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Experimental therapy of human endometrial cancers with a targeted cytotoxic bombesin analog AN-215: Low induction of multidrug resistance proteins

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Abstract

In this study we have investigated the efficacy and toxicity of targeted cytotoxic bombesin (BN) analog AN-215 and its effects on the expression of three multidrug resistance proteins in experimental human endometrial cancers. Nude mice bearing HEC-1A, RL-95-2 and AN3CA tumours were treated with AN-215 and its cytotoxic radical (AN-201). The BN receptor expression in tumours was followed by RT-PCR analysis and radioligand binding assays. Expression of drug resistance proteins MDR-1, MRP-1 and BCRP were measured by realtime PCR. AN-215 significantly ($P < 0.05$) inhibited the growth of HEC-1A, RL-95-2 and AN3CA tumours while AN-201 was ineffective. The expression of BN receptors was demonstrated in all three tumour models. AN-215 caused a lower induction of MDR-1 in HEC-1A and RL-95-2 cancers than AN-201. MRP-1 and BCRP were not induced by AN-215 or AN-201. Thus, targeted chemotherapy with AN-215 powerfully inhibits the growth of human BN receptor-positive endometrial cancers.

Keywords: Targeted chemotherapy; Endometrial cancer; Bombesin/GRP receptor; Targeted cytotoxic bombesin analog AN-215; Multidrug resistance

1. Introduction

Endometrial carcinoma is the most common neoplasm of the female genital tract and in the USA it accounts for about 40,000 new cases and more than 7000 deaths annually [1–3]. If discovered at an early stage, endometrial cancer has a fairly good prognosis [2]. However, in patients with late stage or recurrent disease, survival rates decrease substantially to 18% and 7.7%, respectively [2,4]. Consequently, new therapeutic strategies are needed for advanced disease.

The discovery of molecular characteristics of tumour cells has led to the development of a new treatment strategy known as targeted therapy, the purpose of which is a direct delivery of antineoplastic drugs to cancer cells and a reduction in systemic toxicity. Modern targeted anti-cancer drugs include monoclonal antibodies against surface structures on malignant cells as well as conjugates consisting of receptor specific ligands linked to toxins, radionuclides or chemotherapeutic agents [5]. Higher intratumoural concentrations of antitumour agents produced by targeting may overcome

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chemoresistance of malignant cells and are expected to result in greater therapeutic efficacy.

Cancer cells can develop multidrug resistance (MDR) to a variety of antitumour agents that appear to be structurally and functionally unrelated. One mechanism of action of MDR is the increased efflux of chemotherapeutic agents mediated by transport proteins. The product of the MDR-1 gene, an adenosine triphosphate (ATP)-dependent membrane transporter termed P-glycoprotein (Pgp) and the recently discovered MDR protein 1 (MRP-1) use this mechanism of action [6,7]. Breast cancer resistance protein (BCRP) is another overlapping, but distinct type of MDR, based on drug efflux [8]. Expression of MDR-1 has been detected in most human endometrial cancer specimens as well as in normal endometrial tissue [9–11]. MRP immunoreactivity was detected in normal endometrium and it showed a progressive increase in intensity from endometrial hyperplasia to endometrial carcinoma [12]. Targeting chemotherapeutic drugs directly to tumour cells could overcome MDR based on transmembrane efflux, as it would increase the local concentration of the chemotherapeutic agent.

Specific receptors for bombesin/gastrin releasing peptide (GRP) peptides have been found in various human malignancies and cancer cell lines, including breast, prostatic and ovarian cancers and other tumours [13]. Consequently, we have developed a cytotoxic bombesin analog AN-215 by covalently linking a highly potent derivative of doxorubicin (DOX), 2-pyrrolino-DOX (AN-201) [14] to a bombesin-like carrier, Gln–Trp–Ala–Val–Gly–His–Leu–ψ (CH_2–NH)–Leu–NH_2 [15]. AN-215 shows high affinity to BN/GRP receptors, retains the antiproliferative effect of its cytotoxic moiety [15] and has been successfully used for the experimental therapy of various human cancers, such as prostatic, gastric and other tumours [13].

Receptors for BN/GRP were previously detected in AN3CA, KLE, HEC-1A and Ishikawa human endometrial cancer cells in vitro [16]. Consequently, the current study was designed to investigate the efficacy of targeted chemotherapy with AN-215 in AN3CA, HEC-1A as well as RL-95-2 cancer cell lines in vivo. In addition, we compared the effects of the treatment with AN-215 and the non-targeted cytotoxic radical AN-201 on the expression levels of MDR-1, MRP-1 and BCRP.

2. Materials and methods

2.1. Peptides and cytotoxic radical

Cytotoxic radical 2-pyrrolino-DOX (AN-201) and the cytotoxic bombesin analog AN-215, consisting of 2-pyrrolino-DOX-14-O-hemiglutarate linked to the amino terminal of Gln–Trp–Ala–Val–Gly–His–Leu–ψ (CH_2–NH)–Leu–NH_2 (RC-3094) were synthesised in our laboratory as previously described [15]. Bombesin antagonist RC-3095 (D-Tpi^6, Leu^{13}ψ (CH_2NH)) ^{14}LeuBN was also synthesised in our laboratory [17]. The compounds were dissolved in 5% (w/v) aqueous D-mannitol solution (Sigma, St Louis, MO, USA) before intravenous (i.v.) injection.

2.2. Cell lines

Human endometrial cancer cell lines HEC-1A, RL-95-2 and AN3CA were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were grown at 37 °C in humidified 95% air 5% carbon dioxide atmosphere, passaged weekly and routinely monitored for mycoplasma contamination using a detection kit (Boehringer Mannheim, Mannheim, Germany). All culture media were purchased from Gibco (Grand Island; NY, USA).

2.3. Animals

Five- to six-week-old female athymic nude mice (Ncr *nu/nu*) were obtained from the National Cancer Institute (NCI, Bethesda, MD, USA). The animals were housed in sterile cages under laminar flow hoods in a temperature-controlled room with a 12 h light/12 h dark schedule. They were fed autoclaved chow and provided water ad libitum.

2.4. Experiments

Exponentially growing cells from each cell line were implanted into 5 female donor nude mice by subcutaneous injection of 10^7 cells in both flanks. Tumours resulting after 4 weeks in donor animals were aseptically dissected and mechanically minced. Three mm^3 pieces of tumour tissue were transplanted subcutaneously (s.c.) in the experimental animals by a trocar needle. Tumour volume (length × width × height × 0.5236) and body weight were measured weekly. The total leukocyte count (WBC) was determined with the Unopette microcollection kit (Becton Dickinson, Franklin Lakes, NJ).

At the end of each experiment, animals were sacrificed under anesthesia, tumours were excised and weighed and necropsy was performed. Tumour specimens were snap frozen and stored at −70 °C. The procedures were in accordance with institutional guidelines for the welfare of animals in experimental research. The Institutional Animal Care and Use Committee reviewed the protocols for the animal experiment and gave full approval.

In experiment 1, when HEC-1A tumours had reached a volume of approximately 50 mm^3, mice were assigned to three experimental groups and were given a single i.v. injection of the corresponding agent into the jugular vein on day 1: Group 1 (control) received vehicle solution (5% mannitol) (5 mice); group 2 was injected with cytotoxic