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Hyaluronic acid for treatment of the radiation therapy side effects: a systematic review

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Abstract. – OBJECTIVE: The main limit of radiation therapy is the dose-dependent toxicity to healthy tissues. The 36% of patients exposed to radiotherapy for pelvic malignancies reporting gastrointestinal symptoms as incontinence, pain, mucus discharge, and bleeding (radiation proctopathy). In the cervix cancer, healthy tissues exposed to radiations easily develop inflammation of vaginal mucosa, bleeding and pain and to improve these symptoms, some medical devices were developed. One of the most interesting for its features is undoubtedly the hyaluronic acid. Considering the histological similarity between the vaginal and the rectal mucosa, the application of hyaluronic acid for the radiation proctopathy represents an interesting opportunity.

MATERIALS AND METHODS: We performed a literature search of MEDLINE, EMBASE, PubMed, and Research Gate for studies published up to March 2018. The following combination of medical subject headings, terms and free text words were used: 'hyaluronic acid', 'hyaluronate', 'topical application' and 'radiation proctitis'.

RESULTS: After the screening of titles and abstracts, and using the established criteria, 7 studies were selected for inclusion in the systematic review.

CONCLUSIONS: The clinical use of hyaluronic acid for topical administration in patients with inflammatory conditions at the level of the vaginal and anal mucosa, following radio and chemo-therapies, resulted an innovative approach to help patients in managing the AEs. Hyaluronic acid confirmed its totally safety profile and resulted effective in the inflammation decrease, improving the tissue health and the symptoms related. For all these reasons, we can easily promote the clinical application of hyaluronic acid on inflamed tissues though a substantial work is necessary to investigate more deeply the hyaluronic acid role on this context.

Key Words

Hyaluronic acid, Hyaluronate salt, Radiation therapy, Rectal mucosa, Inflammation.

Introduction

Pelvic malignancies, including prostate cancer, rectal cancer, and cervical cancer often can be subjected to radiation therapy (RT) and the main limit of this treatment is the dose-dependent toxicity to adjacent organs and healthy tissue. For example, in case of radical prostatectomy due to a pT3-4 prostate adenocarcinoma, patients are submitted to a postoperative radiotherapy (RT) as routine approach[1]. This treatment expects the irradiation of bladder, but the proximity of the rectum is a limiting factor in the safe delivery of dose-escalated RT. Radiation toxicity is classified into acute toxicity and late toxicity based on the timing of its occurrence after the completion of RT and toxicity rates, especially in the late-onset form, increase with RT dose[2], decreasing significantly patients QoL[3-5]. Among the adverse events (AEs) due to the radiation toxicity, dysfunctions of intestinal tract are some of the most impactful for patient's quality of life considering that an estimated 1.5 to 2 million cancer survivors presenting these side effects[6]. In 36% of patients subjected to RT for pelvic malignancies a variety of gastrointestinal symptoms were reported and one of the most represented is the radiation proctopathy (RP)[6]. The exposition of pelvic area to RT can be necessary to treat cancer in several organs in the pelvis, such as the anus, rectum, bladder, prostate, gynaecological organs (womb, ovaries, cervix, and vagina), small bowel, and pelvic bones and, in these cases, the risk to develop RP is well-known by physicians. Symptoms related with RP may occur around the time of treatment (early effects) or after a period of time, also many years later (late effects) due to long-term changes secondary to scarring (fibrosis), narrowing (stenosis), and bleeding due to new blood vessel formation (telangiectasia). The most common symptoms related with RP (rectal incontinence, pain, mucus discharge, rectal bleed-

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ing, pruritus, irritation, etc.) are totally comparable with the typical side effects at the vaginal level due to pelvic RT, e.g., for the cervix cancer. Indeed, mucosal inflammation, bleeding and pain, are shared AEs for these 2 kinds of mucosa after a RT. To prevent and counteract these AEs in gynaecology, some medical devices were developed with the aim, on one hand, to reduce inflammation and oxidative stress, regulating immune processes, and, on the other hand, to improve hydration. Thanks to its immunoregulatory and re-hydrating activities, the clinical use of hyaluronic acid (HA) represents one of the most interesting approach to help patients subjected to RT. Considering the histological similarity between vaginal and rectal mucosa and the resemblance of the AEs reported for these two different context after pelvic RT, the HA use for RP could be an interesting hypothesis that have to examine more deeply[7]. HA is a carbohydrate, more specifically a mucopolysaccharide, occurring naturally in all living organisms and consisting of a linear and energetically very stable polyanion formed by 3-D-glucuronic acid and D-N-acetylglucosamine units linked together by alternating β-1,4 and β-1,3 glycosidic bonds[8]. The number of repeat disaccharides in a completed hyaluronan molecule can reach 10,000 or more, with a molecular mass of about 4 million Da and a total extension up to more than 10 μm. In a physiological solution, the backbone of a hyaluronan molecule is stiffened by a combination of the chemical structure of the disaccharide, internal hydrogen bonds, and interactions with the solvent. The axial hydrogen atoms form a non-polar, relatively hydrophobic area while the equatorial side chains form a more polar, hydrophilic face, thereby creating a twisting ribbon structure that traps approximately 1000 times its weight in water. The relatively simple structure of HA is conserved throughout all mammals, suggesting that HA is a biomolecule of considerable importance. In the body, HA occurs in the salt form, hyaluronate, and is found in high concentrations in several soft connective tissues and it is the principal glycosaminoglycan in the body fluids such as synovial fluid, the vitreous humour, and Wharton's jelly of the umbilical cord as well as in skin and mucous membranes[9]. Its biological functions include maintenance of the elastoviscosity of liquid connective tissues, control of tissue hydration and water transport, supramolecular assembly of proteoglycans in the extracellular matrix, and numerous receptor-mediated roles in cell detachment, mitosis, migration, tumour development and metastasis, and inflammation. HA plays several important roles in the organization of extracellular matrix by binding with cells and other components through specific and nonspecific interactions[10]. Despite the numerous functions in which HA is naturally involved, due to its high molecular mass, it is not absorbed once applied to the skin or mucosa, forming a thin, light permeable, invisible, visco-elastic surface film. This reticular layer fixes the moisture on the surface helping to preserve the principal characteristics of a young and healthy tissue, such as smoothness, elasticity, and tone, and supporting the tissue's natural protective mechanism[11]. However, the continuous exposure to reactive oxygen species (ROS) and the potent activity as scavenger of HA induce the depolymerization processes which damage irreversibly these molecules. Oxidative stress results from the metabolic reactions that use oxygen, and it has been defined as a disturbance in the equilibrium status of pro-oxidant/anti-oxidant systems. When additional oxidative events occur, the pro-oxidant systems outbalance the anti-oxidant, potentially producing oxidative damage to lipids, proteins, carbohydrates, and nucleic acids[12]. When this process involves polymeric molecules like HA, it leads to chain breaks and a depolymerization process. In addition, as referred, HA is considered a potent scavenger of •OH, protecting cells from oxidative damages. At level of the oxidation site, HA assumes a radical structure, which may proceed through a series of modifications that affect its polymeric structure, favouring the chain resolution and water loose. In addition to the scavenger activity, HA is also considered an important regulator of inflammatory response able to help tissues healing. In the regulation of the inflammatory processes CD44 is undoubtedly one of the most important HA receptors. Indeed, CD44 is found on many cell types involved in inflammation, including leukocytes, chondrocytes, fibroblasts, and endothelial and epithelial cells and has been implicated in various processes such as lymphocyte recruitment though the precise mechanism remains unclear[13]. Hyaluronan, which is a hygroscopic macromolecule and its solutions are highly osmotic, forms a scaffold that several sulfur proteoglycans bind to. Such structures can reach large size and are able to trap large quantities of water and ions, providing hydration and tissue turgidity. Data from some studies pointed out that in the oral mucosa this property either enables the control of tissue hydration during inflammation process or it allows the response to tissue injury, which results

in ulcer formation. As its structure doesn't exhibit species specificity or tissue specificity, its pure form doesn't have any allergizing or immunogenic properties[14,15]. Up to date, HA is widely used in several other branches of medicine and neither contraindications nor interactions with drugs are reported[16-22]. Its very extensive use in the world is a consequence of its high level of safety. HA is widely used in aesthetic medicine as a filling material for folds and creases and to enlarge some parts of human body (such as lips, breasts, buttocks, etc.), it is also employed for the treatment of arthritis[17,22] and a lot of studies on the effect of HA in the lung disease are available in literature, as well[23]. Among the most interesting HA applications, the formulations developed for topical administration in patients with inflammatory conditions due to RT, e.g., at the level of the vaginal and anal mucosa, represent a truly innovative approach to help patients in managing the AEs related to these therapies, supporting them throughout the duration of the oncological treatment. At this regard, the aim of this work was to systematically review the published literature regarding the therapeutic activities of HA in topical formulations, highlighting the safety and the tolerability of this molecule, with attention for HA based treatments of ano-rectal tissues exposed to RT.

Materials and Methods

We performed a literature search of MEDLINE, EMBASE, PubMed, and Research Gate for studies published in English up to March 2018. We used the following combination of medical subject headings, terms and free text words: 'hyaluronic acid', 'hyaluronate', 'topical application' and 'radiation proctitis'. In addition, reference lists of scientific articles were reviewed to identify additional eligible studies. We followed the PRISMA checklist for meta-analysis (Figure 1)[24]. 596 scientific papers were evaluated, 59 studies were selected excluding those based on a clinical

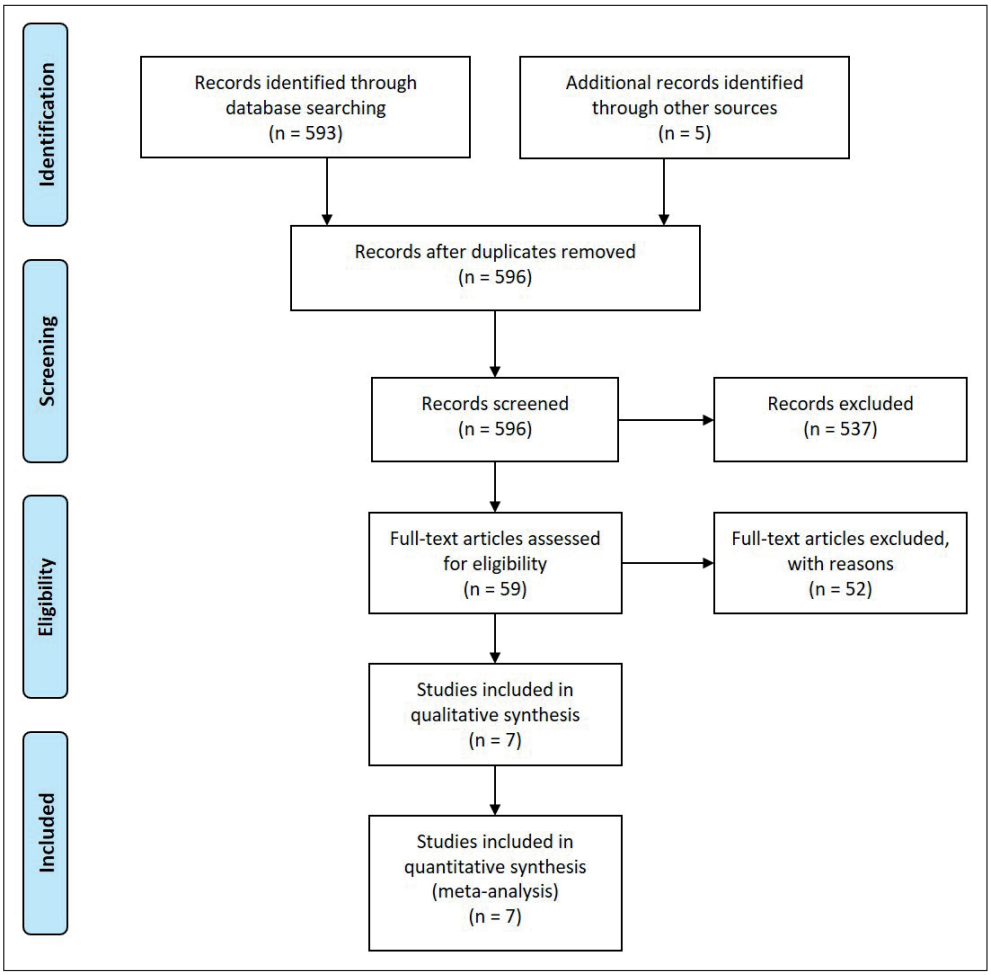

Figure 1. Flowchart showing selection of articles included in the review following the PRISMA checklist.

application of the HA as drug delivery system or for a mechanical activity. Among these 59 studies, only 7 randomized controlled trials (RCTs) investigating the activity of HA in the prevention or treatment of inflammatory conditions were reviewed and identified as eligible studies. Articles were critically reviewed for their eligibility by two reviewers (F P and D C). Among all collected articles, RCTs were identified by reading titles, abstracts and study design to select relevant studies for inclusion/exclusion criteria. Inclusion criteria restricted the search to: (a) inflammation regulatory activity of HA, (b) topical treatments. Exclusion criteria were: (a) clinical application of HA as drug delivery system or for a mechanical activity, (b) study population lower than 30 patients, (c) review papers (d) animal or cell culture works (e) and articles on scientific journals with an impact factor lower than 1. An arbitrary scale of scores from 0 (lowest score) to 5 (highest score) for each criterion was fixed, considering as our target of interest the ano-rectal mucosa reporting AEs due to pelvic RT. An average score of 3.5 points was defined as threshold and only studies that exceeded this value were considered for this analysis.

Results

After the screening of titles and abstracts, and using the established criteria, reports were selected for inclusion in the systematic review (Table I). Starting from these researches a scoring matrix was laid out (Table II). Below we provided a description of the studies that exceeded the threshold value and, therefore, were considered scientifically valid for this systematic review. The studies selected have evaluated the HA activities on two physiologically similar tissues, the ano-rectal and the vaginal mucosae[7]. Relatively to the rectal mucosa, Joksimovic et al[25] analyzed the HA activities on an inflammatory condition of this area such as haemorrhoids. Haemorrhoids are vascular cushions suspended in the anal canal, which play an important role in the maintenance of fecal continence. Their position is due to the presence of connective tissue and the fragmentation of this last just like of the extracellular matrix supporting haemorrhoids, due to age and the passage of hard stools, induces the descent of these cushions until the protrusion[26]. Willis et al[27] leads back the pathophysiology of haemorrhoids to a disorder of collagen metabolism, reporting an important reduction of collagen in the hemorrhoidal disease. Moreover, in these cases is reported a mucosal inflammation and consequently anal pain, bleeding and protrusion[28]. The application of creams and gels can be useful to protect the rectal mucosa helping passage of hard stools, though the adoption of products able to induce the physiological repairing processes would be an optimal choice. At this regard, Joksimovic et al[25] evaluated the activity, safety and tolerability of a gel containing HA in the hemorrhoidal disease. In this randomized, double blind, parallel group, placebo-controlled clinical study, a total number of 36 patients, suffering from haemorrhoids (grade 1°-3°), were enrolled (18 for HA gel treatment arm and 18 for the placebo gel treatment arm). The treatment lasted 14 days by which time, for the patients treated with HA gel, all symptoms (pain, inflammation, pruritus and bleeding) resulted markedly reduced as reported both by the patients and the investigators. According to the extracellular matrix degradation hypothesis for the haemorrhoids pathogenesis[26], the exogenous administration of HA, on one hand, reduces the symptoms, and, on the other hand, may contribute to rebuild the structures anchoring the haemorrhoids. Furthermore, the clinical data obtained by this trial confirm the safety and tolerability of HA treatment, as shown both by the global judgement of the investigators and by the absence of any side effect. Another interesting study, relative to the HA clinical application, was performed by Dinicola et al[29], who evaluated the ability of HA to counteract the onset of AEs due to radiation toxicity in patients with cervical cancer (CC) and exposed to pelvic RT and brachytherapy (BRT). Cervical cancer (CC) is the fourth most common cancer in women, the first one in developed countries. There are many factors which impact to the primary treatment of patients with CC as the staging, tumour characteristics, comorbidity, and patient[30]. Usually, the CC standard therapy is based on surgery. However, RT has proven to be fundamental for the CC treatment as adjuvant therapy of surgery, especially for larger and more advanced tumours, both for cancer treatment and recurrence prevention. Unfortunately, radiotherapic approach is characterized by a lot of side effects inducing a heavy worsening of quality of life. Among these, there are some vaginal alterations strongly associated with RT, like the vaginal atrophy and the subsequent inflammation of vaginal mucosa, two conditions characterized by several signs such as irritation, oedema, burning, itching and dyspareunia[31].

Table I. Clinical studies included - (AE) Adverse Events; (AH) Adeinodal Hypertrophy; (AOM) Acute Otitis Media; (BS) Baseline; (CHL) Conductive Hearing Loss; (CON) Control Group; (D) day; (DB) Double blind; (d/m) days/month; (EST) Estradiol; (HA) Hyaluronic Acid; (LMW-HA) Low Molecular Weight - Hyaluronic Acid; (M) months; (NaCl-Sol) Sodium Chloride Saline Solution; (NDN) Nasal Douche Nebulizer; (OME) Otitis Media with Effusion; (PBO) Placebo; (POP) Population; (PT) Patients; (RAOM) Recurrent Acute Otitis Media; (RD) Randomized; (RT) Radiotherapy; (SB) Single blind; (SD) Standard Deviation; (VAS) visual analogue scale; (W) weeks.

Authors	Patients	Disease	Therapy	Endpoints	Results	AE
Joksimovic et al[25]	36 PT: 18 HA treated; 18 PBO treated (3)	Haemorroids (4)	Gels applied of 1-1.5 g every 12 h for 14 Ds (3)	Primary: pain intensity, bleeding, pruritus, irritation, safety and tolerability Secondary: global assessment of the disease (5)	HA improves all symptoms. The difference between pre-post treatments was highly statistical significant relatively to pain during evacuation, pruritus, irritation and visible bleeding by the patient evaluation and for any symptom reported by the investigators (pain, inflammation and visible bleeding). (5)	No AE (5)
Dinicola et al[29]	45 PT: 23 HA treated; 22 CON (3)	Vaginal atrophy (RT - cervix cancer) (4)	2/D suppositories, for 4 Ms (5)	Primary: vaginal atrophy and related symptoms (3)	The 1° biopsy revealed mucosal inflammation, fibrosis and cellular atypia in 100% of PT. The 2° biopsy showed differences between the 2 groups: 81% of the CON showed mucosal inflammation, 40% more pronounced than in the HA group. HA group pre-post treatment: incidence of bleeding (14% *vs.* 56%), mucositis (14% *vs*. 50%), pain (27% *vs* 62%). Histologically, significant differences between HA-treated PT and CON: inflammation (23% *vs*. 75%); fibrosis (18% vs. 56%); atypia (23% *vs*. 62%). (3)	No AE (5)
Chen et al[32]	144 PT: 72 HA treated; 72 EST treated (5)	Vaginal atrophy (menopause) (3)	1 application of 5 g of HA or 0.5 g of EST twice a W (3)	Primary: improvement of vaginal dryness Secondary: improvement of other vaginal symptoms (4)	At BS, VAS ± SD of vaginal dryness symptoms was 5.76 ± 1.88 in the HA group and 5.26 ± 1.82 in the EST. At the final visit: the scores were 0.90 ± 1.18 and 0.62 ± 1.06, respectively (improvements of 84.44 ± 20.60% and 89.42 ± 17.21% respectively). No differences in HA vs. EST for secondary endpoint were reported. (4)	HA: 4 AEs EST: 6 AEs (3)
Ekin et al[39]	42 PT: 21 HA treated; 21 EST treated (3)	Vaginal atrophy (menopause) (3)	1/D EST tablet for 2 Ws, then 1 EST tablet twice per W or 1/D HA tablet for 8 Ws (4)	Primary: dryness, soreness, irritation and dyspareunia Secondary: assessments of epithelial atrophy, vaginal pH and cytology (3)	Significant relief of symptoms detected in both study groups ($p < 0.001$) after 8 weeks of treatment. A significant decrease of epithelial atrophy was detected in both groups after treatment. The vaginal pH was significantly decreased in both groups after treatment (more prominent in the EST group). Vaginal maturation values were significantly improved in both study groups after 8 weeks of treatment, (mean maturation value significantly higher in the EST group). (3)	No AE (5)

Continued

Table I *Continued*. Clinical studies included (Continued) - (AE) Adverse Events; (AH) Adeinodal Hypertrophy; (AOM) Acute Otitis Media; (BS) Baseline; (CHL) Conductive Hearing Loss; (CON) Control Group; (D) day; (DB) Double blind; (d/m) days/month; (EST) Estradiol; (HA) Hyaluronic Acid; (LMW-HA) Low Molecular Weight - Hyaluronic Acid; (M) months; (NaCl-Sol) Sodium Chloride Saline Solution; (NDN) Nasal Douche Nebulizer; (OME) Otitis Media with Effusion; (PBO) Placebo; (POP) Population; (PT) Patients; (RAOM) Recurrent Acute Otitis Media; (RD) Randomized; (RT) Radiotherapy; (SB) Single blind; (SD) Standard Deviation; (VAS) visual analogue scale; (W) weeks.

Authors	Patients	Disease	Therapy	Endpoints	Results	AE
Torretta et al[41]	116 PT: 58 HA treated; 58 CON (5)	Chronic ear inflammation (3)	3 ml of 0.9% NaCl-Sol 1/D or 9 mg of HA in 3 ml of a 0.9% NaCl-Sol 1/D. 15 Ds/M, for 3 Ms (2)	Primary: improving of signs of middle ear effusion (2)	Prevalence of PT with impaired tympanometry at the end of the follow-up period was significantly lower in the study group but not in the CON. The reduction in the prevalence of PT with CHL and those with moderate CHL was again significant in the HA group, but not in the CON. The mean auditory threshold had also significantly improved by the end of treatment in the study group but not in the CON. (2)	No AE (5)
Torretta et al[42]	103 PT divided in 2 groups: 54 in HA treated group, 49 in the CON (5)	Chronic adenoiditis associated with RAOM and OME (3)	3 ml of 0.9% NaCl-Sol 1/D or 9 mg of HA in 3 ml of a 0.9% NaCl-Sol 1/D. 15 Ds/M, for 3 Ms (2)	Primary: recurrency of AOM episodes and endoscopic outcomes (2)	Significant reduction in the mean number of AOM episodes after in the HA group (reduction 0.8 ± 0.4 per month). The mean number of episodes without tympanic membrane perforation was significantly lower than at BS in the HA group (reduction 0.6 ± 0.3 per month). HA reduced the prevalence of PT with severe AH, an obstructed Eustachian tube orifice, post-nasal drip, dyschromic and swollen nasal mucosa, moderate-severe turbinate hypertrophy, and nasopharyngeal, meatal, and anterior nasal secretions. (2)	No AE (5)
Liguori et al[43]	134 PT divided in 2 groups: 70 in HA treated group, 64 in the CON (5)	Skin inflammation (3)	HA cream or PBO cream application: first one 1-2 h after the morning RT, second in the evening. Treatment for 6 Ws (2)	Primary: Status of skin Secondary: Physician judgement on efficacy and tolerability (2)	No difference between the HA and the PBO groups at BS. Upgrading of the irradiated skin scores in the PBO group after 1 week of RT. In the HA group, a statistically significant difference was observed, after a 2-week observation period and lasting, as in the PBO group, until week 10. Statistical comparison in favour of the HA group at week 3 and throughout the RT treatment (from week 3 until week 7). A significant difference between the 2 treatments was still present at the first 2 controls of the follow-up period. Tolerability results show that both treatments were very well tolerated. (2)	PBO: 4 AE HA: 1 AE (3)

Table II. Scoring matrix of clinical studies included.

Authors	Total Score	Average Score
Joksimovic et al[25]	25	4.16
Dinicola et al[29]	23	3.83
Chen et al[32]	22	3.67
Ekin et al[39]	21	3.5
Torretta et al[41]	19	3.16
Torretta et al[42]	19	3.16
Liguori et al[43]	17	2.83

In the most serious cases, the patients can't complete the medical treatment and are obliged to interrupt it. Thanks to its structural and functional properties, HA more and more has caught the attention of physicians relatively to the hydrating products used for the vaginal atrophy treatment. Thanks to its several activities, HA represent a valid option to support this kind of patients. In this context, Dinicola et al[29] had designed this study as a prospective randomized clinical trial involving 45 women all suffering from CC. For each of them surgery, RT and chemotherapy were used to eradicate the cancer, to avoid recurrences and to act as adjuvant therapy. After surgery, all patients were treated with 4 weeks of RT and 4 weeks of BRT concomitantly with chemotherapy. The women were divided in 2 groups: in the HA group 23 women were treated with two suppositories of a product containing HA per day for 4 months. For the first two months the HA treatment was simultaneous to RT and BRT. In the control group, 22 patients did not undergone to any treatment during RT. To evaluate the efficacy of HA treatment three biopsies were performed. The typical symptoms of vaginal atrophy and stenosis were observed in all cases initially. Indeed, the presence of mucosal inflammation, fibrosis and cellular atypia in 100% of patients were reported by the histological assessment of the 1st biopsy. Relatively to the objective patient assessment, no anomalies resulted, and all women showed absence of bleeding, itching or burning at the vaginal mucosa. At the end of the RT treatment the 2nd biopsy was performed, showing significant differences ($p<0.05$) between the 2 groups of patients: 81% patients of the control group still showed mucosal inflammation while in the HA treated group was recorded a halved value relatively to the inflammation. Comparable differences ($p<0.05$) were found also for the fibrosis of the corium and relatively to the cellular atypia. Moreover, patients treated with HA showed a reduced incidence of bleeding (14% *vs.* 56%), mucositis (14% *vs.* 50%) and pain (27% *vs.* 62%). 2 months later the end of RT, at the 3rd biopsy, a further improvement was reported, as shown by the histological evaluation that demonstrated a significant difference between HA-treated patients and controls, relatively to inflammation (23% *vs.* 75%), fibrosis (18% *vs.* 56%), and cellular atypia (23% *vs.* 62%). No adverse event related with the HA treatment was reported during clinical trial. The use of topical hydrating products such as HA can be useful for Dinicola et al[29] to guarantee a protective and preventive action on the vaginal mucosa exposed to RT. Both histological findings and clinical recording support the beneficial effect that HA had on the vaginal mucosa after RT treatment. Treatment with vaginal suppositories containing HA have promoted an excellent healing with epithelial regeneration and proliferation while maintaining a good tropism, a vaginal elasticity and a sufficient lubrication. In the study of Chen et al[32], an evaluation of safety and efficacy of a treatment with HA vaginal gel on vaginal dryness in post-menopausal women compared with oestradiol (EST) cream in postmenopausal women was performed. This symptom is reported by about 14 million women aged 18 and over. About the 90% of menopausal and postmenopausal women reports vaginal dryness, describe it as a bother[33,34]. Actually, one of the most important therapeutic approach to treat the vaginal dryness is, undoubtedly, the topical application of products to hydrate the vaginal mucosa. The treatment of vaginal mucosa with oestrogen represents today an effective therapy to reverse the atrophic vaginal changes and improving symptoms[35-37]. Furthermore, many patients avoid this kind of formulation for a possible relationship, emerged in the last years, between these molecules and the onset of cancer or heart diseases[38]. In this context, an HA treatment could be representing an important alternative for the hydrating power of HA and for its role in the immunity regulation. For this reason, Chen et al[32] tested the efficacy of HA to demonstrate a non-inferiority compared to EST cream in the treatment of vaginal dryness symptoms. In this RCT, 144 participants were included; all were under 70 years old, had been naturally or surgically postmenopausal for more than 6 months, had symptoms of vaginal dryness due to various causes, and had no contraindications to locally applied oestrogen. Patients were randomly assigned in a 1:1 ratio either to the HA treated group or to the EST treated group. In the two

groups, treatments were applied every 3 days. To the HA treated group. 30-g aluminium tube with a vaginal applicator, providing a dose of 5 g, were provided. Instead, to the EST treated group was supplied a 15-g vial with a prefilled applicator, which provides a dose of 0.5 g. A dosage of one application twice a week was used for both treatments. No statistical differences were emerged between the two groups for any parameter nor did the analysis of the vaginal microecology laboratory examinations, vaginal pH value, and endometrial thickness. Although a higher improvement percentage of the vaginal dryness was reported in the EST treated group than in the HA group at the two visits, the lower limit of mean difference did not exceed the 15% noninferiority margin, demonstrating a comparable activity between these two treatments. Also, the improvement rate in vaginal itching, dyspareunia, and burning sensation were assessed and the differences between the two groups resulted no statistically significant for each symptom evaluated, confirming the ability of these two treatments to reduce the severity of vaginal dryness symptoms. Relatively to safety, 13 AEs were reported in total throughout the clinical trial. 7 AEs occurred in the HA group and, among these, only 4 cases could be potentially related to the HA treatment. In 2 cases vulvovaginal candidiasis (VVC) were found, while, in 1 case, the laboratory examination revealed a bacterial vaginosis (BV) but in all 3 cases AEs had mild severity with no clinical symptoms. In the last case potentially correlated with HA treatment, patient had VVC that resolved after receiving treatment. Relatively to the EST treated group, 6 AEs occurred, and, among these, 2 events were probably correlated with the treatment. In 1 case, laboratory examination revealed a mild BV with no clinical symptoms, an AE resolved receiving no relevant treatment. In the other subject, vaginal itching was reported, for this reason received concomitant medication and this symptom was resolved at the end of the study. In conclusion, data of this study highlight the substantive safety of these two treatments. Also, in the last study reported, Ekin et al[39] have compared the effectiveness of HA or EST vaginal tablets for the treatment of atrophic vaginitis in post-menopausal women. 42 postmenopausal women were involved and subsequently randomized to take 25 μg EST in vaginal tablets, initially, daily for 14 days and, subsequently, 1 tablet twice per week, or 5 mg of HA for 8 weeks. The symptoms evaluation was performed by a self-assessed 4-point scale determining also the degree of epithelial atrophy as, none, mild, moderate and severe. Moreover, vaginal pH and maturation index were evaluated and compared in the two groups. At the beginning of the treatment, the vaginal symptoms comparison between the two groups was not significantly different. After 8 weeks of treatment, instead, an improvement of symptoms was significantly reported in both study groups. Particularly, a higher relief of vaginal symptoms was recorded in the EST treated group. Relatively to the epithelial atrophy, after the treatment, a significant decrease was detected in both groups. A significant reduction of the vaginal pH was also reported in both groups after treatment. Relatively to the vaginal maturation, values significantly improved in both groups were also reported after the treatment. No AEs were reported during this trial, confirming the safety profile of these two treatments. Moreover, considering the improvement of vaginal symptoms, epithelial atrophy, vaginal pH and vaginal maturation reported in both groups as well as safety profile emerged in this study, the clinical application of HA for the treatment of chronic inflamed tissues gains even more credibility.

Discussion

All human tissues exposed to the air, like skin, airways, gastrointestinal routes or vaginal milieu, represent border areas but, above all, points of contact with environment. For this reason, their integrity is fundamental to allow a healthy communication between these two "worlds". When tissues fail this activity, an insane and conflictual relationship, characterized by inflammatory conditions. Tissues inflamed show similar features: redness, pain, atrophy, tissue thinning, and bleeding are some of the shared AEs by these tissues. Unfortunately, an inflamed tissue can be an unavoidable consequence of fundamental therapies as RT. Indeed, the interaction of ionizing radiation with any living organism induces a variety of responses and stress factors in the organism (e.g., DNA damage response, DNA repair, pro-inflammatory pathway initiation, free radical production like reactive oxygen and nitrogen species), either in the irradiated area both in the whole body through systemic (non-targeted) effects. Currently, the prevalent idea is that radiation exposure can only augment a pro-immunogenic phenotype and that healthy

tissue exposed to radiation are injured directly or indirectly through non-targeted effects. Among these effects, the radiation derived inflammation is, undoubtedly, the main driver of subsequent late effects including genomic instability. Inflammatory response is initiated by ionizing radiation usually very shortly after exposure with the primary function of controlling damage and repairing lesions. AEs arise when inflammation sustains for a long time after the completion of radiation treatment and subsequently turns from acute response to chronic late effect(s)[40]. At this regard, the development of therapies able to prevent or limit chronic inflammation, could allow to the patients to complete therapies reducing AEs. To reach this goal, some medical devices were developed attempting, on one hand, to reduce the inflammation and the oxidative stress, regulating the immune processes, and, on the other hand, to improve hydration. Thanks to its immunoregulatory and re-hydrating activities, the clinical use of HA represents one of the most interesting approach to help patients, especially during RT. The relatively simple structure of HA is conserved throughout all mammals, suggesting that HA is a biomolecule of considerable importance. In the body, HA occurs in the salt form, hyaluronate and is found in high concentrations in several soft connective tissues and body fluids such as: synovial fluid, vitreous humour, Wharton's jelly of the umbilical cord, skin and mucous membranes[9]. Its biological functions include maintenance of the elastoviscosity of liquid connective tissues, control of tissue hydration and water transport, supramolecular assembly of proteoglycans in the extracellular matrix and numerous receptor-mediated roles in cell detachment, mitosis, migration, tumour development, metastasis, and inflammation. HA plays several important roles in the organization of extracellular matrix through specific and nonspecific interactions with cells and/or other components[10]. The continuous exposure to reactive oxygen species (ROS) and the potent activity as scavenger of HA induce the depolymerization processes which damage irreversibly these molecules. Oxidative stress results from the metabolic reactions that use oxygen, and it has been defined as a disturbance in the equilibrium status of pro-oxidant/anti-oxidant systems. When additional oxidative events occur, the pro-oxidant systems outbalance the anti-oxidant, potentially producing oxidative damage to lipids, proteins, carbohydrates, and nucleic acids[12]. When this process involves polymeric molecules like HA, it leads to chain breaks and a depolymerization process. In addition, as referred, HA is considered a potent scavenger of •OH, protecting cells from oxidative damages. At level of the oxidation site, HA assumes a radical structure which may proceed through a series of modifications that affect its polymeric structure, favouring the chain resolution and water loose. In addition to the scavenger activity, HA is also considered an important regulator of inflammatory response able to help tissues healing. Data from some studies pointed out that in the oral mucosa this property either enables the control of tissue hydration during inflammation process or it allows the response to tissue injury, which results in ulcer formation. As its structure doesn't exhibit species specificity or tissue specificity, its pure form doesn't have any allergizing or immunogenic properties[14,15]. Up to date, HA is widely used in several other branches of medicine and neither contraindications nor interactions with drugs are reported[16-22]. Its very extensive use in the world is a consequence of its high level of safety. HA is widely used in aesthetic medicine as a filling material for folds and creases and to enlarge some parts of human body (such as lips, breasts, buttocks, etc.), it is also employed for the treatment of arthritis[17,22] and a lot of studies on the effect of HA in the lung disease are available in literature, as well[23]. Among the most interesting HA applications, the formulations developed for topical administration in patients with inflammatory conditions, e.g., at the level of the vaginal and anal mucosa, due to radio and chemo-therapies, represent a truly innovative approach to help patients in managing the AEs related to these therapies, supporting them throughout the duration of the oncological treatment. To evaluate the efficacy and the safety of this therapies a systematically review of the published literature regarding the therapeutic activities of HA in topical formulations were performed, with attention for treatments with HA suppositories of the ano-rectal tissues exposed to RT. Obviously, considering the aim of the HA clinical application, even more the safety profile of this kind of therapy is fundamental. At this regard, in all studies considered in this review, HA confirmed its totally safety profile. Only 4 AE on 134 patients exposed to HA were reported (2.98% of cases) and this percentage is reduced even more considering also the study which didn't exceed the threshold score of 3.5 on the arbitrary scale of scores 0-5 (5 AE on 316 patients – 1.58%). Moreover the 4 cases reported only potentially could be related to the HA treatment and anyway in 3 of 4

cases a mild severity was recorded without clinical symptoms. Relatively to the efficacy HA was used in comparison both with placebo treatments and with standard therapies as made with the EST treatment. In all cases, independently both from the pharmaceutical form (gel or tablets) and from the tissues treated, HA resulted effective in the inflammation decrease, improving the tissue health and the symptoms related. Interestingly, from all papers, it was emerged not only HA hydrating properties but, above all, its regulation activities on the immunity system.

Conclusions

From the analysis of the scientific literature, the potentialities of HA as support therapy in patients exposed to pelvic radiotherapy clearly emerge. The clinical use of hyaluronic acid for topical administration in patients with inflammatory conditions at the level of the vaginal and anal mucosa, following radio and chemotherapies, is resulted an innovative approach able to help patients in managing the AEs. As previously reported, HA confirmed its totally safety profile and resulted effective in the inflammation decrease, improving the tissue health and the symptoms related. For all these reasons, we can easily promote the clinical application of hyaluronic acid on inflamed tissues though a substantial work is necessary to investigate more deeply the hyaluronic acid role on this context. Undoubtedly, even though these studies are really encouraging, they are still very few. For this reason, larger studies and, particularly, RCT will be necessary to investigate the HA role on this context.

Conflict of Interests

The Authors declare that they have no conflict of interests

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