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Safety, Pharmacokinetics and Biodistribution Studies of a β-galactoside Prodrug of Doxorubicin for Improvement of Tumor Selective Chemotherapy

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Anthracycline antibiotics, particularly doxorubicin (DOX) and daunorubicin, have been used extensively in the treatment of human malignancies. However, cardiotoxicity and multidrug resistance are significant problems that limit the clinical efficacy of such agents. Rational design to avoid these side effects includes strategies such as drug targeting and prodrug synthesis. The DOX prodrug N-(β-D-glucopyranosylbenzyloxycarbonyl)-doxorubicin (prodrug 1) was synthesized for specific activation by β-galactosidase, which is expected to release in necrotic areas of tumor lesions. Described here is the safety, pharmacokinetics, and biodistribution studies of a β-galactoside prodrug of DOX. In vivo safety evaluation was done in the Ehrlich Ascites Carcinoma (EAC) tumor model. The dose of DOX was 8 mg/kg and the dose of prodrug was 8 mg/kg and 24 mg/kg of DOX equivalents. Our results on cytotoxicity, which demonstrated compression in the number of EAC cells and their viability, substantiate these data. Prodrug 1 was safe up to a dose of 24 mg/kg of DOX equivalents in EAC mice. The pharmacokinetics and biodistribution of prodrug (300 mg/kg) in normal mice were determined and compared with DOX (20 mg/kg). Administration of DOX in normal mice resulted in a peak plasma concentration of 19.45 μM (t = 30 minutes). Prodrug injection resulted in 3- to 16-fold lower concentrations in the tissues of normal mice. As it is more polar, lower levels were observed in tissues and plasma in contrast to the parent compound DOX. In vivo safety studies have shown that prodrug 1 had a maximum tolerated dose compared with DOX and led to improved pharmacokinetics in normal mice.

Keywords cancer chemotherapy; doxorubicin prodrug; safety; pharmacokinetics; biodistribution; ADEPT

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INTRODUCTION

Doxorubicin (DOX) is an anticancer agent with a wide spectrum of activity. Cumulative dose-related cardiotoxicity, however, is a major side-effect of DOX, in addition to acute toxicities, such as myelosupression, nausea, and vomiting (Chabner, Allegra, Curt, & Calabresi, 1996). Reducing the toxicities associated with DOX and retention of their activity remain a challenge in the field of cancer chemotherapy. The success of DOX, and its limitations in clinical use, has directed research endeavors for the development of analogues of DOX with an improved therapeutic index. Of the available analogues, only epidoxorubicin appears to have a reduced cardiotoxicity with retention of antitumor activity and is in use in cancer chemotherapy (Danesi, Fogli, Gennari, Conte, & DelTacca, 2002).

For many years, efforts have been made to improve the selectivity and efficacy of chemotherapy using nontoxic prodrugs that are preferentially converted into active anticancer agents at the tumor site where the enzyme is expressed at high levels (prodrug monotherapy [PMT]; (Bosslet, Czech, & Hoffmann, 1995) or by tumor-associated antigen-specific monoclonal antibody-enzyme conjugate (antibody-directed enzyme prodrug therapy [ADEPT]; Senter & Springer, 2001; Harikrishna, Rao, & Krishna, 2003). These enzymes are called as lysosomal hydrolases. The specific activity of several lysosomal enzymes in tumors is elevated compared with that in the normal tissues from which the tumors are derived. Elevated enzyme levels in tumor tissue have been reported for β-galactosidase, and this enzyme can only be detected in very low concentrations in the circulation and may be exploited for the specific activation of galactoside prodrugs in tumor tissue

(Michael & Tannock 1993; Krishna, Sperker, Fritz, & Klotz, 1999).

Prodrug 1 (Figure 1) is a derivative of DOX in which the galactose moiety is linked to the DOX via a carbamate spacer. We have previously shown in vitro that a galactoside derivative of DOX, such as prodrug 1, was stable in phosphate buffer. It was relatively nontoxic and could only be activated to active DOX by β-galactosidase. The galactoside-DOX was found to be highly hydrophilic with impaired ability to enter cells (Harikrishna, Raghavendra, Venkateshwarlu, Rao, & Krishna, 2007). As a follow-up, the present study examines the in vivo toxicity evaluation in the Ehrlich Ascites Carcinoma murine tumor model for maximum tolerated dose (MTD). The pharmacokinetics and distribution of DOX and prodrug 1 were measured in plasma and nontumor-bearing mouse organs.

MATERIALS AND METHODS

Prodrug 1 has been characterized in which DOX moiety was linked to the galactose via a carbamate spacer with an aromatic center. Stock solutions of DOX and prodrug 1 were prepared in sterile water and stored at −20ºC.

Animal Stocks

Female Swiss albino mice aged 5 to 6 weeks and weighing 24 to 26 g were obtained from the Experimental Animal Care Centre (National Institute of Nutrition, Hyderabad, India). The experimental protocol involving use of animals was approved by the Institutional Animal Care and Use Committee at Kakatiya University. The animals were fed with a standard diet and water ad libitum. Mice were housed in groups at controlled laboratory conditions of temperature (22 ± 1ºC), relative humidity, and light/dark cycle (12 hours/12 hours). All the animal studies were conducted according to acceptable standard procedures.

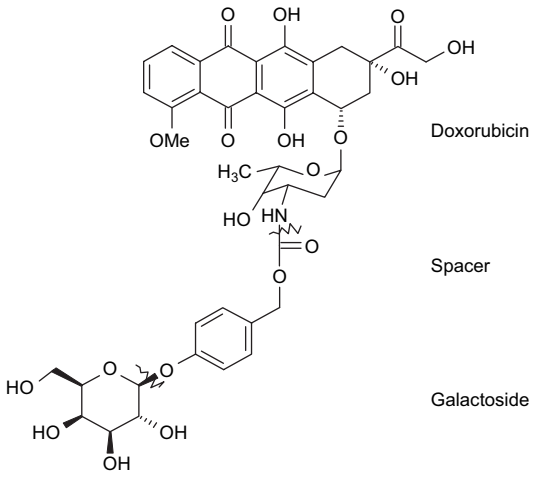

FIGURE 1. Chemical structure of prodrug 1 (N-[β-D-glucopyranosyl-benzyloxycarbonyl]-doxorubicin).

Safety Evaluation in EAC Tumor Model

Implantation of Ehrlich Ascites Carcinoma Cells in the Peritoneal Cavity of Mice

Ehrlich ascites carcinoma cell lines were maintained by serial transplantation in female Swiss albino mice every 8 days. A total of 40 mice were randomly allotted to different control and treatment groups (8 mice in each group). Four mice in each group were used for the evaluation of cytotoxicity, hematological, and biochemical parameters, and the remaining mice were used to study the changes in body weight and survival period. Each mouse was injected intraperitoneally with 2.5×10^6 EAC cells except those in the negative control group, and this was counted as day zero. Treatments were started 6 days after tumor implantation.

Dose and Mode of Administration

The dose of DOX was 8 mg/kg and the dose of prodrug 1 consisted of 8 mg/kg and 24 mg/kg of DOX equivalents. Fresh aqueous solution of the drug and prodrug were administered through the tail vein to different treatment groups once daily three times with a 3 day interval.

Experimental Groups

Experimental groups of mice consisted of the following: group 1, negative control (devoid of EAC-cell implant), saline; group 2, positive control (EAC-implant), saline; group 3, DOX, 8 mg/kg; and groups 4 and 5, prodrug 1 (8 mg/kg equivalent DOX) and (24 mg/kg equivalent DOX). In each treatment group, four animals were killed 24 hours after the last dose. Peritoneal fluid samples from each mouse were collected in vials and immediately processed for the observation of viability and cytotoxicity in EAC cells with a hemocytometer under a microscope using the dye-exclusion technique. Hemoglobin content, red blood cell (RBC) counts, and white blood cell (WBC) counts were measured from freely flowing tail vein blood. Differential WBC leukocyte count was carried out from Leishaman stained blood smears of normal, EAC-control, and treated groups, respectively. Serum glutamate pyruvate transaminase and glutamate oxaloacetate transaminase and alkaline phosphatases were determined using a portion of the blood collected. The remaining mice in each group were kept to check the mean survival time (MST) of the tumor bearing hosts (Matsuoka, Sugimachi, Kuwano, & Yano, 1989; Qureshi et al., 1993).

Evaluation of Body Weight Changes and Survival

Antitumor effect of DOX and prodrug were assessed by observation of changes with respect to body weight, ascites tumor volume, packed cell volume, viable and nonviable tumor cell count, MST, and percentage increase in life span (% ILS).

MST of each group containing four mice were monitored by recording the mortality daily for 6 weeks and % ILS was calculated using the following equation (Mazumder, Gupta, Maiti, & Mukherjee, 1997; Gupta, Mazumder, Rath, & Mukhopadhyay, 2000).

$$\text{MST} = (\text{day of first death} + \text{day of last death})/2$$

$$\text{ILS}(\%) = \times 100$$

Estimation of Cell Viability

One hundred µl of the cell suspension was added to 100 µl of the trypan blue dye solution. This resulted in a 1:1 dilution of the cell suspension. The mixture was allowed to stand for 5 minutes, but no longer than 15 minutes, before counting. Ten µl of the dye-cell suspension mixture was carefully poured into one side of the counting chamber. The cells were examined through a microscope with a 10× objective. The cell count was done in four large squares (four corner squares). A separate count was maintained for viable and nonviable cells.

Calculations

$$\text{Cells/ml} = \text{average count per square} \times \text{dilution factor}$$

$$\text{Total viable cells} = \text{cells/ml} \times \text{original volume of cell suspension}$$

$$\text{Cell viability percent} = \frac{\text{Total viable cells (unstained)}}{\text{Viable cells + dead cells (stained)}} \times 100$$

Statistical Analysis

Data are presented as mean ± standard deviation. Statistical comparisons were made by using analysis of variance (ANOVA) with significance defined as $p < .05$. Newman-Keuls analyses were done by using Student's t-test.

Distribution and Pharmacokinetics in Tissues and Plasma of Normal Mice

Swiss albino mice were randomly allotted to different groups of six animals each and were injected intravenously (iv) with 20 mg/kg DOX and 300 mg/kg of prodrug 1 in water. After 6 hours of administration of DOX and prodrug 1, heart, liver, kidney, lung, and brain tissues were removed in each group. After blood sampling, heart, liver, kidney, lung, and brain tissues were collected and immediately stored at −80°C until preparation for high-performance liquid chromatography (HPLC) analysis. A 30 to100 mg amount of frozen tissue sample was suspended in 400 µL of 50 mM ascorbic acid buffer with a pH of 4.5. Protein and DNA were denatured by adding 50 µL of 3 M $AgNO_3$ and by mixing the resulting suspension for 10 minutes at room temperature. The excess of silver ions was precipitated with 50 µL of 3 M NaCl. After adding 1.25 ml of acetonitrile-methanol (2:1 v/v) the suspension was mixed for 10 minutes at room temperature and separated by centrifugation (11,000 g, 5 minutes). A 20 µL aliquot of the clear supernatant was analyzed by HPLC (Houba et al., 1999; Houba et al., 2000; De Graaf et al., 2004).

For the study of DOX and prodrug 1 in the plasma samples, mice were injected with DOX and prodrug 1 at a dose of 20 mg/kg and 300 mg/kg. Blood samples were collected at different time points ranging from 30 minutes to 24 hours. (0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 12.0, and 24.0 hours). For analysis of DOX and prodrug in the plasma samples, plasma (10 µL) was diluted in ice cold methanol (140 µL), incubated at –20 °C for at least 10 minutes, and centrifuged (13,000 g, 5 minutes). To the supernatant, (100 µL) 12 mM H_3PO_4 (25 µL) was added. Samples were stored at –20 °C until HPLC analysis. A 20 µL aliquot of the clear supernatant was analyzed by the HPLC.

HPLC Analysis

The HPLC (SHIMADZU, MD, USA) apparatus consisted of a pump (LC-10AT VP) and UV-Visible detector (SPD-10A VP). For analysis of DOX and prodrug 1 content in plasma and tissues, 20 µL samples were loaded on a reversed-phase column (Phenomenex C_{18}, stainless steel column of 25 cm length, 4.6 mm diameter, packed with porous silica spheres of 5 µ diameter, 100A° pore diameter). Elution was done with eluents in an isocratic run (1 ml/minute of 65% ammonium acetate buffer [0.05 M, pH 4.0] and 35% acetonitrile with UV detection at 254 nm). Peak area ratios were used to calculate the concentrations of DOX and prodrug 1. The detection limit was 100 nM (pro)drug.

Method Validation

Specificity

The degree of interference by endogenous plasma constituents with DOX and prodrug 1 was evaluated by inspection of the chromatogram derived from processed blank and spiked plasma samples, and also from processed blank samples injected during each analytical run.

Calibration Curve

Calibration standards at the concentrations of 0.05, 0.1, 0.25, 0.5, 1.0, and 2.5 µM were extracted and assayed as mentioned above. The calibration curve was constructed based on percent peak area of the pro(drug). Calibration curve was plotted everyday.

The method of residuals used on the semi logarithmic plot of concentrations following intravenous administration versus time indicated that the data could best be described by two exponential disposition in all animals. Data were analyzed with the WinNonLin (Version 4.1 Pharsight Corporation) software

program in order to calculate half-life times and Area Under Curve (AUCs). Differences in drug concentrations per time point were evaluated with the Student's t-test.

RESULTS AND DISCUSSION

In Vivo Safety Evaluation

The results of in vivo study are presented in Tables 1 through 3.

Effect on Mean Survival Time and Tumor Growth

The EAC cell-bearing mice showed a significant ($p < .01$) increase in body weight compared with the nontumor-bearing mice. Treatment with DOX (8 mg/kg) and prodrug 1 at the highest dose tested checked the increase in body weight of EAC cell-bearing mice and this effect was statistically significant ($p < .01$; $p < .05$). Percentage survival of EAC cell-bearing mice was reduced to half on the 15th day after implantation and no animal survived beyond the 22nd day. DOX treatment increased the mean survival period to 25 days. Prodrug 1 has increased the mean survival period in a dose-dependent manner. DOX treatment significantly reduced the total number of EAC cells in the peritoneal fluid compared with the number of EAC cells obtained from mice in the control group. The prodrug treatment also reduced the viability in EAC cells. The reduction in viability was statistically significant at the higher doses of prodrug, being $p < .05$. Tumor volume and packed cell volume were decreased in a dose-dependent manner compared with that of the EAC control group (Table 1).

Effect on Hematological Parameters

Hemoglobin content and RBC count in the EAC control group was decreased compared with the normal group. Treatment with prodrug at higher doses increased the hemoglobin content and RBC count to more or less normal levels. There was no significant decrease in the WBC count. In the differential count of WBC, increase of neutrophils and the lymphocyte count decreased in the EAC control group. Treatment with prodrug at different doses changed these altered parameters to more or less normal (Table 2).

Effect on Biochemical Parameters

Prodrug 1 at both the doses marginally altered the Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), and alkaline phosphatase levels (Table 3).

TABLE 1
Evaluation of Body Weight Changes, Survival, and Cell Viability

Parameters	Control	Doxorubicin	Prodrug 1 (1 eq)	Prodrug 1 (3 eq)
Body weight/g	32.0 ± 3.12	22.0 ± 3.57[a]	28.0 ± 3.96	25.0 ± 4.67[b]
Mean survival time/days	18.0 ± 2.14	25.0 ± 2.58[a]	21.0 ± 3.54[b]	23.0 ± 3.52[b]
Increase life span/%	–	38.8 ± 5.24[a]	16.6 ± 2.65	27.7 ± 4.72[a]
Tumor cell volume/ml	31.0 ± 4.18	6.00 ± 0.95[a]	20.0 ± 5.71[b]	13.0 ± 2.97[a]
Packed cell volume/ml	4.30 ± 0.89	1.60 ± 0.29	4.0 ± 2.65	3.00 ± 1.43
Viable cell count/10^{10} cells/L	26.0 ± 4.25	0.30 ± 1.29[a]	16.0 ± 3.85[b]	12.0 ± 2.47[b]
Dead cell count/10^{10} cells/L	1.6 ± 0.94	4.70 ± 1.61	1.4 ± 0.48	1.5 ± 0.63

Four mice were used in each group. Treatments were significantly different at $p < .05$ (ANOVA). In Newman-Keuls analysis, groups DOX, prodrug 1 (1 eq), and prodrug 1 (3 eq) were statistically compared with control group.

[a]$p < .01$;

[b]$p < .05$ (Student's *t*-test).

TABLE 2
Estimation of Biochemical Parameters

Parameters $U.L^{-1}$	Normal	Control	Doxorubicin	Prodrug 1 (1 eq)	Prodrug 1 (3 eq)
SGPT	50.0 ± 4.42	65.0 ± 2.16	32.0 ± 1.32[a]	58.0 ± 4.84	64.0 ± 4.63
SGOT	28.0 ± 1.41	32.5 ± 0.44	24.0 ± 2.28	22.0 ± 1.67	23.0 ± 1.26
ALP	320 ± 22.8	500 ± 14.1	305 ± 10.4[a]	403 ± 10.3	350 ± 14.1[a]

Four mice were used in each group. Treatments were significantly different at $p < .05$ (ANOVA). In Newman-Keuls analysis, groups DOX, prodrug 1 (1 eq), and prodrug 1 (3 eq) were statistically compared with control group.

[a]$p < .05$ (Student's *t*-test).

TABLE 3
Estimation of Hematological Parameters

Parameters	Normal	Control	Doxorubicin	Prodrug 1 (1 eq)	Prodrug 1 (3 eq)
Hemoglobin/g %	11.8 ± 0.30	7.00 ± 0.14	12.6 ± 0.22[a]	7.80 ± 0.16	10.8 ± 0.14[a]
Platelet count	1.60 ± 0.10	2.18 ± 0.07	1.80 ± 0.06	2.20 ± 0.10	1.80 ± 0.06
RBC/$10^{15}\cdot L^{-1}$	6.50 ± 0.48	2.40 ± 0.41	3.80 ± 0.42	4.00 ± 0.40	5.50 ± 0.58
WBC/$10^{12}\cdot L^{-1}$	4.70 ± 0.20	20.4 ± 0.16	12.4 ± 0.30[b]	12.0 ± 0.34[b]	10.5 ± 0.27
Monocyte/%	1.80 ± 0.14	3.00 ± 0.34	2.00 ± 0.14	3.00 ± 0.14	2.00 ± 0.22
Neutrophil/%	17.0 ± 1.40	70.83 ± 2.56	66.0 ± 3.27	65.0 ± 3.37	50.0 ± 3.68[a]
Lymphocyte/%	80.0 ± 7.01	23.0 ± 2.82	28.0 ± 1.41	30.0 ± 1.67	40.0 ± 2.09[a]

Four mice were used in each group. Treatments were significantly different at $p < .05$ (ANOVA). In Newman-Keuls analysis, groups DOX, prodrug 1 (1 eq), and prodrug 1 (3 eq) were statistically compared with control group.
[a]$p < .05$;
[b]$p < .01$ (Student's *t*-test).

Biodistribution of DOX and Prodrug 1 in Tissues of Normal Mice

The biodistribution of DOX (20 mg/kg) and prodrug 1 (300 mg/kg) in different tissues was done iny normal mice. After 6 hours of administration of DOX, the highest concentration was measured in the heart (727.18 μmol.g^{-1}) followed by the liver (483.07 μmol.g^{-1}), lungs (150.49 μmol.g^{-1}), kidneys (104.48 μmol.g^{-1}), and brain (46.99 μmol.g^{-1}). After prodrug administration, peak concentrations were highest in the heart (209.97 μmol.g^{-1}) followed by the lungs (23.55 μmol.g^{-1}), liver (29.22 μmol.g^{-1}), kidneys (20.43 μmol.g^{-1}), and brain (13.48 μmol.g^{-1}). Prodrug injection resulted in 3- to 16-fold lower concentrations in these tissues (Figure 2).

Pharmacokinetics of DOX and Prodrug 1 in Plasma

Administration of DOX resulted in a peak concentration of the drug of 19.45 μM (t = 30 minutes; Figure 3) in plasma and an elimination half-life time of 30.13 hours. After administration of prodrug 1 in normal mice, it reached a plasma peak concentration of 11.95 μM (t = 30 minutes; Figure 3) and was eliminated with a half-life time of 30.0 hours (Table 4).

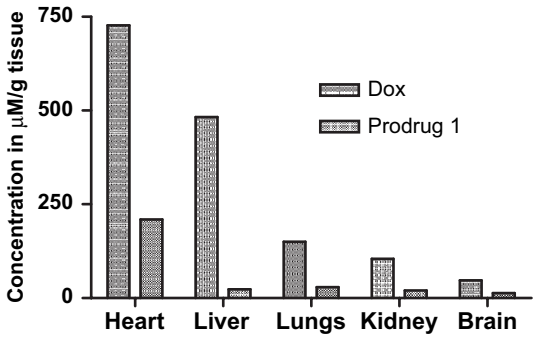

FIGURE 2. Tissue distribution studies of DOX and prodrug 1.

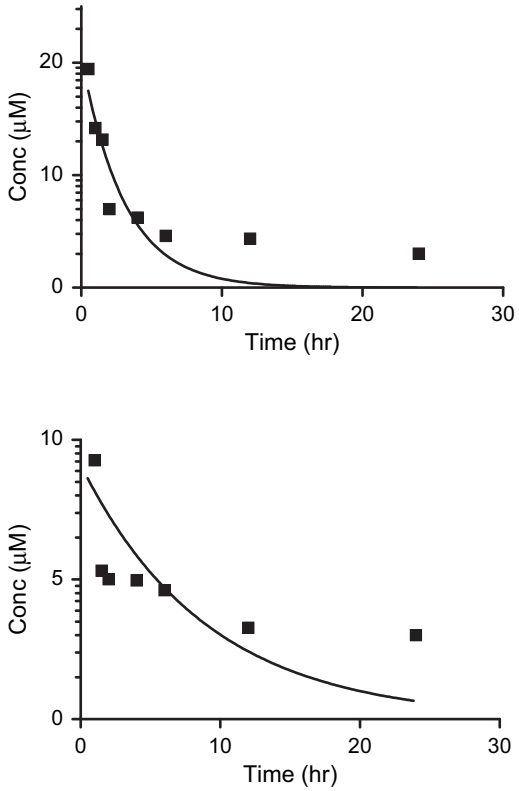

FIGURE 3. Plasma concentration time profile of DOX and prodrug 1 in normal mice.

Prodrug 1 administration resulted in a 10-fold increase in volume of distribution compared with DOX.

DOX is an effective antitumor agent and can rapidly diffuse through cell membranes into cells of both normal and malignant tissues. DOX prodrug was synthesized for use in ADEPT and its in vitro stability, release kinetics, and cytotoxicity, were evaluated. We showed that the prodrug is more than 100 times less cytotoxic than the DOX against HeLa and MCF-7 cell

TABLE 4
Pharmacokinetic Parameters of DOX and Prodrug 1

Treatment	AUC (μM/hr/ml)	C_{max} (μM)	Cl (ml/min)	AUMC ($\mu M/h^2/ml$)	MRT (hr)	$t_{1/2}$ (min)	V_d (Lit)
DOX	262.30	27.40	0.19	10340.83	39.42	30.13	1.78
Prodrug 1	234.67	20.89	1.27	10374.85	44.20	33.00	10.24

lines (Harikrishna, Raghavendra, et al., 2007). As this is relatively nontoxic and would only exert cytotoxic effects upon enzyme activation, it has to be delivered at high levels to the tumor site. This is why we selected the murine tumor model to show the safety of prodrug compared with the parent drug DOX. Furthermore, the synthesis of prodrug 1 is much more efficient than that of reported prodrugs (Bakina, Wu, Rosenblum, & Farquhar, 1997; Florent et al., 1998; Leenders et al., 1999).

Reliable criteria for judging the value of any anticancer agents is the prolongation of the life span of animals (Hogland, 1982). A decrease in tumor volume and viable tumor cell count finally reduced the tumor burden and enhanced the life span of EAC-bearing mice. In cancer chemotherapy, the major problems are of myelosuppression and anemia (Maseki, Nishiagaki, Hagishara, Tomada, & Yagi, 1954; Price & Greenfield, 1958). The anemia encountered in tumor-bearing mice is mainly due to reduction in RBC or hemoglobin percentage, and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions. Treatment with prodrug 1 at higher doses brought back near to normal levels and there was no death of animals observed. This indicates that prodrug 1 has no toxic effects up to 24 mg/kg of DOX equivalents.

We further investigated whether the concentrations of prodrug 1 in in plasma and tissues of nontumor-bearing mice after iv administration were lower than DOX. This information would give an indication whether a higher antitumor efficacy might be expected from prodrug 1. Twenty mg/kg of DOX resulted in 3-fold higher levels of accumulation in the heart compared with prodrug 1. This is due to the dose and route of the administered drug. In pharmacokinetic studies with the prodrug 4-[*bis*-2-chloroethyl)amino]benzoyl-L-glutamic acid, the highest drug:prodrug ratios were found in the tumor after treatment with a monoclonal antibody (mAb) conjugate of the enzyme carboxypeptidase G, leading the authors to conclude that tumor-specific conversion took place (Antoniw et al., 1990). These findings corresponded to greater therapeutic effects with the combination of the targeted conjugate and the prodrug compared with systemic treatment with the parent drug (Springer et al., 1991). In another report, antigen-specific generation of 5-fluorouracil from 5-fluorocytosine was shown to take place using conjugates of the enzyme cytosine deaminase and the mAb L6, leading to a greater intratumoral concentration of 5-fluorouracil compared with that obtained after systemic treatment with the drug (Wallace et al., 1994). Favorable drug distribution was also found after treatment with a beta-glucuronidase mAb fusion protein for the release of DOX (Bosslet, Czech, & Hoffmann, 1994) from a prodrug. DOX is a lipophilic molecule and thus penetrates rapidly into tissues. Therefore, normal tissue DOX levels are relatively high and may account for unfavorable side effects. The hydrophilic galactoside moiety of prodrug 1 prevents rapid diffusion of prodrug into cells. This is confirmed by the high peak plasma concentrations of prodrug and its rapid clearance from the plasma. The rapid clearance of the prodrug may be explained by a smaller distribution volume when compared with that of DOX, caused by its low tissue penetration.

CONCLUSION

In conclusion, in vivo administration of prodrug 1 led to improved pharmacokinetics in terms of clearance and volume of distribution compared with parent drug DOX. Produg 1 had a higher MTD than DOX and, therefore, better antitumor effects might be expected from a lower dose of prodrug 1 than from DOX. These featuers are of clinical interest to target solid tumors. Our next step will be to study the therapeutic efficacy and cleavage profile of prodrug 1 in a panel of different animal tumor models.

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REFERENCES

Antoniw, P., Springer, C. J., Bagshawe, K. D., Searle, F., Melton, R. G., & Rogers, G. T., et al. (1990). Disposition of the prodrug 4-(bis (2-chloroethyl) amino) benzoyl-L-glutamic acid and its active parent drug in mice. *Br. J. Cancer, 62,* 909–914.

Bakina, E., Wu, Z., Rosenblum, M., & Farquhar, D. (1997). Intensely cytotoxic anthracycline prodrugs: glucuronides. *J. Med. Chem., 40,* 4013–4018.

Bosslet, K., Czech, J., & Hoffmann, D. (1994). Tumor-selective prodrug activation by fusion protein-mediated catalysis. *Cancer Res., 54,* 2151–2159.

Bosslet, K., Czech, J., & Hoffmann, D. (1995). A novel one-step tumor-selective prodrug activation system. *Tumor Targeting, 1,* 45–50.

Chabner, B. A., Allegra, C. J., Curt, G. A., & Calabresi, P. (1996). Antineoplastic agents. In J. G. Hardman, L. E. Limbird, P. B. Molinoff, R. W. Ruddon, L. S. Gilman, & A. Goodman (Eds.), *Goodman and Gilman's the pharmacological basis of therapeutics* (pp. 1233–1257). New York: McGraw-Hill.

Danesi, R., Fogli, S., Gennari, A., Conte, P., & DelTacca, M. (2002). Pharmacokinetic-pharmacodynamic relationships of the anthracycline anticancer drugs. *Clin. Pharmacokinet., 41,* 431–444.

De Graaf, M., Nevalainen, T. J., Scheeren, H. W., Pinedo, H. M., Haisma, H. J., & Boven, E. (2004). A methylester of the glucuronide prodrug DOX-GA3 for improvement of tumor-selective chemotherapy. *Biochem. Pharmacol., 68,* 2273–2281.

Florent, J. C., Dong, X., Gaudel, G., Mitaku, S., Monneret, C., & Gesson, J. P., et al. (1998). Prodrugs of anthracyclines for use in antibody-directed enzyme prodrug therapy. *J. Med. Chem., 41,* 3572–3581.

Gupta, M., Mazumder, U. K., Rath, N., & Mukhopadhyay, D. K. (2000). Antitumor activity of methanolic extract of Cassia fistula L seed against Ehrlich ascites carcinoma. *J. Ethanopharmacol., 72,* 151–156.

Harikrishna, D., Raghavendra, S. N., Venkateshwarlu, Y., Rao, A.R., & Krishna, D.R. (2007). β-galactoside prodrugs of doxorubicin for application in antibody directed enzyme prodrug therapy/prodrug monotherapy. *Arch. Pharmacal. Res., 30,* 723–732.

Harikrishna, D., Rao, A. R., & Krishna, D. R. (2003). Selective activation of anthracycline prodrugs for use in conjunction with ADEPT. *Drug News Perspect., 16,* 309–318.

Hogland, H. C. (1982). Hematological complications of cancer chemotherapy. *Semin. Oncol., 9,* 95–102.

Houba, P. H. J., Boven, E., Van Der Meulen-Muileman, I. H., Leenders, R. G., Scheeren, J. W., & Pinedo, H. M., et al. (1999). Distribution and pharmacokinetics of the prodrug daunorubicin-GA3 in nude mice bearing human ovarian cancer xenografts. *Biochem. Pharmacol., 57,* 673–680.

Houba, P. H. J., Boven, E., Van Der Meulen-Muileman, I. H., Leenders, R. G. G., Scheeren, J. W., & Pinedo, H. M., et al. (2000). A novel doxorubicin-glucuronide prodrug DOX-GA3 for tumour-selective chemotherapy: distribution and efficacy in experimental human ovarian cancer. *Br. J. Cancer, 84,* 1–8.

Krishna, D. R., Sperker, B., Fritz, P., & Klotz, U. (1999). Does pH 6 beta-galactosidase activity indicate cell senescence? *Mech. Ageing & Dev., 109,* 113–123.

Leenders, R. G. G., Damen, E. W., Bijsterveld, E. J., Scheeren, H. W., Houba, P. H., & Van Der Meulen-Muileman, I. H., et al. (1999). Novel anthracycline-spacer-beta-glucuronide,-beta-glucoside, and -beta-galactoside prodrugs for application in selective chemotherapy. *Bioorg. Med. Chem., 7,* 1597–1610.

Maseki, M., Nishiagaki, I., Hagishara, M., Tomada, Y., & Yagi, K. (1954). Lipid peroxidation levels and lipid content of serum lipoprotein fractions of pregnant subjects with or without preeclampsia. *J. Clin. Chim. Acta, 41,* 424–426.

Matsuoka, H., Sugimachi, K., Kuwano, H., & Yano, K. (1989). Intratumoural injection of calcium channel blocker enhances cytotoxicity of adriamycin in Ehrlich ascites solid tumour. *Eur. J. Surg. Oncol., 15,* 224–231.

Mazumder, U. K., Gupta, M., Maiti, S., & Mukherjee, M. (1997). Antitumor activity of hygrophilaspinosa on Ehrlich ascites carcinoma and sarcoma-180 induced mice. *Ind. J. Expt. Biol., 35,* 473–477.

Michael, J. B., & Tannock, I. F. (1993). Lysosomes, lysomal enzymes and cancer. *Adv. Cancer Res., 60,* 269–291.

Price, V. E., & Greenfield, R. E. (1958). Anemia in cancer. In: J. P. Greenstein JP, H. A. e. (ed.) Adv. cancer Res., New York, pp 199–200.

Qureshi, S., Al-Harbi, M. M., Ahmed, M. M., Raza, M., Giangreco, A. B., & Shah, A. H. (1993). Evaluation of the genotoxic, cytotoxic, and antitumor properties of Commiphora molmol using normal and Ehrlich ascites carcinoma cell-bearing Swiss albino mice. *Cancer Chemother. Pharmacol., 33,* 130–138.

Senter, P. D., & Springer, C. J. (2001). Selective activation of anticancer prodrugs by monoclonal antibody-enzyme conjugates. *Adv. Drug Deliv. Rev., 53,* 247–264.

Springer, C. J., Bagshawe, K. D., Sharma, S. K., Searle, F., Boden, J. A., & Antoniw, P., et al. (1991). Ablation of human choriocarcinoma xenografts in nude mice by antibody-directed enzyme prodrug therapy (ADEPT) with three novel compounds. *Eur. J. Cancer, 27,* 1361–1366.

Wallace, P. M., Macmaster, J. F., Smith, V. F., Kerr, D. E., Senter, P. D., & Cosand, W. L. (1994). Intratumoral generation of 5-fluorouracil mediated by an antibody-cytosine deaminase conjugate in combination with 5-fluorocytosine. *Cancer Res., 54,* 2719–2723.