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

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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-D-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 as toxic as the parent drug after di-(2-chloroethyl)-4-hydroxyaniline, and 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 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 antibodies can increase the specific targeting of drugs to tumor cells, this method suffers from several drawbacks. Practical limits on drug loading or slow internalization of drug conjugates into cancer cells may result in poor tumor killing (8, 9). In addition, cells expressing low levels of antigen in heterogeneous cell populations may also escape destruction (10).

A new indirect drug-targeting strategy has recently been observed to overcome problems associated with chemoimmunoconjugates while retaining the advantages of selective drug action (11–14). In this method, enzyme rather than drug is linked to an antibody that binds antigen preferentially expressed on the surface of tumor cells. The antibody-enzyme complex is then targeted to tumor cells, thus allowing the conjugated enzyme to accumulate at the cancer site. A latent, nontoxic “pro-drug” is then introduced so that prodrug coming into contact with targeted enzyme at the tumor site can be enzymatically converted to the active parent compound which can then kill the tumor cells. This method may provide some advantages compared with chemoimmunoconjugates, including accumulation of higher drug concentrations at the tumor, less sensitivity to tumor cell heterogeneity, and the possibility of creating defined immunoconjugates through genetic engineering.

We have recently described a relatively nontoxic glucuronide prodrug, BHAMG, that can be enzymatically converted to the parent drug HAM by βG (15). Specific activation of BHAMG at tumor cells can be obtained by targeting βG to tumor cells as an enzyme-Mab conjugate. Fig. 1 illustrates the strategy of targeted-enzyme activation of BHAMG. In this report, we examine the specific in vitro activation of BHAMG at rat hepatoma cells expressing a tumor-associated antigen. Hepatocellular carcinoma is the most common cancer worldwide, causing approximately 1 million deaths each year (16, 17). Hepatoma is usually lethal, with a median survival time as short as several months (17), indicating that improved therapies are needed. We demonstrate that rat and human hepatoma cells are sensitive to HAM but not to the prodrug BHAMG. Protein synthesis of hepatoma cells, however, was inhibited by BHAMG after activation by βG. We also show that BHAMG can be specifically activated and preferentially kill antigen-positive hepatoma tumor cells that were previously exposed to a β-G-Mab conjugate.

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 the gift of 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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**BHAMG (prodrug)**

\[ \text{O} - \text{N}^- (n-Bu)_2 \]

\[ \text{CH}_3\text{CH}_2\text{Cl} \]

\[ \text{CH}_3\text{CH}_2\text{Cl} \]

**HAM (active drug)**

\[ \text{N}^- \]

\[ \text{OH} \]

\[ \text{HO} \]

\[ \text{N}^- \]

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

Pattillo (Medical College of Wisconsin, Milwaukee, WI). HepG2 human hepatoma and COLO 205 human colon carcinoma cells were obtained from the American Type 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 100 \( \mu \)g/ml streptomycin. AS-30D cells were cultured in Dulbecco's modified Eagle's medium (Gibco BRL) supplemented as above.

**\( \beta \)-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-\( \beta \)G was formed by linking \( \beta \)-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 \( \beta \)G. Lysylated \( \beta \)G was dissolved in PBS (3 mg/ml) and passed through Sephadex G-25. Free thiol groups were measured (21), and \( \beta \)G 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, deoxynucleated by boiling and sparging with nitrogen.

**Purification and Characterization of \( \beta \)-Glucuronidase-antibody Conjugate.** RH1-\( \beta \)G was purified in a two-step process. Uncoupled \( \beta \)G 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, with NaOH) for 1 h at 37°C. The reaction was terminated by adding 12 M \( \text{N}^- \) NaOH to each well. The absorbance at 340 nm was measured in a microtiter assay. Serial dilutions of \( \beta \)G standard or sample (20 \( \mu \)l/well) were added to wells of 96-well microtiter plates containing 200 \( \mu \)l reaction buffer (100 mM acetic acid, 50 mM bis-tris, 50 \( \mu \)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 \( \mu \)l 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-nitropheno1 and UDP-glucuronic acid as substrates (27). Briefly, 125 \( \mu \)l cell homogenate, 65 \( \mu \)M 50 mM UDP-glucuronic acid, and 65 \( \mu \)M 2 mM p-nitrophenol were added to 400 \( \mu \)l 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 \( \beta \)G activity.

Glutathione S-transferase activity was measured by using reduced glutathione and 1-chloro-2,4-dinitrobenzene as substrates. One hundred \( \mu \)l cell homogenate, 40 \( \mu \)l 50 mM glutathione, and 40 \( \mu \)l 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 \( e^{340} \text{ of 9.6 mm}^{-1} \text{cm}^{-1} \) (28).
Cell Growth Rate. The growth rate of tumor cells in vitro was determined by trypanosmitizing 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. Comparison of IC50 values revealed that BHAMG was over 1000 times less toxic than HAM to AS-30D rat hepatoma cells (Fig. 2A) and about 150-fold less toxic to HepG2 human hepatoma cells (Fig. 2B) after 24 h of drug exposure. The simultaneous addition of βG (10 units/well) and BHAMG to tumor cells resulted in a cytotoxic effect equal to that of HAM alone, indicating that cleavage of the glucuronide functional group converted BHAMG to HAM (Fig. 2). Addition of βG alone did not affect [3H]leucine incorporation into cellular protein (data not shown).

Table 1 summarizes the effects of HAM and BHAMG on cellular protein synthesis in several cell lines. AS-30D cells were most sensitive to HAM with a mean IC50 value of 0.85 μM. Other cell lines were more resistant to HAM, with CaSki human cervical carcinoma cells being the most resistant (IC50, 53.5 μM). Prodrug latency, a measure of the difference in toxicities between prodrug and the parent compound, was also greatest for AS-30D cells; BHAMG was an average of 1280 times less toxic than HAM to AS-30D cells. The effect of 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...
ronsyltransferase in whole cell lysates were measured as described in "Materials and Methods.”

Mab-ßG Conjugate. To test the feasibility of specifically activating BHAMG at antigen-positive hepatoma tumor cells, ßG was conjugated to Mab RH1 by a stable thioether linkage. We previously linked ßG to the F(ab’)2 fragment of an IgG, Mab Rh1.Animating BHAMG at antigen-positive hepatoma tumor cells, ßG was more sensitive to BHAMG, compared by ICSO values, than HepG2, Colo 205, or CaSki cells (P < 0.10, 0.005, and 0.005, respectively).

Specific Activation of Prodrug. The specific activation of BHAMG at antigen-positive AS-30D cells was examined by preincubating cells with 1 or 10 µg/ml RH1-ßG and subsequently exposing the cells to varying concentrations of BHAMG for 24 h. Preincubation of AS-30D cells with 1 µg/ml RH1-ßG decreased the IC50 of BHAMG by about 200-fold (Fig. 6A). In contrast, RH1-ßG 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-ßG concentration to 10 µg/ml. At this concentration of RH1-ßG, BHAMG was about as potent as HAM, with an IC50 of <0.75 µM.

Table 2. Enzyme activities of tumor cells

<table>
<thead>
<tr>
<th>Cell</th>
<th>Doubling time (h)</th>
<th>10^9 × enzyme activity (µmol/h-cell)</th>
<th>10^9 × 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>

*S Results are mean values of duplicate samples. SD of mean values are also shown.

Fig. 3. Enzyme and antibody activities of RH1-ßG. A, enzyme activity of ßG before (C) or after (0) coupling to Mab RH1, measured by monitoring the release of p-nitrophenol from p-nitrophenyl ß-D-glucuronide at 405 nm. B, enzyme-linked immunosorbent assay measurement of antigen-binding activities of Mab RH1 (0), or RH1-ßG (C, 0) to antigen-positive AS-30D cells (0, 0) or antigen-negative HepG2 cells (0, 0). Bars, SE of triplicate determinations. C, combined immunosorbent and ßG activity measurement of RH1-ßG. RH1-ßG was incubated in wells coated with AS-30D cells and then assayed for ßG activity using p-nitrophenyl ß-D-glucuronide as the substrate.
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Fig. 4. In vitro activation of BHAMG by RH1-βG. AS-30D (A) or HepG2 (B) cells were preincubated with different concentrations of RH1-βG for 30 min at room temperature. Cells were then washed once with PBS and exposed to 90 μM BHAMG (60 μg/ml) for 24 h. The cellular protein synthesis rate was measured 24 h later and is compared to the protein synthesis rate of control cells exposed to 90 μM BHAMG only. Bars, SE of triplicate determinations.

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 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-monoconal 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 appears 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. AS-30D cells preincubated with RH1-βG were at least 200 times more sensitive to BHAMG than untreated AS-30D cells. Elevation of βG enzyme activity at tumor cells by the localization of Mab-βG conjugate also allowed differentiation of target and nontarget cells, demonstrated by the specific killing of antigen-positive AS-30D but not antigen-negative HepG2 cells by combined treatment with RH1-βG and BHAMG.

Preliminary results indicate that tumor cells expressing lower levels of GST, an important family of detoxification enzymes, are more susceptible to HAM than cells with high GST activity. Both rat and human hepatoma cell lines expressed lower GST activities and were more sensitive to HAM or βG-activated prodrug than either colon or cervical carcinoma cells tested. This result is in agreement with reports that tumor cell resistance to alkylating agents is often associated with high GST activities (35, 36). Other factors, however, such as the rate of DNA damage repair, may also be important in determining cellular sensitivity to HAM. No correlation was found between cell sensitivity to HAM and cellular activity of UDPGT, a family of enzymes important in xenobiotic conjugation and detoxification (37). A high level of cellular βG, on the other hand, appeared to be associated with cell sensitivity to BHAMG, suggesting that glucuronide prodrugs can be converted to parent drug by high levels of endogenous βG. BHAMG, however, was several orders of magnitude less toxic than HAM to AS-30D cells which expressed the highest βG activity of the cells examined, indicating that endogenous βG was ineffective at activating BHAMG in vitro.

The application of targeted-enzyme activation of prodrugs to cancer chemotherapy may solve some of the problems associated with the direct linkage of antineoplastic agents to Mabs. Chemoimmunoconjugate drug loading, even when using linkers such as dextran or albumin, appears to be limited to less than 100 drug molecules/antibody (7, 38, 39). Insufficient drug may be internalized into cancer cells to totally eradicate the tumor (8, 9). A single Mab-enzyme conjugate, in contrast, can generate a great number of drug molecules at the tumor site, increasing the chance of attaining therapeutic concentrations of drug. Activated prodrugs have a low molecular weight and should diffuse more readily into the tumor mass (40). They should also be less sensitive to antigen heterogeneity, since prodrug activated at the surface of antigen-positive tumor cells can in principle diffuse to neighboring antigen-negative tumor cells (10). Chemoimmunoconjugates are also difficult to standardize and require extensive characterization. Mab-enzyme conjugates, in contrast, lend themselves to genetic engineering. A fusion protein between immunoglobulin variable chains and enzyme could be produced on a large scale as a standard product (41). This type of chimeric molecule should also clear more rapidly from the blood pool (42) and be less likely to induce antigen modulation (43).

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-amidase conjugate (44) and the conversion of 5-fluorocytosine into the antineoplastic agent 5-fluorouracil by a cytotoxic deaminase-Mab conjugate (45). Bagshawe and coworkers (12) have developed glutamic acid prodrugs which could be converted to toxic bis-chlorobenzoic acid mustards by carboxypeptidase G2. These prodrugs were able 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).

βG-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-hydroxyllepticine (50), 4'-epidoxorubicin (51), 1-naphtol (52), and AZT (3'-azido-3'-deoxythymidine) (53). These studies and our own results showing the low toxicity of BHAMG to cells expressing high endogenous βG activity support the hypothesis that glucuronide prodrugs should be resistant to premature activation by endogenous βG in vivo. These studies also suggest that activated prodrug not taken up by tumor cells may be converted to the glucuronide conjugate after passing through organs 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 glucuronyl 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 vivo 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 vitro 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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