Enhanced Uptake of Doxorubicin to Bronchial Carcinoma: β-Glucuronidase Mediates Release of Doxorubicin from a Glucuronide Prodrug (HMR 1826) at the Tumor Site

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ABSTRACT

Lack of tumor selectivity is a severe limitation of cancer chemotherapy. Consequently, reducing dose-limiting organ toxicities such as the cardiac toxicity of doxorubicin (Dox) is of major clinical relevance. Approaches that would facilitate a more tumor-selective anticancer therapy by using nontoxic prodrugs that are converted to active anticancer agents at the tumor site have been the subject of intensive research. One potential method to overcome the cardiac toxicity of Dox is to apply a nontoxic, glucuronide prodrug (HMR 1826) from which Dox is released by the action of β-glucuronidase, an enzyme present at high levels in many tumors. Using a recently developed, isolated, perfused human lung model, we compared the uptake of Dox into normal lung and lung tumors after 2.5-h lung perfusion with doxorubicin (n = 8) and with the novel doxorubicin glucuronide prodrug (n = 8). Dox showed a poor uptake into lung tumors compared with normal lung (mean Dox concentration at the end of perfusion: 1.78 ± 3.11 (median, 0.66) µg/g versus 22.03 ± 10.4 (median, 18.5) µg/g; P < 0.001). However, after perfusion with HMR 1826, the level of Dox in tumor tissue was about 7-fold higher than after perfusion with Dox itself [14.04 ± 12.9 (median, 12.9) µg/g versus 1.78 ± 3.11 (median, 0.66) µg/g, P < 0.05, n = 8]. In vitro experiments showed a significantly higher β-glucuronidase expression and activity in the tumors. The extent of in vitro cleavage of HMR 1826 by homogenized lung tissue was closely related to the content of β-glucuronidase (r = 0.9834, P < 0.0001). When d-saccharolactone, a specific inhibitor of β-glucuronidase, was added to the perfusate containing HMR 1826, no accumulation of Dox in lung tissue was seen. These data indicate that the high Dox levels achieved in the tumors with HMR 1826 resulted from cleavage of the prodrug by β-glucuronidase at the tumor site. Thus, the problem of poor Dox uptake into lung tumors could be circumvented by applying the doxorubicin glucuronide prodrug. Several lines of evidence based on both ex vivo and in vitro results indicate that the approach described using a glucuronide prodrug may be useful in facilitating more selective delivery of chemotherapy to tumors in humans.

INTRODUCTION

One of the major limitations of conventional chemotherapy is its lack of tumor selectivity, resulting in severe dose-limiting adverse effects. Although toxicities such as bone marrow suppression can now be treated effectively, specific organ toxicities for which no effective therapies exist remain a problem with many anticancer agents. Dox is an anthracycline anticancer agent that is effective in acute leukemias, malignant lymphomas, and a number of solid tumors, including small cell carcinoma of the lung (1). A major complication of DOX chemotherapy is congestive heart failure, the occurrence of which is related to the total cumulative dose of the drug (1). Reducing cardiac toxicity associated with systemic DOX treatment is, therefore, of major clinical relevance.

A large number of studies have focused on developing prodrug strategies that enable selective delivery of cytotoxic agents to the tumor, by using nontoxic prodrugs that are converted to active anticancer agents in high concentrations, preferentially at the tumor site (2–7). One possible approach to overcome the problem of dose-limiting cardiac toxicity of DOX is to apply a nontoxic prodrug from which the active moiety is released by the action of an enzyme present at high levels in tumor tissue. N-[4-β-Glucuronol-3-nitrobenzoxycarbonyl]doxorubicin (HMR 1826), a derivative of DOX in which the glucuronic acid moiety is bound to the anthracycline residue via a synthetic spacer, shows a markedly reduced systemic toxicity compared to DOX (3). This prodrug is cleaved by β-glucuronidase (EC 3.2.1.31), yielding a DOX-spacer derivative that spontaneously decomposes, thereby releasing DOX. Preliminary in vivo studies in animals suggest that the DOX prodrug can be applied to facilitate a more tumor-selective chemotherapy (3, 4).

Because it is difficult to study intratumoral pharmacokinetics of anticancer drugs in vivo in humans, a suitable model is needed to assess the potential of approaches to increase tumor selectivity of cancer chemotherapy. We have recently described, for the first time, an isolated perfused human lung model in which the physiological conditions can be maintained ex vivo near those in vivo for 2–3 h (8). This system lends itself well to studies on the uptake and distribution of anticancer agents into normal lung and lung tumors. Such a model has not been used previously to study the disposition of anticancer agents in human lung and lung tumors, a topic about which very little is known (9). Furthermore, the pharmacokinetics of DOX and the HMR 1826 prodrug in human lung have not been characterized in detail.

Isolated ex vivo or in vivo perfusion of animal lungs with a DOX-containing perfusate has been studied extensively in recent years to develop methods to deliver DOX selectively to lung tumor (10–17). Although doxorubicin was readily taken up into normal lung tissue, animal studies that involved measurement of doxorubicin levels in lung tumors after isolated lung perfusion showed that the concentration of doxorubicin was much lower in tumor tissue than in normal tissue (18, 19). In preliminary clinical studies, doxorubicin was taken up into both normal human lung and lung tumors during isolated in vivo lung perfusion; however, doxorubicin levels were in most cases lower in lung tumors than in normal tissue (12, 20).

Using the isolated perfused human lung model (8), we have directly compared the uptake of doxorubicin into normal lung and lung tumors after perfusion of lung preparations with doxorubicin itself and with the HMR 1826 prodrug in separate sets of experiments. As the generation of doxorubicin from this glucuronide prodrug is mediated...
by β-glucuronidase, in vitro experiments were carried out to study the activity of β-glucuronidase in the same tissue samples that were analyzed for drug levels. In this report, we demonstrate that administration of HMR 1826 leads to enhanced tumor levels of doxorubicin and that this selectivity results from the action of β-glucuronidase.

**MATERIALS AND METHODS**

**Chemicals.** All solvents used were of HPLC quality; chemicals were of analytical grade. Doxorubicin, doxorubicin-aglycone, doxorubicin-7-desoxyaglycone, doxorubicinol-aglycone, doxorubicinol-7-desoxyaglycone, and epirubicin were generous gifts from Pharmacia Biotech, Inc., Farmatalia (Freiburg, Germany). HMR 1826 was synthesized according to Jacques et al. (21). The monoclonal antibody 2156/215 against human β-glucuronidase was described previously (22).

**Patients and Lung Preparations.** Patients with a bronchial tumor underwent a standard thoracotomy. Each patient signed a written informed consent before operation, and the use of resected human lungs for perfusion was approved by a local Ethics Committee. Patients were randomized into two groups whose lung preparations were perfused either with doxorubicin (one female, seven males; average age, 62.1 ± 6.2 years; five squamous cell carcinomas, one adenocarcinoma, one adenosquamous carcinoma, and one other non-small cell lung cancer) or HMR 1826 (one female, seven males; average age, 55.4 ± 7.6 years; three squamous cell carcinomas, two adenocarcinomas, and three adenosquamous carcinomas).

**Perfusion Procedure.** The lobe preparations were perfused as described previously (8). In brief, following operation the arteries were cannulated, and the bronchus was connected to a bronchial tube. After the lung was rinsed through the arterIALIZDe with 1000 ml of perfusion buffer (85 mm NaCl, 4.0 mm KCl, 2.5 mm CaCl2, 1.0 mm MgCl2, 2.5 mm KH2PO4, 25 mm NaHCO3, 5.5 mm glucose, and 5% albumin, pH 7.4), it was placed within the perfusion apparatus in a tempered waterbath (37°C) and ventilated using a respirator (Engström Erica 2, Engelström Elektromedizin GmbH, Munich, Germany) with air and CO2 to maintain physiological pH 7.2–7.4. The perfusion buffer containing either 5 μg/ml doxorubicin or 400 μg/ml HMR 1826 was pumped from a reservoir through a heat exchanger, a blood filter, and a bubble trap, and was delivered through a valve into one to three segmental arteries. After leaving the opened veins, perfusate flowed back to the reservoir, which was held at 37°C. The standard flow rate of the perfusate was 300 ml/min, but it was adjusted to obtain a pulmonary arterial pressure ranging from 20 to 25 mmHg if necessary.

**Sample Preparation.** During perfusion, samples were taken from the perfusate (at 5, 10, 15, 20, 25, 30, 40, 50, 60, 80, 100, 120, 140, and 150 min) and from the peripheral lung tissue (before perfusion started, and at 7, 18, 35, 70, 110, and 150 min) and were immediately frozen in liquid nitrogen. Immediately after perfusion, the lung preparations were examined by a pathologist, and samples were obtained from the tumor and surrounding normal lung tissue and frozen in liquid nitrogen. Samples were stored at −80°C until analysis.

**Determination of Drug Concentrations in Tissue and Perfusate.** Frozen tissue samples were homogenized using a Microdismembrator S (B. Braun Biotech International, Melsungen, Germany). Homogenized tissue samples were suspended in an ascorbate buffer (50 mm ascorbic acid, 10 mm D-saccharolactone, pH 4.5). After adding epirubicin as an internal standard, proteins were precipitated with a solution of silver nitrate (final concentration, 0.3 M) and vortexed for 2 min. The excess of silver ions was precipitated with sodium chloride. A mixture of methanol and acetonitril (1:2 v/v) was added, and the sample was centrifuged. The clear supernatant was analyzed directly by HPLC.

**RESULTS**

**In Vitro Cleavage of HMR 1826.** Protein contents of tissue homogenates were determined according to the method of Lowry et al. (23). Incubation mixtures contained 1.13 μg of homogenate and 50 μl of assay buffer (200 mm sodium acetate (pH 5), 10 mm EDTA, 0.01% (w/v) BSA, 0.1% (v/v) Triton X-100, and 100 μM HMR 1826). Duplicate or triplicate incubations were carried out at 37°C for 2 h, which was within the linear time range of the enzyme reaction (deviations of the mean were below 10%). The enzymatic reaction was stopped by adding 150 μl of a mixture of methanol and acetonitril (1:2, v/v). Doxorubicin concentrations were determined as described above. Specific β-glucuronidase activity was expressed in nanomoles doxorubicin liberated from HMR 1826 per hour per mg total protein.

**Western Blotting and Densitometry.** Western blot analysis was performed as described previously (24). Briefly, 50 μg of tissue homogenate were subjected to 10% SDS gels. The electrophoretic pattern was transferred to a nitrocellulose membrane (1:2, v/v). Doxorubicin concentrations were determined as described above. Specific β-glucuronidase activity was expressed in nanomoles doxorubicin liberated from HMR 1826 per hour per mg total protein.

**Immunohistochemical Staining for Factor VIII.** As a measure of tumor vascularization, factor VIII immunostaining was performed by the ABC technique (avidin-peroxidase technique). Factor VIII antibody (polyclonal rabbit anti-human von Willebrand factor; Dako Diagnostika GmbH, Hamburg, Germany) was diluted 1:400. Paraffin sections were pretreated with Prenose 0.1%. Peroxidase was developed with diamobenzidine/H2O2. Two independent investigators classified the samples into weak, moderate, strong, and very strong vascularized tumors.

**Statistical Analysis.** All data are presented as means ± SD. In addition, median values are presented for comparison of tumor concentrations of doxorubicin following perfusion with doxorubicin or HMR 1826. Unpaired t tests were used to compare tissue and tumor concentrations. Statistical calculations and linear regression analysis were done with GraphPad Prism (GraphPad Software, Inc., San Diego, CA).

**DISCUSSION**

**Isolated Lung Perfusion.** To assess the stability of the lung preparations during perfusion and the quality of the perfusion experiments, we applied appropriate evaluation criteria that have been outlined in detail previously (8). Only perfusion experiments in which physiological and biochemical conditions that approximated those in vivo could be maintained were included. The mean net weight gain of the eight lung preparations perfused with doxorubicin and HMR 1826 was 25 ± 20% and 18 ± 14%, respectively (P not significant).

**Disposition of Doxorubicin and HMR 1826 in Normal Lung.** The uptake of doxorubicin into normal lung tissue and the corresponding decline in the perfusate doxorubicin concentration after perfusion of eight lung preparations with doxorubicin are depicted in Fig. 1. The mean doxorubicin concentration in the perfusate decreased in a biphasic manner from the initial value of 5 μg/ml to 1.38 ± 0.5 μg/ml during the 2.5-h perfusion period with half-lives of 32.6 ± 18.7 and 114.9 ± 57.8 min, respectively. None of the major metabolites of doxorubicin (doxorubicinol, doxorubicin-aglycone, doxorubicinol-7-desoxyaglycone, doxorubicinol-aglycone, and doxorubicinol-7-desoxyaglycone, doxorubicinol-aglycone, and doxorubicinol-7-des-
Disposition of Doxorubicin and HMR 1826 in Lung Tumors.

Doxorubicin concentrations measured at the end of the perfusion (after 2.5 h) demonstrated that the uptake of doxorubicin into the tumor tissue after perfusion with doxorubicin itself was poor, resulting in a mean tumor tissue doxorubicin concentration of less than one-tenth of that achieved in normal tissue (1.78 ± 3.11 (median, 0.66) µg/g versus 22.03 ± 10.4 (median, 18.5) µg/g; *P < 0.001, n = 8; Fig. 3). Using immunohistochemical staining for factor VIII, we discriminated between tumors with weak and moderate vascularization and tumors with strong and very strong vascularization. A significant difference was found between the concentration of doxorubicin in the tumor after perfusion with doxorubicin depending on the extent of vascularization of the tumor; tumors with better vascularization showed higher doxorubicin concentrations (Fig. 4).

At the end of perfusion, the tumor tissue exhibited a decreased uptake of HMR 1826 compared to normal lung tissue (49.7 ± 70.3 (median, 39.8) µg/g versus 197.8 ± 62.8 (median, 180) µg/g; *P < 0.001). Following perfusion with HMR 1826, the mean concentration of doxorubicin in tumor tissue was about 7-fold higher than after perfusion with doxorubicin itself (14.04 ± 12.9 versus oxyaglycone) was detected in the perfusate. The mean concentration of doxorubicin in normal lung tissue steadily increased during the perfusion and appeared to approach a plateau level when the perfusion was stopped after 2.5 h. The final concentration of doxorubicin in normal lung tissue averaged 22.03 ± 10.4 µg/g (median, 18.5 µg/g).

After perfusing eight lung preparations with the prodrug HMR 1826, the mean doxorubicin concentration in normal lung tissue increased in a linear manner as a function of the perfusion time and was 15.08 ± 5.06 µg/g (median, 16.2 µg/g) at the end of perfusion (Fig. 2). However, the mean tissue concentration of HMR 1826 showed a rapid initial increase and appeared to reach a plateau level during the second hour of the perfusion. In fact, the pattern of uptake of HMR 1826 into normal lung seemed to be similar to the uptake of doxorubicin after perfusion with doxorubicin itself. In contrast to doxorubicin, HMR 1826 showed no accumulation in tissue but reached a steady-state level after a perfusion time of 50 min (Fig. 2). The final concentration ratio of tissue versus perfusate was 0.57 ± 0.09 for perfusion with HMR 1826 compared to 16.1 ± 4.6 for perfusion with doxorubicin (*P < 0.01). Following administration of HMR 1826, the final concentration of doxorubicin in perfusate was 1.0 ± 0.35 µg/ml at 150 min (Fig. 2).
The mean \(\beta\)-glucuronidase activity as determined by in vitro cleavage of HMR 1826 was significantly higher in tumor tissue homogenate than in normal lung tissue homogenate (225.2 ± 107.1 versus 96.9 ± 36.1 nmol/h/mg protein, \(P < 0.01\)). In good accordance with this observation, following perfusion with HMR 1826, the ratio of the tissue concentration of doxorubicin versus HMR 1826 at the end of the perfusion (after 2.5 h) was more than 2-fold higher in tumor tissue than in normal tissue (0.244 ± 0.146 versus 0.088 ± 0.028; \(P < 0.05\)). A highly significant correlation \((r = 0.9834; \ P < 0.0001)\) was observed between the content and in vitro activity of \(\beta\)-glucuronidase in a set of eight samples of tumor tissue obtained from the lung preparations after perfusion with HMR 1826. However, there was no direct association between the cleavage of HMR 1826 in situ, as assessed by the ratio of doxorubicin and HMR 1826 tissue concentrations, and the activity of \(\beta\)-glucuronidase in either normal or tumor tissue at the end of the perfusion. In a control experiment, \(\alpha\)-saccharolactone, a specific inhibitor of \(\beta\)-glucuronidase, was added to the perfusate to a final concentration of 10 mm. This concentration leads to a complete inhibition of \(\beta\)-glucuronidase activity in vitro. Despite a concentration of 400 \(\mu\)g/ml HMR 1826 in the perfusate, the level of doxorubicin in the perfusate did not increase during the perfusion, and no accumulation of doxorubicin in lung tissue was seen. The ratio of doxorubicin to HMR 1826 in tumor tissue as an in situ measure of \(\beta\)-glucuronidase activity in the tumor decreased by a factor of 35 when \(\alpha\)-saccharolactone was added to the perfusate (0.212 ± 0.244 versus 0.006). The final doxorubicin concentration in the perfusate was 0.2 \(\mu\)g/ml. These data indicate that the doxorubicin taken up into normal tissue and tumor tissue after administration of HMR 1826 originated from cleavage of HMR 1826 by \(\beta\)-glucuronidase.

**DISCUSSION**

Very little is currently known about the pharmacokinetics of anticancer agents in tumors (9). Importantly, the results of a recent clinical trial, using a novel approach to investigate drug uptake into tumors in vivo, directly support the premise that the efficacy of cancer chemotherapy is dependent on the intratumoral drug concentration (25). In this study involving 34 patients with a wide variety of tumors, a close and highly significant association was found between the ability of a tumor to accumulate fluorouracil, as assessed by fluorine-19 nuclear magnetic resonance spectroscopy, and the cytotoxic efficacy of the drug (25). The disposition of anticancer agents in tumors is thus a pivotal issue. Because it is exceedingly difficult to characterize intratumoral pharmacokinetics of drugs in vivo, an appropriate experimental model is needed for this purpose.

The isolated perfused human lung model recently developed and validated in our laboratories is a suitable tool to investigate drug uptake into normal lung as well as lung tumors ex vivo (8). This system allows perfusion of human lung preparations for up to 3 h in physiological and biochemical conditions that closely resemble those in vivo. Importantly, on the basis of the results obtained from more than 50 perfusion experiments with drug-free perfusates (8), there was no evidence of acute lung injury as a result of perfusion with doxorubicin in this study. Doxorubicin concentrations used in our study are in the same order of magnitude as peak levels reached during chemotherapy (26).

The results of this study showed that doxorubicin was readily taken up into normal lung during isolated lung perfusion with a doxorubicin-containing perfusate. However, uptake of doxorubicin into lung tumors was poor, resulting in a final mean tumor tissue drug concentration that was less than one-tenth of that reached in normal tissue. These findings are in good accordance with the results of animal studies and the preliminary data available in humans (12, 20). Therefore, besides biological resistance, unfavorable intratumoral pharmacokinetics may cause a poor response to cancer chemotherapy. The higher lung doxorubicin levels as compared with tumor tissue are probably related to greater blood flow and more extensive vascularization in lung parenchyma than tumor. Tumors are known to vary greatly in the extent of their vasculature as well as in their blood flow rates (27, 28), which is likely to lead to a high variability in drug uptake into tumors. This is also evidenced by the wide range of doxorubicin tumor levels and the relation between doxorubicin accumulation and tumor vascularization observed in the present study. A completely different picture was evident after perfusion of another set of lung preparations with the doxorubicin prodrug HMR 1826; similar doxorubicin concentrations were achieved in tumor and normal tissue and, remarkably, the final mean concentration of doxorubicin in tumor tissue was about 7-fold higher than after perfusion with doxorubicin itself. HMR 1826 concentrations used in our study are in a range that has been achieved in vivo. Following administration of 120 mg/kg to Macaque monkeys, plasma concentrations of 700 \(\mu\)g/ml were achieved without any detectable side effects (5).

In line with previous studies that indicate that the content of \(\beta\)-glucuronidase is high in cancer tissue in at least some cases of prostate, kidney, and breast (Refs. 29 and 30 and references therein), the mean \(\beta\)-glucuronidase activity was significantly higher in tumor tissue than in normal lung tissue in the present study. The slowly increasing levels of doxorubicin in the tissue during perfusion with HMR 1826 indicated constant release of doxorubicin from the prodrug. Furthermore, the ratio of the tissue concentration of doxorubicin to that of HMR 1826 after perfusion with HMR 1826 was significantly higher in tumor tissue than in normal tissue. Moreover, an experiment with the specific \(\beta\)-glucuronidase inhibitor, \(\alpha\)-saccharolactone, showed that the possibility of nonspecific cleavage of the prodrug could be excluded. These results demonstrate the presence of significant \(\beta\)-glucuronidase activity in the tumors studied and indicate that the high doxorubicin levels achieved in the tumors after perfusion with HMR 1826 resulted from cleavage of the prodrug by \(\beta\)-glucuronidase inside or in the vicinity of the tumor mass. The doxorubicin concentration achieved in the perfusate at the end of the experiment averaged 1.0 \(\mu\)g/ml. Administration of \(\alpha\)-saccharolactone reduced this level to 0.2 \(\mu\)g/ml, thereby indicating \(\beta\)-glucuronidase-mediated cleavage to be responsible for the doxorubicin concentration in the perfusate. Under in vivo conditions, both distribution and clearance processes will decrease this doxorubicin concentration to the subtoxic range.

The localization and regulation of expression of \(\beta\)-glucuronidase can be, together with the prodrug concentration, the key factors that modulate the release of doxorubicin from the glucuronide prodrug. The variability in the activity of \(\beta\)-glucuronidase in kidney and liver is high (31, 32), but the localization of this enzyme in the lung and lung tumors has not been characterized systematically. Furthermore, it

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5 K. Bosslet, personal communication.
is not known how β-glucuronidase is regulated in humans. If the mechanisms that control the level of β-glucuronidase in specific tumor tissues were known, it might be possible to increase the level of the enzyme and thereby increase tumor selectivity of glucuronide prodrugs. The observation that there was no direct association between the cleavage of HMR 1826 in situ (as assessed by the ratio of doxorubicin and HMR 1826 concentrations in samples taken at the end of the perfusion) and the activity of β-glucuronidase in either normal or tumor tissue indicates that, apart from β-glucuronidase activity, other factors such as localization of β-glucuronidase modulate the extent of liberation of doxorubicin by β-glucuronidase-mediated cleavage. In contrast, β-glucuronidase content was positively correlated with the extent of HMR 1826 cleavage in homogenized lung tissue in vitro. As a hydrophilic prodrug, HMR 1826 did not accumulate in tissue (Fig. 2) and was not taken up by cells in vitro (33). Consequently, lysosomal β-glucuronidase should not play an important role in the liberation of doxorubicin from HMR 1826. The presence of enzymatically active “extralysosomal” β-glucuronidase was demonstrated in tumor cells exhibiting necrosis by enzyme histochemical methods (4).

In recent years, several groups have tried to develop prodrug systems that would enable selective delivery of anticancer agents to the tumor tissue, thereby reducing drug concentrations in nontarget organs and minimizing systemic toxicity (reviewed in Refs. 6 and 7). Another widely investigated approach to facilitate regional chemotherapy is in vivo isolated animal lung perfusion with doxorubicin (10-13, 15-17). Because of the good efficacy of doxorubicin against sarcoma (34), isolated lung perfusion with doxorubicin has been explored primarily as a possible means of treating metastatic sarcoma localized in pulmonary tissue. A preliminary clinical trial indicates that isolated lung perfusion is technically feasible also in humans and may be a potential method to deliver high doses of anticancer agents to tumors (20). In this pilot study, eight patients with metastatic sarcoma to the lung or diffuse bronchialoalveolar carcinoma of the lung underwent isolated lung perfusion with chemotherapy added to the lung perfusate (doxorubicin in six patients and cisplatin in two). Analysis of lung and tumor tissue samples taken immediately after perfusion demonstrated significant levels of drug in both primary and metastatic tumors, while the amount of drug entering the systemic circulation was minimal. The doxorubicin level was in most cases somewhat lower in the tumor than in normal lung (20).

Still another possibility to improve the tumor selectivity of cancer chemotherapy would be to modulate the activity of drug-metabolizing enzymes. Bosslet et al. (3) recently presented a two-component system, consisting of a fusion protein and the doxorubicin glucuronide prodrug. The fusion protein consists of a humanized antibody directed against a tumor-specific surface antigen (carcinoembryonic antigen) and human β-glucuronidase (2). In vivo studies in nude mice bearing human carcinoembryonic antigen-expressing tumor xenografts demonstrated that this system is well suited to perform selective tumor therapy, i.e., to increase drug concentration in the target tissue and reduce it in nontarget tissues (3). The present results suggest that administration of the doxorubicin prodrug alone may also be a potential approach to improve tumor selectivity of therapy with doxorubicin, as compared with systemic doxorubicin administration. However, because β-glucuronidase is an ubiquitous enzyme, there is the possibility that doxorubicin may be released from the prodrug also in nontarget tissues. The results obtained by Bosslet et al. (3), suggesting that tumor selectivity may be further enhanced by supplying β-glucuronidase in the form of a fusion protein, are of importance in this regard.

In summary, the results obtained in the present study showed that the poor uptake of doxorubicin into lung tumors during isolated lung perfusion with doxorubicin was markedly improved when a perfusate containing the novel doxorubicin glucuronide prodrug was used for perfusion. In vitro experiments demonstrated a higher activity of β-glucuronidase in tumor tissue and strongly suggest that the good uptake of doxorubicin into tumors after prodrug administration resulted from β-glucuronidase-mediated cleavage of the glucuronide prodrug at the tumor site.

Finally, and more generally speaking, approaches using nontoxic, glucuronide prodrugs may be useful in facilitating tumor-selective delivery of chemotherapy. In general, the delivery of drugs different from doxorubicin as glucuronide prodrugs is possible, if the drugs contain groups allowing the coupling to the appropriate spacer. One limiting factor in clinical application, however, is that glucuronide prodrugs require i.v. administration. In the case that the level of endogenous β-glucuronidase activity in the target tissue is not sufficient, β-glucuronidase may be supplied in the form of a fusion protein to ensure high levels of β-glucuronidase in the tumor (3).

REFERENCES


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