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Neopterin as a Monitoring Parameter for Treatment with BCG in Superficial Bladder Cancer

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INTRAVESICAL BACILLUS Calmette-Guerin (BCG) therapy has been demonstrated to be one of the most effective treatments for both prophylaxis and cure of superficial transitional cell carcinoma (STCC) of the bladder [1–5]. The mechanism by which BCG mediates antitumour activity is still unclear [6–10]. The local application usually results in an immune reaction [8, 11]. Clinical investigations have shown that immune activation can be qualified by measuring soluble immune reaction products in serum and urine, such as interleukins, interferon-γ and tumour necrosis factor. We therefore investigate neopterin in serum and urine after intravesical therapy with BCG.

30 patients with high-risk STCC were treated once a week with intravesically instilled BCG. We used 75 mg BCG Pasteur Paris. Instillations were retained for 2 h. Urine and blood samples were collected before therapy (baseline) and 4, 24, 48 and 96 h after each instillation of BCG during the 6 weeks of therapy. Neopterin was measured with commercially available radioimmunoassays. Friedman's test was used to determine whether concentrations at five different times were equal. Differences between two different times were tested with the Wilcoxon signed rank test. Statistical analysis for the groups was conducted with the median values.

Statistically significant changes were found in neopterin concentrations of serum and urine. Highest serum neopterin concentrations were found 48 h after BCG instillation and were significantly higher than concentrations before BCG, 4 h and 24 h after BCG ($P = 0.028$) (Figure 1). Urinary neopterin concentration was also significantly higher 48 h after treatment with BCG (Figure 2).

The mechanisms by which BCG mediates antitumour activity have not been clearly established. Ratliff's hypothesis is that antitumour response induced by instillation of BCG is followed by induction of the appropriate immunological events which lead to tumour destruction [12]. Neopterin, a non-specific marker, is released by activated macrophages, which are known to represent a potent effector system for elimination of tumour cells. Huber and associates demonstrated that macrophages,

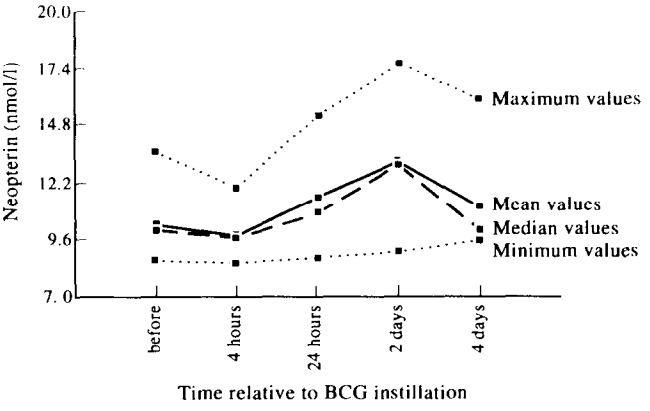

Figure 1.

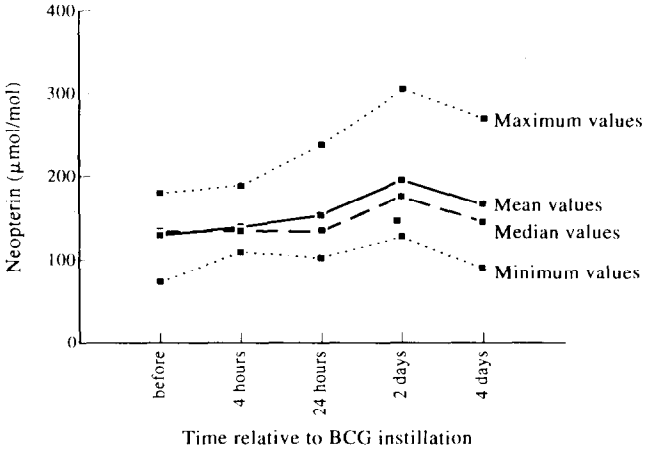

Figure 2.

when exposed to interferons, release large amounts of neopterin [13]. There is no literature on neopterin in serum measured during intravesical BCG therapy. Similar to the results of Huber, we observed statistically significant changes in serum neopterin after instillation with BCG, which is interesting since it indicates a systemical reaction after only an endovesical treatment. Elevated urinary neopterin levels in patients with bladder cancer are well known, but are not elevated in stage T1 [14]. Although neopterin is a non-specific marker for diagnosis of bladder cancer, it seems to be valuable as a parameter of therapy: 71% of our patients, all responders, showed a significant peak of neopterin in serum and urine after each administration of BCG, with no enhancement of neopterin after 6 weeks, although we used BCG only in a low-dose regimen. The question now remains as to whether successful BCG treatment is dose-dependent. We feel that the individual immunological situation of the patient may be responsible for the success of BCG therapy. The individual dose should possibly correspond with neopterin in serum and urine as a parameter for monitoring treatment course.

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1. Debruyne FMJ, Denis L, Van der Mejden APM. EORTC Genitourinary Group Monograph, 6. BCG in superficial bladder cancer. *Prog Clin Urol* 1989, **310**, 98–101.
2. Kelley DR, Haaff EO, Becich M. Prognostic value of purified

protein derivate skin test and granuloma formation in patients treated with intravesical BCG. *J Urol* 1986, **135**, 268–271.
3. Lamm DL. BCG—immunotherapy for bladder cancer. *J Urol* 1985, **134**, 40–47.
4. Mack D, Jakse G. Topische BCG Therapie des *in situ* Karzinoms der Harnblase. *Urologe A* 1987, **25**, 22–25.
5. Morales A, Eidinger D, Bruce A. Intracavitary BCG in the treatment of superficial bladder cancer. *J Urol* 1976, **116**, 180–185.
6. De Jong WH, Steerenberg PA, Ruitenberg EJ. BCG and its use cancer immunotherapy. In Den Otter W, Ruitenberg EJ, eds. *Tumor Immunology—Mechanisms, Diagnosis, Therapy*. Amsterdam, Elsevier, 1987, 283–287.
7. Hamma MG Jr, Snodgrass MJ, Zbar B, Repp HJ. Histopathology of tumor regression after intralesional injection of *Mycobacterium bovis*: IV Development of immunity of tumor cells and BCG. *J Natl Cancer Inst* 1987, 51–54.
8. Ratliff TL, Gallen DP, Catalona WJ. Requirement of a thymus-dependent immune response for BCG-mediated antitumor activity. *J Urol* 1987, **137**, 155–158.
9. Ratliff TL. Role of the immune response in BCG for bladder cancer. *Eur Urol* 1992, **2**, 17–21.
10. Van der Meijden APM, De Jong WH, De Boer EC, Steerenberg PA, Debruyne FMJ, Ruitenberg EJ. Immunological aspects of intravesical administration of Bacillus Calmette Guerin in the guinea pig. *Urol Res* 1989, **17**, 47–50.
11. Van der Meijden APM, Steerenberg PA, Van Hoogstraaten IMW, *et al.* Immune reactives in patients with superficial bladder cancer after intradermal and intravesical treatment with BCG. *Cancer Immunol Immunother* 1989, **28**, 287–292.
12. Ratliff TL. Mechanisms of action of intravesical BCG for bladder cancer. EORTC Genitourinary group Monograph. 6. BCG in superficial bladder cancer. *Prog Clin Biol* 1989, **10**, 107–111.
13. Huber C, Batcheler JR, Fuchs D, *et al.* Immune response associated production of neopterin release from macrophages preliminary under the control of IFN-gamma. *J Exp Med* 1984, **60**, 310–316.
14. Frick J, Aulitzky W, Fuchs D, *et al.* The value of urinary neopterin as an immunological parameter in patients with malignant tumors of the genitourinary tract. *Urol Int* 1985, **40**, 155–159.

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Soluble Intercellular Adhesion Molecule-1 in Melanoma Patients Treated with Liposomes Containing Muramyl Tripeptide

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A SOLUBLE form of intercellular adhesion molecule-1 (sICAM-1) has been recently identified in patients with malignant melanoma [1]. It has been demonstrated that inflammatory cytokines can modulate the cellular expression of ICAM-1 [2] and the shedding of this molecule by cells [3–5]. To our knowledge, few data exist on serum sICAM-1 levels in cancer patients treated with immunomodulators [6]. Liposomes containing muramyl tripeptide (MLV MTP-PE) can activate monocytes from cancer patients *in vitro* and *in vivo*, making them cytotoxic for tumour cells, and increasing the serum levels of inflammatory cytokines, such as tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6) [7–10]. The purpose of the present study was to evaluate the levels of sICAM-1 and their possible correlation with serum inflammatory cytokine levels in melanoma patients treated with MLV MTP-PE. The sera from 9 patients with metastatic melanoma, treated with MLV MTP-PE 4 mg intravenously (i.v.) twice a week for 12 weeks, were tested using an ELISA system to detect sICAM-1, TNF-α, IL-6, IL-1β and interferon-γ (IFN-γ) before, 2 and 24 h after the first, 12th and 24th infusion of MLV MTP-PE. Baseline sICAM-1 levels were elevated in all 9 patients (median 540 ng/ml; range 400–1030). Two hours after the first infusion, serum sICAM levels were similar to baseline values; the median value was 565 ng/ml (range 250–940). Twenty-four hours after the first infusion of MLV MTP-PE, we observed an increase in sICAM-1 levels in 6 patients, a decrease in 1 and stable values in 2, and the median value at this point was 720 ng/ml (range 410–1820; $P=0.060$).

At the 12th infusion, all of the 7 evaluable patients had elevated baseline sICAM-1 levels (median 500 ng/ml: range 415–1080), and 2 h later the median value was 450 ng/ml (range 330–1440). Twenty-four hours after the 12th infusion, sICAM-1 increased in 3 patients and did not change in 4 patients; the median value was 790 ng/ml (range 495–1650; $P=0.069$).

At the 24th infusion, baseline and 2-h median values of sICAM-1 were similar (650 and 630 ng/ml, respectively) in the 6 evaluable patients. At 24 h, sICAM-1 increased in 4 of the 6 evaluable patients and remained stable in 2; the median value, which at this time point was significantly higher than baseline levels, was 802 ng/ml (range 510–1450; $P=0.045$). In order to analyse the variations in sICAM-1, the patients were arbitrarily divided into two groups according to the clinical behaviour of their tumours: 4 patients had stable disease (all lesions, 2 patients; some lesions, 2 patients; group A); and 5 patients had progressive disease (group B). In group A, sICAM-1 levels remained stable or showed a modest increase during treatment (except in 1 patient, who exhibited a substantial variation after the 12th infusion) (Figure 1a). In contrast, very high levels of sICAM-1 were observed in group B, at the beginning of the study therapy in 1 patient and after the first infusion in 3 other patients; these values remained high until the 24th infusion (Figure 1b).

In the majority of patients, TNF-α and IL-6 increased in 2 h after the first infusion, but not therafter. IFN-γ was never detected; IL-β was detectable in a few cases, but only before the infusions.

In conclusion, baseline levels of sICAM-1 were elevated in all patients, and increased further during treatment in patients with more aggressive disease. In our experience, no correlation was found between sICAM-1 and inflammatory cytokines. There were no differences in the cytolytic activity of monocytes or in the number and intensity of inflammatory episodes (fever or an increase in acute phase reactants) between the two groups of patients and, moreover, their cytokine production was also similar. Consequently, we may speculate that the higher levels of sICAM-1 observed in patients with progressive disease could

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