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Selective cytotoxicity against human tumor cells by an anti–gastric cancer monoclonal antibody–mitomycin C conjugate

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ABSTRACT An anti–gastric cancer monoclonal antibody, MGb_2, was chosen to prepare MGb_2–mitomycin C (MMC) conjugate. Four to five molecules of MMC were introduced into each molecule of antibody with the antibody activity well retained. The conjugate showed a highly selective cytotoxicity upon human gastric cancer cells KATO–Ⅲ. In the 48–h exposure test, the cytotoxic effect of MGb_2–MMC upon target cells was similar to that of free MMC, but much greater than that of normal mouse immunoglobulin–MMC conjugate. Instead, the MGb_2–MMC showed a statistically less cytotoxic effect upon non–target cells. Imaging and biodistribution studies indicated that the MGb_2 was still well localized in tumor tissue after its conjugation with MMC.

KEY WORDS monoclonal antibodies; mitomycins; immunologic cytotoxicity; cultured tumor cells; stomach neoplasms

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Mitomycin C (MMC), an antibiotic, has

potent antitumor activity and has been used in the treatment of gastric cancer. However, its clinical use is limited by its detrimental effect on normal cells.[1] In order to improve therapeutic efficacy while minimize toxic effect, MMC was linked to an anti-gastric cancer monoclonal antibody, MGb_2, prepared in our laboratory. The MGb_2-MMC conjugate was found to exhibit selective cytotoxicity on human gastric cancer cells KATO-Ⅲ *in vitro*.

MATERIALS AND METHODS

Cells and tumor model The cells used in this study were of the human gastric cancer cell line KATO-Ⅲ (target cells) and the human normal embryonic lung cell line SL_7 (non-target cells). We used RPMI 1640 completed with 10% new born calf serum as the culture medium. SGC 7901 cloned human gastric carcinoma was maintained by sc transplantation in Swiss-nu / nu mice. Three mice each bearing a tumor 35 mm × 32 mm in size 2 wk after the transplantation were used for the *in vivo* study.

Monoclonal antibody MGb_2 The monoclonal antibody $MGb_2(IgG_1)$ was prepared in BALB c mice with respective hybridoma clones established in the Laboratory of Gastroenterology of Xijing Hospital[2] and purified from the ascitic fluid by $(NH_4)_2SO_4$ precipitation and ion-exchange chromatography on DEAE-cellulose. The purified MGb_2 was essentially free from non-IgG protein on SDS-polyacrylamide gel with the purity being 92.60%.

Preparation of MGb_2-MMC conjugate MGb_2-MMC conjugate was prepared according to the method essentially as described before[3]. MMC concentration in the conjugate was determined by measuring the absorbance at 363 nm (E, 23 000). The amount of IgG was determined by protein-dye binding method[4] with immuno-affinity purified MGb_2 as standard. Normal mouse immunoglobulin-MMC conjugate was prepared by the same procedure.

Immunoreactivity of MGb_2-MMC conjugate Indirect enzyme-linked immunosorbent assay was employed to evaluate the antigen-binding capacity of the conjugate. The cancer cells KATO-Ⅲ were distributed in a tissue culture plate with 40 flat-bottom wells and fixed with 0.25% glutaraldehyde. Different dilutions of conjugate and free antibody in 0.1 ml was added. Following incubation at 37℃ for 1 h, rabbit anti-mouse Ig antibody with horseradish peroxidase labeled was added. Substrate was added 1 h later and the A_{495} nm was recorded.

Cytotoxicity of conjugate The tetrazolium (MTT) colorimetric assay was employed in the cytotoxicity test with the cancer cells and cell line SL_7 as described previously[5]. The cytotoxic effects of MGb_2-MMC conjugate upon target cells and non-target cells were compared with that of free MMC and normal mouse immunoglobulin-MMC conjugate.

Iodination of MGb_2-MMC conjugate MGb_2-MMC conjugate was labeled with ^{125}I by the chloramine-T method to a specific activity of 59.16 kBq / μg.

Imaging and biodistribution study For imaging and biodistribution study, SGC 7901-bearing Swiss athymic nude mice were injected ip ^{125}I-MGb_2-MMC conjugate (2.59×10^3 kBq / mouse). After 8 d the mice had SPECT (single photon emission computed tomography) taken and tissue samples were taken and assayed for radioactivity. The result of analyses was expressed as the ratio of the radioactivity / mg tumor to the radioactivity / mg blood or tissue (T./ NT). The antigen-binding activity of conjugate in the serum collected was measured by indirect immunofluorescence method.

RESULTS

Preparation of the conjugate and its immunoreactivity By reaction of 15-20 times

molar excess of MMC reactive ester with MGb_2 (6.5 mg / ml), 4–5 molecules of MMC were introduced into each molecule of antibody. The immunoglobulin recovery rate reached 85–90 %.

The result of enzyme-linked immunosorbent assay was shown in Tab 1. The antibody retained its activity well during the conjugate preparation. Even at the concentration of 1 nmol / L, the conjugate was still bound to tumor cells, the result of which being quite similar to that of unmodified antibody.

Tab 1. Enzyme-linked immunosorbent assay of binding of anti-gastric cancer monoclonal antibody MGb_2-mitomycin C conjugate and free MGb_2 to human gastric cancer cell line KATO-Ⅲ.

Concentration (nmol / L)	Absorbance at 495 nm	
	MGb_2-MMC	MGb_2
100	1.00	1.20
10	0.63	0.81
1	0.16	0.24
0.1	0.04	0.07

Cytotoxic effects of MGb_2-MMC conjugate The 48-h cytotoxic effects of MGb_2-MMC conjugate, free MMC and normal mouse immunoglobulin-MMC conjugate on target cells and non-target cells were shown in Fig 1. The cytotoxicity of MGb_2-MMC on the cell line KATO-Ⅲ was similar to that of free MMC, but much greater than that of normal mouse immunoglobulin-MMC conjugate ($P<0.05$). Compared with free MMC, the MGb_2-MMC conjugate showed much less cytotoxic effect on non-target cells, the result of which being quite similar to that of normal mouse immunoglobulin-MMC conjugate ($P>0.05$).

***In vivo* studies** Tab 2 showed the tissue distribution of ^{125}I-MGb_2-MMC in Swiss nude mice bearing SGC 7901. The distribution of the labeled conjugate in tumor was much higher than that in other tissues, indicating that the conjugate was taken up by the tumor preferentially. SPECT imaging confirmed the result of biodistribution study. Tumors were clearly visualized by ^{125}I-MGb_2-MMC without using substraction technique. Immunofluorescence study showed that on d 8 after ip injection the conjugate in the circulating blood was still bound to tumor cells.

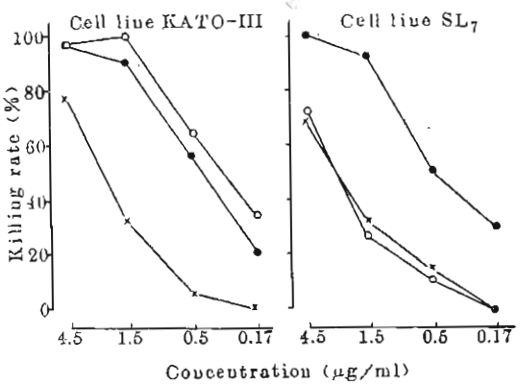

Fig 1. 48-h cytotoxic effects of antibody MGb_2-mitomycin C (O), free mitomycin C (●) and normal mouse immunoglobulin-mitomycin C (×).

Tab 2. Tissue distribution of ^{125}I-MGb_2-mitomycin C conjugate in Swiss nude mice bearing human gastric carcinoma xenografts 8 d after ip. $n=3$

Tissue	dpm / mg tumor / dpm / mg blood or tissue
Heart	3.07; 3.95; 3.20
Liver	5.44; 4.00; 2.80
Kidney	3.82; 3.73; 2.60
Spleen	9.17; 4.99; 3.50
Lung	1.59; 3.24; 1.96
Stomach	6.80; 5.10; 3.97
Intestine	10.10; 9.76; 8.80
Blood	0.84; 0.70; 0.82

DISCUSSION

An reactive ester method was chosen to prepare MGb_2-MMC conjugate. According to the method described as above, 4–5 molecules of MMC were introduced into each molecule of antibody while the antigen-binding capacity of the antibody well retained during

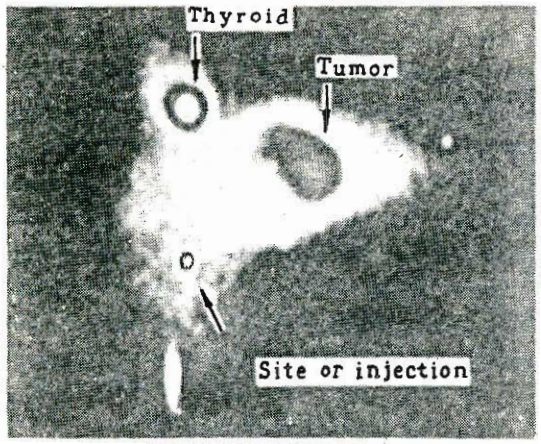

Fig 2. Single photon emission computed tomography of a swiss nude mouse bearing SGC 7901 human gastric carcinoma injected with ^{125}I–MGb_2–mitomycin C conjugate.

the conjugation. The MGb_2–MMC exhibited highly selective cytotoxic effect upon human gastric cancer cell line KATO–Ⅲ. We once chose MG11, another anti–gastric cancer monoclonal antibody, to prepare antibody–MMC conjugate. The cytotoxic effect of the conjugate was weaker than that of MGb_2–MMC. This may be due to the difference of expression of the corresponding antigens on tumor cells, suggesting that the cytotoxic effects of the conjugates on target cells be mediated by the antibodies chosen.

In vivo behaviour of antibodies is closely related to the methods of coupling cytotoxic agents and antibodies. Different methods of linkage may affect greatly the *in vivo* distribution of the antibodies.[6,7] The result of imaging and biodistribution studies showed that MGb_2 was still well localized in the tumor tissue after conjugation with MMC. Compared with unmodified antibody, no obvious increase in the uptake of the labeled conjugate by liver was observed, suggesting that reactive ester method be suitable for the linkage of antibody with MMC. The *in vivo* study on the therapeutic effect of MGb_2–MMC conjugate is underway.

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胃癌单抗–丝裂霉素 C 结合物对肿瘤细胞选择性杀伤作用

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提要 采用活性酯法制备鼠抗人胃癌单克隆抗体 MGb_2–丝裂霉素 C(MMC)结合物, 于每一抗体分子中引入 4–5 个分子 MMC 抗体活性仍保存良好, MGb_2–MMC 结合物对人胃癌细胞 KATO–Ⅲ具有高度选择性杀伤作用, 免疫显像及生物学分布研究结果表明, 抗体与 MMC 交联后, 在体内肿瘤组织定位能力无明显改变.

关键词 单克隆抗体; 丝裂霉素类; 免疫细胞毒性; 培养肿瘤细胞; 胃肿瘤