Interactions of BCG with urothelial tumor cells in immunotherapy for superficial bladder cancer
Bevers, R.F.M.

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Mycobacterial adherence and BCG treatment of superficial bladder cancer

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² Laboratory of Pathology, National Institute of Public Health and Environmental Protection (RIVM), Bilthoven, The Netherlands

Abstract

The importance of adherence of BCG (Bacillus Calmette-Guérin) to the bladder wall as an initiator of the processes leading to the BCG-induced antitumor activity is still controversial. A study was initiated addressing this subject by an experimental procedure modulating BCG adherence using pre-treatment with pentosan polysulphate (PPS), a polysulphated polysaccharide with glycosaminoglycan (GAG) like properties and reported bacterial anti-adherence properties to the bladder mucosa. Furthermore, PPS is applied as a drug to treat chronic and radiation induced cystitis. It was reasoned that
application of PPS during BCG treatment might prevent cystitis, a common side effect. However, nothing is known about a potential interaction of PPS with the effectiveness of BCG treatment. The results obtained with guinea pigs receiving prior to each of the 6 weekly instillation with BCG-RIVM (1 x 10^7 cfu) an intravesical pre-treatment with 10 mg PPS in 1 ml for 0.5 hours indicated an enhancement of the PPD skin reaction, inflammatory response and number of iliac lymph node cells after instillation 6 compared to non-pretreated animals. These results, contrary to the expected, were supported by the indication of an increased binding of [3H]uracil-labeled BCG to the bladder after PPS pre-treatment. To explain these results, the binding of PPS to the bladder wall and BCG were quantitated spectrophotometrically with DMB (dimethylmethylen blue). After administration of 40 µg, 80 µg, and 10 mg in appropriate volumes into the rat guinea pig & human bladder 0.9 ± 0.3, 4.3 ± 1.1 µg, and 5.7 ± 1.8 mg PPS (n > 5) were recovered respectively, showing a strong adherence. Furthermore, in contrast to commonly found bacteria associated with cystitis, a significant adherence of PPS to mycobacteria was observed: 1.7, 3.6, and 3.1 µg/mg dry weight of BCG Connaught, RIVM and Pasteur respectively and 0.2, 0.3, 0.7, and 0.0 µg/mg dry weight of E. coli, Streptococcus faecalis, Klebsiella pneumoniae, and Proteus respectively.

In conclusion, the present results could explain: (a) the inflammatory reaction associated with BCG therapy by a binding of BCG to the GAG-layer of the bladder and (b) the increased BCG response in guinea pigs after enhancing this binding by pre-treatment with PPS. It is tempting to speculate about the possibility to increase the effectiveness of BCG treatment by PPS.

**Introduction**

Intravesical instillation of BCG (Bacillus Calmette-Guérin) has been recognized as an effective (adjuvant) therapy for superficial bladder cancer. As a treatment for residual Ta/T1, grade 1-3 tumors the overall complete response rate is approximately 50%, while for Tis tumors this figure appeared to be 55-90% (Herr et al., 1987; Reitsma, et al., 1989). Although repeated bladder instillation of BCG is well tolerated, side effects have been reported, including local (90%) and systemic (3%) inflammation (Lamm, et al., 1986). However, severe complications are rare. The immunological mechanisms responsible for the effectiveness of BCG therapy in bladder cancer still remain to be established (Ratliff, 1989). Occurrence of (long-term) adherence of BCG to the bladder is controversial, although binding to fibronectin of the bladder mucosa has been proposed as a major initial factor (Ratliff, 1989; Steerenberg, et al., 1989).
In order to study the adherence mechanism of BCG to the bladder wall and in an attempt to reduce adverse effects of BCG, a study was initiated using pentosan polysulphate (PPS). PPS is a polysulphated polysaccharide, which have been reported to protect the bladder wall against bacteria and various urinary (harmful) chemicals (Parson, et al., 1980; Parson, 1982). Covering the bladder PPS forms a polyanionic layer, resembling the natural GAG-layer of the uninjured bladder mucosa (Parson, et al., 1990; Hurst, et al., 1987). Currently nothing is known about the interference of PPS with the effectiveness of BCG as an antitumor modality.

In the present study, the interaction of PPS with BCG-associated reactions was investigated in the guinea pig after intravesical PPS treatment prior to BCG instillations. Furthermore, adhesion of PPS to the bladder wall was measured in the guinea pig, rat and human. Adherence of PPS to bacteria was tested for different BCG-preparations and some commonly found bacteria associated with cystitis.

Materials and methods

**BCG-Induced Effects in the Guinea Pig**

BCG-RIVM (RIVM, Bilthoven, The Netherlands) was administrated intravesically in female guinea pigs as described previously (van der Meijden, et al., 1989). Briefly, BCG-RIVM, containing 1 x 10^7 cultivable particles/1 ml buffered saline, was instilled for 0.5 hours into the empty bladder in anaesthetized animals. BCG was instilled once weekly for 6 consecutive weeks. Control animals were treated similarly with a placebo preparation. In animals, receiving PPS (Fibrezym; Bene, Germany) prior to BCG, the weekly pretreatment consisted of a 10 µg PPS in 1 ml buffered saline instilled into the bladder for 0.5 hours, immediately followed by BCG treatment.

The PPD (purified protein derivate of Mycobacterium tuberculosis) skin reaction was performed by injecting 20 µg PPD-RIVM intradermally. At 24 hours after the PPD injection the diameter of the skin reaction was measured. After 6 weeks the animals were sacrificed. The bladders were excised, fixed and prepared for histological examination. The number of infiltrates in the bladder wall was noted. The iliac lymph nodes were also resected. They were weighed and the number of cells per 2 iliac lymph nodes was counted (van der Meijden, et al., 1989).

**Adherence of PPS to the Bladder**

PPS was instilled into the bladder at a concentration depending on the organism. After 0.5 hours the bladders of the guinea pigs and rats were
emptied by gentle pressing on the pubic region. In humans, voided urine was collected after 0.5 hours. The amount of PPS in the collected urine was determined.

**Adherence of PPS to Bacteria**

BCG-RIVM, BCG-Pasteur, BCG-Connaught, *Escherichia coli*, *Streptococcus faecalis*, *Klebsiella pneumoniae*, and *Proteus* were grown in standard culture media, respectively. At the end of the exponential growth phase the bacteria were centrifuged at 4500 rpm for 5 minutes, washed and resuspended in appropriate volumes of PBS. After incubation (0.5 hours) with various concentrations PPS the bacterial suspension was centrifuged and the remaining PPS was determined in the supernatant.

**Determination of PPS**

PPS was determined spectrophotometrically at 525 nm with DMB (dimethylmethylen blue) and PPS as a standard curve, modified after de Jong et al. (1989).

**Results**

**Effects of PPS on BCG-induced reactions in the guinea pig**

The effects of pretreatment with PPS on the BCG-induced reaction in the guinea pig were determined after instillation 6. After this instillation all BCG-treated animals showed a positive PPD skin reaction and an increased number of bladder infiltrates and number of iliac lymph node cells compared to the placebo group. The data observed for these parameters after pretreatment with PPS followed by BCG treatment suggest a PPS-induced enhancement of the BCG-associated responses (Table 1).

<table>
<thead>
<tr>
<th>Table 1. The influence of PPS pretreatment on BCG-induced effects in guinea pigs at instillation 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
</tr>
<tr>
<td>PPD skin reaction (mm)</td>
</tr>
<tr>
<td>Number of infiltrates</td>
</tr>
<tr>
<td>Weight of 2 iliac lymph nodes (mg)</td>
</tr>
<tr>
<td>Number of cells/2 iliac lymph nodes (x 10^6)</td>
</tr>
<tr>
<td>Binding of [3H] Uracil labeled BCG to the bladder (cpm x 10^3)</td>
</tr>
</tbody>
</table>
**Adherence of PPS to the bladder wall and bacteria**

In order to explain this unexpected enhancement of BCG-associated reactions by PPS, the adherence of PPS to the bladder wall of the guinea pig and to BCG were determined. In addition, adherence to the bladder of the rat and human were measured. The results, presented in Table 2, showed a strong adherence of PPS to the bladder of the investigated organisms. In agreement with this observation, an increased binding of $[^3H]$uracil-labeled BCG was indicated after pretreatment with PPS compared with BCG instillation alone. As shown in Table 3, the binding of PPS to the three Mycobacteria preparations was approximately 2 to 4 μg PPS/mg dry weight of bacteria. In contrast, the investigated bacteria, commonly found in the bladder, did not bind significant amounts of PPS, with the possible exception of *K. pneumoniae*.

**Table 2. Adherence of PPS to the bladder of the guinea pig, rat, and human**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Instilled Adherence</th>
</tr>
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<tbody>
<tr>
<td>Guinea pig (n=5)</td>
<td>80 μg / 1 ml 76 ± 1 μg</td>
</tr>
<tr>
<td>Rat (n=7)</td>
<td>40 μg / 1 ml 39 ± 1 μg</td>
</tr>
<tr>
<td></td>
<td>80 μg / 1 ml 40 ± 3 μg</td>
</tr>
<tr>
<td>Human (n=3)</td>
<td>10 mg / 100 ml 4 ± 2 mg</td>
</tr>
</tbody>
</table>

**Table 3. Binding of PPS to various bacteria**

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>PPS adherence (μg/mg dry weight [n=3])</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCG-RIVM</td>
<td>3.6 ± 0.3</td>
</tr>
<tr>
<td>BCG-Pasteur</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td>BCG-Connaught</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Streptococcus faecalis</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Proteus</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>

**Discussion**

Following the data reported in literature, characterizing PPS as a bacterial anti-adherence agents (Parson, et al., 1980; Parson, et al., 1990), it was reasoned that PPS may be useful to prevent or at least reduce the side effects occurring during BCG therapy of superficial bladder cancer (Lamm, et al.,
1986). However, nothing is known about the potential interference of PPS with BCG therapy. Consequently, the effects of PPS on BCG-associated reaction were studied in an animal model system, the guinea pig (van der Meijden, et al., 1989). Furthermore, prevention/ reduction of the BCG-induced inflammatory reaction by PPS could jeopardize a recently developed hypothesis about the working mechanism of BCG (Schamhart, et al., in press 1991). Within this hypothesis, it was suggested that an early, recurrent, transient inflammatory reaction, reflected by elevated IL-6 titers, is an obligate condition that increases the probability of a T-cell mediated or delayed type hypersensitivity (DTH) reaction reflected by urinary IL-2 levels.

The observed binding of PPS to the bladder wall by direct measurements is in agreement with the indirect observations of others (Parson, et al., 1990; Hurst, et al., 1987), showing a PPS-related reduction of bacterial adherence to the bladder wall. Although at present not statistically significant, an unexpected finding of this pilot study was the enhancement of BCG-induced reactions, PPD skin test number of infiltrates and iliac lymph node cells. Accepting the reduction of bacterial adherence to the bladder by PPS, diminished BCG-induced reactions were expected (Parson, et al., 1980; Parson, 1982).

However, determination of PPS adherence to bacteria for the first time seems to explain these conflicting observations. The absence of PPS adherence to gram positive/negative bacteria commonly found during cystitis is in accordance with the reported bacterial anti-adherence properties of PPS. In contrast, the relatively strong binding of PPS to Mycobacteria may (indirectly) anchor BCG to the bladder wall. This possibly results in an enhancement of BCG-induced reactions. In conclusion, it is tempting to speculate about the possibility to increase the effectiveness of BCG therapy by pretreatment with PPS.
References
