

Correlation between radioactivity and chemotherapeutics of the ^{111}In -VNB-liposome in pharmacokinetics and biodistribution in rats

Wen-Chuan Lee^{1,*}
Chih-Hsien Chang^{2,3,*}
Chih-Min Huang¹
Yu-Tse Wu¹
Liang-Cheng Chen²
Chung-Li Ho²
Tsui-Jung Chang²
Te-Wei Lee²
Tung-Hu Tsai^{1,4}

¹Institute of Traditional Medicine, School of Medicine, National Yang-Ming University, Taipei, ²Division of Isotope Application, Institute of Nuclear Energy Research, Taoyuan, ³Department of Biomedical Imaging and Radiological Sciences, National Yang-Ming University, Taipei, ⁴Department of Education and Research, Taipei City Hospital, Taipei, Taiwan

*These authors contributed equally to this work

Background: The combination of a radioisotope with a chemotherapeutic agent in a liposomal carrier (ie, Indium-111-labeled polyethylene glycol pegylated liposomal vinorelbine, [^{111}In -VNB-liposome]) has been reported to show better therapeutic efficiency in tumor growth suppression. Nevertheless, the challenge remains as to whether this therapeutic effect is attributable to the combination of a radioisotope with chemotherapeutics. The goal of this study was to investigate the pharmacokinetics, biodistribution, and correlation of Indium-111 radioactivity and vinorelbine concentration in the ^{111}In -VNB-liposome.

Methods: The VNB-liposome and ^{111}In -VNB-liposome were administered to rats. Blood, liver, and spleen tissue were collected to determine the distribution profile of the ^{111}In -VNB-liposome. A liquid chromatography tandem mass spectrometry system and gamma counter were used to analyze the concentration of vinorelbine and radioactivity of Indium-111.

Results: High uptake of the ^{111}In -VNB-liposome in the liver and spleen demonstrated the properties of a nanosized drug delivery system. Linear regression showed a good correlation ($r = 0.97$) between Indium-111 radioactivity and vinorelbine concentration in the plasma of rats administered the ^{111}In -VNB-liposome.

Conclusion: A significant positive correlation between the pharmacokinetics and biodistribution of ^{111}In radioactivity and vinorelbine in blood, spleen, and liver was found following administration of the ^{111}In -VNB-liposome. The liposome efficiently encapsulated both vinorelbine and Indium-111, and showed a similar concentration-radioactivity time profile, indicating the correlation between chemotherapy and radiotherapy could be identical in the liposomal formulation.

Keywords: Indium-111, vinorelbine, liposome, pharmacokinetics, radiochemotherapy

Introduction

Vinorelbine is a semisynthesized vinca alkaloid belonging to the Catharanthus alkaloid group.¹ Vinca alkaloids are potent anticancer agents that act by binding to tubulin and preventing tubulin assembly into microtubules, that can ultimately lead to mitotic inhibition and induction of apoptosis.² Vinorelbine has been approved as a treatment for various cancers, including metastatic breast cancer and nonsmall cell lung cancer.^{3,4} Vinorelbine is better tolerated than other vinca alkaloids because of a lower propensity for axonal microtubules to cause neurotoxicity.⁵ Many studies have put effort into maintaining the vinorelbine concentration surrounding tumor cells in order to improve the anticancer activity of vinorelbine.^{6,7} Liposomal encapsulation is practical for vinorelbine to extend its circulation time and to increase its accumulation in tumor tissue.

Correspondence: Tung-Hu Tsai
Institute of Traditional Medicine,
School of Medicine, National Yang-Ming
University, 155, Li-Nong Street Section 2,
Taipei 112, Taiwan
Email thtsai@ym.edu.tw

The liposome is a drug carrier system that may prolong drug retention in the blood circulation. Liposomes consist of phospholipid bilayers and have an aqueous cavity in the inner phase, which can be a stable shelter for pharmacologic agents, including chemotherapeutic drugs used in cancer therapy, antisense oligonucleotides used in gene therapy, peptides used in the treatment of infectious diseases, antigens that stimulate an immune response, and radiopharmaceuticals used for targeting diagnosis and therapy.^{8–11} The application of a liposomal drug delivery system in cancer therapy has many advantages, such as increasing drug stability in vivo, enhancing drug bioavailability, and targeting the site of action.^{12,13}

Radiolabeling technology has been utilized for drug development to evaluate the biodistribution and pharmacokinetics of investigational new drugs. In addition, radioisotopes can be used for diagnostic and therapeutic purposes.^{10,14,15} Nowadays, finding the optimal strategy for cancer therapy is still a challenge.¹⁶ Concurrent or sequential combination of chemotherapy and external beam radiotherapy is recognized as a standard therapeutic procedure for treating many cancers.^{17,18} The rationale for combining various therapeutic modalities is to expand the therapeutic index by synergistic drug effects and reducing the overlapping spectrum of side effects or toxicity.¹⁹ Radionuclide therapy integrated into anticancer drug-loaded nanocarrier delivery systems may provide a potential therapeutic strategy for cancers.²⁰

Previous reports on the encapsulation of Indium-111 into the vinorelbine liposome (¹¹¹In-VNB-liposome) and Rhenium-188 (¹⁸⁸Re-DXR liposome) have shown that combination therapy can be realized and provides better tumor-targeting therapeutic activity.^{21–28} There are still no reports correlating the pharmacokinetics of a radiolabeled tracer and vinorelbine concentration. In this investigation, both the pharmacokinetics and biodistribution of the vinorelbine liposome (VNB-liposome) and ¹¹¹In-VNB-liposome are discussed. Results from two experiments show a correlation between vinorelbine concentration and Indium-111 radioactivity.

Materials and methods

Materials

Methanol, ammonium formate, and formic acid were purchased from Merck (Darmstadt, Germany). Vincamine, heparin sodium, and 8-hydroxyquinoline (oxine) were obtained from Sigma-Aldrich (St Louis, MO). Aerrane (isoflurane) was purchased from Baxter (San Juan, Puerto Rico), and

vinorelbine was obtained from Orient Europharma (Taipei, Taiwan). The VNB-liposome (NanoVNB) was kindly provided by the Taiwan Liposome Company (Taipei, Taiwan). Deionized water (Millipore, Bedford, MA) was used throughout the entire experiment.

Preparation of VNB-liposome

Preparation of liposomes and the VNB-liposome has been previously described.^{21,29} Briefly, PEGylated liposomes were prepared from distearoyl phosphatidylcholine, cholesterol, and PEG-DSPE (molar ratio 3:2:0.045). Small unilamellar vesicles (100 nm in diameter) were produced by a combination of the standard thin-film hydration method, the freeze-thaw method, and repeated extrusion. The extraliposomal salt was removed by a Sephadex™ G-50 column (Bio-Rad, Hercules, CA) and elution with histidine-sucrose buffer (pH 6.0). Vinorelbine, an anticancer agent was encapsulated into nanoliposomes using a polyanionic gradient. After removing the extraliposomal salt using a Sephadex G-50 column, vinorelbine was added immediately to the solution at a concentration of 3.5 mg/10 μmol of phospholipid. The mixture of liposomes and vinorelbine was incubated in a water bath at 60°C for 30 minutes with agitation at 100 rpm. After loading, the liposomal vinorelbine was sterilized by 0.2 μm filtration and stored at 4°C–6°C before use. The mean particle size of the VNB-liposome and the concentration of vinorelbine in the VNB-liposome was 95.2 ± 4.9 nm and 2.08 mg/mL, respectively.

Preparation of ¹¹¹In-VNB-liposome

The method used to label VNB-encapsulated PEGylated liposomes with ¹¹¹In-oxine has been detailed in a previous report.²⁵ Briefly, ¹¹¹In-oxine residue was dissolved in 20 μL of ethanol, added to 80 μL of distilled water, and then incubated with 2 mL PEGylated VNB-liposomes for 30 minutes at 37°C. About 100 μL of reaction solution were loaded onto a column (40 × 8 mm, Bio-Rad) containing Sephadex G-50 fine gel and eluted with normal saline. The labeling efficiency was determined by dividing the radioactivity of the PEGylated VNB-liposome fractions after separation by total radioactivity before separation. The particle size of ¹¹¹In-VNB-liposome (after the radioactivity decay to background) was determined using an ultraviolet-visible spectrophotometer (V-530; Jasco, Tokyo, Japan). The radiochemical purity values for the ¹¹¹In-VNB-liposome were all greater than 90%. The average particle size of the ¹¹¹In-VNB-liposome was 102 ± 6.9 nm, which is similar to that of VNB-liposome (95.2 ± 4.9 nm).

Experimental animals

Male Sprague-Dawley rats (National Yang-Ming University Animal Center, Taipei, Taiwan), were housed on a 12-hour light and 12-hour dark cycle. Free access to food (Laboratory Rodent Diet 5001, PMI Feeds, Richmond, IN) and water was allowed at all times. All animal protocols were approved by the Institutional Animal Care and Use Committee at National Yang-Ming University (Taipei, Taiwan) and the Institute of Nuclear Energy Research (Taoyuan, Taiwan). The rats weighed 250 ± 10 g. The animals were separated into two groups, one for pharmacokinetic experiments and the other for biodistribution experiments.

For the pharmacokinetics, ten rats (five rats per group) were anesthetized with 1.5% isoflurane, and each rat was given the VNB-liposome (containing vinorelbine 0.3 mg/kg) or ^{111}In -VNB-liposome (vinorelbine 0.3 mg/kg, Indium-111 2.22 MBq per rat) through the tail vein. A 0.3 mL blood sample was collected as blank plasma prior to drug administration, and further blood samples were collected at 0.25, 1, 4, 24, 48, and 72 hours after drug administration. Following blood collection, radioactivity was measured using a Cobra II auto-gamma counter (1470 Wizard Gamma Counter; Wallac, Turku, Finland). The percentage of injected dose per mL (% ID/g) was calculated by comparison with standards representing the injected dose per animal. Plasma samples were stored at -20°C before analysis.

For the biodistribution study, 30 rats (three rats at each time point in each group) were anesthetized with 1.5% isoflurane, and each rat was given the VNB-liposome (containing vinorelbine 0.3 mg/kg) or ^{111}In -VNB-liposome (vinorelbine 0.3 mg/kg, Indium-111 2.22 MBq per rat) through the tail vein. At 1, 4, 24, 48, and 72 hours following injection, the rats were sacrificed by CO_2 asphyxiation and the liver and the spleen were collected, rinsed with normal saline, weighed, and the radioactivity was measured using the Cobra II auto-gamma counter. The %ID and the %ID/g were calculated by comparison with standards representing the injected dose per animal. The data were expressed as the mean \pm standard deviation. The organs were preserved at -20°C for further treatment and vinorelbine analysis.

Sample preparation

Protein precipitation was used to extract vinorelbine from the rat plasma samples. Briefly, 240 μL of methanol with 10 ng/mL of vincamine (as the internal standard) was added to 80 μL of collected plasma, and the mixture was vortexed for 10 minutes then centrifuged at 16,000 g and 4°C for 10 minutes. The supernatant was collected and dried using

a centrifugal vaporizer. The dried sample was reconstituted with 80 μL of 80% methanol, and filtered using a 0.2 μm filter (Millipore, Millex[®]-GV, Bedford, MA). The filtrate was analyzed using a liquid chromatography tandem mass spectrometry system. The standard samples were prepared in the same protein precipitation method by spiking stock solution (20 μL vinorelbine standard) in plasma (80 μL). The mixed sample was then added to 300 μL of methanol with 10 ng/mL vincamine and followed the previous procedure, but was reconstituted with 100 μL of 80% methanol. The final filtrate was also analyzed using a liquid chromatography tandem mass spectrometry system.

Liver and spleen samples were extracted using a solid-phase extraction cartridge (Oasis HLB, 1 mL, 10 mg). The organ samples were first homogenized (Polytron PT-MR 2100, Kinematica AG, Lucerne, Switzerland) with 50% methanol (5:1 v/w for spleen and 3:1 v/w for liver) at 20,000 rpm for 10 minutes. The supernatant was obtained by centrifugation at 10,000 rpm for 10 minutes. A 100 μL supernatant sample was mixed with 100 μL of vincamine (5 ng/mL) and 800 μL of 1% formic acid. The mixture was loaded into a solid-phase extraction cartridge, washed with 10 mM ammonium formate, 20% methanol in 10 mM ammonium formate, and eluted with 90% methanol in 10 mM ammonium formate. The collected elution was dried using a centrifugal vaporizer, reconstituted with 100 μL of 80% methanol, and filtered using a 0.2 μm filter (Millipore). The filtrate was analyzed using a liquid chromatography tandem mass spectrometry system. The standard samples were prepared by the solid-phase extraction method using a spiking stock solution (20 μL vinorelbine standard) in plasma (80 μL). The sample was mixed using the same solid-phase extraction preparation method. The final reconstituted sample was also analyzed using the liquid chromatography tandem mass spectrometry system.

Liquid chromatography-tandem mass spectrometry

The system consisted of a Waters 2690 Alliance LC with an automatic liquid chromatographic sampler and injector and a Micromass Quattro Ultima tandem quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with an electrospray ionization interface which was in acquired positive mode. Ultrapure argon was used as the collision gas, and high-pure nitrogen was used as cone gas. Multiple-reaction monitoring analysis was used for quantitation and the samples were quantified using peak area. The multiple-reaction monitoring transitions were m/z 779.2 to

m/z 122.0 for vinorelbine and m/z 355.2 to m/z 337.2 for vincamine, which was used as the internal standard. The electrospray ionization-tandem mass spectrometry parameters were set as follows: capillary voltage, 3.0 kV; source temperature, 110°C; desolvation temperature, 350°C; cone gas flow, 100 L/hour; and desolvation gas flow, 500 L/hour. To obtain optimal responses, the cone voltage was set at 30 V, and the collision energy was adjusted to 20 eV for vinorelbine and 45 eV for the internal standard, vincamine. MassLynx 3.5 (Micromass) software was used for data processing. A Phenomenex Luna C18 column (5 μ m and 50 \times 4.6 mm) maintained at an ambient temperature was used to separate the vinorelbine. The mobile phase consisted of 70% methanol and 30% 10 mM ammonia acetate with 0.8% formic acid, and the flow rate was 0.2 mL/minute. The injection sample volume was 5 μ L.

Method validation

Calibration curves were established using blank samples (plasma, liver, and spleen) spiked with different amounts of vinorelbine. Stock solution diluted with 50% methanol was used to form a series of concentrations from 25 to 2500 ng/mL. The concentration-response relationship for this method indicated linearity over a concentration range of 5–500 ng/mL, with a coefficient of determination (r^2) of at least 0.999. The intra-assay and interassay variabilities were determined by quantitating six replicates at concentrations of 5, 10, 20, 50, 100, 200, and 500 ng/mL on the same day and consecutive days, respectively. The limit of detection was defined as a signal-to-noise ratio of 3, the lower limit of quantitation was defined as 10, and the lowest concentration of the linear regression defined the limit of quantitation. The accuracy (bias%) was calculated from the mean value of observed concentration (C_{obs}) and the nominal concentration (C_{nom}) as follows: accuracy (%) = $[(C_{obs} - C_{nom})/C_{nom}] \times 100$. The relative standard deviation (RSD) was calculated from the observed concentrations as follows: precision (%) = $[\text{standard deviation (SD)}/C_{obs}] \times 100$. Accuracy and precision values within $\pm 20\%$ covering the actual range of experimental concentrations were considered acceptable.

Three sets of samples were prepared to evaluate the matrix effect and the recovery of the quantitative bioanalytical method:³⁰

- Neat sample – samples were prepared using a vinorelbine standard solution diluted with normal saline to the target concentrations of 5, 50, and 500 ng/mL.
- Postextraction fortification – samples were prepared by spiking appropriate concentrations of standard solutions

of vinorelbine to the postextracted blank plasma sample, with target concentrations of 5, 50, and 500 ng/mL.

- Pre-extraction fortification – samples were prepared by spiking appropriate concentrations of standard solutions of vinorelbine to the pre-extracted blank plasma sample with target concentrations of 5, 50, and 500 ng/mL. These samples were further processed by the protein precipitation methods before analysis. By comparing the peak areas of set 1 and set 2, the data allow determination of the matrix effect, which represents ion suppression or enhancement association. By comparing the peak areas of set 2 and set 3, the data enable determination of the recovery sample treatment procedure.

Pharmacokinetic parameters and statistical analysis

The pharmacokinetic parameters were calculated by WinNonlin (v 5.0.1; Pharsight Corporation, Mountain View, CA) and included half-life ($t_{1/2}$), maximum concentration (C_{max}), and area under the concentration-time curve (AUC). All data are presented as the mean \pm standard deviation or the mean \pm standard error of the mean. The unpaired t -test was used for group comparisons. Values of $P < 0.05$ were considered significant. Coefficient of correlation (r) was used to estimate the correlation between radioactivity of Indium-111 and vinorelbine concentrations in this study.

Results

Method validation

Selectivity was confirmed by a chromatogram of a blank sample and a blank sample spiked with vinorelbine. Under the given conditions, vinorelbine was eluted at a retention time of 2.7 minutes for the plasma sample and 2.2 minutes for liver and spleen samples, and there was no interference at the same retention time. Liquid chromatography tandem mass spectrometry images of vinorelbine in plasma, liver, and spleen are shown in Figure 1. Calibration curves for vinorelbine were shown to have linear regression in the concentration range of 5 to 500 ng/mL for plasma, liver, and spleen, and a coefficient of determination (r^2) > 0.999 for all curves, demonstrating good linear regression in the concentration range tested. Table 1 demonstrates that intraday and interday precisions (RSD%) were less than 13.5%, and accuracies (RE%) for intraday and interday assays were less than 16.0%. The signal-to-noise ratio of 3, defined as the limit of detection, was 0.25 ng/mL; that of 10, defined as the lower limit of quantification, was 0.83 ng/mL; and the lowest concentration of the linear regression, defined as the limit of

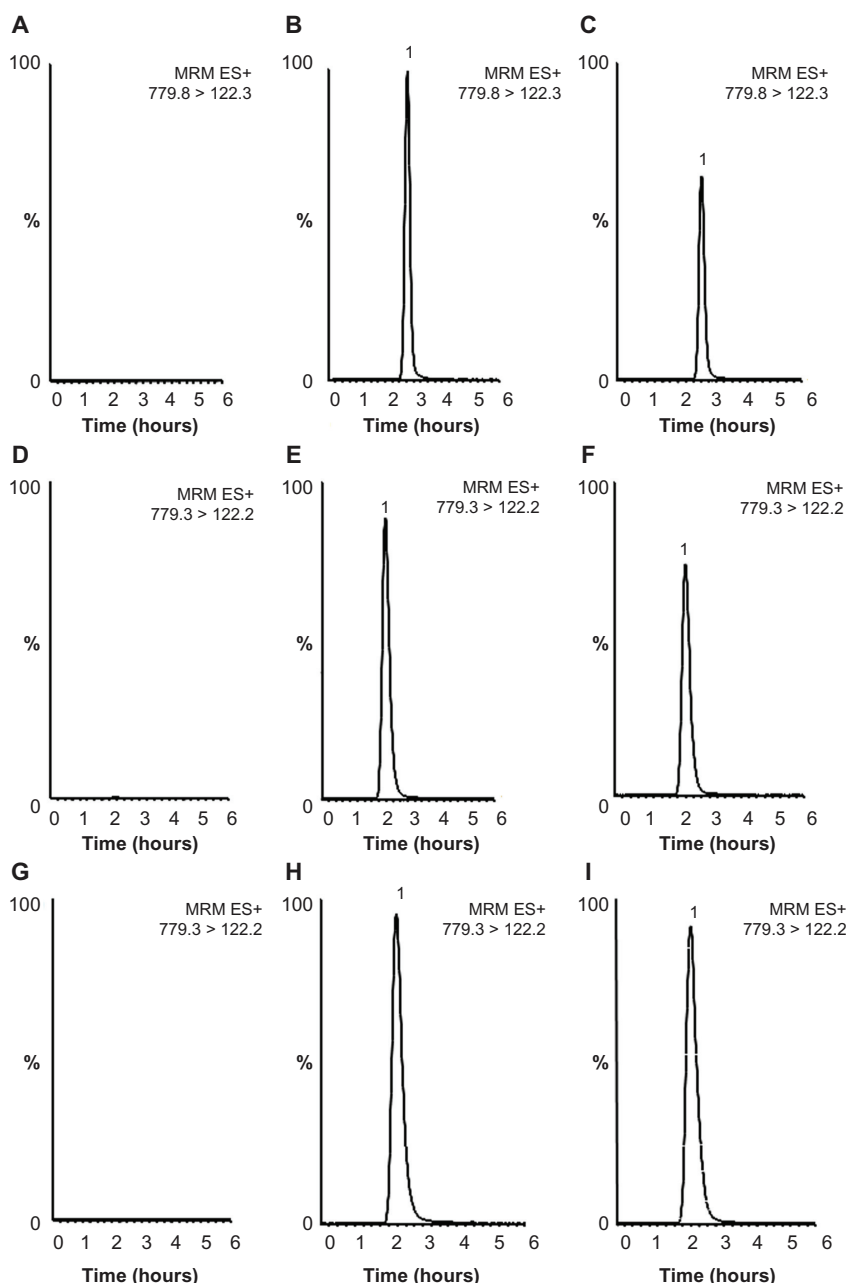


Figure 1 Typical liquid chromatography tandem mass spectrometry images of vinorelbine. (A) Blank plasma sample, (B) blank plasma spiked vinorelbine sample (200 ng/mL), (C) real plasma sample (156 ng/mL, $t = 24$ hours), (D) blank liver sample, (E) blank liver spiked vinorelbine sample (200 ng/mL), (F) real liver sample (141 ng/mL, $t = 4$ hours), (G) blank spleen sample, (H) blank spleen spiked vinorelbine sample (500 ng/mL), (I) real spleen sample (424 ng/mL, $t = 1$ hour).

Note: The maximal signal intensities for plasma, liver and spleen samples were $9.0 \times e5$, $1.3 \times e6$, and $8.5 \times e5$, respectively.

quantification, was 5 ng/mL. The matrix effect and recovery for determination of vinorelbine are summarized in Table 2, and these results show that the detection and extraction methods for these samples were reliable and acceptable.

Pharmacokinetics of VNB-liposome

The vinorelbine concentration-time profile in the rat is shown in Figure 2. It is clear that in rats administered 0.3 mg/kg of the VNB-liposome, the concentration of vinorelbine

gradually decreased over 24 hours, and the concentration of vinorelbine at the 24-hour sampling point was 204 ± 64 ng/mL. According to the time-concentration profile, the pharmacokinetic parameters were estimated as: $t_{1/2} = 4.8 \pm 1.2$ hours; $C_0 = 6.27 \pm 2.68$ $\mu\text{g/mL}$; $AUC_{0-24\text{h}} = 65.5 \pm 26.6$ $\text{h} \cdot \mu\text{g/mL}$. These data are listed in Table 3.

The concentration of vinorelbine in the liver and spleen versus time was also observed in this investigation, and the results are shown in Figure 3. The concentration of

Table 1 Intraday and interday assay for accuracy and precision for determination of vinorelbine

Intraday				Interday			
C _{nom} (ng/mL)	C _{obs} (ng/mL)	RSD (%) ^a	Bias (%) ^b	C _{nom} (ng/mL)	C _{obs} (ng/mL)	RSD (%) ^a	Bias (%) ^b
Plasma							
5	5.0 ± 0.6	12.0	0.0	5	5.8 ± 0.6	10.3	16.0
10	10.0 ± 0.2	2.0	0.0	10	10.9 ± 0.8	7.3	9.0
20	20.6 ± 0.2	1.0	3.0	20	21.2 ± 1.5	7.1	6.0
50	49.9 ± 1.3	2.6	-0.2	50	48.8 ± 0.0	0.0	-2.4
100	100.1 ± 0.7	0.7	0.1	100	97.9 ± 0.4	0.4	-2.1
200	199.8 ± 1.8	0.9	-0.1	200	196.7 ± 3.3	1.7	-1.7
500	500.7 ± 2.5	0.5	0.1	500	498.1 ± 5.1	1.0	-0.4
Liver							
5	5.2 ± 0.7	13.5	4.0	5	5.4 ± 0.1	1.9	8.0
10	10.2 ± 1.0	9.8	2.0	10	9.0 ± 0.8	8.9	-10.0
20	20.2 ± 1.0	5.0	1.0	20	20.8 ± 1.7	8.2	4.0
50	50.5 ± 0.4	0.8	1.0	50	48.0 ± 2.9	1.9	-4.0
100	99.9 ± 1.5	1.5	-0.1	100	101.1 ± 1.4	1.4	1.1
200	198.4 ± 2.2	1.1	-0.8	200	201.7 ± 2.7	1.3	0.8
500	500.6 ± 0.9	0.2	0.1	500	499.8 ± 0.6	0.1	0.0
Spleen							
5	5.8 ± 0.2	3.4	16.0	5	4.8 ± 0.6	12.5	-4.0
10	9.9 ± 0.6	6.1	-1.0	10	10.0 ± 0.5	5.0	0.0
20	19.3 ± 1.5	7.8	-3.5	20	19.1 ± 0.5	2.6	-4.5
50	50.7 ± 3.3	6.5	1.4	50	50.2 ± 1.0	2.0	0.4
100	101.4 ± 3.0	3.0	1.4	100	101.7 ± 6.9	6.8	1.7
200	197.3 ± 5.3	2.7	-1.3	200	199.4 ± 7.3	3.7	-0.3
500	501.1 ± 3.1	0.6	0.2	500	500.2 ± 3.4	0.7	0.0

Notes: Data are expressed as mean ± standard deviation (n = 3). ^aRSD (%) = [standard deviation/C_{obs}] × 100; ^bbias (%) = [(C_{obs} - C_{nom})/C_{nom}] × 100.

Abbreviation: RSD, relative standard deviation.

vinorelbine in these two organs could be maintained for at least 24 hours. The results also show that the concentration of vinorelbine in the spleen was significantly higher than that in the liver ($P < 0.05$). From the concentration-time profile of these two organs, two parameters could be obtained: $C_{\max} = 0.35 \mu\text{g/g}$, $\text{AUC}_{0-24 \text{ h}} = 5.26 \text{ h} \cdot \mu\text{g/g}$ and $C_{\max} = 2.47 \mu\text{g/g}$, $\text{AUC}_{0-24 \text{ h}} = 47.0 \text{ h} \cdot \mu\text{g/g}$ for the liver and spleen, respectively.

Pharmacokinetics of ¹¹¹In-VNB-liposome

The vinorelbine concentration-time profile of the rats administered ¹¹¹In-VNB-liposome (0.3 mg/kg vinorelbine, Indium-111 2.22 MBq/rat) was similar to that in rats administered 0.3 mg/kg VNB-liposome (Figure 2). After administration of the ¹¹¹In-VNB-liposome, the concentration of vinorelbine still reached $116 \pm 77 \text{ ng/mL}$ at 24 hours. From the time-concentration profile, the pharmacokinetic parameters were obtained: $t_{1/2} = 3.3 \pm 1.3 \text{ hours}$; $C_0 = 7.15 \pm 1.60 \mu\text{g/mL}$; $\text{AUC}_{0-24 \text{ h}} = 44.5 \pm 31.0 \text{ h} \cdot \mu\text{g/mL}$. These data are listed in Table 3. The radioactivity-time profile of the rats is demonstrated in Figure 2. Radioactivity could be detected in rat plasma 24 hours after administration of the ¹¹¹In-VNB-liposome,

which was $0.7610 \pm 0.3003 \text{ \%ID/mL}$, and based on the profile, the pharmacokinetic parameters obtained were $C_0 = 8.88 \pm 0.43 \text{ \%ID/mL}$ and $\text{AUC}_{0-24 \text{ h}} = 115.46 \pm 16.83 \text{ h} \cdot \text{\%ID/mL}$.

The concentration of vinorelbine in the liver and spleen versus time are shown in Figure 3. The results were similar to that of rats administered the VNB-liposome. From the concentration-time profile of these two organs, the pharmacokinetic parameters could be calculated as $C_{\max} = 0.19 \mu\text{g/g}$, $\text{AUC}_{0-24 \text{ h}} = 3.54 \text{ h} \cdot \mu\text{g/g}$ and $C_{\max} = 2.86 \mu\text{g/g}$, $\text{AUC}_{0-24 \text{ h}} = 57.5 \text{ h} \cdot \mu\text{g/g}$ for liver and spleen, respectively. The radioactivity could also be detected, and based on the profile, the pharmacokinetic parameters were calculated as: $C_{\max} = 4.8 \text{ \%ID/g}$, $\text{AUC}_{0-24 \text{ h}} = 87.7 \text{ h} \cdot \text{\%ID/g}$ and $C_{\max} = 21.5 \text{ \%ID/g}$, $\text{AUC}_{0-24 \text{ h}} = 418 \text{ h} \cdot \text{\%ID/g}$, respectively.

Radiolabeled tracer and vinorelbine concentration in ¹¹¹In-VNB-liposome

The radioactivity-time profile clearly matched the vinorelbine concentration-time profiles in ¹¹¹In-VNB-liposome experiments (Figure 2). This result showed that liposome encapsulated concurrently with Indium-111 and vinorelbine did not affect

Table 2 Matrix effect and recovery for the determination of vinorelbine

Concentration (ng/mL)	Matrix effect (%)	Recovery (%)
Plasma		
5	99.3 ± 13.7	95.3 ± 19.5
50	102.1 ± 7.2	103.9 ± 6.8
500	82.0 ± 4.6	90.9 ± 6.4
Average	94.4 ± 10.9	96.7 ± 6.6
Liver		
5	102.0 ± 2.2	85.4 ± 8.2
50	95.4 ± 3.4	84.3 ± 2.4
500	88.0 ± 2.6	79.2 ± 3.2
Average	95.2 ± 7.0	82.9 ± 3.3
Spleen		
5	93.0 ± 5.3	69.0 ± 2.6
50	97.2 ± 2.8	72.4 ± 2.0
500	94.5 ± 4.4	80.0 ± 3.7
Average	94.9 ± 2.1	73.8 ± 5.6

Notes: Data are expressed as mean ± standard deviation (n = 3). Set 1 = neat sample (vinorelbine prepared in normal saline); set 2 = postextraction fortification; set 3 = pre-extraction fortification; matrix effect (%) = (mean area of set 2/mean area of set 1) × 100; Recovery (%) = (mean area of set 3/mean area of set 2) × 100.

blood pharmacokinetic parameters of vinorelbine, which was evidenced by the similar vinorelbine concentration-time profiles between the ¹¹¹In-VNB-liposome group and VNB-liposome group. From the result of vinorelbine concentration as a function of radioactivity profiles (Figure 4), the linear regression between radioactivity and vinorelbine concentration in rats administered with ¹¹¹In-VNB-liposome was $y = 725.98x - 688.13$, where $r = 0.97$, showing that the radioactivity and vinorelbine concentration was in good correlation.

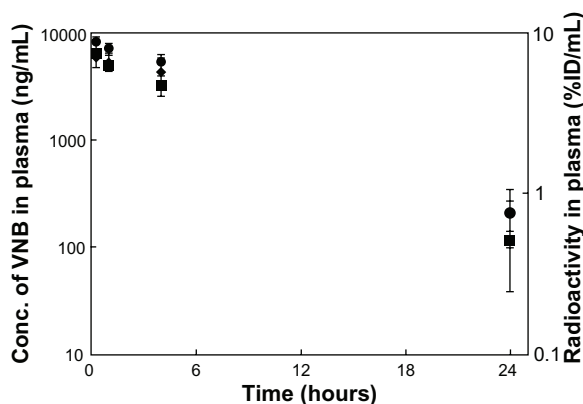


Figure 2 Vinorelbine concentration-time curve and radioactivity-time curve in plasma. ♦ Vinorelbine concentration of the VNB-liposome (vinorelbine 0.3 mg/kg) intravenous administration group, ■ vinorelbine concentration of ¹¹¹In-VNB-liposome (vinorelbine 0.3 mg/kg, Indium-111 2.22 MBq/rat) intravenous administration group, and ▲ radioactivity of ¹¹¹In-VNB-liposome (vinorelbine 0.3 mg/kg, Indium-111 2.22 MBq/rat) intravenous administration group.

Note: Data are expressed as the mean ± standard error of the mean (n = 5 for each group).

Abbreviation: VNB, vinorelbine.

Table 3 Pharmacokinetic parameters of vinorelbine in plasma of rats administered with VNB-liposome (0.3 mg/kg VNB) and ¹¹¹In-VNB-liposome (0.3 mg/kg vinorelbine, 2.22 MBq/rat)

	VNB-liposome	¹¹¹ In-VNB-liposome
Blood		
t _{1/2} (hours)	4.8 ± 1.2	3.3 ± 1.3
C ₀ (μg/mL)	6.27 ± 2.68	7.15 ± 1.60
AUC _{0-24h} (h * μg/mL)	65.5 ± 26.6	44.5 ± 31.0
Liver		
C _{max} (μg/g)	0.35	0.19
AUC _{0-24h} (h * μg/g)	5.26	3.54
Spleen		
C _{max} (μg/g)	2.47	2.86
AUC _{0-24h} (h * μg/g)	47.0	57.5

Notes: Data are expressed as the mean ± standard deviation. Pharmacokinetic parameters: t_{1/2}, half life; C₀, extrapolated blood concentration at time 0; AUC_{0-24h}, area under concentration-time curve; C_{max}, maximum drug concentration. Blood sample: n = 5; liver and spleen: n = 3 for each sampling time (1, 4, and 24 hours). Mean concentrations at 1, 4, and 24 hours constructed the time-concentration profile of liver and spleen to obtain C_{max} and AUC_{0-24h}.

Abbreviation: VNB, vinorelbine.

Discussion

The characteristics of the ¹¹¹In-VNB-liposome including pharmacokinetics, biodistribution, histology, and molecular imaging, have been reported.²¹⁻²⁵ In addition to these pharmacological studies, ¹¹¹In-VNB-liposomal combination therapy can provide better tumor-targeting therapeutic efficacy in C26 colorectal carcinoma-bearing mouse models.^{21,22,24} In this

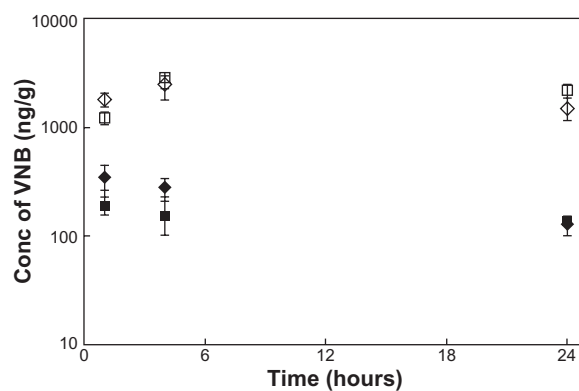


Figure 3 VNB concentration-time curve and radioactivity-time curve in liver and spleen. ♦ Vinorelbine concentration of liver in the group of VNB-liposome (vinorelbine 0.3 mg/kg) intravenous administration; ◊ Vinorelbine concentration of spleen in the group of VNB-liposome (vinorelbine 0.3 mg/kg) intravenous administration; ■ vinorelbine concentration of liver in the group of ¹¹¹In-VNB-liposome (vinorelbine 0.3 mg/kg, 2.22 MBq/rat) intravenous administration; □ vinorelbine concentration of spleen in the group of ¹¹¹In-VNB-liposome (vinorelbine 0.3 mg/kg, 2.22 MBq/rat) intravenous administration; ● radioactivity of liver in the group of ¹¹¹In-VNB-liposome (vinorelbine 0.3 mg/kg, 2.22 MBq/rat) intravenous administration; ○ radioactivity of spleen in the group of ¹¹¹In-VNB-liposome (vinorelbine 0.3 mg/kg, 2.22 MBq/rat) intravenous administration.

Note: Data are expressed as mean ± standard error of the mean (n = 3 for each time point per group).

Abbreviation: VNB, vinorelbine.

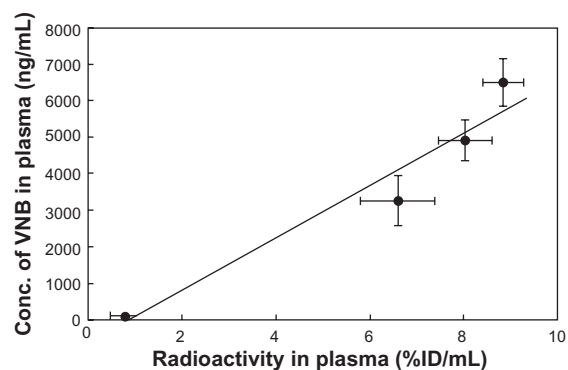


Figure 4 Correlation of pharmacokinetics between radiolabeled tracer and vinorelbine concentration in ^{111}In -VNB-liposome (vinorelbine 0.3 mg/kg, 2.22 MBq/rat) post intravenous administration.

Note: Data are expressed as mean \pm standard error of the mean ($n = 5$ for each time point per group).

Abbreviation: VNB, vinorelbine.

study, we have further shown a correlation between radioactivity and chemotherapeutics of the ^{111}In -VNB-liposome in the pharmacokinetics and biodistribution in the rat. The improved kinetic properties of vinorelbine attributable to the liposomal carrier were evidenced by an extended circulation time in rat blood and increased accumulation in the reticuloendothelial system (ie, liver and spleen). The concentration of vinorelbine rapidly decreased from approximately 7.5 $\mu\text{g}/\text{mL}$ to 0.2 $\mu\text{g}/\text{mL}$ within 4 hours of intravenous administration of vinorelbine 20 mg/kg in mice.³¹ Different formulations, such as phosphatidylserine liposome and lipid microspheres, have been designed to improve the pharmacokinetics of vinorelbine and overcome the problem of its rapid elimination from the circulation.³² Our liposomal vinorelbine formulation had improved in vivo pharmacokinetic properties, achieving the highest concentration at 24 hours (204 ± 64 ng/mL) with approximately one thirtieth of the dose administered (0.3 mg/kg, Figure 2).

Liposomal drug delivery systems have been studied extensively as a method to increase the therapeutic index of chemotherapy.^{33,34} Our previous work has demonstrated that liposomal doxorubicin has a better ability to penetrate the blood-brain barrier.²⁹ Nanoliposomes, which are double-membrane lipid vesicles with a particle size from 10 nm to 100 nm, are important carriers capable of packaging drugs in various drug delivery applications via the enhanced permeability and retention effect at leaky tumor sites. The reticuloendothelial system is the main turnover organ for liposomal drug delivery.^{35,36} Several investigations have indicated high uptake of liposome drug delivery systems in reticuloendothelial organs such as the liver and spleen.^{24,37} Our previous study has demonstrated that reticuloendothelial

system-rich organs, ie, the liver and spleen, are the major sites of uptake of liposomal vinorelbine, which is evidenced by calculating the accumulation of radioactivity.²⁴ In this work, the average concentrations of vinorelbine in blood, liver, and spleen were 204 ± 64 ng/mL, 126 ± 26 ng/g, and 1499 ± 345 ng/g, respectively, at 24 hours after administration of liposomal vinorelbine 0.3 mg/kg (Figure 3). The concentration of vinorelbine in the spleen was 7.3-fold higher than the concentration in blood.

A significant positive correlation between doxorubicin concentration (ng/mg) and $^{99\text{m}}\text{Tc}$ radiotracer (%ID/g) in tumor tissue has been reported previously.³⁸ The combined therapeutic efficacy of the ^{111}In -VNB-liposome has been evaluated in colorectal carcinoma-bearing mice.^{22,23} Indium-111 is a radionuclide commonly used for scintigraphic imaging ($t_{1/2}$ 2.81 days, 172 and 247 keV photon emission), emitting 14.7 Auger electrons (mean energy 0.46 keV) on average per decay, and may also be suitable for radiotherapy.³⁹ Our current study found a good pharmacokinetic correlation between radioactivity and vinorelbine concentration in rats administered the ^{111}In -VNB-liposome. A significant positive correlation ($r = 0.97$) between vinorelbine concentration (ng/mL) and the Indium-111 radiotracer (%ID/g) in plasma was observed (Figure 4). Our work demonstrates that the biodistribution of radiolabeled tracer correlates well with chemotherapeutic vinorelbine, which can be used to estimate the distribution profile of vinorelbine in a liposomal formulation (^{111}In -VNB-liposome) and for drug uptake in vivo. However, characterization of the vinorelbine versus tracer uptake relationship still needs further validation.

In conclusion, this investigation identified a valid and reliable liquid chromatography tandem mass spectrometry method to determine the concentration of vinorelbine in rat plasma, liver, and spleen after administration of the VNB-liposome and ^{111}In -VNB-liposome in rats. We found that the nanoliposomal formulation improved the kinetic properties of vinorelbine and prolonged the circulation time in rat blood. Vinorelbine in an Indium-111-encapsulated liposome exhibits a correlated concentration/radioactivity-time profile, providing evidence that the strategy of combinatorial chemoradiotherapy is practical in vivo.

Acknowledgments

Funding for this study was provided in part by research grants (NSC99-2113-M-010-001-MY3, and NSC99-2628-B-010-008-MY3) from the National Science Council, Taiwan, grant TCH 10001-62-007 from Taipei City Hospital, Taiwan, and 982001INER067 from the Institute of Nuclear

Energy Research, Taiwan. We also thank Yun-Long Tseng from the Taiwan Liposome Company for preparation of the VNB-liposome.

Disclosure

The authors report no conflicts of interest in this work.

References

- Potier P. The synthesis of navelbine prototype of a new series of vinblastine derivatives. *Semin Oncol*. 1989;16(2 Suppl 4):2-4.
- Jordan MA, Thrower D, Wilson L. Mechanism of inhibition of cell proliferation by vinca alkaloids. *Cancer Res*. 1991;51(8):2212-2222.
- Weber BL, Vogel C, Jones S, et al. Intravenous vinorelbine as first-line and second-line therapy in advanced breast cancer. *J Clin Oncol*. 1995;13(11):2722-2730.
- Gridelli C, De Vivo R. Vinorelbine in the treatment of non-small cell lung cancer. *Curr Med Chem*. 2002;9(8):879-891.
- Drummond DC, Noble CO, Guo Z, et al. Improved pharmacokinetics and efficacy of a highly stable nanoliposomal vinorelbine. *J Pharmacol Exp Ther*. 2009;328(1):321-330.
- Baweja M, Suman VJ, Fitch TR, et al. Phase II trial of oral vinorelbine for the treatment of metastatic breast cancer in patients > or = 65 years of age: an NCCTG study. *Ann Oncol*. 2006;17(4):623-629.
- Okouneva T, Hill BT, Wilson L, Jordan MA. The effects of vinflunine, vinorelbine, and vinblastine on centromere dynamics. *Mol Cancer Ther*. 2003;2(5):427-436.
- Allen TM, Cullis PR. Drug delivery systems: entering the mainstream. *Science*. 2004;303(5665):1818-1822.
- Phillips WT, Goins B, Bao A. Radioactive liposomes. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*. 2009;1(1):69-83.
- Ting G, Chang CH, Wang HE, Lee TW. Nanotargeted radionuclides for cancer nuclear imaging and internal radiotherapy. *J Biomed Biotechnol*. 2010;953537.
- Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. *Nat Rev*. 2005;4(2):145-160.
- Allen TM, Brandeis E, Hansen CB, Kao GY, Zalipsky S. A new strategy for attachment of antibodies to sterically stabilized liposomes resulting in efficient targeting to cancer cells. *Biochim Biophys Acta*. 1995;1237(2):99-108.
- Drummond DC, Meyer O, Hong K, Kirpotin DB, Papahadjopoulos D. Optimizing liposomes for delivery of chemotherapeutic agents to solid tumors. *Pharmacol Rev*. 1999;51(4):691-743.
- Hamoudeh M, Kamleh MA, Diab R, Fessi H. Radionuclide delivery systems for nuclear imaging and radiotherapy of cancer. *Adv Drug Deliv Rev*. 2008;60(12):1329-1346.
- Ting G, Chang CH, Wang HE. Cancer nanotargeted radiopharmaceuticals for tumor imaging and therapy. *Anticancer Res*. 2009;29(10):4107-4118.
- Dancey JE, Chen HX. Strategies for optimizing combinations of molecularly targeted anticancer agents. *Nat Rev*. 2006;5(8):649-659.
- Choy H, Kim DW. Chemotherapy and irradiation interaction. *Semin Oncol*. 2003;30(4 Suppl 9):3-10.
- Jain RK. Normalizing tumor vasculature with anti-angiogenic therapy: a new paradigm for combination therapy. *Nat Med*. 2001;7(9):987-989.
- Huber PE, Bischof M, Jenne J, et al. Trimodal cancer treatment: beneficial effects of combined antiangiogenesis, radiation, and chemotherapy. *Cancer Res*. 2005;65(9):3643-3655.
- Wong JY. Systemic targeted radionuclide therapy: potential new areas. *Int J Radiat Oncol Biol Phys*. 2006;66 Suppl 2:S74-S82.
- Chow TH, Lin YY, Hwang JJ, et al. Therapeutic efficacy evaluation of ¹¹¹In-labeled PEGylated liposomal vinorelbine in murine colon carcinoma with multimodalities of molecular imaging. *J Nucl Med*. 2009;50(12):2073-2081.
- Chow TH, Lin YY, Hwang JJ, et al. Diagnostic and therapeutic evaluation of ¹¹¹In-vinorelbine-liposomes in a human colorectal carcinoma HT-29/luc-bearing animal model. *Nucl Med Biol*. 2008;35(5):623-634.
- Chow TH, Lin YY, Hwang JJ, et al. Improvement of biodistribution and therapeutic index via increase of polyethylene glycol on drug-carrying liposomes in an HT-29/luc xenografted mouse model. *Anticancer Res*. 2009;29(6):2111-2120.
- Lee WC, Hwang JJ, Tseng YL, et al. Therapeutic efficacy evaluation of ¹¹¹In-VNB-liposome on human colorectal adenocarcinoma HT-29/luc mouse xenografts. *Nucl Instrum Meth A*. 2006;569:497-504.
- Lin YY, Li JJ, Chang CH, et al. Evaluation of pharmacokinetics of ¹¹¹In-labeled VNB-PEGylated liposomes after intraperitoneal and intravenous administration in a tumor/ascites mouse model. *Cancer Biother Radiopharms*. 2009;24(4):453-460.
- Chang YJ, Chang CH, Yu CY, et al. Therapeutic efficacy and microSPECT/CT imaging of ¹⁸⁸Re-DXR-liposome in a C26 murine colon carcinoma solid tumor model. *Nucl Med Biol*. 2010;37(1):95-104.
- Chen LC, Chang CH, Yu CY, et al. Pharmacokinetics, micro-SPECT/CT imaging and therapeutic efficacy of ¹⁸⁸Re-DXR-liposome in C26 colon carcinoma ascites mice model. *Nucl Med Biol*. 2008;35(8):883-893.
- Chen MH, Chang CH, Chang YJ, et al. MicroSPECT/CT imaging and pharmacokinetics of ¹⁸⁸Re-(DXR)-liposome in human colorectal adenocarcinoma-bearing mice. *Anticancer Res*. 2010;30(1):65-72.
- Hsieh YJ, Chang CH, Huang SP, et al. Effect of cyclosporin A on the brain regional distribution of doxorubicin in rats. *Int J Pharm*. 2008;350(1-2):265-271.
- Wu YT, Huang CM, Lin CC, et al. Determination of melamine in rat plasma, liver, kidney, spleen, bladder and brain by liquid chromatography-tandem mass spectrometry. *J Chromatogr A*. 2009;1216(44):7595-7601.
- Semple SC, Leone R, Wang J, et al. Optimization and characterization of a sphingomyelin/cholesterol liposome formulation of vinorelbine with promising antitumor activity. *J Pharm Sci*. 2005;94(5):1024-1038.
- Webb MS, Johnstone S, Morris TJ, et al. In vitro and in vivo characterization of a combination chemotherapy formulation consisting of vinorelbine and phosphatidylserine. *Eur J Pharm Biopharm*. 2007;65(3):289-299.
- Hong RL, Huang CJ, Tseng YL, et al. Direct comparison of liposomal doxorubicin with or without polyethylene glycol coating in C-26 tumor-bearing mice: is surface coating with polyethylene glycol beneficial? *Clin Cancer Res*. 1999;5(11):3645-3652.
- Kobayashi S, Sakai T, Dalrymple PD, Wood SG, Chasseaud LF. Disposition of the novel anticancer agent vinorelbine ditartrate following intravenous administration in mice, rats and dogs. *Arzneimittelforschung*. 1993;43(12):1367-1377.
- Choice E, Masin D, Bally MB, Meloche M, Madden TD. Liposomal cyclosporine. Comparison of drug and lipid carrier pharmacokinetics and biodistribution. *Transplantation*. 1995;60(9):1006-1011.
- Gabizon A, Isacson R, Libson E, et al. Clinical studies of liposome-encapsulated doxorubicin. *Acta Oncol*. 1994;33(7):779-786.
- Gabizon A, Shmeeda H, Barenholz Y. Pharmacokinetics of pegylated liposomal doxorubicin: review of animal and human studies. *Clin Pharmacokinet*. 2003;42(5):419-436.
- Kleiter MM, Yu D, Mohammadian LA, et al. A tracer dose of technetium-99 m-labeled liposomes can estimate the effect of hyperthermia on intratumoral doxil extravasation. *Clin Cancer Res*. 2006;12(22):6800-6807.
- Kereiakes JG, Rao DV. Auger electron dosimetry: report of AAPM Nuclear Medicine Committee Task Group No. 6. *Med Phys*. 1992;19(6):1359.

International Journal of Nanomedicine**Dovepress****Publish your work in this journal**

The International Journal of Nanomedicine is an international, peer-reviewed journal focusing on the application of nanotechnology in diagnostics, therapeutics, and drug delivery systems throughout the biomedical field. This journal is indexed on PubMed Central, MedLine, CAS, SciSearch®, Current Contents®/Clinical Medicine,

Journal Citation Reports/Science Edition, EMBase, Scopus and the Elsevier Bibliographic databases. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/international-journal-of-nanomedicine-journal>