BORON-10 CANCER THERAPY—II

Session Organizer: Albert H. Soloway (Ohio State)

All Papers Invited

BNL--39114

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1. Boronated Monoclonal Antibody Conjugates for Neutron Capture Therapy. *Donald C. Borg, John J. Elmore, Jr. (BNL), Soldano Ferrone (NY Med Coll)*

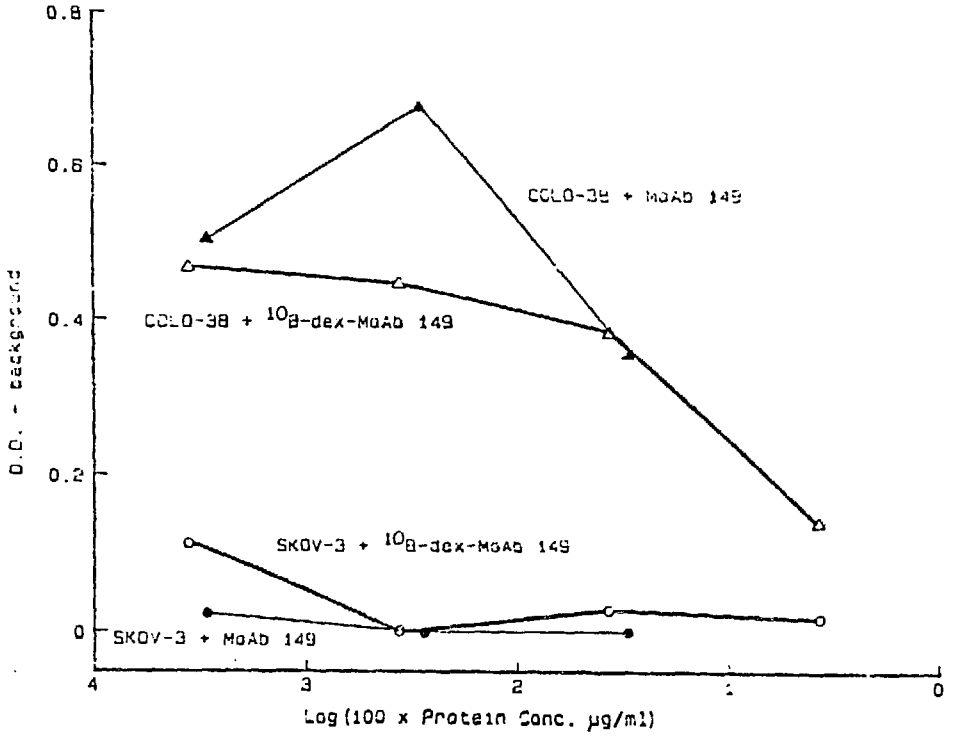
INTRODUCTION

Monoclonal antibodies (MoAbs) to tumor-associated antigens are attractive for concentrating ^{10}B in cancer tissue, in part because neutron capture therapy (NCT) is not disadvantaged by the hours to days required to optimize tumor:background concentration ratios of MoAbs or their $F(ab')_2$ or Fab fragments. Since direct coupling of ^{10}B compounds in amounts sufficient for radiotherapy appears to inactivate MoAbs, we used dextran intermediate carriers to provide high levels of ^{10}B per MoAb while modifying fewer amino acid residues.

DESCRIPTION

Three to five periodate-oxidized dextrans (molecular weight ~40000) were attached to anti-IgM polyclonal (IgG) antibodies, followed by coupling of decachlorocarboranylmethylbenzylamine, a benzylamine derivative of decachlorocarborane ("decloc") synthesized by D. Gabel (University of Bremen). With ~1000 boron atoms per IgG, 60 to 65% of the complexes retained antigen-binding capacity on an IgM-sepharose affinity column.

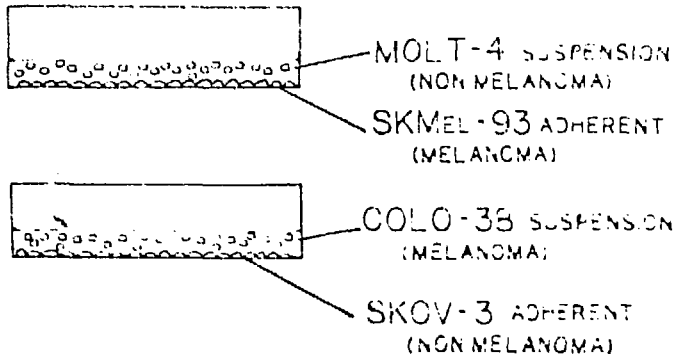
Reactions at pH 6.0 achieved high boron:antibody ratios with dextrans, 15 to 25% of whose glucose residues were previously oxidized by periodate. Sodium cyanoborohydride reduced Schiff's bases formed but not unreacted dextran aldehydes. An anthrone color reaction showed 2 to 5 dextrans/IgG, but an *o*-phthalaldehyde assay indicated more extensive modification of epsilon amino groups. Oxidized dextran-antibody complexes isolated by chromatography were then



ELISA OF AN ACTIVE ^{10}B-DEXTRAN-MONOCLONAL ANTIBODY CONJUGATE (MoAb 149)

Reactivity in the ELISA assay of MoAb 149.53 (filled symbols) and ^{10}B-dextran MoAb 149.53 (open symbols) with Colo-38 melanoma cells (triangles) and SKOV-3 ovarian carcinoma cells (circles). Ordinate is optical density from peroxidase coupled antimouse antibody reaction with substrate minus background of cells incubated with mouse Ig.

Fig. 1.



DOUBLE CELL "COMPETITIVE" BINDING TEST

This assay was developed to minimize the effect of nonspecific binding of boronated complexes to cells. In a 5 ml volume ^{10}B-dextran-MoAb-149 was incubated with Colo-38 melanoma cells, which grow predominantly in suspension, and plated SKOV-3 ovarian carcinoma cells. After incubation and several washings in a reciprocal binding test with ^{10}B-dextran-MoAb-149 to SKOV-3 [illegible] anchorage dependent cells incubated with Molt-4 lymphoblastic cells which grow in suspension. The suspended cells were decanted, spun down and washed, and [illegible] taken for FUSA or for lysis and ^{10}B assay (using the neutron activation [illegible]). The adherent cells were scraped into suspension and treated similarly.

Fig. 2. Double cell incubation [illegible]

reacted with "deeloc" benzylamine and sodium cyanoborohydride and finally reduced by sodium borohydride.

A track-etch neutron radioactivation assay demonstrated extensive boron binding to the Colo 38 human melanoma cell line *in vitro*, using boronated dextran conjugates of MoAbs to the human high molecular weight-melanoma associated antigen (HMW-MAA). Four to six times as many anti HMW MAA MoAbs 225.28S and 763.74T conjugated with dextran and 500 to 1400 boron atoms bound to melanoma cells as to control cells, with 5 to 20 μg of boron per gram of cells. However, ELISA and competition binding assays showed seriously reduced specific binding of these boron-dextran-antibody complexes to Colo-38 cells. Anti-HMW-MAA MoAb 149.53 tolerated the required chemical modifications better. MoAb 149.53 conjugated with dextran and ^{10}B enriched "deeloc" benzylamine and incubated simultaneously with melanoma and non-melanoma cells [one adherent and [illegible] other in suspension (Fig. 1)] showed good binding to melanoma cells by ELISA (Fig. 2) and high specific uptake of boron (see Table I).

TABLE I

^{10}B Content (μg B-10^9 cells) in Double Cell Incubations with ^{10}B-Dextran-MoAb-149

Suspended Colo-38 Melanoma Cells Versus Anchored Nonmelanoma SKOV-3		Anchored SK-Mel-93 Melanoma Cells Versus Suspended Nonmelanoma Molt-4	
Colo-38	SKOV-3	SK-Mel-93	Molt 4
19.4	−0.2	19.9	2.1

Results of the Double Cell "Competitive" Binding Test

The number of ^{10}B-dextran-MoAb 149.53 complexes bound per melanoma cell, based on ^{10}B content, was >4 × 10^6. (The amount added was about seven times this value.) Nonmelanoma cells, either adherent or in suspension, accumulated 10% or less of the amounts bound to melanoma cells. Ratios of binding of boronated antibody to melanoma cells versus control cells of >10 and ^{10}B levels of ~20 μg/g of melanoma cells are within the ranges calculated for successful NCT.

SIGNIFICANCE

The amounts and concentration ratios achieved (see Table I) are within the ranges required in tumor for successful ^{10}B NCT. Although these are among the most encouraging findings regarding [illegible] conjugates for NCT obtained to date, concentrations of boronated antibodies *in vivo* are likely to be far lower than those *in vitro*. There is also a need for more robust and consistent conjugation protocols, and trials of NCT on cultured cells and distribution and neutron irradiation studies in animals remain to be done. As this summary was submitted, a revised protocol provided ^{10}B-MoAb 149.53 conjugated to dextrans *previously* boronated with "deeloc" benzylamine, and results with this material will be reported

DISCLAIMER