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CHEMO- AND RADIOTHERAPY INDUCED URINARY SIDE EFFECTS: THE ROLE OF GLYCOSAMINOGLYCANS

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Chemo- and radiotherapy-induced urinary side effects: the role of glycosaminoglycans

The annual EAU Congress at Madrid this year from March 20 to 24 was an enormous success with more than 14,000 participants from over 100 countries, offering a forum for clinicians with a variety of different expertise to exchange data and ideas in areas of clinical relevance. One of these subareas was chemo- and radiotherapy-induced cystitis. Treatment-related cystitis is a pathology that is drawing increased attention from urologists for several reasons. Chemo- and radiotherapy-induced cystitis has been reported with varying percentages during treatment, generally in around 20-30% of cases. This high percentage is still observed despite best prophylactic measures, which further highlights their relative inefficacy. The condition can be the source of considerable discomfort for sufferers, with symptoms including dysuria, increased urinary frequency and urgency and haematuria, which may become chronic. In the most severe cases, it is associated with significant morbidity, hospitalisation and even death.

This relatively common complication of chemotherapy and radiotherapy is a challenging condition to treat, especially considering the paucity of established guidelines on its optimal definition and management. There are few evidence-based clinical studies demonstrating the efficacy of treatments currently in use. The most commonly used therapies include intravesical treatments such as sodium hyaluronate, chondroitin sulphate and prostaglandins, systemic treatments such as hyperbaric oxygen therapy, oestrogen, sodium pentosan polysulphate, recombinant factor VII or VIII and aminocaproic acid. In more severe cases of non-responders, surgery may be indicated. Considering the range of therapeutic options available at present, there is still the objective need for a more effective approach based on solid clinical evidence.

In recent years, therapy with glycosaminoglycans (GAG) has substantially broadened the therapeutic options for chemo- and radiotherapy-induced cystitis. This relatively new approach is gaining favour among urologists due to its encouraging efficacy and tolerability profile. The scientific programme at EAU 2015 included a satellite session entitled ‘Chemo- and radiotherapy-induced urinary side effects: The role of glycosaminoglycans’, which provided urologists with an overview of the pathophysiology of treatment-induced bladder injury, treatment options and the available evidence for GAG therapy in the management of the condition. These are all important aspects given that urologists are likely to see an increase in the number of cases of chemo- and radiotherapy-induced cystitis as the global burden of cancer is predicted to rise. Thus, this symposium - hosted by the Institut Biochimique SA (IBSA) - offered the possibility to gain increased knowledge of the condition and its treatment with the aim of helping clinicians better understand and manage it. The present supplement to Urologia summarises the presentations made by leading experts during that symposium, which I had the privilege of chairing.

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THE ROLE OF GLYCOSAMINOGLYCANS (GAGs)

Chemotherapy and pelvic radiotherapy-induced bladder injury

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ABSTRACT

An understanding of the basics of the anatomy of the bladder mucosa is essential to better understand the pathophysiology of chemo-and radiotherapy-induced cystitis. Following an overview of bladder anatomy and the definitions and causes of bladder injury, the mechanisms of cyclophosphamide (CP)-induced bladder injury are discussed as a specific example.

Keywords: Chemo- and radiotherapy-induced cystitis, Glycosaminoglycans, Therapy

Functional anatomy of the bladder mucosa

The wall of the bladder has three layers that are histologically well-defined (Fig. 1) (1). The first is the mucosa, or innermost portion, followed by the muscularis propria and the adventitia/serosa layer. The mucosa is in turn composed of the urothelium, basement membrane and lamina propria. It also contains sparse smooth muscle cells and blood vessels. The basal membrane separates the mucosa from the urothelium. The urothelium itself is composed of three layers. A basal cell layer is attached to the basement membrane. Below the superficial apical layer lies an intermediate layer. As is well known, the urothelium plays a key role in maintaining the barrier function of the bladder. On the apical layer a GAG layer is present, which is composed of glycosaminoglycans. The GAG layer is thought to have a number of functions, including protection of the urothelium from urinary microorganisms and toxins. Umbrella cells on the apical surface have a unique asymmetric unit membrane that contains several proteins, notably uroplakins, occludins and claudins that are present in tight junctions (1). All these structures contribute to the barrier function of the bladder mucosa. There is also recent evidence suggesting that administration of liposomes may play a role in coating the surface of the urothelium, which may help in restoring the barrier function of the urothelium (1). During distension of the bladder, the shape of the superficial epithelium changes considerably. As part of this process, there is an increase in cellular exocytosis and decreased endocytosis, which adds membrane to the apical cell surface. This process allows for increases in bladder volume without loss of barrier function. There is evidence to suggest that during stretch-induced exocytosis there is increased expression of several signalling molecules including epidermal growth factor receptor (EGFR). The increased expression of EGFR likely augments vesicular traffic and increases exocytotic activity (1). All these processes allow the bladder to adapt to differences in urine volume without compromising its barrier function.

During bladder filling, a so-called ‘sensory web’ is involved, which refers to the cascade of transduction mechanisms underlying the activation of afferent fibres. The sensory web includes the urothelium, the afferent and efferent nerves and the interstitial cells of the lamina propria.Interstitial cells serve as the functional link between the urothelium, afferent nerves and detrusor muscle. As part of the physiological process of bladder filling, ‘crosstalk’ between these structures through various mediators ensures normal function. In fact, the urothelium responds to physical and chemical stimuli releasing signalling molecules and transfers complex information to and from the nervous system. In this way, it is able to modulate detrusor function either directly or indirectly (1). Thus, the urothelium acts as a functional and dynamic barrier.

In recent years, several pathways which form parts of the sensory web have been studied (Fig. 2) (1). These pathways involve acetylcholine, adenosine triphosphate (ATP), adenosine and nitric oxide (NO). Acetylcholine is an important signalling molecule that is released in the epithelium and appears to target nicotinic and muscarinic receptors. In this way, acetylcholine modulates the release of both ATP and NO and depending on the type of muscarinic receptor; the effects of acetylcholine may be either excitatory or inhibitory. Acetylcholine can activate both nicotinic and muscarinic receptors on afferent nerves and myofibroblasts to modulate...
sensory signalling, which directly affects bladder contraction. Thus, acetylcholine in the urothelium can facilitate and inhibit afferent signalling.

In addition to serving as the main source of energy of the cell, ATP plays an important role in normal detrusor physiology (2). ATP modulates exocytosis and endocytosis via P2X and P2Y receptors. ATP is also able to signal the degree of bladder filling to the central nervous system via P2X and P2Y receptors. Lastly, ATP is associated with detrusor contraction. Abnormalities in release of ATP have been linked to several pathologies, including interstitial cystitis, urinary urgency and incontinence.

Adenosine is an additional signalling molecule that acts in response to stretch that accompanies bladder filling. It is thought to modulate sensory afferent function and contraction of detrusor smooth muscle cells (2). In fact, stretch induces the release of adenosine by targeting adenosine receptors A1, A2 and A3, which in turn inhibit the release of ATP. Adenosine also modulates afferent nerve activity, while smooth muscle cells are relaxed by adenosine, via A2b receptors. Lastly, NO pathways appear to play a key role in regulating normal bladder function. Stretch induces release of NO and is thought to be mediated through the protein transient receptor potential vanilloid 1 (TRPV1). NO formed by endothelial nitric oxide synthase has been linked with an increase in the barrier function by desensitisation of afferent nerves and attenuation of detrusor contraction. NO from inducible nitric oxide synthase, on the other hand, has also been linked to disruption of barrier function via sensitisation of afferent nerves.

**Chemotherapy- and pelvic radiotherapy-induced haemorrhagic cystitis**

Haemorrhagic cystitis (HC) can be induced by both radio- and chemotherapy, and can be either acute or chronic (3, 4). The condition often results in storage-type lower urinary tract
symptoms and haematuria. It is generally thought that damage to the GAG layer coating the urothelium is the initial trigger for development of HC. Haematuria may range from non-visible to gross haematuria with visible clots causing urinary tract obstruction. It is often graded as mild, moderate or severe depending on the degree of pain and haematuria (5). HC is defined as acute when present during treatment and within 3-6 months following the end of treatment. It is considered chronic when it is present for more than 6 months after the conclusion of therapy. The incidence of HC varies with the type of treatment received. For systemic chemotherapy, busulfan, CP, idarubicin, ifosfamide, paclitaxel/carboplatin commonly induce HC, while doxorubicin, epirubicin and mitomycin C are frequently reported causes of HC in patients undergoing intravesical chemotherapy (4). Chemotherapy-induced HC is reported in <10% to up to 35% of patients undergoing chemotherapy, while radiation-induced HC is reported in 5-10% of patients.

Recently proposed mechanisms of CP-induced bladder injury

CP is an antineoplastic agent used since 1958 for the treatment of Hodgkin’s disease, solid tumours, Wegener’s granulomatosis, rheumatoid arthritis and systemic lupus erythematosus (3, 6, 7). Many cases of CP-induced HC have been reported. Bladder injury in CP-induced HC appears to occur through a complex mechanism (Fig. 3). In the liver and possibly kidney, CP is metabolised to phosphoramide mustard (PAM) and acrolein after which it is renally exported to the bladder where it comes into contact with umbrella cells (3). Although PAM was the primary metabolite, it was acrolein which played the role as the causative agent in CP induced HC. Acrolein causes injury to the GAG layer resulting in loss of barrier function. Acrolein has two mechanisms of action related to its toxicity. It firstly increases the production of reactive oxygen species, and secondly is able to cleave proteins. This induces activation of the nuclear factor-κB apoptotic pathway. The superoxide peroxynitrate radical breaks DNA, while energy sources such as NAD and ATP are deleted. As a result of these latter mechanisms, necrosis occurs resulting in cell death.

Several studies have recently investigated the mechanism of action of CP-induced cystitis in detail, mostly using an experimental rat model. The study by Kim et al investigated the protective effects of diallyl disulphide against CP-induced acute urotoxicity in rats (8). CP-induced severe HC was found to be associated with increased urinary bladder epithelial cell apoptosis, protein expression of nuclear factor erythroid 2-related factor-2 (Nrf-2), NAD(P)H:quinone oxidoreductase-1 (NQO-1) and heme oxygenase-1 (HO-1). Moreover, significant decreases in glutathione content and catalase, glutathione-S-transferase and glutathione reductase activities were observed along with an increase in the level of malondialdehyde content, which suggests that CP-induced bladder injury is mediated, at least in part, through oxidative stress.

This mechanism is somewhat different to that proposed for radiotherapy-induced cystitis (9, 10). In fact, radiation causes breaks in DNA leading to activation of DNA repair genes and radiation-induced apoptosis. In addition, radiation penetrates the muscle causing endarteritis, which compromises the blood supply leading to tissue hypoxia. This has the effect of detrusor being replaced with fibroblasts, with the result that the bladder has decreased compliance and capacity.

As noted by Lee et al in their review, the CP metabolite acrolein causes apoptosis and increases expression of EGFR in urothelial cells (3). The EGFR in urine can then initiate cell proliferation by binding to the EGFR, which leads to formation of a hyperplastic urothelium that maintains the barrier function, although it is depleted in umbrella cells.

Several studies have examined the beneficial effects of several agents in CP-induced cystitis. Kyung et al studied uroplakin II expression in the urinary bladders from CP-induced rat cystitis following intravesical administration of epinephrine (11). Interestingly, rats treated with epinephrine showed less submucosal oedema and haemorrhage, and preserved expression of uroplakin II. This demonstrates that epinephrine may have a protective effect against CP-induced cystitis, which involves expression of uroplakin II.

Funahashi et al examined the effects of intravesical application of rebamipide on bladder inflammation and overactivity in CP-induced cystitis in rats (12). Histological analysis showed that bladder inflammation in CP-treated rats was inhibited by rebamipide, and expression of myeloperoxidase, IL-1β, IL-6 and TNF-α was also suppressed. Inflammation was associated with upregulation of the expression of nociceptive receptors and afferent sensitivity. Furthermore, rebamipide was found to inhibit the urotoxicity of acrolein. Considering these findings, these authors suggested that rebamipide may represent a novel treatment option that warrants further development.

Zhang et al examined the function of P2X3 and NK1 receptor antagonists on CP-induced cystitis in rats (13). In CP-induced HC, expression of P2X3 and NK1 receptors was increased in the urothelium and suburothelium. However, perfusion of antagonists of P2X3 and NK1 receptors decreased receptor expression and improved bladder function. Lastly, Ho et al assessed the effect of hyaluronic acid (HA) on
urine nerve growth factor (NGF) in CP-induced cystitis (14). HA instillation significantly increased the intercontraction interval as measured by cystometrogram, and also decreased the urinary levels of NGF. There was only sparse coating of HA on the bladder mucosa. These authors suggested that the improvements in cystitis might be related to the NGF signalling pathway and provide further support for the possibility that HA may be an effective treatment for CP-related bladder overactivity.

Conclusions

- The urothelium has sensory web function in addition to its barrier function in normal micturition.
- Bladder injury caused by many chemotherapeutic agents and radiotherapy results in voiding dysfunction and haematuria.
- The mechanism is complex and consists of DNA breaks and induction of apoptosis through oxidative stress.
- Radiotherapy causes progressive vascular endarteritis which leads to tissue hypoxia, fibrosis and decreased bladder capacity.
- Loss of uroplakin II expression and the GAG layer, together with increased expression of the P2X3 and NK1 receptors, urothelial NO formed by iNOS, inflammation and NGF activity may play a role in CP-induced HC.

Disclosures

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References

THE ROLE OF GLYCOSAMINOGLYCANS (GAGs)

Treatment of bladder urothelium injury

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ABSTRACT

The management of patients with cystitis-related symptoms due to urinary tract infection, bladder pain syndrome (BPS) or radio/chemo-induced cystitis remains challenging. A component in the pathophysiology of these symptoms relates to the fact that the urothelium is a highly metabolically active structure and that alterations in this structure can give rise to a variety of symptoms.

Keywords: Glycosaminoglycans, Chemo- and radiotherapy induced cystitis, Therapy

Symptoms of urothelial dysfunction

Many of the symptoms suffered by patients with BPS/interstitial cystitis (BPS/IC) are believed to be related to dysfunction of the glycosaminoglycan (GAG) layer, which alters its barrier function (1, 2). Furthermore, the urothelium has a range of regulatory functions that are controlled via neurotransmitters such as NO, ATP, acetylcholine and adenosine as detailed previously. These factors act to provide the urothelium with a semi-permeable barrier function. Dysfunction in the regulatory function of the urothelium gives rise to symptoms of overactive bladder syndrome and pain, as seen in BPS/IC and chemo and radiotherapy-induced haemorrhagic cystitis (HC). As stated above, urothelial dysfunction can be associated with a variety of factors, including BPS/IC, trauma, urinary tract infection and chemotherapy and radiotherapy.

Significant progress has been made into better understanding the pathophysiology of these conditions (Fig. 1). Several important hypotheses have been developed, which are related to autoimmunity, traumatic abrasion of the GAG layer and mast cell activation. In animal models, mast cells induce cystitis pain and regulate bladder pathophysiology through the actions of histamine and tumour necrosis factor (TNF), respectively (3). The ESSIC (International Society for the Study of BPS) identifies mast cell infiltrates in detrusor muscle as a diagnostic criterion for BPS/IC. However, it is increasingly recognised that low-grade, occult infection due to fastidious microorganisms is also related to the pathophysiology of these symptoms. Finally, GAG alterations are another key component in the symptoms of urothelial dysfunction.

Glycoaminoglycans are complex carbohydrates based on saccharides (Fig. 2). They are a highly heterogeneous group of compounds. Their final structure is modulated by enzymes, and as a consequence, they have diverse biological roles. For example, they form the basis of heparin, have essential functions in synovial tissue and joints and have an important membrane function in the bladder. In fact, the urothelium is coated with a GAG layer that is composed of hyaluronic acid, heparins and chondroitin sulphate (CS, Fig. 3). When the GAG layer is depleted or damaged, a number of protective functions are compromised. The barrier function is lost (or at least depleted) and urothelial permeability is increased. The altered permeability can result in an inflammatory response, with hypersensitisation of nerves. As a consequence, patients may present with symptoms of pain, frequency, urgency and haematuria. The symptoms are variable in terms of both severity and duration and symptoms can vary greatly between individuals.

Haemorrhagic cystitis

Haemorrhagic cystitis symptoms also present as a variable clinical spectrum and it is important to clarify both the severity and duration of symptoms. It is also important to exclude other causes of haematuria and pain, as HC can mimic conditions such as urinary tract infection, BPS/IC and pelvic malignancies.

HC is generally graded from grade 1 (non-visible haematuria) to grade 4 (gross haematuria and clot formation) (2). The condition can also be classified as early or late-onset. Acute HC often presents with mild to severe dysuria, increased urinary frequency and urgency, with various grades of haematuria. Chronic HC, in addition to the symptoms of acute HC, may result in potentially irreversible urinary symptoms, sphincter dysfunction, reduced bladder capacity and urothelial ulceration. Basic clinical assessment should include a comprehensive clinical history, physical examination, urinalysis and urine culture, as well as cystoscopy. The diagnosis may also require additional investigations, including urine cytology and imaging, such as computed tomography urogram or magnetic resonance imaging.

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Fig. 1 - Pathophysiology of bladder diseases.

Fig. 2 - Structures of some disaccharide monomers that form glycosaminoglycans.

Fig. 3 - Distribution of GAG components in the bladder wall.
Treatment of bladder urothelium injury

**TABLE I** - Summary of studies on intravesical monotherapy for chemotherapy and radiotherapy-induced cystitis

<table>
<thead>
<tr>
<th>Study design</th>
<th>Patients, n</th>
<th>Treatment</th>
<th>Efficacy</th>
<th>Adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chondroitin sulphate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hazewinkel (2011)</td>
<td>Comparative (RT for gynae ca)</td>
<td>20</td>
<td>CS vs. no CS</td>
<td>Trend towards better control</td>
</tr>
<tr>
<td>Sodium hyaluronate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shao (2012)</td>
<td>Randomised (RT for pelvic malignancy)</td>
<td>36</td>
<td>SH vs. HBO</td>
<td>75% vs. 50% (12 months) 50% vs. 45% (18 months) (p = ns)</td>
</tr>
<tr>
<td>Sommariva (2010)</td>
<td>Prospective (Chemo for bladder cancer, RT for CaP)</td>
<td>69</td>
<td>SH</td>
<td>Pain ↓ Capacity ↑ (4 w) (p&lt;0.01)</td>
</tr>
<tr>
<td>Delgado (2003)</td>
<td>Retrospective (RT for gynaecological cancer)</td>
<td>90</td>
<td>SH vs. no SH</td>
<td>RT toxicity: 1.24 vs. 0.71 (p&lt;0.004)</td>
</tr>
<tr>
<td>Samper (2009)</td>
<td>Retrospective (BT for gynaecological cancer)</td>
<td>95</td>
<td>SH vs. no SH</td>
<td>Bladder toxicity: 2% vs. 13% (p&lt;0.05)</td>
</tr>
</tbody>
</table>

**Treatment of haemorrhagic cystitis**

As with many diseases, prevention is the best strategy (1, 2) and several prophylactic measures are commonly implemented during chemotherapy and radiotherapy. These include hyperhydration, bladder irrigation and the use of mesna (Uromitexan®). However, in general, the results of prophylactic treatment of HC are poor.

Both systemic and intravesical therapies are used for treatment of established HC (Fig. 4). Systemic therapies include hyperbaric oxygen (HBOT), oestrogens, sodium pentosanpolysulphate, recombinant factor VII or VIII and aminocaproic acid. Intravesical therapies have a greater evidence base than systemic therapies, and are usually reserved for symptomatic and refractory cases. These therapies include CS and sodium hyaluronate (HA). Prostaglandins, formalin and alum have also been used infrequently and the evidence to support use of these latter agents is limited.

**The potential role of glycosaminoglycans in cystitis**

More recently, GAG replenishment therapy with CS, heparin, HA and a new combination of CS and HA (ialuril®, IBSA) have been used. Table I summarises a summary of the most relevant investigations involving intravesical monotherapy for chemotherapy and radiotherapy-induced cystitis. Most of the available studies are comparative or retrospective, rather than randomised and/or controlled. One exception is the study by Shao et al (4). This trial randomised 36 patients undergoing radiotherapy for gynaecological malignancies to receive either HA or HBOT. It found no significant differences between the two groups in terms of haematuria, voiding frequency or visual analogue scale (VAS) pain at 6, 12 and 18 months after treatment, except for a decreased frequency of voiding at 12 months in the HA group.

A smaller number of studies have investigated the efficacy of intravesical dual therapy (Tab. II). A single randomised study has been carried out in which 28 women with recurrent bacterial cystitis who were randomised to either intravesical instillation of HA/CS or antibiotic prophylaxis using sulfamethoxazole (5). The HA/CS group was found to have significant improvement in the number of urinary tract infections (UTIs) 3-day voiding, VAS pain, quality of life, frequency symptoms (pelvic pain and urinary/frequency (PUF) symptom scale) and maximum cystometric capacity (MCC). However, this was a small study with a limited number of patients and a relatively short follow-up of 12 months.
Thus, although there are limitations in the available clinical data supporting the use of intravesical HA/CS therapy, the results from these early studies are encouraging. In addition, the safety profile of these therapies has been shown to be favourable, and no significant adverse events have been documented. There is also emerging evidence that combination therapy with HA/CS appears to have a durable response, lasting up to 3 years. However, these studies have similar limitations and further research is needed.

**Conclusions**

- GAG replenishment therapy is being increasingly used in patients with HC, as well as in those with a variety of other chronic bladder symptoms.
- The available data are encouraging, but limited.
- Dual intravesical therapy with HA and CS preparations can relieve symptoms and may alter the disease process and prognosis of HC.
- Other treatments, such as HBOT and pentosan polysulphate, are also available, but their use is supported by very limited clinical data.

**Disclosures**

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**References**

New horizons for GAG therapy in the management of urothelial damage

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ABSTRACT
Urothelial defects may manifest as various types of signs of symptoms such as frequency and urgency, haematuria and pain. Both prevention and treatment of urothelial damage is fundamental. Many currently adopted treatments lack robust clinical data and are associated with variability in management strategies. Glycosaminoglycan (GAG) therapy is one of the most promising therapies in treatment of chemotherapy and radiotherapy-induced urothelial defects, and has been investigated in both animal models and clinical studies.

Keywords: Glycosaminoglycans, Chemo and radiotherapy induced cystitis, Therapy

Effect of HA + CS instillation on cyclophosphamide-induced cystitis in a rat model

The effects of instillation of hyaluronic acid + chondroitin sulfate (HA + CS) have been studied in a rat model of cystitis induced by CP (San Raffaele Hospital and URI, unpublished data on file). In this controlled study, on day 0, following catheter implantation, rats were instilled postsurgically with HA + CS or saline. On day 1, cystitis was induced by CP, and 4 hours later, intravesical instillation (30 min exposure) with HA + CS or saline was performed. The following day, an additional intravesical instillation was made with either HA + CS or saline, and on day 3, animals were assessed by cystometry and pain behaviour test (30 min exposure to acetic acid and evaluation of pelvic licking) prior to sacrifice and histological evaluation. The main parameters examined were micturition volume and interval in addition to area under the curve of detrusor pressure during voiding (AUC) and non-voiding bladder contractions (NVBCs). As expected, treatment with CP reduced micturition volumes and intervals compared with untreated rats. Compared with animals that administered saline, HA + CS significantly (p<0.05) increased the micturition interval vs. saline-treated rats from about 275 s to roughly 400 s (Fig. 1). Micturition volume was also significantly increased from about 500 µl to around 750 µl. Considering AUC and NVBC, functional markers of overactive bladder, treatment with HA + CS was associated with significant decreases in both parameters vs. saline (Fig. 1). Cumulative licking time following exposure to acetic acid, a surrogate marker for pain, was likewise decreased by about one-half in rats treated with HA + CS compared with saline (45 vs. 100 s; p = 0.0063). Figure 2 shows the histological evaluation of urothelium where it can be seen that HA + CS had a protective effect against CP-induced urothelial damage. Thus, in this animal model, bladder instillations with HA + CS show positive effects in reducing CP-induced bladder injury, as also supported by histological evidence.

Clinical studies on GAG replenishment therapy

A total of four clinical studies have been published on the use of GAG therapy in patients with different types of chemo or radio-induced cystitis.

HA + CS in late radiation tissue cystitis

Sommariva et al studied the effects of intravesical HA + CS in patients with symptomatic late radiation tissue cystitis (1). In this 12-month, prospective, longitudinal, non-randomised, investigative pilot study, patients were treated with intravesical instillations of HA + CS, the frequency of which depended on the severity of haematuria. Patients with severe haematuria received daily instillations 5 days/week in the first month, 3 days/week in the second month, 2 days/week in the third month, once weekly in months 4-6, every 2 weeks in months 7-8, every 3 weeks in months 9-10 and monthly/bimonthly for 1 year. Patients without or with occasional haematuria received...
Starting from a mean baseline of 66.9 ml, bladder capacity significantly increased to 101.9 at 3 months and to 174.4 at 12 months (p<0.05 for both vs. baseline). Voiding frequency also significantly decreased from 14.6/day at baseline to 10.5 at month 3 and 8.8 at month 12 (p<0.001 for both vs. baseline). In addition, significant increases were observed at months 3 and 12 in terms of improvement in the quality of life as measured by the EQ-5D and EQ-5D VAS (Fig. 3). Thus, in this study, intravesical co-administration of HA and CS improved bladder function and symptoms in patients with late radiation tissue cystitis. The association of HA and CS also improved quality of life in this group of patients.

**HA + CS in treatment of nocturia due to postradiation bladder pain**

After radiotherapy for prostate cancer, up to 50% of patients can have nocturia (2). Giannessi et al investigated a group of patients with nocturia related to postradiation bladder...
Changes in quality of life measured with the EQ-5D (A) and EQ-5D VAS (B) following treatment with intravesical instillation of HA + CS in patients with late radiation tissue cystitis.

This study evaluated the impact of HA + CS on symptoms and bother related to nocturia in men with bladder pain syndrome. Twenty-three consecutive patients with a mean age of 67.9 years with bladder pain syndrome due to pelvic irradiation for locally advanced prostate cancer (16 treated with radical prostatectomy along with radiotherapy and seven with radiotherapy alone) were included. Patients underwent intravesical administration of HA + CS weekly for the first month, and subsequently after 6, 8 and 12 weeks. Nocturia was assessed by item 3 (Q3) of the Interstitial Cystitis Symptoms Index (ICSI, the ‘symptom’ nocturia) and item 2 (Q2) of the Interstitial Cystitis Problem Index (ICPI, the ‘bother’ nocturia). Both questionnaires were self-administered immediately after radiotherapy and at the end of treatment with HA + CS (12 weeks) to evaluate symptoms.

Mean ± SE of the mean pre and post-treatment ICSI-Q3 was 2.13 ± 0.28 and 1.61 ± 0.21 (% of Delta pre-post: -24.4%, p = 0.001) (Fig. 4). At logistic regression, both age and baseline ICSI-Q3 had a negative impact on this item (r = 0.293, p = 0.011 and r = 0.970, p = 0.000). Mean ± SE of the mean pre and post-treatment ICPI-Q2 was 1.87 ± 0.26 and 1.30 ± 0.25 (% of Delta pre-post: -30.5%, p = 0.016). Therefore, in this challenging group of patients with post-radiation bladder pain syndrome, HA + CS was effective in reducing nocturia and related bother. Nonetheless, further studies are needed to confirm the result of this pilot study.

**HA + CS in Bacillus Calmette–Guérin induced chemical cystitis**

Bacillus Calmette-Guérin (BCG)-induced chemical cystitis unresponsive to conventional therapies represents a considerable challenge for clinicians. Histologically, it is characterised by an intense inflammatory reaction involving the lamina propria associated with non-specific reactive atypia of the overlying urothelium that may be partially or entirely denuded. The study by Imperatore et al investigated treatment of BCG-induced chemical cystitis unresponsive to traditional treatments with intravesical administration of HA + CS (4). This was a retrospective chart review of patients with BCG-induced grade 2 chemical cystitis who had failed conventional therapies performed for at least 2 months according
to the International Bladder Cancer Group recommendations and who underwent intravesical instillations with HA + CS. Grade 2 chemical cystitis was defined as severe and/or more than 48 h cystitis according to the WHO grading scale. Data on follow-up collected at 8 weeks, 6 months and 1 year intervals using a 3-day voiding diary and VAS scores for patient-reported measures of urinary urgency and pain were collected together with adverse events.

A total of 20 patients (nine males and 11 females) were identified. Chemical cystitis arose after a mean of three BCG instillations (range: 1-5). First-line therapy for cystitis consisted of quinolone antibiotics and anti-inflammatory agents in all cases. All patients suspended BCG instillations and received intravesical instillations (40 ml) of HA + CS once weekly for 8 weeks. HA + CS was well tolerated in all patients, and no adverse effects were recorded. At all follow-up times, significant improvements were recorded. At all follow-up times, significant improvements were recorded in VAS scores for pain and urgency, voids/24 h and urine volume per void (Tab. I). Significant improvements were observed throughout the 12-week study period. These authors concluded that intravesical instillation of HA + CS is an efficacious strategy for refractory BCG-induced chemical cystitis with results that appear to be long-lasting.

### TABLE I - Outcome measures recorded before treatment and at follow-up in patients with BCG-induced chemical cystitis unresponsive to traditional treatment and treatment with HA + CS

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>8 weeks follow-up</th>
<th>6 months follow-up</th>
<th>1-year follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAS score for pain, mean (range)</td>
<td>7.2 (6-8)</td>
<td>4.2 (3-7)*</td>
<td>4.4 (3-7)*</td>
<td>4.2 (2-6)*</td>
</tr>
<tr>
<td>VAS score for urgency, mean (range)</td>
<td>7.8 (7-9)</td>
<td>4.7 (3-7)*</td>
<td>4.5 (3-8)*</td>
<td>4.3 (2-5)*</td>
</tr>
<tr>
<td>Voids/24 h, n, mean (range)</td>
<td>15.4 (12-18)</td>
<td>9.6 (8-13)*</td>
<td>9.1 (7-12)*</td>
<td>9.0 (7-11)*</td>
</tr>
<tr>
<td>Urine volume per void ml, mean (range)</td>
<td>85.8 (25-150)</td>
<td>194.1 (80-300)*</td>
<td>210.9 (80-300)*</td>
<td>220.7 (90-310)*</td>
</tr>
</tbody>
</table>

*p < 0.05 vs. baseline.

**Fig. 5 - Results of VAS, IPSS and bladder diaries at baseline and at the end of BCG induction cycle.**

**HA in combination with Bacillus Calmette–Guérin**

Although BCG is considered to be an effective treatment to reduce recurrence and progression of non-muscle invasive bladder cancer (NMIBC), it is also associated with local treatment-related side effects that can lead to discontinuation or interruption. Topazio et al investigated whether sequential administration of HA could reduce the side effects related to BCG (5). A total of 30 consecutive subjects undergoing BCG intravesical administration for high-risk NMIBC were randomised to receive either BCG alone or BCG and HA. VAS was used to evaluate bladder pain; International Prostate Symptom Score (IPSS) and number of micturitions per day were evaluated in the two groups before and after 6-weekly instillations. Patients were also evaluated at 3 and 6 months by cystoscopy and urine cytology.

Mean VAS for pain was significantly lower in the group receiving the combination of BCG and HA (Fig. 5; p = 0.04). Post vs. pre-treatment differences in VAS for pain, IPSS and number of daily micturitions were all significantly lower in the group receiving BCG and HA. At 6-month follow-up, three patients in the BCG and four patients in the BCG and HA group
presented with recurrent pathology. Thus, this preliminary data suggest that HA may have a role in reducing the local side effects of BCG, and that additional studies are warranted on the basis of these encouraging results. On the basis of the results in other studies with HA + CS, the question can also be raised as to whether the addition of HA + CS to BCG would be even more effective than the addition of HA alone.

**Clinical levels of evidence for GAG replenishment therapy**

Considering the available evidence, only the study by Topazio et al has a somewhat high level of evidence (2b, individual cohort study or low-quality randomised controlled trials; <80% follow-up). However, that study had a small number of patients who were followed for a relatively short period. The remaining studies described herein have a level of evidence of 4 (case-series and poor quality cohort and case-control studies). Thus, additional studies are needed to validate the promising clinical results obtained to date with GAG replenishment therapy.

**Conclusions**

- The experimental rationale for HA + CS is sound.
- Preliminary clinical evidence for GAG therapy is encouraging.
- The safety profile favours and supports the need for randomised clinical trials.

**Disclosures**

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**References**


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