Specific Activation of Glucuronide Prodrugs by Antibody-targeted Enzyme Conjugates for Cancer Therapy1

Shing-Ming Wang,2 Ji-Wang Chern, Ming-Yang Yeh, Joyce Co Ng, Edward Tung, and Steve R. Roffler3

Institute of Biomedical Sciences, Academia Sinica, Taipei 11529 [S-M. W., J. C. N., E. T., S. R. R.], and Institute of Pharmacy and Medical Laboratories [J-W. C.] and Department of Microbiology and Immunology [M-Y. Y.], National Defense Medical Center, Taipei 10764, Taiwan, Republic of China.

ABSTRACT

Cancer chemotherapy may be improved by increasing antineoplastic drug specificity for tumor cells. We have synthesized a glucuronide prodrug that can be enzymatically converted to an antineoplastic agent at tumor cells that are able to bind β-glucuronidase-monoclonal antibody conjugates. The glucuronide prodrug BHAMG, the tetra-n-butyl ammonium salt of (p-di-2-chloroethylaminophenyl-β-o-glucopyranosid) uronic acid, was 150 times less toxic than the parent drug, N,N-di-(2-chloroethyl)-4-hydroxyaniline, to HepG2 human hepatoma cells and over 1000-fold less toxic than the parent drug to AS-30D rat hepatoma cells in vitro. In the presence of β-glucuronidase, BHAMG was activated and became toxic as the parent drug N,N-di-(2-chloroethyl)-4-hydroxyaniline. A conjugate (RH1-βG) was formed by linking β-glucuronidase to a monoclonal antibody which binds to an antigen expressed on the surface of AS-30D cells. The concentration of BHAMG causing 50% inhibition of AS-30D cellular protein synthesis was reduced over 1000-fold, from >770 μM to <0.74 μM after these cells were preincubated with RH1-βG. Specificity of BHAMG activation at antigen-positive cells was shown by monoclonal antibody RH1 blocking of RH1-βG conversion of BHAMG to toxic drug and by the inability of BHAMG to be converted to active drug when antigen-negative control cells were preincubated with RH1-βG. Our results show that the targeted-β-glucuronidase activation of BHAMG can increase the specificity of chemotherapy for rat hepatoma in vitro and suggest that the targeted activation of glucuronide prodrugs may be useful for cancer therapy.

INTRODUCTION

Chemotherapy is an important treatment modality for many cancers, although its use is often palliative rather than curative (1). The basic limitation of chemotherapy is the physiological similarity between normal and tumor cells (1). Cancer chemotherapy is thus often terminated due to normal tissue toxicity and associated side effects such as leukopenia; immunosuppression; and pulmonary, cardiac, and neurotoxicities (2). We and others have attempted to increase drug specificity by linking antineoplastic agents to monoclonal antibodies that bind to tumor-associated antigens preferentially expressed on the surface of tumor cells (3-7). While direct conjugation of drugs to antibody conjugates through genetic engineering.

MAterials and METHODS

Reagents and Cells. HAM and BHAMG were synthesized as described (18). Structures were confirmed by nuclear magnetic resonance and melting point determination. UDP-glucuronic acid, p-nitrophenyl β-d-glucuronide, p-nitrophenol, glucaro 1,4-lactone, reduced glutathione, 1-chloro-2,4-dinitrobenzene, and β-glucuronidase (EC 3.2.1. 31) from Escherichia coli (type X-A) were purchased from Sigma Chemical Company (St. Louis, MO). Sephadex G-25 gel was from Pharmacia LKB Biotechnology (Uppsala, Sweden). SMCC was from Pierce Chemical Company (Rockford, IL). [3H]Leucine (50 Ci/mmol) was purchased from ICN Biomedicals, Inc. (Costa Mesa, CA). AS-30D rat hepatoma cell line (19) was generously provided by Dr. J. P. Chang (Institute of Zoology, Academia Sinica, Taipei, Taiwan, ROC). CaSki human cervical carcinoma cells were kindly provided by Dr. R. A.

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1 Supported by grants and allocations from the National Science Council and Academia Sinica, Taipei, Taiwan, Republic of China.
2 Present Address: Division of Colon and Rectal Surgery, Tri-Service General Hospital, Taipei, Taiwan, Republic of China.
3 To whom requests for reprints should be addressed.

4 The abbreviations used are: BHAMG, tetra-n-butyl ammonium salt of HAMG; HAMG, glucuronide prodrug of p-hydroxy aniline mustard; HAM, p-hydroxyaniline mustard [N,N-di-(2-chloroethyl)-4-hydroxyaniline]; βG, β-glucuronidase; Mab, monoclonal antibody; RH1-βG, conjugate of RH1 antibody with β-glucuronidase; PBS, phosphate-buffered saline (0.14 M NaCl, 2.7 mM KCl, 1.5 mM KH2PO4, 8.1 mM Na2HPO4); SMCC, succinimidyl 4-(N-maleimidomethyl) cyclohexane 1-carboxylate; UDPGT, uridine 5′-diphosphoglucuronyl transferase; GST, glutathione S-transferase; IC50, concentration of test sample causing 50% inhibition of cellular protein synthesis.

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Fig. 1. A glucuronide prodrug system. Mab-ßG conjugate is first allowed to bind antigen that is preferentially expressed on the tumor cell membranes, resulting in accumulation of ßG at the cancer site. The glucuronide prodrug BHAMG is subsequently activated by ßG to the potent alkylating agent HAM which can kill the tumor cell.

Pattullo (Medical College of Wisconsin, Milwaukee, WI). HepG2 human hepatoma and COLO 205 human colon carcinoma cells were obtained from the AmericanType Culture Collection (Rockville, MD). Human cells were maintained in RPMI 1640 (Gibco BRL, Grand Island, NY) supplemented with 5% heat-inactivated fetal bovine serum, 100 units/ml penicillin, and 10 μg/ml streptomycin. AS-30D cells were cultured in Dulbecco’s modified Eagle’s medium (Gibco BRL) supplemented as above.

ß-Glucuronidase Conjugation to Monoclonal Antibody. Mab RH1 is a murine IgG2a monoclonal antibody developed in our laboratory that binds strongly to AS-30D cells but does not bind HepG2 cells. RH1-ßG was formed by linking ß-glucuronidase to Mab RH1 via a thioether bond. A maleimido group was first introduced into the immunoglobulin molecule with the heterobifunctional cross-linking agent SMCC. A 7-fold molar excess of SMCC dissolved in dioxane (3 mg/ml) was added to Mab RH1 (5-10 mg/ml) in PBS for 45 min at 37°C. Excess SMCC was removed by gel filtration on Sephadex G-25, and the number of maleimido groups was measured (20). Modified RH1 antibody was then reacted with thiol groups present in ßG. Liphylized ßG was dissolved in PBS (3 mg/ml) and passed through Sephadex G-25. Free thiol groups were measured (21), and ßG was mixed with derivatized IgG, concentrated by ultrafiltration, and reacted overnight at 4°C. All coupling reactions were performed in PBS containing 1 mM EDTA, deoxygenated by boiling and sparging with nitrogen. Purification and Characterization of ß-Glucuronidase-Antibody Conjugate. RH1-ßG was purified in a two-step process. Uncoupled ßG was removed from the conjugate by protein A-Sepharose affinity chromatography. Free Mab RH1 was then removed by ion exchange chromatography on a DEAE 5 PW high-performance liquid chromatography column (Waters) by eluting with a linear gradient of NaCl in 20 min triethanolamine, pH 7. 0. Eluted conjugates were concentrated by ultrafiltration, and after adding 1 mg/ml human serum albumin they were filter sterilized and stored at -7°C. Protein concentrations were measured by the bicinchoninic acid assay (22). Apparent molecular weight of the enzyme-antibody conjugate was calculated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blot analysis (23, 24). The antigen-binding activities of Mab RH1 and RH1-ßG were determined by enzyme-linked immunosorbent assay using whole AS-30D cells coated on 96-well plates as antigen (7). ßG enzyme activity was measured in a microtiter assay. Serial dilutions of ßG standard or sample (20 μl/well) were added to wells of 96-well microtiter plates containing 200 μl reaction buffer (100 mM acetic acid, 50 mM bis-tris, 50 mM triethanolamine, pH 7.0, with NaOH) and 20 μl of 40 mM ß-nitrophenyl ß-D-glucuronide. Plates were incubated at 37°C for 30 min before addition of 12 μl of 1 N NaOH to each well. The absorbance of the wells was immediately read at 405 nm in a Molecular Devices (Menlo Park, CA) microplate reader. Antigen-binding and enzyme activities of RH1-ßG were also measured simultaneously by first incubating RH1-ßG in plates coated with AS-30D cells for 1 h at 37°C and then carrying out the ßG enzyme activity assay after washing plates three times with PBS.

In Vitro Activity of Prodrug and Conjugate. Protein synthesis of cell cultures was measured as described (24). Briefly, AS-30D or HepG2 cells were plated overnight in 96-well microtiter plates at 20,000 cells/well. Serial dilutions of HAM or BHAMG in medium containing 5% fetal calf serum were added to cells for 1 or 24 h at 37°C. Cells were subsequently washed once with sterile PBS, incubated until hour 48 in fresh medium, and then pulsed for 2 h with [3H]leucine (1 μCi/well) in leucine-free medium. The radioactivity of trichloroacetic acid-precipitated protein was measured in a Beckman LS 6000 series liquid scintillation counter.

BHAMG conversion to cytotoxic HAM was tested by incubating AS-30D and HepG2 cells with BHAMG plus ßG (10 units/well) for 24 h at 37°C. One unit of ßG can hydrolyze 1 μmol p-nitrophenol ß-D-glucuronide in 1 h at 37°C. Cells were washed once with PBS and incubated in fresh medium for an additional 24 h before measuring the rate of protein synthesis. The in vitro activation of BHAMG by RH1-ßG was examined by preincubating plates AS-30D and HepG2 cells with the indicated concentration of conjugate for 30 min at room temperature. After washing cells once with PBS, BHAMG was added, and the assay was carried out as described above.

Competitive blocking of RH1-ßG with Mab RH1 F(ab')2 fragments was also examined. RH1 F(ab')2 (50 μg/ml) was added as described (25) except for increasing the pepsin:antibody ratio to 40:1 (wt/wt) and digesting for 1 h, was added with 1 μg/ml RH1-ßG to AS-30D or HepG2 cells for 30 min at room temperature. Cells were then washed once with PBS and exposed to 90 μM BHAMG for 24 h at 37°C. Fresh medium was added to the cells for 24 h before measuring cellular protein synthesis rate. All experiments were performed in triplicate.

Enzyme Activities. Enzyme activities of cell homogenates were measured. Cultured cells were trypsinized, washed twice with PBS, and transferred to 20 mM bis-tris, pH 6.0, containing 0.1% (v/v) triton X-100 for 45 min at 4°C. Cells were broken in a glass Dounce homogenizer, and homogenates were frozen at -76°C or immediately assayed for enzymatic activity.

ßG activity was measured using p-nitrophenyl glucuronide as substrate (26). One hundred μl of cell homogenate and 50 μl of 40 mM p-nitrophenyl glucuronide were added to 300 μl reaction buffer (100 mM acetic acid, 50 mM bis-tris, 50 mM triethanolamine, pH adjusted to 7 with NaOH) for 1 h at 37°C. The reaction was terminated by adding 500 μl 0.5 M trichloroacetic acid and heating to 100°C for 5 min. Samples were clarified by centrifugation, and 0.7 ml was then transferred to a 3-ml cuvette. Sample pH was adjusted to >11 by addition of 250 μl of 1 N NaOH and 1 ml distilled water. After mixing, absorbance was measured in a Beckman DU-70 spectrophotometer at 405 nm. Specific activities were calculated from a standard curve of absorbance versus p-nitrophenol concentration.

UDPGT activity was measured in a similar fashion using p-nitrophe

ol and UDP-glucuronic acid as substrates (27). Briefly, 125 μl of cell homogenate, 65 μl 50 mM UDP-glucuronic acid, and 65 μl 2 mM p-nitrophenol were added to 400 μl reaction buffer (80 mM sodium phosphate, pH 7.0, 8.0 mM glucurono-1,4-lactone, 0.8 g/liter Triton X-100). After reaction at 37°C for 1 h, samples were processed as above for ßG activity.

Glutathione S-transferase activity was measured by using reduced glutathione and 1-chloro-2,4-dinitrobenzene as substrates. One hundred μl cell homogenate, 40 μl 50 mM glutathione, and 40 μl 50 mM 1-chloro-2,4-dinitrobenzene (in ethanol) were added to 1.8 ml buffer (80 mM sodium phosphate, pH 7). The absorbance at 340 nm was detected in a spectrophotometer every 10 s for 2 min using a kinetic data program of the Beckman DU-70 spectrophotometer. The slope of the linear curve was determined by least squares regression. Specific GST activities were calculated assuming an ε340 of 9.6 mM-1 cm-1 (28).
Cell Growth Rate. The growth rate of tumor cells in vitro was determined by trypsinizing cells from triplicate wells of 6-well plates and counting viable cells once a day for 5 days. Doubling times were calculated from the slope of log (cell number) versus time as determined by least-squares regression.

RESULTS

β-Glucuronidase Activation of Glucuronide Prodrug. BHAMG was designed as a glucuronide prodrug of the potent alkylating agent HAM. The effect of BHAMG and HAM on several tumor cell lines was determined by measuring [3H]-leucine incorporation into the protein of cells after drug exposure time on cell cytotoxicity was also examined in AS-30D cells. HAM and BHAMG were both about 2 times more toxic in a 24-h exposure assay compared to a 1-h exposure (Table 1). Drug latency, however, was relatively insensitive to drug exposure time (1300 versus 1160 for 1-h and 24-h exposure times, respectively).

Endogenous Enzyme Activities of Cells. Cell line sensitivities to HAM or BHAMG plus βG varied by nearly 100-fold. Variation of cellular sensitivity to HAM or BHAMG was hypothesized to be due to the relative activities of the endogenous detoxification enzymes GST and UDPGT and the prodrug-activating enzyme βG. Table 2 summarizes specific enzyme activities in whole cell homogenates prepared from the cell lines shown in Table 1. The sensitivity of cells to HAM appeared to be inversely related to cellular GST activity. Cells with lower GST activities (AS-30D and HepG2) were more sensitive to HAM, while cells expressing high GST activity (COLO 205 and CaSki) were relatively resistant to HAM. Linear regression analysis of HAM IC50 values versus the GST activity of these cells gave a positive correlation coefficient of 0.976. No correlation was found between cell sensitivity to HAM and UDPGT activity or cell growth rate. The sensitivity of AS-30D cells to intermediate concentrations of BHAMG (20% inhibition of protein synthesis at 100 μM BHAMG) is likely due to the high βG activity of these cells. The βG activity of AS-30D cells was significantly greater than that of other cells shown in Table 2 (P < 0.005). Similarly, AS-30D cells were significantly more

![Graph](image-url)

Fig. 2. In vitro growth inhibition of hepatoma cells by HAM and BHAMG. AS-30D rat hepatoma (A) and HepG2 human hepatoma (B) cells were exposed to HAM (○), BHAMG (△), or BHAMG plus 10 units βG (■) for 24 h, washed with PBS, and then incubated in fresh medium for an additional 24 h. The cellular protein synthesis rate of drug-treated cells is compared to that of untreated control cells at 48 h. Note that the scales of the abscissas are different. Bars, SE of triplicate determinations.

Table 1 In vitro effect of HAM and BHAMG

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Mean IC50 (μM)</th>
<th>BHAMG IC50 (μM)</th>
<th>BHAMG + βG IC50 (μM)</th>
<th>Latency (BHAMG/HAM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS-30D</td>
<td>0.85 ± 0.15 (5)</td>
<td>1090 ± 180 (4)</td>
<td>0.69 ± 0.25 (4)</td>
<td>1280</td>
</tr>
<tr>
<td>1 h exposure</td>
<td>1.18 ± 0.08 (2)</td>
<td>1370 ± 200 (2)</td>
<td>0.55 ± 0.09 (2)</td>
<td>1160</td>
</tr>
<tr>
<td>24 h exposure</td>
<td>0.62 ± 0.12 (3)</td>
<td>809 ± 9 (2)</td>
<td>0.82 ± 0.58 (2)</td>
<td>1300</td>
</tr>
<tr>
<td>HepG2</td>
<td>7.9 ± 1.6 (8)</td>
<td>1185 ± 138 (7)</td>
<td>10.8 ± 2.9 (9)</td>
<td>150</td>
</tr>
<tr>
<td>Colo 205</td>
<td>37 ± 6.4 (3)</td>
<td>1880 ± 18 (3)</td>
<td>15.9 ± 2.5 (7)</td>
<td>51</td>
</tr>
<tr>
<td>CaSki</td>
<td>53.5 ± 2.2 (3)</td>
<td>2790 ± 190 (2)</td>
<td>126 ± 42 (3)</td>
<td>52</td>
</tr>
</tbody>
</table>

* Unless otherwise indicated, cells were exposed to drugs for 24 h.

* Latency is the ratio of mean IC50 values for BHAMG to HAM.

* Numbers in parentheses, number of independent assays, each carried out in triplicate, used to determine mean values. SEMs are also indicated.

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sensitive to BHAMG, compared by IC50 values, than HepG2, Colo 205, or CaSki cells (P < 0.10, 0.005, and 0.005, respectively).

Mab-βG Conjugate. To test the feasibility of specifically activating BHAMG at antigen-positive hepatoma tumor cells, βG was conjugated to Mab RH1 by a stable thioether linkage. We previously linked βG to the F(ab’)2 fragment of an IgG, Mab (Fig. 5, Lane a). Blocking of RH1-βG with excess Mab RH1 F(ab’)2 did not affect the protein synthesis of antigen-negative HepG2 cells exposed to BHAMG (Fig. 5, Lanes c and d).

The ability of RH1-βG to specifically activate BHAMG at antigen-positive AS-30D cells was also examined by preincubating cells with 1 or 10 μg/ml RH1-βG and subsequently exposing the cells to varying concentrations of BHAMG for 24 h. Preincubation of AS-30D cells with 1 μg/ml RH1-βG decreased the IC50 of BHAMG by about 200-fold (Fig. 6A). In contrast, RH1-βG was ineffective at potentiating the activity of BHAMG at HepG2 cells (Fig. 6B). Table 3 shows that BHAMG toxicity to AS-30D cells was further increased by raising the RH1-βG concentration to 10 μg/ml. At this concentration of RH1-βG, BHAMG was about as potent as HAM, with an IC50 of <0.75 μM.

Specific Activation of Prodrug. The specific activation of BHAMG at antigen-positive AS-30D cells was examined by first incubating cells with different concentrations of RH1-βG for 30 min, washing the cells, and then exposing the cells to 90 μM BHAMG for 24 h. Fig. 4A shows that protein synthesis was reduced by up to 95% in AS-30D cells preincubated with RH1-βG and then exposed to BHAMG. Even at a RH1-βG concentration of only 60 ng/ml, protein synthesis of BHAMG-treated AS-30D cells was inhibited by 44% compared to cells not exposed to RH1-βG. RH1-βG activation of BHAMG was specific for antigen-positive cells; preincubation of antigen-negative HepG2 cells with RH1-βG did not increase the toxicity of BHAMG to these cells (Fig. 4B).

RH1-βG specificity for AS-30D cells was further verified by a competition assay. The addition of 50 μg/ml Mab RH1 F(ab’)2 during the preincubation of AS-30D cells with 1 μg/ml RH1-βG protected the cells from BHAMG; cellular protein synthesis was inhibited by only 30% (Fig. 5, Lane b) compared to 90% inhibition of protein synthesis in the absence of competing antibody fragment (Fig. 5, Lane a). Blocking of RH1-βG with excess Mab RH1 F(ab’)2 did not affect the protein synthesis of antigen-negative HepG2 cells exposed to BHAMG (Fig. 5, Lanes c and d).

Table 2. Enzyme activities of tumor cells

<table>
<thead>
<tr>
<th>Cell</th>
<th>Doubling time (h)</th>
<th>10^6 × enzyme activity (μmol/h-cell)</th>
<th>10^6 × GST activity (μmol/min-cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS-30D</td>
<td>21</td>
<td>10.1 ± 0.6</td>
<td>7.6 ± 0.1</td>
</tr>
<tr>
<td>HepG2</td>
<td>25</td>
<td>1.5 ± 0.1</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>Colo 205</td>
<td>20</td>
<td>2.7 ± 0.1</td>
<td>11.4 ± 0.3</td>
</tr>
<tr>
<td>CaSki</td>
<td>24</td>
<td>2.6 ± 0.2</td>
<td>0.26 ± 0.37</td>
</tr>
</tbody>
</table>

*Results are mean values of duplicate samples. SD of mean values are also shown.*
TARGETED-ENZYME ACTIVATION OF A GLUCURONIDE PRODRUG

DISCUSSION

The impetus for examining targeted-enzyme activation of glucuronide prodrugs for cancer therapy came from earlier reports that mice bearing well-established PC5 plasma tumors containing high levels of β-glucuronidase were cured by treatment with aniline mustard (30–32). Aniline mustard was apparently converted to HAM and then to HAMG \textit{in vivo} in the liver of treated mice. HAMG was subsequently converted to highly cytotoxic HAM by endogenous βG present at the tumor site (31). Clinical trials using aniline mustard for cancer chemotherapy (33, 34), however, were disappointing, likely due to insufficient activity of βG in most human tumors (33). We hypothesized that targeting βG to the cancer site could allow specific activation of glucuronide prodrugs at tumor cells. Our results show that it is possible to specifically kill cancer cells expressing tumor-associated antigen by first targeting a β-glucuronidase-monoclonal antibody conjugate to tumor cells to elevate the activity of βG and then treating the cells with a glucuronide prodrug.

The purpose of converting a prodrug to an antineoplastic agent at tumor cells but not normal tissues is to increase the specificity and lower the toxicity of cancer chemotherapy. The generation of local high concentrations of drug at tumor sites...
could increase the killing of cancer cells with a commensurate decrease in normal tissue toxicity. Several conditions, however, must be met for this strategy to be feasible. The most basic requirements are that (a) the prodrug should be less toxic than the corresponding parent drug; (b) prodrug can be converted under defined conditions into the active parent compound; and (c) tumor and normal cells should display sufficient differences in the cellular property used to activate prodrug to parent drug.

The specific activation of BHAMG by Mab-targeted βG appeared to meet these criteria. BHAMG was over 1000 times less toxic than HAM to tumor cells but could be enzymatically converted to HAM by βG. More importantly, by linking βG to a Mab against rat hepatoma cells, sufficient βG was targeted to antigen-positive cells to activate BHAMG to HAM in vitro. More importantly, by linking βG to a Mab against rat hepatoma cells, sufficient βG was targeted to antigen-positive cells to activate BHAMG to HAM in vitro.

Several conditions, however, could increase the killing of cancer cells with a commensurate decrease in normal tissue toxicity. Several conditions, however, could increase the killing of cancer cells with a commensurate decrease in normal tissue toxicity. Several conditions, however, could increase the killing of cancer cells with a commensurate decrease in normal tissue toxicity.}

Besides the specific activation of BHAMG described here, other targeted-enzyme-activated prodrugs have been described. Senter and colleagues (13, 14) investigated the activation of phosphorylated drug derivatives with Mab-alkaline phosphatase conjugates. They were able to demonstrate the regression of human lung adenocarcinoma xenografts in nude mice treated with Mab-alkaline phosphatase conjugates followed by mitomycin phosphate administration (14). Phosphorylated prodrug alone and in combination with a control Mab-alkaline phosphatase conjugate also delayed tumor growth. The same group has also described the activation of a doxorubicin prodrug with a Mab-penicillin-V-amilodase conjugate (44) and the conversion of 5-fluorouracil into the anticancerous agent 5-fluorouracil by a cytotoxic adeninase-Mab conjugate (45).

Bagshawe and coworkers (12) have developed glutamic acid prodrugs which could be converted to toxic bis-chloroazobenzene acid mustards by carboxypeptidase G2. These prodrugs were unable to inhibit or eliminate human choriocarcinoma (12) or colon carcinoma (46) xenografts in nude mice after treatment with antibody-carboxypeptidase G2 conjugates. A cephalosporin-Vinca alkaloid prodrug activated by a β-lactamase-antibody fragment conjugate has also been described (47).

Beta-G-activated glucuronide prodrugs possess potential advantages over other enzyme-prodrug combinations for cancer therapy. βG concentration in human serum is very low (26), suggesting that glucuronide prodrugs should be stable in the blood after i.v. administration. Although several organs, including the liver, gastrointestinal tract, spleen, and lung, do contain endogenous βG (48, 49), mammalian tissues also express UDPGT, a class of xenobiotic detoxification enzymes that can reverse the reaction catalyzed by βG (27, 37). In studies carried out in rodents and humans, glucuronide conjugates were major metabolites of several drugs including aniline mustard (32), 9-hydroxyellipticine (50), 4'-epidoxorubicin (51), 1-naphthol (52), and AZT (3'-azido-3'-deoxythymidine) (53). These studies and our own results showing that glucuronide prodrugs show a low toxicity in vivo. These studies also suggest that activated enzyme anticancer prodrugs are used to target tumor cells with a higher specificity than the corresponding parent compound. Glucuronide conjugates can also be recovered from tissues containing high UDPGT activities. Also, because βG is an endogenous enzyme, it may be possible to target human βG to tumor cells, reducing the chance of inducing an immune response against the Mab-enzyme conjugate in humans, a potential problem with conjugates containing exogenous enzyme. In addition, although βG is highly specific for the glucuronol residue of glucuronide conjugates, it has little specificity for the conjugated aglycone (26), suggesting that a wide variety of glucuronide prodrugs could be used for cancer therapy. Glucuronide prodrugs also appear to be less toxic than similar prodrugs (19, 46).

In summary, we have demonstrated that the glucuronide prodrug BHAMG is much less toxic than the corresponding parent compound HAM to several tumor cell lines, including both human and rat hepatoma cells. A monoclonal antibody-βG conjugate was constructed and shown to preferentially accumulate at cancer cells that express tumor-associated antigen. Antigen-positive tumor cells were also specifically killed by BHAMG.
after cells were exposed to antibody-βG conjugate. These results show that a glucuronide prodrug of low toxicity can be converted to a highly toxic drug in vitro at tumor cells in which βG activity has been elevated. Taken together, these results suggest that targeted βG activation of glucuronide prodrugs is potentially useful for cancer therapy. More work is required, however, to examine the behavior of βG-activated glucuronide prodrugs in vivo and to address questions such as the mechanism of reduced glucuronide prodrug toxicity, the stability of glucuronide prodrugs in vivo, and the effect of antibody internalization on the ability of conjugates to activate prodrug at tumor cells. Only when these and other questions are answered can the potential of this strategy be realized.

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