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Cytotoxicity of Anti-CALLA Monoclonal Antibody Conjugates to Methotrexate

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It is believed that development of any system which could selec tively deliver a chemotherapeutic agent to the cancer cells in desired concentrations, without allowing it to interact with the healthy cells, would be highly desirable. To achieve this goal, a wide variety of natural and synthetic carrier systems have been developed. However very few systems have demonstrated significant quantitative benefit over the conventional counterparts. In fact, monoclonal antibody mediated drug delivery is the only system which has demonstrated some selectivity in cancer chemotherapy. Since the development of hybridoma technique by Köhler and Milstein (1975), the effort of using MoAb has become more and more widespread and several approaches have been examined including drug immunoconjugates, immunotoxins, drug-loaded immunomicrospheres and immunoliposomes (Baldwin and Byers, 1985; Reisfeld and Sell, 1985; Rodwell, 1988; Wick and Siegal, 1988; Quash and Rodwell, 1989; Lee *et al.*, 1990) . This paper describes the effect of methotrexate conjugated to anti-common acute lymphoblastic leukemia antigen (CALLA) monoclonal antibodies on human lymphoblastic leukemia cell lines grown in vitro.

Monoclonal antibody 269-65 (IgG_1) was obtained in ascites form from the hybridoma cell line secreting anti-CALLA monoclonal antibody (MoAb) in BALB/c mice (Lee *et al.*, 1990). Ascites fluid was fractionated by ammonium sulfate precipitation and affinity chromatography on protein-A Sepharos CL-4B (Pharmacia, Sweden). The MoAb were shown to undergo binding reactions with NALM-6, Daudi and RPMI 8402 cells, whereas no reactivity was observed with the K562, MOLT4 and Jukart cell lines. In this study, Daudi and K562 were employed as antigen-positive and antigen-negative cells, respectively.

MoAb-MTX conjugates was synthesized by reaction of active ester of MTX and MoAb (Wong, 1993) as follow; A solution of 22.7 mg methotrexate (MTX, Sigma, U.S.A.), 11.35 mg dicyclo-hexylcardodiimide (Sigma, U.S.A.) and 6.33 mg N-hydroxysuccinimide (Sigma, U.S.A.) in 1 ml dry dimethylformamide (DMF, Fisher, U.S.A.) was stirred at room temperature for 12 hrs in dark room under nitrogen purging. To complete precipitation, the reaction flask was kept at −20℃ for 60 min, then filtered off and washed with cold DMF. The clear orange supernatant solution of MTX-active ester was used for coupling to MoAb. Cold DMF (0.2 ml) was added dropwise to a solution of 10 mg MoAb in 2.0 ml phosphate buffer solution (PBS, pH 7.2) over 1 min period and stirred for 30 min. Then cold MTX-active ester solution in DMF was added to the MoAb solution, and then stirred for 1.5 hrs and centrifuged. The supernatant was subject to gel filtration using Sephadex G-25 (Pharmacia, Sweden) to get the MTX-MoAb conjugate (Wong, 1993). The conjugate fraction monitored at 370 nm was dialyzed against PBS extensively and freeze dried to stored at 4℃. With molar absorption coefficients of MoAb and MTX at 280 and 370 nm, respectively, the conjugation molar ratio of MoAb to MTX was calculated as 1 : 10.

The effects of continuous exposure of two target cell lines, an antigenic cells Daudi and a non-antigenic cells K562 to test MoAb-MTX conjugates were observed. When cells enter the logarithmic phase of proliferation, two ml of each cell type (5×104 cells/ml) in complete RPMI 1640 media, supplemented with 10% heat-inactivated fetal calf serum (Sigma, U.S.A.) were seeded into 24-well microplates (Bellco, U.S.A.). Subsequently, various amount of MoAb-MTX conjugates, MTX alone and mixture of MTX and MoAb were added. The incubation was performed at 37℃ for 15 min with gentle agitation, followed by culturing at 37℃ under 10% atmospheric CO_2 for a week. The number of viable cells in each of the wells was determined daily by trypan blue dye exclusion method.

Fig. 1 shows the cytotoxic effect of MoAb-MTX conjugate on the cell lines grown in vitro, when 60 μl of one of the following two preparations was added: i) MoAb-MTX conjugate in PBS solution containing 0.22 μmol of MTX conjugated to 0.011 μmol of MoAb, ii) PBS solution. As shown in Fig. 1, MoAb-MTX conjugate had specific growth inhibiting effect on the antigenic cells Daudi and made no effect on the non-antigenic cell K562 over control groups. Preparations of MoAb alone, MTX alone and mixture of MTX and

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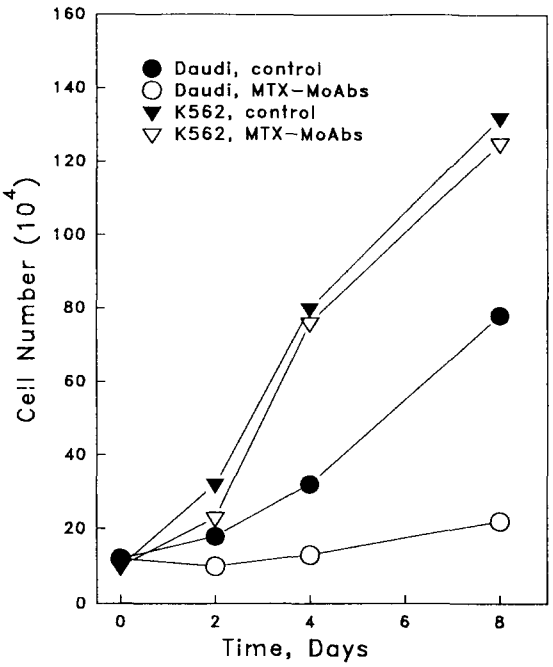

Fig. 1. Cytotoxicity of anti-CALLA monoclonal antibody conjugate to methotrexate on antigenic cell Daudi and non-antigenic cell K562 in vitro (Mean value of 3 experiments).

MoAb containing same amount of MTX and/or MoAb were also examined. No significant effect of above three preparation was shown on both Daudi and K562 cell growth comparing with control (data not shown).

In conclusion, anti-CALLA monoclonal antibody conjugates to methotrexate were prepared by active ester method. From the comparative cytotoxicity test between MoAb-MTX conjugate, MoAb alone, MTX alone and mixture of MTX and MoAb including control on both antigenic Daudi and non-antigenic K-562 cells, the specific cell growth inhibition effect of MoAb-MTX conjugate on antigenic cells was confirmed. The in vivo effect of MoAb-MTX conjugate on tumor cell growth in athymic mice are currently in progress.

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