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Urothelial Cancer

Celecoxib has Potent Antitumour Effects as a Single Agent and in Combination with BCG Immunotherapy in a Model of Urothelial Cell Carcinoma

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Abstract

Objectives: Prostaglandin E_2 (PGE_2) is a potent immune modulator and known to suppress both tumour antigen-specific helper T (T_H1) cells and the generation of cytotoxic T lymphocytes (CTLs). We hypothesised that a combination of the cyclooxygenase 2 (COX-2) selective inhibitor celecoxib and intravesical bacillus Calmette-Guérin (BCG), an effective tumour immunoprophylaxis and ablative therapy for non–muscle-invasive bladder cancer, would be more effective than BCG alone.
Methods: We assessed urinary levels of PGE_2 in humans receiving BCG and in a murine model of urothelial cell carcinoma (UCC). The cytokine response to BCG plus celecoxib was assessed in murine dendritic cells (DCs) in vitro and tumour ablation was assessed in an orthotopic MBT2 murine bladder cancer model.
Results: Administration of intravesical BCG resulted in elevated urinary PGE_2 levels in patients with high-grade superficial UCC and in a mouse model of UCC. In vitro, activation of DCs with BCG stimulated COX-2 up-regulation and release of the archetypal tolerogenic factors, PGE_2 and interleukin 10. In a superficial mouse model of UCC, combination of celecoxib and intravesical BCG therapy resulted in increased tumour infiltration of $CD4^+$ T cells and improved efficacy when compared to BCG alone. Further, celecoxib demonstrated marked antitumour efficacy when administered in the absence of BCG immunotherapy.
Conclusions: This study demonstrates that a combination strategy involving BCG immunotherapy and celecoxib may be more therapeutically beneficial than stand-alone intravesical therapy.

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1. Introduction

Intravesical immunotherapy with bacillus Calmette-Guérin (BCG) is an effective adjuvant therapy for high-grade non–muscle-invasive urothelial cell carcinoma (UCC) and remains a first-line ablative therapy for carcinoma in situ (CIS). A recent meta-analysis of BCG trials indicated that effective immunotherapy may reduce progression to muscle-invasive disease [1]. Intravesical BCG therapy is associated with significant toxicity related to local inflammatory events and, although the therapeutic advantage of BCG is clear, treatment is associated with long-term recurrence and cancer progression dictating the need to improve efficacy.

Activation of the immune system, particularly the activation and tumour infiltration of $CD4^+$ and $CD8^+$ T cells has been shown to be critical in mediating the host antitumour immune response to BCG [2]. Interleukin 12 (IL-12) plays a pivotal role in the host immune response to BCG by directly stimulating $CD8^+$ and interferon-γ (IFN-γ)-secreting $CD4^+$ helper T (T_H1) cells [3]. Expression of cytokines can be measured in urine and following BCG therapy a T_H1 cytokine profile, with high expression of IFN-γ, IL-12, and IL-2, is associated with successful clinical response to BCG, whereas high levels of IL-10 and IL-6, indicative of a T_H2 response, are associated with BCG failure [4,5]. The importance of the T_H cytokine response is highlighted in a syngeneic MB49 orthotopic model of UCC in which IL-$12^{-/-}$ and IFN-$\gamma^{-/-}$ mice show a reduced survival compared to wild-type and the efficacy of BCG therapy is markedly increased in IL-$10^{-/-}$ mice [6].

Overexpression of cyclooxygenase 2 (COX-2), one of two isoenzymes responsible for the production of prostaglandin E_2 (PGE_2), has been well described in UCC and several other human cancers including breast, cervix, colon, lung, renal cell, and hepatocellular [7]. PGE_2 is frequently elevated at tumour sites and has been implicated as a potent immunomodulator in numerous model systems. In addition to its immunosuppressive effects, PGE_2 has also been demonstrated to support cellular proliferation, migration, and angiogenesis and to enhance apoptotic resistance [8–11]. PGE_2 can potently modulate dendritic cell (DC) function leading to T-cell anergy and even immune tolerance to tumour-associated antigens (TAAs). We previously reported that COX-2 inhibition was able to reverse the potentially tolerogenic phenotype originating in BCG-activated DCs by inhibiting PGE_2-induced IL-10 up-regulation [12]. In this system, decreased IL-10 levels led to enhanced DC–IL-12 expression. The current study was designed to assess whether combination of a COX-2 inhibitor with BCG would be more effective than BCG alone in an orthotopic model of urothelial carcinoma in immunocompetent mice.

2. Materials and methods

2.1. Measurement of urinary PGE_2

Urinary PGE_2 levels were assessed by enzyme-linked immunosorbent assay (ELISA; Cayman Chemical, Ann Arbor, MI, USA) and corrected for dilution by reference to creatinine (Cayman Chemical) according to a previously described method [13]. PGE_2 levels were measured in pooled clean-catch urine specimens from mice that were maintained as described below. PGE_2 levels were also measured in 29 patients (25 men, 4 women) diagnosed with grade 2–3, superficial UCC (Ta–T1) or CIS or both. The mean age of the patients was 73 yr (range: 61–102 yr).

2.2. Generation of DCs from murine bone marrow

Femurs and tibiae were removed from euthanised 8- to 10-wk-old female CD1 mice (Harlan UK, Oxon, United Kingdom). Bones were sterilised by brief washing in 70% (vol/vol) ethanol and the marrow flushed out using complete medium (RPMI-1640 supplemented with 10%, wt/vol, foetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine [Sigma, Dorset, United Kingdom]) using a 21-gauge needle attached to a 1-ml syringe. Following centrifugation, red blood cells were lysed by washing in 0.15 M ammonium chloride, 0.01 mM ethylenediaminetetraacetic acid (EDTA), and 10 mM sodium bicarbonate. Cells were suspended in complete medium and seeded into either 24-well or 6-well tissue culture plates at seeding densities of 2×10^5 or 2×10^6 cells/well, respectively. These cultures were supplemented with 10 ng/ml recombinant murine granulocyte-macrophage colony-stimulating factor (GM-CSF) and 10 ng/ml recombinant murine IL-4 (PeproTech EC, London, United Kingdom) and incubated at 37 °C in a humidified atmosphere containing 5% (vol/vol) CO_2 for 7 d. On the third and fifth days of culture the medium and nonadherent cells were carefully removed from each well and replaced with fresh cytokine-containing medium. Fresh medium and cytokines were also added prior to all stimulations.

2.3. Stimulation of DCs with BCG

Seven-day-old DC cultures were provided with fresh RPMI 1640 culture medium containing fresh cytokines (10 ng/ml GM-CSF, 10 ng/ml IL-4); experimental wells were further supplemented with 1.25×10^5 colony-forming units (cfu)/ml BCG (ImmuCyst™, Connaught strain, Pasteur Merieux Connaught, Toronto, ON, Canada) or lipopolysaccharide (LPS) at 100 ng/ml. All DC cultures were stimulated at 37 °C for 72 h.

2.4. Analysis of COX-2 expression by Western blot

Proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) based on the