Disposition of a murine monoclonal antibody vinca conjugate (KS1/4-DAVLB) in patients with adenocarcinomas

The pharmacokinetics of a murine monoclonal antibody vinca conjugate (KS1/4-DAVLB) was investigated in 13 patients with adenocarcinomas who received single intravenous doses ranging from 40 to 250 mg/m² and in three patients who were administered 1.5 mg/kg every 48 to 72 hours for up to 15 doses. Five patients in the single-dose study also received 100 μCi of [³H]-KS1/4-DAVLB. Overall mean values for the pharmacokinetic variables were as follows: elimination half-life, 31.5 hours; distribution volume, 4.43 L; and clearance, 0.09 L/hr. KS1/4-DAVLB demonstrated linear elimination kinetics in both the single- and multiple-dose studies. Significant concentrations of KS1/4-DAVLB were noted in a pleural effusion. Ten percent of the radioactive dose was recovered in the urine and 20% in the feces over a 5-day period. Small molecular weight vinca species were detected in the feces but not in the serum. (CLIN PHARMACOL THER 1990;47:36-41.)

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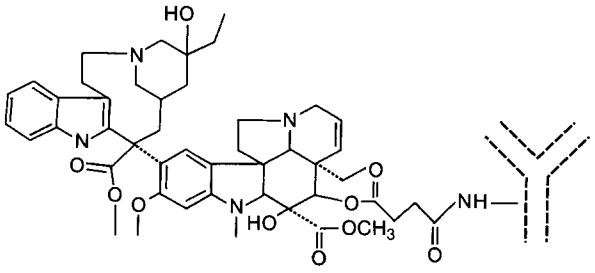
Several studies have examined the potential therapeutic value of unmodified monoclonal antibodies (MOABs) as tumor therapy by use of in vivo models of human tumor growth and in human clinical trials.[1-5] Additional reports have used conjugates of MOABs with radionuclides, plant and microbial toxins, and oncolytic agents such as methotrexate, adriamycin, and the vinca alkaloids.[6-9] These efforts have sought to test the concept of using the MOAB as a site-directed targeting agent for tumors in humans.

The IgG2a murine monoclonal antibody KS1/4 recognizes a 40,000 Da cell surface glycoprotein found in high density in the tumor cell membrane of a human lung, colon, rectal, pancreatic, and ovarian adenocarcinomas.[10-12] The KS1/4 antigen is also expressed on the cell surface of a variety of normal tissues, including the epithelium of the gastrointestinal tract, kidney tubular cells, bile and pancreatic ductal epithelium, bronchial and alveolar epithelium, ovary epithelium, and sweat ducts.[10]



Structure of KS1/4-DAVLB. Four to six desacetylvinblastine molecules are covalently bonded by means of hemisucinate linkers to epsilon amino groups of lysine residues.

The conjugate KS1/4-DAVLB, containing 4 to 6 molecules of desacetylvinblastine (DAVLB) bound covalently to KS1/4 by means of hemisuccinate linkers (see Structure), retains a high degree of reactivity with the tumor-associated antigen. Preclinical pharmacology

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Table I. KS1/4-DAVLB dose schedule for patients enrolled in the single-dose study

Patient No.	*KS1/4-DAVLB Dose*			
	(mg/m²)	*(mg)*	*(mg vinca)*	*Infusion time (hr)*
1	40	56	1.4	2.0
2	40	60	1.5	2.0
3	80	168	4.3	2.0
4	80	44	3.6	2.0
5	140	137	3.5	4.5
6	140	175	4.5	1.5
7	140	196	5.0	2.0
8	140	252	6.4	2.0
9	140	293	7.4	2.0
10	250	350	8.9	2.0
11	250	500	12.7	4.2
12	250	525	13.3	6.2
13	250	550	13.9	7.6

experiments demonstrating the antitumor properties of KS1/4-DAVLB have been reported.[13] This study describes the pharmacokinetic characteristics of KS1/4-DAVLB in patients who were administered single and multiple intravenous infusions of the drug.

PATIENTS AND METHODS

Single-dose study. Six women and seven men with adenocarcinoma of the lung (Stage III), colon, or rectum (stage D) participated in this study. Patients were 40 to 76 years old and weighed from 104 to 230 pounds. Life expectancy of at least 2 months and a Karnofsky performance value of at least 60% were required for enrollment. Adequate marrow function (Hb > 10 gm/dl; white blood cell count >4000/mm³; platelet count > 100,000/mm³), liver function (bilirubin < 2.5 mg/dl), and kidney function (creatinine < 2.5 mg/dl) were also required. The patients were informed of the nature and objectives of the studies and gave written consent. The studies were approved by the Indiana University Institutional Review Committee and by the Methodist Hospital Institutional Review Board (Indianapolis, Ind.).

Each dose of KS1/4-DAVLB was infused over a 1.5- to 2-hour time period. The infusion vehicle was normal saline solution that contained human albumin (5 gm/100 ml). Patients fasted from midnight until completion of the infusion. The dosing schedule for this study is shown in Table I.

At the end of the 2-hour infusion of KS1/4-DAVLB, a trace dose of tritium-labeled KS1/4-DAVLB (100 μCi, 3.2 mg, label in the vinca molecule) was given as a bolus to patients 10 and 11. For patients 5, 6, and 9, the tritium-labeled KS1/4-DAVLB was added to the main dose and administered over the entire infusion.

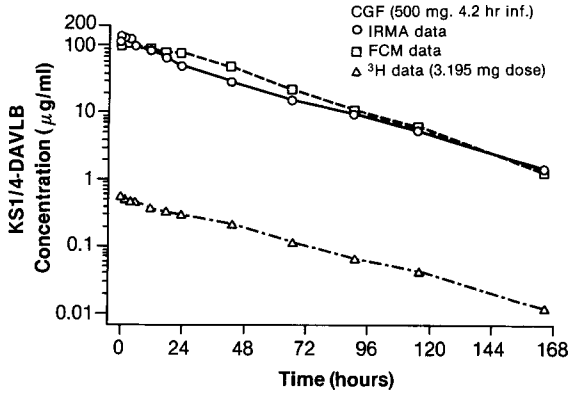

Fig. 1. Concentration-versus-time data for patient 3 after intravenous infusion of KS1/4-DAVLB.

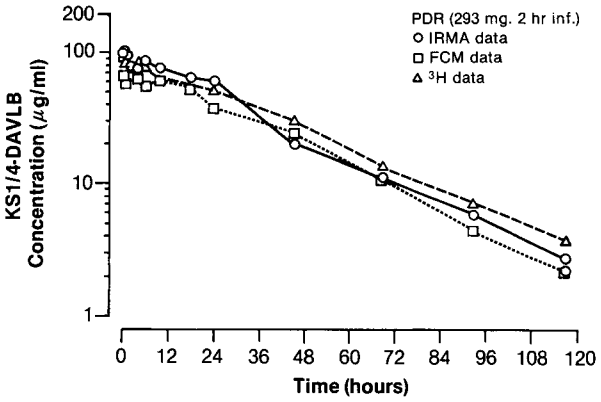

Fig. 2. Concentration-versus-time data for patient 9 after intravenous infusion of KS1/4-DAVLB.

Serial blood samples were obtained from each patient for up to 7 days after the KS1/4-DAVLB infusion. The serum concentration of KS1/4-DAVLB was measured by means of immunoradiometric (IRMA) and flow cytometric assays (FCM). In the IRMA assay, goat anti-mouse immunoglobin covalently bonded to acrylic microspheres, is incubated with human serum containing KS1/4-DAVLB. This complex is mixed with ^{125}I-labeled goat anti-mouse immunoglobin and the radioactivity binding to the microspheres measured. This assay does not require that a functional antigen binding site be present on the mouse immunoglobulin. Details of the IRMA assay have been published.[14] The FCM assay consisted of a modification of the method described by Marder et al.[15] Dilutions of serum samples

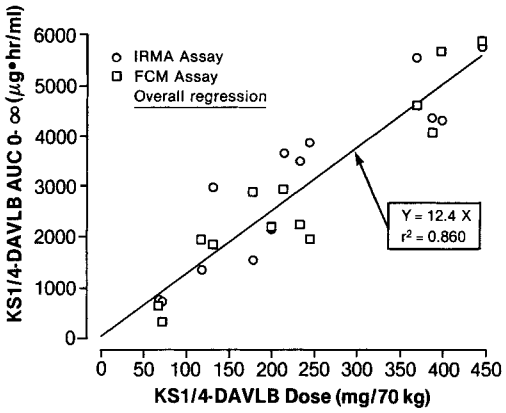

Fig. 3. KS1/4-DAVLB AUC versus weight-normalized dose after single intravenous infusions.

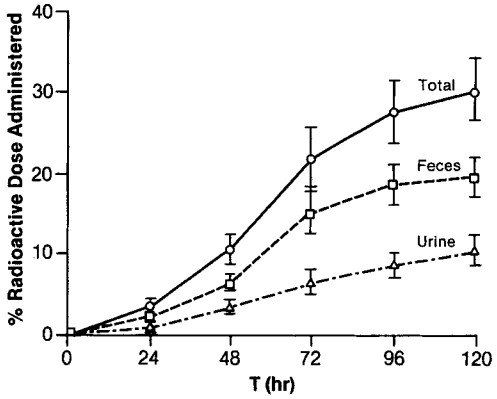

Fig. 4. Recovery of urinary and fecal radioactivity after administration of 100 μCi tritium-labeled KS1/4-DAVLB.

Table II. Mean values of pharmacokinetic variables for KS1/4-DAVLB after a single intravenous infusion

Assay	*Elimination $t_{1/2}$* (hr)*	*Systemic clearance (L/hr)*	*Volume of distribution (L)*
IRMA (n = 13)	33.5 ± 12.0	0.085 ± 0.027	4.60 ± 2.77
FCM Assay (n = 13)	29.8 ± 9.02	0.097 ± 0.034	4.26 ± 1.19
[^{3}H] (n = 5)†	33.6 ± 6.47	0.104 ± 0.036	5.34 ± 2.44

Data are mean values ± SD.
IRMA, Immunoradiometric assays; FCM, flow cytometric.
*Harmonic mean.
†Values were determined from the serum radioactivity content.

from patients infused with KS1/4-DAVLB, normal serum controls, and standard KS1/4 dilutions were added to paraformaldehyde fixed P3-UCLA cells (human adenocarcinoma cell line, 8 × 10e5/tube). These cells highly express the KS1/4 membrane antigen. Thus, KS1/4-DAVLB must be immunoreactive with the antigen for this assay to measure the presence of this immunoconjugate in human serum. The mixture was incubated at 4° C for 30 minutes, centrifuged, washed, and the cell pellet resuspended in 0.1 ml of fluorescein-conjugated sheep anti-mouse IgG antisera. This mixture was incubated at 4° C for 30 minutes in the dark, centrifuged, and the cells washed. Aliquots of the cells were analyzed for fluorescence intensity by use of an EPICS C flow cytometer (Coulter, Hialeah, Fla.). The mean channel of fluorescence was determined for each sample and the standard curve and the unknown concentrations were determined by use of a computer program (RIASYS, Eli Lilly & Company) (Indianapolis, Ind.).

Urine and fecal samples from each patient who was administered radioactive KS1/4-DAVLB were collected for up to 5 days after dosing. Aliquots of blood serum (Isolab, Inc., Akron, Ohio) and the radioactivity was determined in a Beckman LS 3801 liquid scintillation system (Beckman Instruments, Fullerton, Calif.). Aliqouts of 1/1 aqueous fecal homogenate were combusted with a Packard Tri Carb Model B 306 sample oxidizer (Packard Instrument Company, Inc., Downers Grove, Ill.). Determination of radioactivity in the combusted samples was similar to that described above for serum and urine. Radioactivity was converted to microgram equivalents of KS1/4-DAVLB per volume of biologic sample on the basis of the calculated specific activity of the administered drug.

Serum KS1/4-DAVLB concentration data were analyzed by model-dependent pharmacokinetic methods. A one- or two-compartment open pharmacokinetic model was fitted to the individual concentration versus time data by use of an iterative nonlinear regression program, NONLIN84.[16] The estimated values of the coefficients were corrected for the length of the intravenous infusion, and corrected values for the coefficients and the fitted values of the exponents were used to calculate values of pharmacokinetic variables.[17]

Multiple-dose study. Three male patients, ranging in

pounds, were administered multiple doses of KS1/4-DAVLB. The KS1/4-DAVLB dosing schedule for these patients was 1.5 mg/kg administered every 48 to 72 hours for nine to 15 doses. Blood samples were obtained before and after completion of each infusion for measurement of the serum concentrations of KS1/4-DAVLB.

RESULTS

Single-dose study. The serum concentrations of KS1/4-DAVLB obtained by the IRMA and FCM assays were highly correlated with an r value of 0.93 and a slope of 1.1.

Figs. 1 and 2 show semilogarithmic plots of concentration-versus-time data for two patients. Patient 11 (Fig. 1A) received ^{3}H-KS1/4-DAVLB as an intravenous bolus after completion of the main infusion. The KS1/4-DAVLB serum concentration estimated from radioactivity content was therefore calculated on the basis of labeled dose administered (3.2 mg). The serum KS1/4-DAVLB concentrations measured by IRMA and FCM methods were similar for the entire time profile. The slope of the ^{3}H concentration-versus-time curve is also similar to that observed with the other two methods. Patient 9 (Fig. 2) received ^{3}H-labeled KS1/4-DAVLB mixed with the total unlabeled dose. Therefore, the KS1/4-DAVLB serum concentrations estimated from radioactivity content were calculated on the basis of total dose administered (293 mg). The serum KS1/4-DAVLB concentrations were comparable among the three methods of analysis.

Mean pharmacokinetic variables calculated from serum concentration data obtained by use of the three analytic methods are shown in Table II. No significant differences were detected among mean values of these variables for the three methods. Overall mean values for the IRMA and FCM assays were as follows: elimination half-life ($t_{1/2}$), 31.5 hours; systemic clearance, 0.09 L/hr; and volume of distribution, 4.43 L. For those patients whose data were described by use of a two-compartment model (6 of 13 patients), the range of distribution $t_{1/2}$ values was 1.4 to 5.7 hours (all assay methods).

Fig. 3 shows a plot of the KS1/4-DAVLB AUC versus weight-normalized KS1/4-DAVLB dose. The AUC versus dose relationship was linear (r^2 = 0.860), suggesting that KS1/4-DAVLB elimination kinetics were linear in the range of doses administered.

Mean total urinary and fecal recovery of a radiolabeled dose of KS1/4-DAVLB was only 30% over the 5-day collection period (Fig. 4). Two thirds of the recovered dose was found in the feces and one third was found in the urine. Preliminary experiments that used

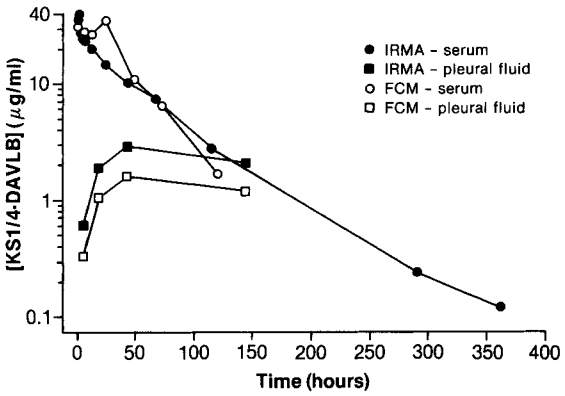

Fig. 5. Pleural fluid KS1/4-DAVLB concentration.

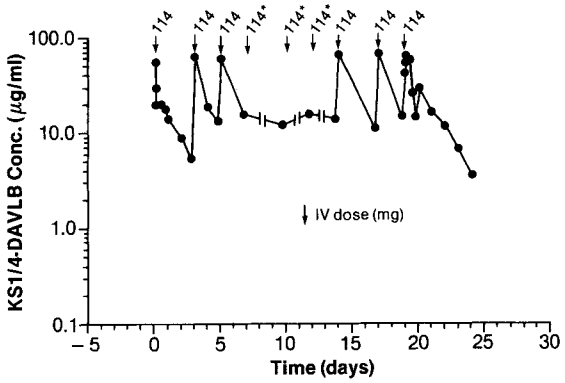

Fig. 6. Serum concentrations of KS1/4-DAVLB during a multiple dose schedule. *Peak blood samples not available for analysis.

lecular weight of the radioactive material in the feces was consistent with free desacetylvinblastine or metabolite(s). Less than 1% of the radioactivity in the serum could be accounted for by a free vinca species.

Patient 3 had a malignant pleural effusion from which serial samples were obtained for measurement of KS1/4-DAVLB concentration. The pleural fluid concentrations of KS1/4-DAVLB relative to serum values are shown in Fig. 5. Peak pleural fluid concentration was approximately 2 μg/ml, occurring about 45 hours after drug administration. This concentration was maintained for up to 150 hours after drug infusion.

Multiple dose study. Nine 114-mg doses of KS1/4-DAVLB were administered to a patient participating in the multiple-dose study. The peak and trough concentrations of KS1/4-DAVLB for this patient are shown in Fig. 6. Steady state was achieved by the third infusion, and subsequent values did not change during repeated infusions of KS1/4-DAVLB. Similar observations were

doses. For these three patients, the steady-state peak concentrations ranged from 55 to 65 μg/ml and the trough concentrations ranged from 10 to 20 μg/ml.

DISCUSSION

KS1/4-DAVLB pharmacokinetic parameters in patients are similar to values found for another murine IgG2a immunoglobulin, 17-1A, when it was infused into patients with metastatic gastrointestinal cancer, except the 17-1A $t_{1/2}$ of about 18 hours appears to be shorter than the $t_{1/2}$ found for KS1/4-DAVLB.[18] The addition of vinca molecules to the immunoglobulin molecule may reduce the rate of metabolism of the conjugate relative to unconjugated protein. The mechanism by which murine immunoconjugates are eliminated in human beings is not known; however, it is possible that cells of the reticuloendothelial system—particularly those in the liver—are responsible for the initial degradation of these molecules. The fecal recovery of radioactivity as low molecular weight vinca species indicates that the vinca is removed from the immunoglobulin. The vinca may then be further metabolized, followed by biliary excretion of parent drug and metabolites.

The decay of serum radioactivity after the administration of [^{3}H]-vinca labeled KS1/4-DAVLB was similar to the concentration decay of KS1/4-DAVLB measured by immunologic assays. The lack of recovery of small molecular weight compounds in the serum indicates that any free vinca formed does not appear in the circulation to any significant degree.

Only a total of about 30% of the radioactive dose was recovered over a 5-day period. The lack of quantitative recovery of radioactivity could indicate persistent binding of the conjugate to normal tissues that express the antigen or to tumor tissue. However, only about 30% of an intravenous [^{3}H] dose of free vinblastine is recovered over a 6-day period.[19] The recovery pattern of radioactivity in humans is similar to that observed in the rhesus monkey,[20] fisher rat,[20] and tumor-bearing nude mouse[21] after administration of single doses of KS1/4-DAVLB. In the rhesus monkey, approximately 50% of a radioactive dose is recovered after 7 days (80% of that is recovered in the feces). Similar results were obtained in the rat and mouse. The distribution volume also approximated the blood volume in these species. The $t_{1/2}$ was longer in the nude mouse ($\simeq$ 90 hours), and rat ($\simeq$ 60 hours). These results are not surprising because one would anticipate a slower rate of clearance after administration of a mouse immunoglobulin to rodent species.

Multiple doses of KS1/4-DAVLB did not result in enhanced or diminished clearance of the compound because peak and trough values remained constant during multiple dosing (Fig. 6). This result is similar to that reported earlier for 17-1A.[18] None of the three patients studied after multiple dosing developed a significant antimouse antibody response during the time period that drug was administered. The effect of a human antibody response (HAMA) on the clearance of KS1/4-DAVLB is not known at this time.

Significant concentrations of KS1/4-DAVLB were found in a malignant pleural effusion. This observation indicates that KS1/4-DAVLB can penetrate into third space tumor compartments.

In summary, KS1/4-DAVLB has a rather long $t_{1/2}$ (31.5 hours), a low systemic clearance (0.1 L/hr), and an apparent volume of distribution approximating blood volume (4 to 5 L).

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