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European Association of Urology

From Lab to Clinic

Antitumor Activity of the Antimicrobial Peptide Magainin II against Bladder Cancer Cell Lines☆

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Article info

Article history:
Accepted December 19, 2005
Published online ahead of print on January 6, 2006

Keywords:
Magainin
Antimicrobial peptides
Bladder cancer
Cell proliferation
Cytotoxicity

Abstract

Objective: Magainin II belongs to a family of antimicrobial peptides and has been shown to exhibit antibiotic activity in a wide range of organisms. Recent studies have also reported a significant antitumor effect of magainin II against various cancer cell lines and tumor mice models. In this study, we evaluated the cytotoxic and antiproliferative potency of magainin II in bladder tumor cells and normal fibroblasts.
Methods: The antiproliferative and cytotoxic effect of magainin II was quantified by colorimetric WST-1-, bromodeoxyuridine (BrdU)-, and lactic dehydrogenase (LDH) assays in three bladder cancer cell lines (RT4, 647V, and 486P) and in the murine fibroblast cell line 3T3 as well as in a primary culture from human fibroblasts. The median inhibitory concentration (IC_{50}) values were determined for each assay, representing the concentration at which cell viability was reduced by 50%. Scanning electron microscopy (SEM) was used to visualize the morphologic effects of magainin II on bladder tumor cells and fibroblasts.
Results: Magainin II inhibited cell proliferation of bladder cancer cells in a dose-dependent manner. The average IC_{50} of magainin II against all bladder cancer cell lines was 198.1 μM (range, 52.4–484.03 μM) for the WST-1 assay and 75.2 μM (range, 31.0–135.3 μM) for the BrdU assay. The normal murine and human fibroblast cell lines were not affected by magainin II and their IC_{50} could not be determined at the concentrations of magainin II tested. LDH release was increased in all bladder tumor cell lines in the presence of magainin II, whereas normal fibroblasts showed no cell lysis. SEM demonstrated lethal membrane perforation by peptide pore formation in bladder cancer cells, but not in fibroblasts.
Conclusion: Magainin II peptide exerts cytotoxic and antiproliferative efficacy by pore formation in bladder cancer cells but has no effect on normal murine or human fibroblasts. Magainin II may offer a novel therapeutic strategy in the treatment of bladder cancer with potentially low cytotoxic effects on normal cells.

☆ This work was supported by the Deutsche Forschungsgemeinschaft (LE 1213/1-1) and Stiftung zur Förderung der Medizinischen Forschung, Universität Kiel.
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0302-2838/$ – see back matter doi:10.1016/j.eururo.2005.12.043

1. Introduction

Patients with superficial bladder cancer experience tumor recurrences in 50–70% of cases within 5 yr after transurethral resection [1]. Particularly, patients with high-grade tumors and carcinoma in situ are at risk to develop progressive disease and even experience cancer-related death [2,3]. To reduce the rate of tumor recurrence and progression, various chemotherapeutic drugs such as mitomycin and epirubicin as well as the immunotherapeutic agent bacillus Calmette-Guérin (BCG) have been used for intravesical application. Although intravesical treatment regimens are well established, their therapeutic efficacies have been disappointing with respect to long-term outcome. A meta-analysis by Böhle et al. demonstrated that 38.8% of patients treated with BCG and 46.4% of patients treated with mitomycin C developed tumor recurrences within the overall median follow-up time of 26 mo [4]. A second meta-analysis by Sylvester et al. also concluded that the risk of tumor progression could only be reduced when patients were treated exclusively with long-term maintenance BCG therapy [1]. However, long-term BCG treatment bears moderate to severe side effects, including febrile episodes, arthritis, and the risk of sepsis. The toxicity profile of BCG has a significant impact on treatment protocols, because only 16% of patients in the BCG maintenance group received the full course of treatment [5]. The identification and development of new intravesical agents that have a different mechanism of action than the currently available drugs are therefore highly desirable to reduce toxicity and improve long-term outcome for patients with superficial bladder cancer.

Magainin II provides promising antineoplastic activity, which renders it potentially useful as an agent for intravesical bladder tumor therapy. Magainin II belongs to a family of antimicrobial peptides and was originally isolated from the skin of the African clawed frog, *Xenopus laevis* [6]. These peptides are important components in the innate host defense response in a wide range of organisms, from bacteria to humans [7]. Magainins have been shown to exhibit antibiotic activity on both Gram-positive and Gram-negative strains of bacteria as well as on fungi and protozoa [8]. Magainin II has an amphiphilic α-helical structure that enables it to target nonpolar lipid cell membranes where it can form ion-permeable channels in the membrane, leading to depolarization, irreversible cytolysis, and finally to cell death [9,10]. Besides their well-known antimicrobial activity, recent studies have also reported a significant cytotoxic effect of magainin II against a wide range of cancer cell lines including melanoma, breast and lung cancers as well as lymphomas and leukemias [11–14]. In vivo, magainin peptides have been shown to improve survival of animals with ascites-producing tumors [12]. Furthermore, in a subcutaneous xenograft model of melanoma tumor growth in nude mice, local treatment of magainin II completely ablated the tumor [13]. More importantly, the main advantage of magainins is their selectivity for neoplastic versus normal cells. Magainin peptides are toxic to cancer cells at concentrations lower than that required to lyse peripheral blood lymphocytes, erythrocytes, and normal fibroblasts [9]. Additionally, magainins are highly resistant to serum proteolysis [15].

Our aim was to evaluate the potency of the peptide magainin II as an anticancer drug in bladder tumor cells. We investigated the antitumor activity of magainin II on three different bladder cancer cell lines, as measured by lactic dehydrogenase (LDH) assays to determine cytotoxicity as well as WST-1 and bromodeoxyuridine (BrdU) assays to examine the effects on cell proliferation. Furthermore, using electron microscopy we document the morphologic changes on the cell membrane bladder cancer cells treated with magainin II.

2. Materials and methods

2.1. *Magainin II*

Lyophilized magainin II was purchased from Bachem (Heidelberg, Germany) and was reconstituted in serum free RPMI 1640 (Sigma, Taufkirchen, Germany) or Dulbecco modified Eagle medium (DMEM; Gibco BRL, Gaithersburg, MD), respectively.

2.2. *Cell lines*

Three established human bladder cancer cell lines (RT4 pathologic grade 1, 647V grade 2, and 486P grade 4), obtained from the American Type Culture Collection (Rockville, MD) were used in this study. Cell lines were cultured as monolayers in 75-cm^2 flasks in RPMI 1640 medium (Sigma, Taufkirchen, Germany) supplemented with 10% fetal calf serum (FCS). The normal mouse fibroblast cell line 3T3 was purchased from Deutsche Sammlung für Mikrobiologie und Zellkultur (DSMZ, Braunschweig, Germany). Fibroblasts were grown as monolayers in DMEM (Gibco BRL, Gaithersburg, MD) supplemented with 10% FCS. Primary fibroblasts from human gingival tissue samples were isolated using a standard protocol described elsewhere [16]. Fibroblasts were grown as monolayers in DMEM (Gibco BRL, Gaithersburg, MD) supplemented with 10% FCS.