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Potent antitumor effects of a novel actinomycin D analog Leu^{5}AMD

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Abstract

Leu^{5}AMD ([D-Val^2, L-MeLeu^5]$_2$ AMD) is a novel actinomycin D (AMD) analog, in which both *N*-methylvalines were replaced by *N*-methylleucines. In the present study, an attempt has been made to investigate the effects of Leu^{5}AMD on the proliferation of human gastric carcinoma cell line SGC-7901. The results showed that Leu^{5}AMD inhibited the proliferation and induces apoptosis in SGC-7901 cells in a dose-dependent manner. Apoptosis induced by Leu^{5}AMD was further confirmed by annexin V-FITC/PI dual staining assay. After treatment with Leu^{5}AMD, the loss of mitochondrial potential and the decrease of bcl-2 gene expression were observed in apoptotic cells, suggesting that Leu^{5}AMD may be involved in mitochondria and bcl-2 related apoptotic pathway. In addition, the *in vivo* antitumor effects of Leu^{5}AMD on S-180 bearing mice and the acute toxicity on healthy mice were investigated. Treatment with Leu^{5}AMD markedly suppressed the growth of Sarcoma xenograft. These results suggest that Leu^{5}AMD may be used as a promising chemotherapeutical agent for patients affected by gastric carcinoma and other solid cancer.

Keywords: Actinomycin D; Analog; Apoptosis; Antitumor; SGC-7901 cell

1. Introduction

Actinomycin D (AMD) is a chromopeptide consisting of a phenoxazinone planar chromophore with two pentapeptide rings attached. It is one of the most widely studied anticancer antibiotics which generate a wide variety of biochemical and pharmacological effects [1,2]. It has been clinically used in

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the treatment of certain cancers [3–5]. Due to its significant biological activity, there is great interest in the modification of AMD by directed biosynthesis, partial synthesis and total synthesis in order to increase the selectivity against certain cancers [6,7]. In many cases, replacements of amino acid residues in the cyclic depsipeptides of AMD have been found to make such analogs inactive or less active. However, it has been demonstrated by Mauger et al. that the analog in which L-Mevaline replaced by L-Meleucine shows equal antitumor activity to AMD at approximately 100-fold lower concentrations [8]. This compound was identified as Leu^{5}AMD.

Gastric carcinoma (GC) is one of the most common tumor types in the world, and it is frequently lethal, having only ~20% 5-year survival rate [9]. Even though the prognosis of patients with advanced gastric carcinoma seems to have been improved as the result of the standardization of surgical techniques and recent advances in multimodal adjuvant therapy, the 5-year postoperative survival rate is still forbidding low [10,11]. In addition, advanced gastric carcinoma does not generally respond to conventional chemotherapy or radiotherapy. So developing new chemotherapeutical agents is an attractive job for the treatment of gastric carcinoma.

To evaluate the potency of the novel AMD analog Leu^{5}AMD as an anticancer drug in gastric carcinoma, we investigate its inhibitory effects on the proliferation of SGC-7901 cell line (a human gastric carcinoma cell line) by MTT assay, and its induction of apoptosis by cell morphology observation and Annexin V binding assay. The change of mitochondria potential and the bcl-2 expression were analyzed by flow cytometry. Furthermore, we also investigated the antitumor effects on Sarcoma S-180-bearing mice and evaluated the acute toxicity of Leu^{5}AMD on healthy mice.

2. Materials and methods

2.1. Reagents

Leu^{5}AMD was synthesized by the same route described previously with minor modification [8]. In brief, Leu^{5}AMD was synthesized from C terminal to N terminal in solution phase to form linear pentapeptide and cyclized by Bop-Cl/Et_3N in DCM. Condensation on pentapeptide lactone with BMNBCA, followed by catalytic reduction controlling oxidation by $K_3Fe(CN)_6$. The homogeneity of the products was checked by thin-layer chromatography on silica-gel plates. The intermediates and the final products of Leu^{5}AMD were confirmed by NMR and Mass spectra analysis. The molecular formulas of AMD and Leu^{5}AMD are illustrated in Fig. 1.

2.2. Cell lines and culture conditions

Human gastric adenocarcinoma cell line SGC-7901 was grown in RPMI 1640 medium (Gibco–BRL, USA). Cells were maintained at 37 °C in humidified air with 5% CO_2. Media were supplemented with penicillin (50 U/ml), Streptomycin (50 μg/ml) and 10% fetal bovine serum (FBS) (Minhai biotech, China).

2.3. Animals

Kunming mice (Grade II, Certificate No. 9700047) were provided by the Animal Center of Lanzhou University (Lanzhou, China). The animals (20 ± 1 g, 8–10 weeks old) were housed at a room temperature of approximately 22 ± 1 °C and 50–60% relative humidity with circadian light rhythm of 12 h, and given standard sterile diet pellets and tap water according to institutional guidelines.

2.4. Cell proliferation assay

SGC-7901 cells (5×10^3 cells/well) were seeded overnight in 96-well plates and incubated for 24, 48, 72, 96 h in the absence or presence of various concentrations (1–100 nM) of Leu^{5}AMD. The assay was initiated by adding

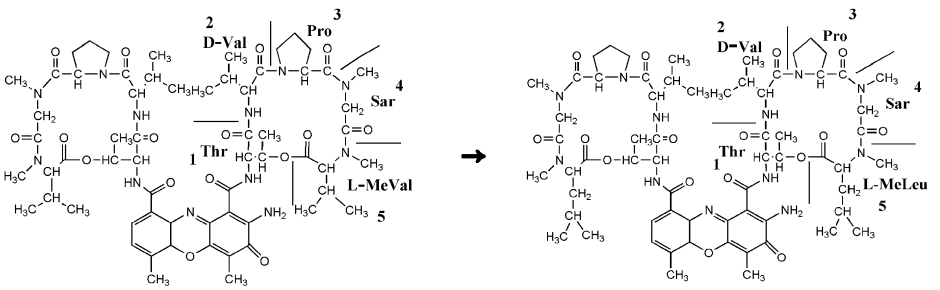

Fig. 1. Chemical structures of AMD and Leu^{5}AMD.