In vivo and in vitro antitumor activity of mitomycin C conjugates at 7-N position through a linker containing thiocarbamate bond with CD10 monoclonal antibody

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Abstract

Through a linker containing thiocarbamate bound to the 7-N position of mitomycin C (MMC), conjugates with a monoclonal antibody to CD10 (NL-1) were prepared, and their antitumor activities were examined. All five conjugates, except one, showed in vitro cytotoxity to two CD10⁺ lymphoid cell lines superior to MMC. The conjugate displaying the highest cytotoxicity was selected and further tested against three CD10⁺ and two CD10⁻ lymphoid cell lines in vitro. The conjugate with NL-1 antibody demonstrated higher cytotoxic activity against CD10⁺ tumor cells than the control conjugate with normal immunoglobulin, while there was no significant difference, when tested against CD10⁻ tumors. The cytotoxic activity of the NL-1 conjugate to CD10⁺ tumors was significantly blocked by NL-1 antibody.

In vivo antitumor activity of the NL-1 conjugate was then tested against a CD10⁺ tumor transplanted to nude mice, and side effects were recorded. The NL-1 conjugate (4 mg/kg) showed an in vivo antitumor effect similar to MMC (2 mg/kg), which is at nearly maximal tolerable dose; the latter induced decreases in numbers of leukocytes and platelets, while the former did not, suggesting less side effect by the NL-1 conjugate. Since MMC demonstrates a broad spectrum of antitumor activity, the conjugate, as such, may be applicable for the treatment of cancer patients.

Abbreviations: ALL: acute lymphocytic leukemia; CML: chronic myelocytic leukemia; h: hour; IgG: immunoglobulin G; MMC: mitomycin C; MMC-D: mitomycin C derivative; PBS: phosphate buffered saline.

Introduction

Mitomycin C (MMC) is a valuable antitumor antibiotic heralding a broad spectrum and strong cytotoxicity against experimental tumors and human neoplastic diseases [1, 2]. Relatively severe side effects, such as myelosuppression, have, however, limited its clinical use. One approach to overcome this problem is to produce new analogues. Thus, derivatives of MMC have been extensively searched for many years [3–7], including those with substituents at 7-N position, because N-7 has a strong association with reduction of quinone ring. Recently, MMC derivatives with symmetrical and unsymmetrical disulfide at 7-N were reported to show cytotoxic activities superior to MMC, one of which, KW2149 (7-N-\{(2-\{(2-(\gamma-L-gultamylamino)ethyl)dithio\})}
ethyl} mitomycin C), is highly water soluble and was finally selected for further evaluation [8, 9].

A second approach to reducing MMC's side effects is to improve tumor targeting resulting from various procedures [10–12], such as preparation of the conjugate of the cytotoxic agents with an antibody [13–15]. Several reports have appeared in which conjugates of MMC with an antibody have been described [16–20]. In all of these conjugates, MMC was bound to the antibody at 1-N position directly or with the use of linkers.

We herein describe the preparation of several conjugates of MMC bound with a CD10 monoclonal antibody (NL-1) [22], in which MMC was bound to the antibody at 7-N position through a linker containing thiacarbamate bond, and investigated in vitro and in vivo antitumor activities of the conjugates, together with their side effects. One of them was found to show a selective in vivo cytotoxicity to a CD10+ tumor with less myelosuppression than MMC.

Materials and methods

Antibody

NL-1 hybridoma producing IgG2a monoclonal antibody to CD10 [common acute lymphocytic leukemia (ALL) antigen, CALLA] was established in our laboratories, and the characterization of this NL-1 antibody was previously described [22]. IgG2a fraction was prepared from ascites of hybridoma bearing BALB/c nude mice (Shizuoka Agriculture Cooperative Association, Hamamatsu, Japan) using Protein A-Sepharose column (Pharmacia, Uppsala, Sweden). Control rabbit non-immune immunoglobulin G (IgG) fraction was purchased and further fractionated with a DEAE Sepharose column (Pharmacia).

Preparation of precursors of antibody-mitomycin C conjugates

N-hydroxysuccinimide (113 mg) and dicyclohexylcarbodiimide (203 mg) were added to a stirred solution of 7-N-\{2-\{(\text{carboxy})\text{ethyl}\}amino\text{carbonylthio}ethyl\}mitomycin C 2a (100 mg) in N, N-dimethylformamide (2 ml) at room temperature and the stirring was continued for 2 hours (h) at room temperature. After precipitated dicyclohexylurea was removed by filtration, the filtrate was concentrated in vacuo. The residue was dissolved in chloroform and was washed with water and saturated aqueous sodium chloride solution. After the organic layer was dried over anhydrous sodium sulfate, it was concentrated in vacuo. The residue was flash-chromatographed on a column of silica gel with chloroform-methanol (9:1, v/v) as eluent to give 7-N-\{2-(succinimidoxycarbonyl)\text{ethyl}\}amino\text{carbonylthioethyl\}mitomycin C 3a (41 mg, yield 35%). It was too unstable to determine the structure spectroscopically. Therefore, its benzylamide derivative, which was prepared by the reaction of 3a with benzylamine, was determined by H-nuclear magnetic resonance (NMR) and mass spectrometry. Other precursors were prepared in a similar manner as described above and their structure was determined by the same method.

Preparation of antibody-mitomycin C conjugates

The solution of precursor 3a (35.8 mg) in dioxane (1.5 ml) was added dropwise to a solution of NL-1 antibody (590 mg) in phosphate buffered saline (PBS) (pH 6.8) (59 ml), and the mixture was stirred gently at room temperature for 30 minutes. The reaction mixture was concentrated down to 10 ml by ultra filtration and the concentrate was subjected to gel filtration on Sephadex G-25 column (1.0 cm × 30 cm, Pharmacia) with PBS (pH 6.8) to give NL-1 antibody-MMC conjugate 4a (703 mg, yield 82%). Further conjugates were prepared as described above (Table 1).

Determination of amount of mitomycin C bound to immunoglobulin

Drug bound to immunoglobulin was determined as 7-N-(2-mercaptoethyl)mitomycin C (MMC-D) spectroscopically by measuring the absorbance at UV absorption maxima at 280 nm and 372 nm