In vivo antitumor effect of methotrexate conjugated to a monoclonal IgM antibody specific for stage-specific embryonic antigen-1, on MH-15 mouse teratocarcinoma*

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Summary. Methotrexate (MTX) was coupled to an IgM monoclonal antibody specific for stage-specific embryonic antigen-1 (SSEA-1), and the resulting immunoconjugate (MTX-anti-SSEA-1) was used for in vivo drug targeting in mice bearing MH-15 teratocarcinoma. Immunoconjugates having an average of 65 mol MTX/mol antibody retained full antigen-binding capacity. Mice bearing well-established tumors (approx. 1 g) were treated i.v. using the immunoconjugate. MTX-anti-SSEA-1 at 15 mg/kg of drug had significant antitumor activity with no significant systemic toxicity. Neither an irrelevant isotype-matched conjugate, MTX-MOPC-104E, prepared from the MOPC 104E myeloma protein, nor free MTX injected alone or with either antibody had any significant antitumor effect. These results indicate that IgMs can be effective drug carriers for tumor targeting in spite of their high molecular mass, and that antigens that are selectively accessible in tumors, even though present in normal tissues, can be suitable targets for in vivo chemoimmunotherapy.

Introduction

A number of attempts have been made to improve the specificity of antineoplastic drugs by coupling them to polyclonal and monoclonal tumor-directed IgG antibodies. While in vitro and in vivo specificity were enhanced, significant losses in drug activity or antigen-binding capacity usually occurred [9].

Methotrexate (MTX) conjugated to the IgM antibody specific for stage-specific embryonic antigen-1 (anti-SSEA-1) showed greater antitumor activity than free MTX in vitro and retained full antigen-binding activity [18]. IgM antibodies may have some advantages for drug targeting. The large number of antigen-binding sites (ten for IgM versus two for IgG) decreases the likelihood that all antigen-binding sites on a molecule will be inactivated by conjugation, hence more drug may be conjugated per mass of antibody. Even if the mass ratio of drug to antibody in the conjugate were identical for IgMs and IgGs, the amount of drug delivered per antibody molecule would be higher for IgMs because of their higher molecular mass. On the other hand, IgMs have a short half-life in vivo, and also might be expected to penetrate more slowly to antigenic sites, because of their higher molecular mass. One purpose of these experiments was therefore to test whether an IgM conjugate that was highly active in vitro might be useful in vivo. Furthermore, in spite of the presence of SSEA-1 determinants on several normal mouse tissues, including kidney, salivary glands, and brain [4, 5], little or no localization to normal organs occurs after injection of radioiodinated anti-SSEA-1 in tumor-bearing mice, although antigenic tumors were labeled [2]. Thus, anti-SSEA-1 offers a suitable model to test whether tumor-associated antigens that are present at equal or higher levels in normal tissues can be used as targets for in vivo therapy.

Materials and methods

Preparation of monoclonal anti-SSEA-1 and myeloma protein MOPC 104E conjugates. Both monoclonal anti-SSEA-1 [20] and myeloma protein MOPC 104E were purified from clarified ascites by Sephacyl S-300 gel filtration chromatography as previously described [18]. MOPC 104E (IgM, λ) was chosen as a control because of its known lack of in vivo targeting in this tumor model [3] (and unpublished data). MTX was conjugated to anti-SSEA-1 and MOPC 104E by a carbodiimide coupling reaction [18]. The resulting MTX-anti-SSEA-1 immunon conjugate contained 65 molecules of MTX/molecule of antibody, the conjugate MTX-MOPC-104E contained 97 molecules of MTX/molecule of myeloma protein, as calculated by the method of Shen et al. [18]. Solutions of MTX-anti-SSEA-1 or MTX-MOPC-104E were stable in storage for more than 1 month at 4 °C and no significant amount of free MTX could be detected.

Antigen-binding activity of MTX-anti-SSEA-1. Anti-SSEA-1 was conjugated to horseradish peroxidase as described [16] except that instead of reacting with fluorescein isothiocyanate, the peroxidase was activated by periodate in 1 mM sodium acetate buffer, pH 4.4, to minimize self-coupling reactions. For enzyme-linked immunosorbent assay (ELISA), serocluster plates (Costar) were coated with MH-15 crude membranes obtained from tissue homogenate by CaCl2 precipitation (1 g tumor/16 96-well plates) [12]. MTX-anti-SSEA-1 and anti-SSEA-1 samples (50 μl)
were diluted progressively in twofold steps with 10% immunoglobulin-free fetal calf serum in phosphate-buffered saline pH 7 (PBS). Conjugated or drug-free anti-SSEA-1 was mixed with an equal volume of peroxidase-conjugated anti-SSEA-1 (2 μg/ml) prior to addition to the wells. Samples were then added to each well and incubated overnight at 5°C. The following morning, plates were washed with 0.05% Tween-20 in PBS to eliminate unreacted antibody. Plates were then developed by adding 50 μl/well of a solution of 0.05 g/1,2,2'-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid) in 0.1 M citric acid, pH 4, containing 0.03% H2O2 [17]. Absorbance was measured using an automated ELISA plate reader (Dynatech MR 580).

Growth of MH-15 teratocarcinomas in mice. The MH-15 mouse teratocarcinoma [20] was grown in 7-week-old Balb/c mice (Charles River Laboratories). Typically, 3 x 10⁶ cells were injected i.m. into the right thigh at time 0. Medium-size tumors (1 g) developed 2 weeks after inoculation, at which time in vivo drug targeting experiments were started. All groups (experimental and control) contained ten mice at the start of the experiments. Tumor cross-sectional diameters were measured using vernier calipers. The perpendicular cross-sectional product recorded tumor size. Tumor weights were calculated using the method of Geran et al. [8]. Results were analyzed using Student’s t-test.

Treatment of tumor-bearing mice with MTX or its conjugates. Mice bearing 1-g tumors were divided into four groups of ten. Group I received PBS as a control, groups II, III and IV received MTX-anti-SSEA-1 at 5, 10 and 15 mg/kg of drug, respectively. Five i.v. injections were given at days 14, 17, 20, 23 and 26 after tumor inoculation. To demonstrate that the effect of MTX-anti-SSEA-1 was due to antibody specificity, MTX was conjugated to a non-specific IgM, MOPC 104E. The MTX-MOPC-104E conjugate was administered at 15 mg/kg of drug. In parallel, MTX (15 mg/kg) was also administered mixed with, but not conjugated to anti-SSEA-1 or MOPC 104E. Additional control groups received either anti-SSEA-1 or MOPC 104E at the dose level corresponding to the conjugates, PBS, or MTX in PBS (15 mg/kg). As in the previous experiment, five i.v. injections were given at days 14, 17, 20, 23 and 26 after tumor inoculation.

Results

Antigen-binding activity of MTX-anti-SSEA-1
Figure 1 shows the competition of anti-SSEA-1 and MTX-anti-SSEA-1 with peroxidase-conjugated anti-SSEA-1 on MH-15 cell membranes. No significant difference was detected; thus, the conjugation of MTX to anti-SSEA-1 at up to 65 mol MTX/mol antibody did not cause a significant loss of antigen-binding activity.

Antitumor activity of MTX-anti-SSEA-1
Figure 2 shows the response of MH-15 mouse teratocarcinomas growing in Balb/c mice to PBS and MTX-anti-SSEA-1 at 5, 10 and 15 mg/kg of drug. No signs of toxicity, i.e. (body weight loss, hair loss or diarrhea) were noted. Eight of ten mice in the 15 mg/kg group and six of ten mice in the 10 mg/kg group responded to the treatment with either tumor growth inhibition or regression. There was no significant response in the 5 mg/kg group. The results obtained in this experiment suggested that MTX-anti-SSEA-1 at 15 mg/kg of drug is effective in controlling the growth of the MH-15 mouse teratocarcinoma.

Specificity of MTX-anti-SSEA-1 against MH-15 tumors
Figure 3 shows the response of MH-15 mouse teratocarcinomas growing in Balb/c mice to MTX-anti-SSEA-1 at 15 mg/kg of drug, to an irrelevant conjugate MTX-MOPC-104E administered at 15 mg/kg of drug, or to free MTX (15 mg/kg) administered mixed with but not conjugated to anti-SSEA-1 or MOPC 104E at the dose level correspond