Dose Optimization of a Doxorubicin Prodrug (HMR 1826) in Isolated Perfused Human Lungs: Low Tumor pH Promotes Prodrug Activation by β-Glucuronidase

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ABSTRACT

HMR 1826 (N-[4-β-Glucuronyl-3-nitrobenzyl-oxycarbonyl]doxorubicin) is a nontoxic glucuronide prodrug from which active doxorubicin is released by β-glucuronidase. Preclinical studies aimed at dose optimization of HMR 1826, based on intratumoral pharmacokinetics, are important to design clinical studies. Using an isolated perfused human lung model, the uptake of doxorubicin into normal tissue and tumors after perfusion with 133 μg/ml (n = 6), 400 μg/ml (n = 10), and 1200 μg/ml (n = 6) HMR 1826 was compared. Extracellular tissue pH was measured, and enzyme kinetic studies were performed in vitro to investigate the effect of pH on the formation of doxorubicin. Extracellular pH was lower in tumors than in healthy tissue (6.46 ± 0.35, n = 8 versus 7.30 ± 0.33, n = 10; p < 0.001). In vitro, β-glucuronidase activity was 10 times higher at pH 6.0 than at neutral pH. After perfusion with HMR 1826, there was a linear relationship between HMR 1826 concentrations in perfusate and normal lung tissue. After perfusion with 133, 400, and 1200 μg/ml HMR 1826, the final doxorubicin concentrations in normal and tumor tissue were 2.7 ± 0.9, 11.1 ± 5.4, and 21.8 ± 8.4 μg/g (p < 0.05 for all comparisons), and 0.7 ± 0.3, 8.6 ± 2.0 μg/g (p < 0.01 versus 133 μg/g), and 8.7 ± 4.9 μg/g, respectively. This agrees with the enzyme kinetic observations of saturation of β-glucuronidase at 400 μg/ml HMR 1826 in the acidic environment of the tumor. Therefore, the escalation of the HMR 1826 dose most likely results in higher circulating concentrations than 400 μg/ml but does not increase the uptake of doxorubicin into tumors and, subsequently, antitumor efficacy. The isolated perfused human lung is an excellent model for preclinical investigations aimed at optimization of tissue pharmacokinetics of tumor-selective prodrugs.

One of the major limitations of conventional chemotherapy is its lack of tumor selectivity, resulting in severe dose-limiting adverse effects. For example, the anthracycline anticancer agent doxorubicin is effective in treatment of acute leukemias, malignant lymphomas, and a number of solid tumors, including small cell carcinoma of the lung (Chabner et al., 1996). However, chemotherapy using doxorubicin is limited by its cardiac toxicity, which leads to congestive heart failure after exceeding a certain cumulative dose.

A promising approach to overcome the problem of the dose-limiting toxicity of doxorubicin is to apply a nontoxic prodrug from which active doxorubicin is released enzymatically at the tumor site (Bosslet et al., 1994; Sinhababu and Thakker, 1996; Desbène et al., 1998). HMR 1826 (N-[4-β-Glucuronyl-3-nitrobenzyl-oxycarbonyl]doxorubicin) (Mürdter et al., 1997), is a glucuronyl-spacer-doxorubicin prodrug, which is activated by β-glucuronidase (EC 3.2.1.31), an enzyme present in high extracellular concentrations in necrotic areas of human cancers (Bosslet et al., 1998). In animal studies, administration of HMR 1826 resulted in markedly increased deposition of doxorubicin in human tumor xenografts and significantly reduced doxorubicin load to normal tissues, compared with administration of doxorubicin. Consequently, chemotherapy with HMR 1826 also resulted in improved efficacy and markedly reduced systemic toxicity in nude mice bearing human tumor xenografts, compared with treatment with doxorubicin (Bosslet et al., 1994,

ABBREVIATIONS: HMR 1826, N-[4-β-glucuronyl-3-nitrobenzyl-oxycarbonyl]doxorubicin; HPLC, high pressure liquid chromatography.
suggesting that the doxorubicin prodrug can be applied to facilitate a more tumor-selective chemotherapy.

In a previous study, we used an isolated perfused human lung model to investigate the uptake of doxorubicin into bronchial carcinoma after perfusion with doxorubicin or HMR 1826 (Mürder et al., 1997). After perfusion with doxorubicin at concentrations comparable with the peak plasma concentration during chemotherapy, tumor concentrations of doxorubicin were low, approximating less than one-tenth of the concentrations reached in normal lung tissue (Mürder et al., 1997), which is in good agreement with preliminary data available in humans (Johnston et al., 1995). In contrast, perfusion with a 50-fold higher molar concentration of HMR 1826 resulted in a 7 times higher uptake of doxorubicin into tumors than perfusion with doxorubicin, whereas the final concentrations of doxorubicin in normal lung tissue were comparable with those after perfusion with doxorubicin itself. Similar concentrations of doxorubicin were reached in tumor and normal lung tissue.

Toxicological animal studies revealed good toleration of HMR 1826, up to blood concentrations in the milligrams per milliliter range (Platel et al., 1999), suggesting that even higher circulating concentrations than the 400 μg/ml HMR 1826 concentration used in our previous lung perfusion experiments can be tolerated well. However, because the release of doxorubicin from HMR 1826 is an enzymatic process, mediated by β-glucuronidase (Mürder et al., 1997), it is likely that the formation of doxorubicin in tumor tissue is not directly proportional to the circulating concentration of the prodrug. Furthermore, the activity of human β-glucuronidase is highest at acidic pH (Paigen, 1989), and solid tumors have been described to possess an acidic environment (Ashby, 1966; Griffiths, 1991; Stubbs et al., 2000). Thus, the kinetics of doxorubicin formation at different prodrug concentrations may be modulated by enzyme activity, drug concentration, and tissue pH.

To facilitate prediction of the optimal dose of HMR 1826 in treatment of lung carcinoma, we investigated the effect of circulating HMR 1826 prodrug concentration on the intratumoral concentrations of active doxorubicin using the isolated perfused human lung model (Linder et al., 1996). In addition, we carried out tissue pH measurements and enzyme kinetic experiments to investigate the relationship between tissue pH and the β-glucuronidase-mediated activation of HMR 1826. In this report, we demonstrate that pH is significantly lower in lung tumors than in healthy lung tissue, and that a change from healthy tissue pH to acidic pH markedly alters the kinetics of HMR 1826 cleavage. Furthermore, we show that increasing perfusate HMR 1826 concentration above 400 μg/ml does not further increase the uptake of doxorubicin into lung tumors because β-glucuronidase is almost saturated in the acidic environment of the tumor.

Materials and Methods

Chemicals. All solvents used were of HPLC quality; chemicals were of analytical grade. Doxorubicin and epirubicin were generous gifts from Pharmacia, Farmitalia (Freiburg, Germany). HMR 1826 was synthesized as described previously (Jacquey et al., 1992).

Patients and Lung Preparations. Twenty-two patients (20 males, 2 females; age (mean ± S.D., range) 58.1 ± 8.1 years, 43–69 years) with a bronchial tumor and undergoing a standard thoracotomy, were included in the study. Each patient signed a written informed consent before operation, and a local Ethics Committee approved the use of resected human lungs for perfusion. Tumors were of the following histological types: 12 squamous cell carcinomas, 6 adenocarcinomas, and 4 adenosquamous carcinomas. Patients were randomized into three groups in which lung preparations were perfused ex vivo either with 133 μg/ml (n = 6; mean age, 59.7 years), 400 μg/ml (n = 10; mean age, 57.1 years), or 1200 μg/ml HMR 1826 (n = 6; mean age, 58.0 years).

Perfusion Procedure. The lobe preparations were perfused ex vivo for 150 min as described previously (Linder et al., 1996, Mürder et al., 1997). In brief, immediately after lung resection, the arteries were cannulated and the bronchus was connected to a bronchial tube. After the lung was rinsed through the arteries with 1 liter 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 water bath (37°C) and ventilated using a respirator (Engström Erica 2; Engström Elektromedizin GmbH, Munich, Germany) with air and CO2 to maintain a physiological pH of 7.2 to 7.4. The perfusion buffer, containing either 133, 400, or 1200 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.

During lung perfusion, pH, pO2, pCO2, K+, and Na+ in the perfusate were monitored continuously with an Eschweiler System 2000-D03 (L. Eschweiler & Co., Kiel, Germany) and registered via a computer system. By addition of CO2, perfusate pH was maintained within the physiological range of about 7.4. Tumor and normal lung tissue temperature and pH were measured using Pt1000 sensors and pH-microelectrodes NS800A (Schott Geräte GmbH, Hofheim, Germany) before perfusion and five to eight times during the perfusion procedure. Lung preparations were weighed before and after perfusion to check for edema formation during perfusion.

Sample Preparation and Determination of Drug Concentrations. Immediately after perfusion, the lung preparations were cut into fragments, and samples were taken from the tumor and normal lung tissue and frozen in liquid nitrogen. Samples were stored at −80°C until analysis.

Tissue concentrations of doxorubicin and HMR 1826 were determined as described previously (Mürder et al., 1998). After homogenization of the frozen tissue samples using Mikrodisembrator S (B. Braun Biotech International, Melsungen, Germany), tissue samples were suspended in an ascorbate buffer. After adding epirubicin as an internal standard, proteins were precipitated with a solution of silver nitrate. The excess of silver ions was precipitated with sodium chloride. A mixture of methanol and acetonitrile (1:2, v/v) was added and the sample was centrifuged. After this, the concentrations of doxorubicin and HMR 1826 in the supernatant were determined by HPLC with fluorescence detection. The intra-assay and interassay coefficients of variation were less than 10% for concentrations in samples from perfusion experiments.

In Vitro Cleavage of HMR 1826. A frozen lung tumor sample not included in the perfusion experiments was homogenized using Mikrodisembrator S. Protein content in the tissue homogenate was determined according to the method of Smith et al. (1985). The incubation medium contained 1.13 μg of protein and 50 μl of assay buffer [200 mM acetate buffer, pH 4.0–6.0; 100 mM phosphate buffer, pH 6.5–7.5; 10 mM EDTA; 0.01% (w/v) bovine serum albumin; 0.1% (v/v) Triton X-100; 0–2000 μM HMR 1826] (Sperker et al., 1997). Incubations were carried out at different pH values (4–7.5) with a substrate concentration of 400 μM HMR 1826 to determine the effect of pH on β-glucuronidase activity. For detailed enzyme kinetic experiments at pH 6.5 and pH 7.5, increasing concentrations of HMR 1826 (0–2000 μM) were used. Duplicate or triplicate incubations were carried out at 37°C for 2 h, which was within the linear time range of the enzymatic reaction. The reaction was stopped by adding 150 μl of a mixture of methanol and acetonitrile (1:2, v/v).
Doxorubicin concentrations were determined as described above. Formation of doxorubicin from HMR 1826 (specific \(-\beta\)-glucuronidase activity) was expressed in units of nanomoles per hour per milligram. The kinetics of doxorubicin formation (\(V_{\text{max}}, K_m\), and the Hill coefficient, \(n\)) were estimated by nonlinear regression with GraphPad Prism software (GraphPad Software Inc., San Diego, CA) using the single-enzyme Michaelis-Menten equation: 
\[ V = V_{\text{max}} \cdot [S]/(K_m + [S]) \]
or the Hill equation: 
\[ V = V_{\text{max}} \cdot [S]^n/[S]^n + [S]^n \]. EC\(_{50}\) values were calculated by the equation: 
\[ EC_{50} = K^{\text{Hill}}. \]

**Statistical Analysis.** All data are presented as mean \(\pm\) S.D. Comparisons of the pH values and drug concentrations between the different perfusate HMR 1826 concentrations were performed using the Kruskal-Wallis test and, when appropriate, a posteriori testing by the Mann-Whitney \(U\) test with Bonferroni correction for multiple comparisons. Linear regression analysis was used for testing the relationship between HMR 1826 concentration in perfusate and the concentration of HMR 1826 in normal lung and lung tumor tissue. Statistical calculations were done with GraphPad Prism. A one-tailed \(p\) value was used, and the level of statistical significance was \(p < 0.05\).

**Results**

**Isolated Lung Perfusion.** All samples included fulfilled the previously outlined evaluation criteria for the stability of the lung preparations during perfusion and the quality of the perfusion experiments (Linder et al., 1996). The mean \((\pm\)S.D., \(n\)) net weight gain due to the formation of edema during isolated lung perfusion was 22% \((\pm 16.8\% , n = 22)\) with no differences between subgroups. Net weight gain did not exceed 66%, which was the predefined acceptance criterion (Linder et al., 1996). Histological examinations of the samples did not reveal any damage of the endothelial cells.

**Tissue pH.** The mean \((\pm\)S.D., \(n\)) perfusate pH during the perfusion procedure was 7.37 \((\pm 0.15, n = 22)\). In some lung preparations the tumor was not accessible by the pH-electrode because of its localization or size. Therefore, tumor tissue pH was determined in eight lung preparations. During the perfusion, tissue pH values remained within 0.3 unit of the initial values in all samples. The pH in tumor tissue was significantly more acidic than pH in normal lung tissue \((6.46 \pm 0.35, n = 8\) versus \(7.30 \pm 0.33, n = 10; p < 0.001)\) (Fig. 1).

**In Vitro Cleavage of HMR 1826.** Activity of \(-\beta\)-glucuronidase (formation of doxorubicin from HMR 1826, 400 \(\mu\)M) in human lung cancer homogenate was higher at an acidic pH than at a neutral pH (Fig. 2A). The maximum activity, observed at pH 5.5, was about 15 times higher than the activity at pH 7.5 \((1582 \pm 190.2\) versus \(100.0 \pm 2.6\) nmol/h/mg, \(n = 3)\). A change of pH from 7.5 (corresponding to extracellular pH in healthy lung tissue) to 6.5 (corresponding to pH in tumor tissue) was accompanied by a more than 4-fold increase in \(-\beta\)-glucuronidase activity \((n = 3)\). In further kinetic studies, the formation of doxorubicin from HMR 1826 was best described by single-enzyme Michaelis-Menten kinetics at pH 7.5 \((K_m = 908 \mu\)M; \(V_{\text{max}} = 282\) nmol/h/mg; \(r^2 = 0.9964)\) (Fig. 2B). However, at pH 6.5, doxorubicin formation displayed a sigmoidal curve best described by the Hill equation \((EC_{50} = 241\) \(\mu\)M; \(V_{\text{max}} = 482\) nmol/h/mg of protein; \(n_H = 1.851; r^2 = 0.9985)\).

**Disposition of HMR 1826 and Doxorubicin in Normal Lung and Lung Tumor Tissue.** In normal lung tissue, the concentration of HMR 1826 reached approximately one-third of the initial concentration in perfusion buffer (Fig. 3A), reflecting the low lipophilicity of the prodrug, its poor penetration into cells, and a minor tendency to accumulate in tissue. An increase of HMR 1826 concentration in perfusion buffer resulted in a roughly proportional increase in final concentrations of HMR 1826 in normal lung tissue (Fig. 3A), as shown by the strong correlation between HMR 1826 concentration in perfusion buffer and lung tissue (linear regression; \(r^2 = 0.857, n = 22; p < 0.0001)\). The doxorubicin concentration reached at the end of perfusion in normal lung tissue increased with increasing initial concentrations of HMR 1826 in perfusate, and averaged \(2.7 \pm 0.9, 11.1 \pm 5.4,\) and \(21.8 \pm 8.4\) \(\mu\)g/g \((p < 0.05\) for all comparisons) in the samples perfused with 133 \(\mu\)g/ml \((n = 6), 400 \mu\)g/ml \((n = 10),\) and 1200 \(\mu\)g/ml \((n = 6)\) HMR 1826, respectively (Fig. 3C). Thus, in contrast to the linear increase of HMR 1826 concentration in normal lung tissue (Fig. 3A), increasing the concentration of HMR 1826 in perfusion buffer from 400 to 1200 \(\mu\)g/ml only doubled the concentration of doxorubicin in normal lung tissue (Fig. 3C).

In lung tumor tissue, the final concentrations of HMR 1826 were only 2.4, 11.7, and 8.0% of the initial concentrations in perfusion buffer following perfusion with 133, 400, and 1200 \(\mu\)g/ml HMR 1826, respectively (Fig. 3B). An increase of HMR 1826 concentration in perfusate resulted in an increase in the final concentration of HMR 1826 in tumor tissue (Fig. 3B), but the correlation between the concentration of HMR 1826 in perfusion buffer and tumor tissue was relatively weak (linear regression; \(r^2 = 0.263, n = 22; p = 0.012)\). In tumor tissue, perfusion with 133 \(\mu\)g/ml, 400 \(\mu\)g/ml, and 1200 \(\mu\)g/ml HMR 1826 resulted in final doxorubicin concentrations of \(0.7 \pm 0.3, 8.6 \pm 2.0\) \(\mu\)g/g \((p < 0.01)\) versus 133 \(\mu\)g/ml, and 8.7 \pm 4.9 \(\mu\)g/g, respectively (Fig. 3D). Thus, although an increase of perfusate HMR 1826 concentration from 133 to 400 \(\mu\)g/ml substantially increased the concentration of doxorubicin in lung tumor tissue, a further increase of HMR 1826 concentration to 1200 \(\mu\)g/ml did not lead to increased uptake of doxorubicin into lung tumor tissue (Fig. 3D) despite a marked increase in the final intratumoral concentration of HMR 1826 \((p < 0.05)\) (Fig. 3B).
As can be seen from Fig. 3, C and D, the ratio of doxorubicin concentration reached in tumor tissue to that in normal lung tissue, reflecting tumor selectivity of the prodrug, seemed to be markedly higher in the 400 \(\mu\)g/ml HMR 1826 samples than in the 133 \(\mu\)g/ml HMR 1826 or 1200 \(\mu\)g/ml HMR 1826 samples. There was a weak negative correlation between tissue pH and the doxorubicin/HMR 1826 concentration ratio (a measure of \(\beta\)-glucuronidase activity) in tumor and normal lung tissue (linear regression; \(r^2 = 0.281, n = 8; p = 0.024\)), supporting the in vitro observation that low pH facilitates formation of doxorubicin from HMR 1826 (Fig. 2).

The present findings regarding in situ bioactivation of HMR 1826 to doxorubicin during isolated perfusion of human lung preparations can be explained by comparison with the enzyme kinetics of the cleavage of HMR 1826 in vitro (Fig. 4, A and B). At the pH value found in normal lung tissue (pH 7.5), 50% of the maximum reaction velocity was reached at 830 \(\mu\)g/ml HMR 1826 (Fig. 4A). However, at the tumor tissue pH value (6.5), 50% of the maximum velocity was obtained at a substrate concentration of 400 \(\mu\)g/ml HMR 1826, and \(\beta\)-glucuronidase was almost saturated (about 80% of \(V_{max}\) reached) at a substrate concentration of 400 \(\mu\)g/ml HMR 1826 (Fig. 4B). Therefore, an increase of perfusate HMR 1826 concentration from 400 to 1200 \(\mu\)g/ml resulted in increased formation of doxorubicin by \(\beta\)-glucuronidase in normal lung tissue (Fig. 4A), but not in lung tumor tissue (Fig. 4B).

**Discussion**

The efficacy of cancer chemotherapy of solid tumors depends on the intratumoral concentration of the active drug (Presant et al., 1994; Price and Griffiths, 1994). It is believed that a correlation exists between dose and response, and therefore, high-dose chemotherapy is commonly used in the treatment of certain cancers (Cripe, 1997; McGuire, 1998). However, in the case of anticancer prodrugs, dose escalation does not necessarily improve the anticancer effects. Above a certain threshold dose, the enzyme responsible for bioactivation of the prodrug may be saturated and, therefore, concen-
different circulating concentrations of HMR 1826. To characterize the tissue pharmacokinetics of HMR 1826 at the isolated human lung perfusion model (Linder et al., 1996), we used appropriate preclinical pharmacokinetic studies to facilitate dose optimization. Because, in contrast to cyclophosphamide, bioactivation of HMR 1826 takes place in the tumor, pharmacokinetic studies aimed at dose optimization of HMR 1826 should be based on characterization of its intratumoral pharmacokinetics. Inasmuch as it is exceedingly difficult to measure intratumoral concentrations of drugs in vivo, we used the isolated human lung perfusion model (Linder et al., 1996) to characterize the tissue pharmacokinetics of HMR 1826 at different circulating concentrations of HMR 1826.

The results of the present experiments with perfused human lungs showed that HMR 1826 was taken up into normal lung with a linear relationship between HMR 1826 concentration in perfusate and lung tissue. However, the correlation between HMR 1826 concentration in perfusate and tumor tissue was relatively weak, which may be due to the high variability in tumor vascularization. Furthermore, an increase of HMR 1826 concentration in the perfusate from 133 to 400 μg/ml increased the concentration of doxorubicin proportionally in normal tissue and more than proportionally in tumor tissue. A further increase of HMR 1826 in the perfusion solution to 1200 μg/ml resulted in higher concentrations of doxorubicin in normal lung tissue but not in the tumor. These data indicate that increasing concentrations of HMR 1826 above 400 μg/ml will probably not improve the anticancer efficacy of HMR 1826 in patients with bronchial carcinoma. However, systemic exposure to doxorubicin will be enhanced, a factor possibly leading to a higher incidence of toxic effects in healthy tissues. Unfortunately, no data of toxicity or doxorubicin release after administration of HMR 1826 to humans are available yet.

In our previous lung perfusion study, the final concentration of doxorubicin in tumor tissue was 7 times higher after perfusion with 400 μg/ml HMR 1826 than after perfusion with doxorubicin itself at a concentration approximating peak levels reached in blood of cancer patients under doxorubicin treatment (Mürder et al., 1997). In the present experiments, even lung perfusion with the lowest HMR 1826 concentration (133 μg/ml) resulted in final doxorubicin concentrations in tumor tissue that were similar to those from perfusion with doxorubicin in the previous study. Furthermore, the tumor to normal lung ratio of doxorubicin was at least 5 times higher after perfusion with all of the three HMR 1826 concentrations used than after perfusion with doxorubicin in the previous study, indicating superior tumor selectivity of the prodrug over a wide concentration range.

An important feature of HMR 1826 with regard to its tumor selectivity is that HMR 1826 itself is not able to pass biological membranes and enter cells (Houba et al., 1996). In healthy tissues, the bioactivating enzyme β-glucuronidase is mainly localized intracellularly in lysosomes (Erickson and Blobel, 1983; Paigen, 1989). In contrast, Bosslet et al. (1998) concluded from enzyme histochemical and immunohistochemical studies that in lung tumors (and some other tumors), β-glucuronidase is present mainly extracellularly, with expression being much higher than in normal lung tissue (Mürder et al., 1997). In the present study we used microelectrodes to determine pH in tumors and healthy tissue. This approach is thought to mainly reflect extracellular pH, although some intracellular fluids may be liberated from injured cells during introduction of electrodes (Akagi et al., 1999). We found that extracellular pH is significantly lower in tumor tissue (6.5) than in normal lung (7.5), which is in agreement with previous studies in different solid tumors (Griffiths, 1991). Furthermore, our enzyme kinetic studies revealed that the rate of doxorubicin formation from HMR 1826 was much higher in the acid environment of tumor tissue. In parallel, saturation of the β-glucuronidase-mediated reaction was achieved at a 4 times lower substrate concentration, at a pH of 6.5 compared with 7.5. Thus, the pH difference between tumor and normal lung tissue readily explains why an increase in perfusate HMR 1826 concentra-

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tion above 400 µg/ml did not result in higher final concentrations of doxorubicin in tumor tissue, but increased the concentrations of the active drug in healthy lung tissue. On the other hand, our data strongly suggest that tissue pH modulates β-glucuronidase activity and that low extracellular tumor pH improves the tumor selectivity of HMR 1826.

At pH 5.0, the activity of β-glucuronidase was about 4 times higher than at pH 6.5, which was the observed tumor pH in our ex vivo studies. Thus, bioactivation as well as tumor selectivity of HMR 1826 can be further improved by decreasing tumor pH. Interestingly, recent data demonstrated that intravenous infusion of glucose could be used to reduce extracellular pH in tumor tissues (Leeper et al., 1998; Akagi et al., 1999). Another approach to increase the tumor-selective release of doxorubicin from HMR 1826 would be to administer a fusion protein consisting of a humanized antibody directed against a tumor-specific surface antigen and human β-glucuronidase (Bosslet et al., 1994). Finally, transfection of the gene encoding for a secreted β-glucuronidase into tumor cells has been demonstrated to increase cytotoxicity (Weyel et al., 2000).

In summary, the present study has demonstrated that extracellular pH is significantly lower in lung tumors than in healthy lung tissue and that this change in pH markedly increases β-glucuronidase-mediated formation of doxorubicin from HMR 1826 in tumors. During ex vivo lung perfusion with HMR 1826, we observed a linear relationship between concentrations of HMR 1826 in perfusate and in both normal lung and tumor tissue. However, increasing the perfusate HMR 1826 concentration above 400 µg/ml is not paralleled by a further increase in the uptake of doxorubicin into lung tumors. These data are in line with in vitro experiments indicating saturation of β-glucuronidase at this concentration and pH. Thus, it is likely that escalation of HMR 1826 dose resulting in circulating concentrations above 400 µg/ml does not increase the concentration of doxorubicin in tumor tissue. In contrast, high doses of HMR 1826 may increase systemic exposure to doxorubicin, possibly leading to a higher incidence of toxic effects in healthy tissues. In conclusion, this study has proved that the ex vivo isolated perfused human lung can predict the appropriate dose of HMR 1826 for treatment of lung cancer based on tissue pharmacokinetics and, therefore, is a suitable model for these kinds of preclinical investigations.

References


Weyel D, Sedlacek HH, Müller R, and Brüsselbach S (2000) Secreted human β-glu-


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