related data at the three time points of pre-O₃T, post-O₃T, and final follow-up. Correlations were assessed using Spearman's correlation coefficient (rho). Comparisons of categorical values were done with the Chi-square test. Statistical significance was set at $P < 0.05$.

Results

Median time lapse between the end of RT and rectal bleeding was five months (range 0–34 months). Median time lapse between the onset of rectal bleeding and commencing O₃T was 11 months (range 1–41 months). Time lapse between the end of RT and commencing O₃T was 16 months (range 8–61 months). Median duration of O₃T was 10 months (range 1–19 months). Median number of O₃T sessions was 38 (range 5–107). Median follow-up post-O₃T was 40 months (range 3–56 months).

A total number of 44 blood transfusions were required (median 4; range 1–11) by 11 of the 17 patients. The lowest hemoglobin value pre-O₃T was a median of 7.25 g/dL (range 5–14 g/dL). Immediately pre-O₃T, the median hemoglobin value was 10.35 g/dL (range 7–14 g/dL), and 13 patients

(76%) were anemic. At the end of O₃T, the median hemoglobin value was 13 g/dL (range 9–15 g/dL) ($P=0.001$) and only six of the 17 patients (35%) were anemic ($P=0.038$) compared with pre-O₃T.

Before undergoing O₃T, 11 patients (65%) had had a total of 43 (median 1; range 0–10) unsuccessful endoscopy treatments performed (argon plasma coagulation and/or topical formalin). Endoscopy was reduced to 17 procedures (median 0; range 0–8; $P=0.063$) during O₃T and to five procedures during follow-up (median 0; range 0–2; $P=0.008$). Overall, the change in the number of endoscopy treatments applied was statistically significant ($P=0.009$).

Pre-O₃T, the median CTCAE toxicity grade was 3 (range 2–4). This was reduced to 1 (range 0–2) by the end of O₃T ($P<0.001$) and to 0 (range 0–1) by the time of the last follow-up ($P<0.001$). Overall, the change in CTCAE toxicity grade was statistically significant ($P<0.001$) (Fig. 1).

CTCAE toxicity grade pre-O₃T was inversely correlated with the time lapse between RT and rectal bleeding ($\rho = -0.580$; $P=0.015$) and low hemoglobin levels ($\rho = -0.554$; $P=0.026$), that is, higher radiation toxicity was related to earlier commencement of rectal bleeding and with the lowest hemoglobin levels reached by the patient before commencing O₃T.

Toxicity grade pre-O₃T was directly correlated with the number of endoscopy procedures required during O₃T ($\rho = 0.516$; $P=0.034$), number of blood transfusions required pre-O₃T ($\rho = 0.481$; $P=0.051$) or during O₃T ($\rho = 0.534$; $P=0.027$), number of O₃T sessions ($\rho = 0.594$; $P=0.025$), and number of months of O₃T ($\rho = 0.648$, $P=0.005$).

Five of the 17 patients (29%) were receiving antiaggregant treatment for cardiovascular disease. Antiaggregant treatment was correlated with older age ($\rho = 0.651$; $P=0.012$), requirement for more blood transfusions pre-O₃T ($\rho = 0.559$; $P=0.038$), and a trend toward anemia by the end of O₃T ($\rho = 0.519$; $P=0.057$).

O₃T was well tolerated and no adverse effects were noted, except soft and temporal flatulence some hours after each session. Results are summarized in Fig. 2.

Discussion

Rectal bleeding is the most significant symptom of radiation proctitis. The toxicity scales we used were those of the CTCAE and focused on hemorrhagic symptoms. There are no specific criteria for the diagnosis and assessment of the different symptoms of radiation proctitis and their impact on patient quality of life.

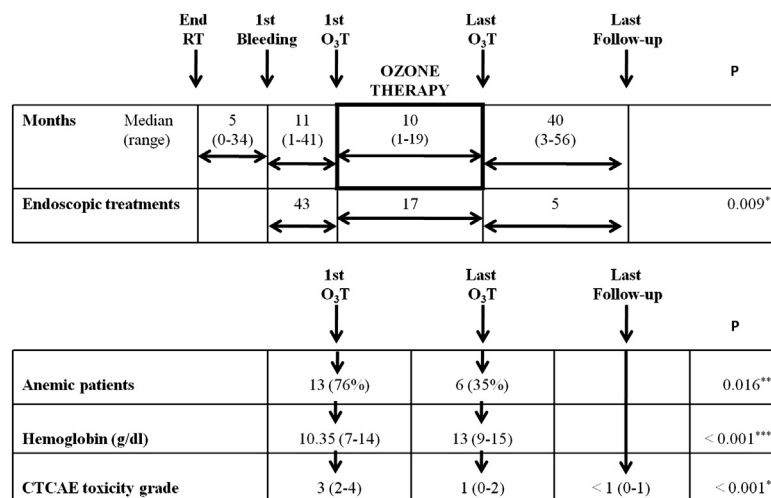


Fig. 2. Summary of outcomes of O₃T. Values are shown as median and range (minimum and maximum values) except for the numbers of endoscopic treatments. *Friedman’s test. **Chi-square test. ***Wilcoxon signed rank test. RT = radiotherapy; O₃T = ozone therapy; CTCAE = Common Terminology Criteria for Adverse Events.

Currently, the European Organization for Research and Treatment of Cancer is in the process of validating a specific questionnaire to address this issue.⁸

Mild symptoms of radiation proctitis may remit spontaneously without specific therapy within six months in up to a third of affected patients.¹ First-level treatment includes laxatives, rectal or oral sulfasalazine, or corticosteroids and, especially, rectal sucralfate suspension enemas.⁹ Second-level treatments for nonresponders are coagulation via endoscopy using bipolar or heater probes¹⁰ or argon plasma and formaldehyde application for the more persistent symptoms.^{11,12} Several studies have described the beneficial effect of HBO,^{13,14} which has been confirmed by a double-blind randomized clinical trial.¹⁵ However, HBO is usually reserved for third-level treatment, and, being cumbersome and difficult to coordinate, it is not readily available in most hospitals. The last option is surgery, which is often associated with higher morbidity and, as such, is reserved for the most adverse clinical conditions. Over the years, several of these first- and/or second-level treatments have been used in our patients.

Acute radiation proctitis reflects early inflammation during, or immediately after, RT. It results from the death of mitotically active intestinal crypt cells. Chronic radiation proctitis commonly refers to rectum toxicity after the first three to six months after RT. It results from lesions in the slowly responding cells of connective tissues and blood vessels, with progressive endarteritis leading to ischemia and hypoxia and, subsequently, to mucosal atrophy and intestinal wall fibrosis.¹⁶

In this disease context, the systemic effect of O₃T confers some valuable effects. O₃T can improve hemorheological parameters of blood¹⁷ and can increase blood flow and oxygenation in hypoxic tissues.^{6,18,19} Details on vascular effects and modulation of the immune-inflammatory response have been summarized recently.³ Standard management of radiation proctitis is based on the effects on blood flow (pentoxifylline),²⁰ oxygenation with HBO,¹³⁻¹⁵ and inflammation (corticosteroids). Additionally, the aforementioned O₃T effects on hemorheological parameters and blood flow augur well for its use in combination with HBO (if/when available) because

the high arterial pO₂ levels obtained by HBO tend to induce vasoconstriction.

The key mechanism of action of systemic O₃T is the production of a "controlled and moderate" oxidative stress. O₃ reacts quickly with many substances including antioxidants and polyunsaturated fatty acids, resulting in lipid oxidation products and an induction of intracellular second messengers, the most important being hydrogen peroxide (H₂O₂) and alkenals (mainly 4-hydroxynonenal, 4-HNE). These second messengers lead to the activation of nuclear transcriptional factors such as nuclear factor erythroid 2-related factor 2, which results in the transcription of antioxidant response elements and subsequent production of antioxidant enzymes including superoxide dismutase, glutathione peroxidase, and heat shock proteins. Additionally, nuclear factor erythroid 2-related factor 2 can lead to the suppression of the nuclear factor kappa B, which has a proinflammatory effect. O₃T can, via the production of a moderate oxidative stress, modulate the immune response by the suppression of nuclear factor kappa B and the induction of other nuclear transcription factors such as nuclear factor of activated T-cells and activated protein-1, together with further modulation of interferons and interleukins. Because inflammation can enhance tissue damage secondary to high oxidative stress (as occurs with radiation-induced side effects), the final anti-inflammatory effect of O₃T can offer an additional beneficial effect that is similar to that achieved with the use of corticoids. The molecular mechanisms of action of O₃ have been described extensively in recent reviews.^{3,21} The enhancement of antioxidant systems by systemic O₃T has been demonstrated in rat models of toxicity mediated by free radicals in the liver²² and kidney.²³ This could be relevant in the treatment of radiation proctitis, as observed with the combination of vitamin E with vitamin C²⁴ or vitamin E with pentoxifylline.²⁰

Finally, a local effect of O₃T probably contributed to the clinical evolution of our patients. There have been some reports on the success of O₃T applications in radiation-induced toxicity.^{2,4-6} In our study, this additional benefit was obtained by the local effect of rectal O₃ insufflations and the topical application of ozonized oil. The ozonized oil does

not penetrate through the mucous membrane but reacts with polyunsaturated fatty acids to induce cellular production of hydrogen peroxide and alkenals, which act as second messengers to improve wound healing.²⁵ Another beneficial action of the local application of O₃T is its bactericidal properties,²⁵ such as in the treatment of public drinking water in several cities. This effect is potentially beneficial, as well, as has been demonstrated with the use of the anti-anaerobic antibiotic metronidazole in radiation proctitis.²⁶

The optimal number of O₃T sessions for the successful treatment of HRP remains to be determined. In our study, the median number of treatment sessions was 38 (range 5–107 sessions) and is similar to reports using HBO (median number between 20 and 80).¹⁵ For example, Mayer et al.¹³ reported 30 sessions (range 13–60 sessions), and Jones et al.¹⁴ reported 40 sessions (range 36–41 sessions). Median follow-up in our patient series was 40 months (range 3–56 months). This is more protracted than the 12 months (range 8–27 months) and the 25 months (range 6–43 months) reported in other studies.^{13,14} Additionally, of note is that our study included patients with more advanced clinical conditions: 18% Grade 2, 65% Grade 3, and 18% Grade 4 toxicity, of which 65% of patients had received laser coagulation therapy. Mayer et al.¹³ described a group with 40% Grade 2 and 60% Grade 3 toxicity, in which only 30% had undergone previous treatment with laser coagulation. Jones et al.¹⁴ described a group with 70% Grade 2 and 30% Grade 3 toxicity. Clarke et al.¹⁵ described a review of studies using HBO for the treatment of radiation proctitis. Many of the patients included in the studies had received HBO for non-bleeding symptoms. Nevertheless, our results compare favorably, although all our patients had been treated for rectal bleeding, a symptom that implies more advanced toxicity (82% had Grade 3 or Grade 4 toxicity) and, theoretically, poorer outcome. They had had a high requirement for endoscopy treatments and blood transfusions. Of note is that HBO requires costly and cumbersome facilities, with limited availability and several limitations such as claustrophobia, headache, vomiting, convulsions, and risk of barotrauma (ears, sinuses, lung), none of which apply to O₃T. Indeed, the mechanism of action

of O₃T is not only dissimilar but also can be potentially complementary to HBO in the treatment of radiation-induced side effects. We do not propose O₃T as a substitute for medical or endoscopic treatments because, as our study shows, O₃T can enhance the effect of the medical or endoscopic management of these patients.

In conclusion, this report of treatment of refractory HRP highlights that the persistent and advanced grades of toxicity were significantly improved using O₃T, even over the long term. We believe that the local application of O₃T can be useful as a complementary treatment in managing HRP and, as such, merits further evaluation in randomized clinical trials.

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References

1. Rodriguez ML, Martin MM, Padellano LC, Palomo AM, Puebla YI. Gastrointestinal toxicity associated to radiation therapy. *Clin Transl Oncol* 2010;12:554–561.
2. Stoker G. The surgical uses of ozone. *Lancet* 1917;26:797.
3. Bocci V, Borrelli E, Travagli V, Zanardi I. The ozone paradox: ozone is a strong oxidant as well as a medical drug. *Med Res Rev* 2009;29:646–682.
4. Clavo B, Gutierrez D, Martin D, et al. Intravesical ozone therapy for progressive radiation-induced hematuria. *J Altern Complement Med* 2005;11:539–541.

5. Clavo B, Santana-Rodriguez N, Lopez-Silva SM, et al. Persistent PORT-A-CATH®-related fistula and fibrosis in a breast cancer patient successfully treated with local ozone application. *J Pain Symptom Manage* 2012;43:e3–e6.
6. Clavo B, Suarez G, Aguilar Y, et al. Syndrome of brain ischemia and hypometabolism treated by ozone therapy. *Forsch Komplementmed* 2011;18:283–287.
7. National Cancer Institute. Common Terminology Criteria for Adverse Events (CTCAE) grading system (version 4.0). Available from http://evsncinhi.gov/ftp1/CTCAE/CTCAE_403_2010-06-14_QuickReference_5x7.pdf. Accessed February 21, 2011.
8. Halkett G, Aoun S, Hayne D, et al. EORTC radiation proctitis-specific quality of life module—pre-testing in four European countries. *Radiother Oncol* 2010;97:294–300.
9. Kochhar R, Patel F, Dhar A, et al. Radiation-induced proctosigmoiditis. Prospective, randomized, double-blind controlled trial of oral sulfasalazine plus rectal steroids versus rectal sucralfate. *Dig Dis Sci* 1991;36:103–107.
10. Jensen DM, Machicado GA, Cheng S, Jensen ME, Jutabha R. A randomized prospective study of endoscopic bipolar electrocoagulation and heater probe treatment of chronic rectal bleeding from radiation telangiectasia. *Gastrointest Endosc* 1997;45:20–25.
11. Karamanolis G, Triantafyllou K, Tsiamoulos Z, et al. Argon plasma coagulation has a long-lasting therapeutic effect in patients with chronic radiation proctitis. *Endoscopy* 2009;41:529–531.
12. Haas EM, Bailey HR, Farragher I. Application of 10 percent formalin for the treatment of radiation-induced hemorrhagic proctitis. *Dis Colon Rectum* 2007;50:213–217.
13. Mayer R, Klemen H, Quehenberger F, et al. Hyperbaric oxygen—an effective tool to treat radiation morbidity in prostate cancer. *Radiother Oncol* 2001;61:151–156.
14. Jones K, Evans AW, Bristow RG, Levin W. Treatment of radiation proctitis with hyperbaric oxygen. *Radiother Oncol* 2006;78:91–94.
15. Clarke RE, Tenorio LM, Hussey JR, et al. Hyperbaric oxygen treatment of chronic refractory radiation proctitis: a randomized and controlled double-blind crossover trial with long-term follow-up. *Int J Radiat Oncol Biol Phys* 2008;72:134–143.
16. Hauer-Jensen M, Wang J, Denham JW. Bowel injury: current and evolving management strategies. *Semin Radiat Oncol* 2003;13:357–371.
17. Giunta R, Coppola A, Luongo C, et al. Ozonized autohemotransfusion improves hemorheological parameters and oxygen delivery to tissues in patients with peripheral occlusive arterial disease. *Ann Hematol* 2001;80:745–748.
18. Clavo B, Catala L, Perez JL, Rodriguez V, Robaina F. Ozone therapy on cerebral blood flow: a preliminary report. *Evid Based Complement Alternat Med* 2004;1:315–319.
19. Clavo B, Perez JL, Lopez L, et al. Effect of ozone therapy on muscle oxygenation. *J Altern Complement Med* 2003;9:251–256.
20. Hille A, Christiansen H, Pradier O, et al. Effect of pentoxifylline and tocopherol on radiation proctitis/enteritis. *Strahlenther Onkol* 2005;181:606–614.
21. Sagai M, Bocci V. Mechanisms of action involved in ozone therapy: is healing induced via a mild oxidative stress? *Med Gas Res* 2011;1:29.
22. Peralta C, Leon OS, Xaus C, et al. Protective effect of ozone treatment on the injury associated with hepatic ischemia-reperfusion: antioxidant-prooxidant balance. *Free Radic Res* 1999;31:191–196.
23. Gonzalez R, Borrego A, Zamora Z, et al. Reversion by ozone treatment of acute nephrotoxicity induced by cisplatin in rats. *Mediators Inflamm* 2004;13:307–312.
24. Kennedy M, Bruninga K, Mutlu EA, et al. Successful and sustained treatment of chronic radiation proctitis with antioxidant vitamins E and C. *Am J Gastroenterol* 2001;96:1080–1084.
25. Valacchi G, Lim Y, Belmonte G, et al. Ozonated sesame oil enhances cutaneous wound healing in SKH1 mice. *Wound Repair Regen* 2011;19:107–115.
26. Cavcic J, Turcic J, Martinac P, et al. Metronidazole in the treatment of chronic radiation proctitis: clinical trial. *Croat Med J* 2000;41:314–318.