Oral low molecular weight hyaluronic acid in the prevention and treatment of radiation-induced cystitis

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ABSTRACT — INTRODUCTION: *Radiotherapies (RT) are considered as the second major therapeutic modalities for localized high-risk prostate cancers. Conformal postoperative radiotherapy in patients with positive resection margins and/or pT3-4 prostate adenocarcinoma is the routine approach. Nevertheless, the proximity of the rectum and bladder is a limiting factor in the safe delivery of dose-escalated RT. Urinary toxicity has a major impact on the post-treatment quality of life of patients who have undergone postoperative RT. The standard therapy of severe radiation cystitis consists in the intravesical administration of hyaluronic acid, a long treatment, not simple for the use of the catheter and extremely suffering for patients. Based on this scientific background, we conducted a non-randomized controlled study to evaluate the impact of oral administration of low molecular weight hyaluronic acid (LMW-HA) as preventive treatment for RT-induced toxicity.*

PATIENTS AND METHODS: A total of 42 patients aged between 55-87 years who underwent RT after prostatectomy, were included in our study. Eighteen patients received 1 tablet of LMW-HA twice a day 1-month before and for all duration of RT; the other 24 patients underwent standard-RT protocol.

RESULTS: Only two people in treated group developed toxicity (11.11%), whereas all patients in control group developed toxicity during RT (100%) ($p \leq 0.0001$). Moreover, after appearance of toxicity, 5 patients from the control group started the treatment with LMW-HA removing completely toxicity.

CONCLUSIONS: In 89% of cases enrolled in the experimental arm, the appearance of acute toxicity during RT was prevented, improving their quality of life. Therefore, oral LMW-HA can be considered an alternative approach in preventing and/or reducing cystitis symptoms in patients undergoing RT.

KEYWORDS
Low molecular weight hyaluronic acid, Hyaluronic acid, Prostate cancer, Radiation-induced cystitis, Toxicity.

INTRODUCTION

Prostate cancer is the most commonly diagnosed cancer among men in the Western countries[1]. Radical prostatectomy is an operation to remove the prostate gland and is most often done when cancer has not spread outside the prostate (localized prostate cancer). Postoperative radiotherapy (RT) in patients with positive resection margins and/or pT3-4 prostate adenocarcinoma is the routine approach

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for prostate cancer, which is considered as the second major therapeutic modalities for localized high-risk prostate cancers. Once the prostate is removed, the patient undergoing RT has a bladder, which must necessarily be irradiated in the case of tumor infiltrating either the surrounding tissue of seminal vesicle or the apex of the prostate. Nevertheless, the proximity of the rectum and bladder is a limiting factor in the safe delivery of dose-escalated RT. Indeed, radiation induced cystitis is a severe complication following RT to the pelvic tumor and it occurs in 3-6% of patients[2]. According to the toxicity criteria of the Radiation Therapy Oncology Group (RTOG) and the European Organization for Research and Treatment of Cancer (EORTC), the symptoms of radiation cystitis vary from minor angioectasias causing hematuria, to severe frequency, dysuria, and reduction of the bladder capacity[3]. Urinary toxicity has a major impact on the quality of life (QoL) of patients who have undergone postoperative RT[4]. Radiation toxicity is classified into acute and late toxicity based on the timing of its occurrence after the completion of RT. Acute toxicity occurs during treatment and after completing RT, while late toxicity occurs from weeks to years after treatment. It is sometimes irreversible and difficult to be treated. Toxicity rates, especially in the late-onset form, increase with RT dose[5], decreasing significantly patients QoL[6-8]. The standard therapy of severe radiation cystitis is the intravesical administration of hyaluronic acid (HA)[9], which consists of using a catheter that can be extremely painful for the patient. HA is a linear polysaccharide present in the extracellular matrix. It is ubiquitous in mammals, playing several physiological roles in tissue hydration and mechanical protection[10]. Due to its versatility and exemplary physicochemical properties such as biocompatibility, biodegradability, nontoxicity and nonimmunogenicity, it finds a wide range of biomedical applications from osteoarthritis, ocular and plastic surgery, to drug delivery[10]. Supplementation of HA by oral ingestion has gained increasing attention. The most important factor in defining the oral uptake of food substances is their absorbability across the intestinal epithelium. How HA passes through the intestinal epithelium has not been elucidated yet. Nevertheless, Hisada et al[11] investigated the intestinal permeability of low molecular-weight hyaluronan (LMW-HA) using Caco-2 cells *in vitro* model and demonstrated that its permeability inversely increases with the molecular size and it is dose-dependent[11]. The aim of our study was to test whether the oral administration of LMW-HA prior and simultaneously to radiation can be an alternative approach in preventing and/or reducing cystitis symptoms as well as improving QoL in patients undergoing RT.

PATIENTS AND METHODS

A total of 42 patients aged between 55-87 years old were recruited from October 2014 to March 2015 in this non-randomized pilot study. Patients were selected according to the following criteria: 1) diagnosis of localized prostate cancer; 2) prostatectomy; 3) prescribed RT protocol. Eligible exclusion criteria: 4) history of acute urinary retention or bladder catheterization; 5) recurrent and persistent urinary tract infections, bladder stones; 6) drug hypersensitivity and refusal; 7) other malignant diseases. An oral informed consent was obtained before entering the study from each patient. The study was conducted following the Ethical principles of the Declaration of Helsinki and national laws. The nature of the study was explained in detail to patients and an oral consent was obtained from all of them. The study was a non-randomized clinical trial. Patients were divided in 2 groups. Eighteen patients received 1 tablet of LMW-HA (IALOS®, Lo.Li. Pharma, Rome, Italy) orally, twice a day (treated group). The treatment started 1 month before RT and was continued for all duration of RT. The other 24 patients followed the standard-RT protocol but did not undergo any preventive treatment (control group). Toxicity was recorded by an experienced surgeon for each patient, and toxicity criteria of the RT were evaluated according to the RTOG/EORCT grading system. The patients were evaluated before RT and weekly during RT for acute morbidity. Patients received the three-dimensional conformal radiotherapy (3D-CRT) by a linear accelerator with a full bladder and empty rectum. To evaluate the dose, the NCCN 2010 guidelines (http://www.nccn.org/) were followed. The total prescribed dose of 70 Gy was delivered in 35 daily fractions (Monday to Friday) to the whole prostatic gland, given in 2 Gy fractions with 18 MV photon beam. Physicians recorded patients' compliance during scheduled medical visits. The study outcome was the impact of treatment on RT-induced toxicity. The focus was on the RT-specific signs and symptoms subdivided into four grades: I, II, III, IV according to the RTOG/EORTC radiation toxicity grading[3].

Statistical Analysis

Significance was estimated by the Fisher exact test for a 2 x 2 contingency table. The two-tailed $p < 0.05$ was considered significant.

RESULTS

At baseline, all the 42 patients showed no toxicity. Each patient started the RT protocol 1-month post operation. Eighteen patients received LMW-HA 1

Table I. RTOG/EORTC radiation induced cystitis toxicity, in the treated and control groups at the end of trial.

		RTOG/EORTC radiation induced cystitis toxicity		*p*-value
		No	Yes	
Received LMW-HA	No	0/24 (0%)	24/24 (100%)	≤ 0.0001
	Yes	16/18 (89%)	2/18 (11%)	

Prevalence of radiation induced cystitis toxicity at the end of the trial. At baseline, all patients had no toxicity. Total patients 42 - treated: 18 patients LMW-HA + RT; controls: 24 patients RT. Significance was estimated by the Fisher exact test for a 2 x 2 contingency table. The two-tailed P value is less than 0.0001.

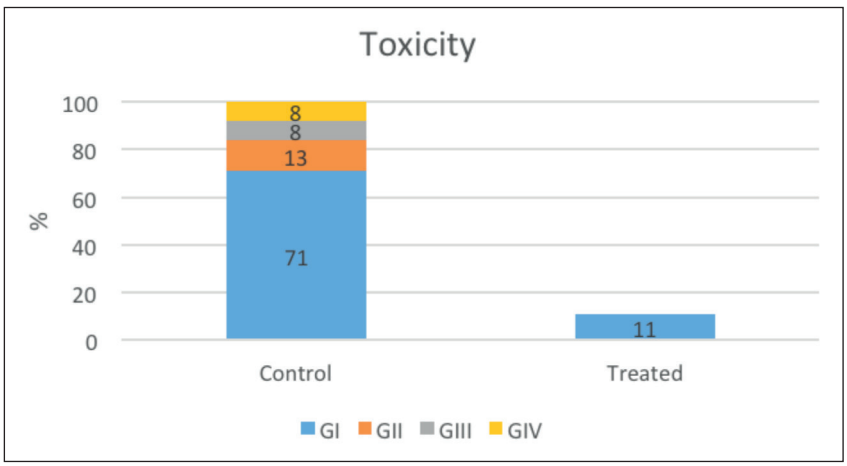
month pre-RT and during RT, the other 24 followed only the RT protocol. No dropouts over the trial period were recorded. Only two among eighteen patients (11%) in the treated group showed toxicity (Grade I), (Table 1, Figure 1). All patients in the control group (100%) showed toxicity from Grade I to Grade IV, (Figure 1). Differences in the response rate between control and the experimental group was extremely statistically significant ($p \leq 0.0001$). In particular, 17 patients showed Grade I, 3 patients Grade II, 2 patients Grade III, and 2 patients Grade IV (Figure 1). Moreover, after appearance of toxicity, 5 patients from the control group started treatment with LMW-HA removing completely toxicity (Data not shown).

DISCUSSION

Data presented herein suggest that patients affected by prostate cancer and undergoing RT, experienced a significant clinical benefit following treatment with LMW-HA. In 89% of patients enrolled in the experimental arm, was prevented the appearance of acute toxicity during RT. Moreover, toxicity was completely reduced after administration of LMW-HA in those patients who started the treatment following appearance of related-symptoms. Keeping in mind that acute and late toxicity are one of the most problematic aspects of RT[12], the prevention of correlated symptoms has caught the attention of clinicians in order to improve patients' QoL. Prostate cancer is one of the most life-threatening disorders in male[13]. Current medical approaches include surgery[14], radiation therapy[15], chemotherapy[16], cryosurgery[17] hormonal therapy[18], and other methods[19]. These approaches are quite effective either as monotherapy or in combination. However, many side effects are recorded during these treatments.

Urinary toxicity severely affects the QoL of patients undergoing postoperative RT. Therefore, the exacerbation by RT of urinary incontinence and urethral stricture formation, which are inherently associated with radical prostatectomy, represents a major issue[20]. Nevertheless, long-term urinary toxicity from postoperative RT has been little investigated. Unlike for the rectum, clinically relevant dose-volume parameters predictive of late genitourinary toxicity are less available, possibly because of the highly variable bladder filling, leading to difficulty in calculating the actual dose the bladder is receiving through treatment[21,3]. Histological features of radiation cystitis include microscopic progressive obliterating endarteritis that leads to mucosal ischemia. The ischemic bladder mucosa then ulcerates causing bleeding. Neovascularity occurs in the damaged areas, which causes the characteristic vascular blush on cystoscopic evaluation[22]. In contrast to acute changes, late radiation injuries are irreversible and progressive. The time interval between the treatment and development of delayed symptoms is inversely

Figure 1. Prevalence of RTOG/EORTC Radiation Toxicity Grading in treated and control groups at the end of trial.



Prevalence of the 4 different grades of toxicity according to RTOG/EORTC3 in both groups expressed as percentage (%).

proportional to the dose received[23]. Intravesical agents such as aluminum potassium sulfate, silver nitrate, formalin, or phenol are available mainly used as treatment for induced-hemorrhagic cystitis but often cause chemical corrosion of the bladder urothelium[24,25]. In general, it is best to manage patients conservatively and intervene only when necessary[26]. Hyperbaric oxygen (HBO) therapy has been extensively investigated in the management of radiation induced injuries[27]. HBO treatment reduces hypoxia and tissue perfusion by enhancing neovascularization[28], which may stimulate tissue repair and prevent infection; however, the high cost of this therapy is probably the cause of its drawback.

HA at a dose of 40 mg/ml solution for 30 min as a weekly intravesical instillation has be found to reduce the incidence of bladder complications by 33% when used as a preventive measure for radiation cystitis[29]. It associates to collagen, fibrin and other matrix molecules concurring to tissues homeostasis. Early response to tissue injury includes formation of temporary matrix rich in hyaluronan and fibrin which supports the influx of fibroblasts and endothelial cells into the wound site[30,31]. HA is found at high concentrations as a native homeostatic form within hydrated tissues such as the vitreous of the eye, articular cartilage, and lymphatics and skin. Due to its hydrophilic, it facilitates migration of cells to new tissue site, while its free-radical scavenging and protein exclusion properties protects cells and extracellular matrix molecules against free radical and proteolytic damage[32]. Many reports have attested the effects of exogenous hyaluronan in promoting wound healing. It has been demonstrated that LMW-HA facilitates the differentiation of many mesenchymal cells that are activated as a normal response following injury, including chondrocytes[33], fibroblasts growth factor (FGF-2) and keratinocyte growth factor (KGF)[34,35], and vascular smooth muscle cells (VSMCs). VSMC differentiation was associated with increased collagen deposition[36]. LMW-HA has shown to exert a reparatory action in dermal excisional wounds, associated with increased expression of CD44 and RHAMM and deposition of type-III collagen in aged mice[37]. Another study showed improved age-related skin function, when HA was administered to patients with skin atrophy in a CD44-dependent manner[38]. Topical administration of LMW-HA also acts as a scavenging agent following xenobiotic treatment, promoting wound healing[39]. In lungs, LMW-HA protected against porcine pancreatic elastase-induced bronchoconstriction[40]. Its protective effect against elastase was confirmed in a second model where aerosolized LMW-HA blocked experimental emphysema induced by intra-tracheal administration of elastase[41].

Moreover, La Galia et al[42] have recently demonstrated in a double blind, placebo-controlled trial, that orally administration of LMW-HA could ameliorate vaginal atrophy as well as improve epithelium thickness and number of epithelial layers in postmenopausal women. Furthermore, treatment with oral LMW-HA was able to alleviate the symptoms improving patients' QoL.

We are aware that administration of a drug to prevent a possible toxicity is not a standard practice and it needs strong motivations and rational drug-economic considerations to be performed. From 30% to 40% of patients present acute urinary toxicity after RT, despite the innovative techniques [43,44].

CONCLUSIONS

Our preliminary results highlight that patients might benefit of an early oral administration of LMW-HA as already demonstrated for others application fields[42]. We believe these are very promising results, because the low incidence of acute toxicity and no late toxicity recorded, implicates a substantial improvement of patients' QoL during RT. Moreover, our treatment seems to have remarkable benefits also in those patients in control group that had reduced toxicity symptoms after administration of LMW-HA. Keeping in mind that the systemic approach to oral administration, in comparison to bladder instillation, constitutes a cost saving for those patients that undergo RT, additional prospective clinical trials are necessary to validate our results.

Conflicts of interest:
All authors declare no conflict of interests.

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