Original Article

Pharmacokinetics and Tissue Distribution of PLGA-PLL-PEG-TF Nanoparticles Loaded with Daunorubicin and Tetrandrine Following Intravenous Injection in the Rats Using LC-MS/MS

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ABSTRACT

Objective: A sensitive, rapid and reliable LC-MS/MS method was developed for simultaneously determination of daunorubicin (DNR) and tetrandrine (Tet) in rat plasma and tissues. **Methods:** The pharmacokinetics and tissue distribution of DNR/Tet-PLGA-PLL-PEG-NPs-Tf (D/T-PPP-NPs-Tf), DNR/Tet-PLGA-PLL-PEG-NPs (D/T-PPP-NPs) and DNR/Tet (D/T) solution were compared following intravenous injection in the rats. **Result:** Compare to D/T solution, the parameters of DNR and Tet in D/T-PPP-NPs-Tf group presented significantly higher AUC ($p<0.01$) and lower V ($p<0.05$). Compared with D/T solution, the distribution of DNR in groups of D/T-PPP-NPs and D/T-PPP-NPs-Tf were higher in liver ($p<0.01$), lung ($p<0.05$), fat ($p<0.05$) and testicle ($p<0.05$), and were lower in heart, spleen, kidney ($p<0.01$), brain and intestine ($p<0.01$). The distribution of Tet in D/T-PPP-NPs and D/T-PPP-NPs-Tf groups were higher in liver, brain and uterus ($p<0.05$), and were lower in heart, spleen, lung, kidney and fat ($p<0.05$) compared with D/T solution. **Conclusion:** The LC-MS/MS method was specific, sensitive, rapid and reliable for the simultaneously determination of DNR and Tet in rat plasma and tissues. Compared with D/T solution, D/T-PPP-NPs-Tf tended to stay in the blood after injection. The distribution of D/T-PPP-NPs and D/T-PPP-NPs-Tf were significantly changed.

Key words: Nanoparticles, Daunorubicin, Tetrandrine, Pharmacokinetics, Tissue Distribution.

INTRODUCTION

Daunorubicin has been proved to be an effective anti-tumor antibiotic, which has been widely used in clinic for the treatment of leukemia.[1] However the clinical application of DNR has been highly restricted by MDR (multidrug resistance) and cumulative cardiotoxicity which is one of its serious side effects.[2] Tetrandrine (Tet) is one kind of bisbenzylisoquinoline alkaloid which has nonselective calcium channel block effect and has shown strong MDR reverse effect.[3-5] In order to increase the therapeutic effect and decrease the toxicity of DNR, a Nano drug delivery system encapsulated DNR and Tet simultaneously was designed in our previous studies.[1-10] The Nano drug delivery system comprised of a multi-polymer composing PLGA, PLL and PEG which has been widely studied recently.[1-12] The DNR and Tet were loaded with a double-emulsion solvent evaporation method.[6] Then Transferrin (Tf) was conjuncted into the surface of the nanoparticles in attempt to get a better targeting antitumor effect. The results of viability tests showed that D/T-PPP-NPs-Tf presented stronger cytotoxicity on K562/ADR cells compared with D/T solution. D/T-PPP-NPs-Tf also showed stronger anticancer effects on nude mice xenografts K562/A02 cells after injection

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compared with D/T solution.[6,7] Based on our previous work, Tf could significantly increase the drug concentration at the tumor tissue compared with D/T-PPP-NPs group, which proved the active targeting antitumor effect of Tf.[7]

The multi-polymer system of PLGA-PLL-PEG is quite new. There was no pharmacokinetic study about it. In this study, we want to investigate the pharmacokinetic characters of D/T-PPP-NPs-Tf in rats. In attempt to study the pharmacokinetics and tissue distribution of DNR and Tet in rats, a sensitive and reliable liquid chromatography with tandem mass spectrometry method (LC-MS/MS) was developed and validated for the quantification of DNR and Tet in rat plasma or tissues.[13-16]

MATERIALS AND METHODS

Materials

Poly (lactic-co-glycolic acid) (PLGA; 50:50 lactic acid: glycolic acid; molecular weight 30 kDa) with carboxylic acid ends of was purchased from Evonik Industries (Frankfurt, Germany). Polyethylene glycol (PEG, Molecular Weight (MW) 4 kDa), dicyclohexyl carbodiimide (DCC), anhydrous dimethylformamide (DMF), N, N-carbonyldiimidazole (CDI) and 4-dimethylaminopyridine (DMAP) were purchased from the Aladdin Company (Shanghai, China). Nε-(Z)-L-lysine-N-carboxyanhydride (NCA) was purchased from the Hanhong Company (Shanghai, China). Daunorubicin(DNR), Doxrubicin(IS) and tetrandrine(Tet) were purchased from Jinan Huifengda Chemical Co., Ltd. (Jinan, China). Transferrin (Tf) was purchased from Sigma-Aldrich Co. (St Louis, MO, USA); HPLC grade acetonitrile was purchased from Wanqing Chemical Reagent Co., Ltd. (Nanjing, China). All other chemicals and reagents were of analytical grade. Sprague Dawley (SD) rats weighing 190–250 g, half male and half female, were obtained from Shanghai Jiesijie experimental animal Co. Ltd. The rats were acclimatized for ten days before experimentation, which were fed with a standard diet. All animal experiments were approved by the Animal Ethics Committee of Nanjing Tech University.

Preparation

ofDNR/Tet-PLGA-PLL-PEG-NPs-Tf(D/T-PPP-NPs-Tf) and DNR/Tet-PLGA-PLL-PEG-NPs(D/T-PPP-NPs)

D/T-PPP-NPs-Tf were prepared using the same procedure which had been reported in our previous study.[1-6-8] The multipolymer comprising of PLGA, PLL and PEG (P-P-P) were synthesized firstly. Then DNR and Tet were loaded with a modified double-emulsion solvent evaporation method (D/T-PPP-NPs). Transferrin (Tf) was conjuncted into the surface of the nanoparticles (D/T-PPP-NPs-Tf) in attempt to get a better targeting antitumor effect. The samples were finally freeze-dried and stored at 4 °C.

The average diameter, zeta potential and polydispersity index (PDI) of the D/T-PPP-NPs-Tf were evaluated using a Zetasizer 3000HS system (Malvern Instruments, UK). The amounts of DNR and Tet encapsulated in the NPs were determined by the LC-20ATvp HPLC (Shimadzu Corporation). Then the entrapment efficiency (EE) and drug-loading efficiency (DL) of DNR/Tet were calculated using methods which we have previously described.[1,2,-6,7]

Analytical methods

A Shimadzu HPLC system was used which equipped with a LC-20AD binary pump, a SIL-20AC auto sampler and a CTO-20AC column oven (Shimadzu Corporation, Kyoto, Japan). The Liquid chromatography was performed on a BDS HYPERSIL-C8 analytical column (100 mm×2.1 mm, particle size 3 μm, Thermo Co., USA) at 40 ℃. The mobile phase consisted of acetonitrile and water containing 0.04% formic acid and 5 mmoL•L-1 ammonium acetate (46:54, v/v). The flow rate was 0.2 mL/min. The detection was performed using an API 4000 Qtrap MS=MS system (Applied Biosystems, Foster City, CA, USA) with a Turbo Ion Spray inlet. The mass spectrometer was operated in the positive ionization mode at an ion source heater temperature of 450°C. The multiple reaction monitoring (MRM) was used to monitor the transitions of the protonated molecular ions at mass-to-charge ratio (m/z): 528→363.1 for DNR, 623.4→381.1 for Tet, and 544.3→397.3 for DOX. The optimized ion spray voltage was 5500 V. Gas 1, gas 2 and curtain gas (nitrogen) were set at 50, 50, 10 psi, respectively. The declustering potential (DP) and collision energy (CE) were 60 V and 20 Ev for DNR, 120 V and 59 Ev for Tet, 68 V and 17 Ev for DOX, respectively. The Chromatographic analysis using LC-MS/MS method was validated for the quantification of DNR and Tet in biological matrix.[16-21]

Specificity

The specificity of the LC–MS / MS method was assessed by comparing chromatograms of six different source blank biological samples with the corresponding spiked biological samples (plasmas or tissue homogenates) at LLOQ (Lowest Limit of Quantitation) level.

Linearity and LLOQ

The calibration curve was prepared by plotting the peak-area ratios of DNR or Tet to the IS (y-axis) versus

the nominal concentration of DNR or Tet (x-axis) using a weighted linear regression. The LLOQ was defined as the lowest concentration of DNR or Tet in the standard curve with the signal-to-noise ratio was 10:1. The values could be detected with an acceptable precision and accuracy which were less than 20%.

Accuracy and precision

The intra-day accuracy and precision were evaluated by analyzing the low, media and high Quality Control (QC) samples of biological samples in the same day. The inter day accuracy and precision were determined from the same samples in three consecutive days. The criteria for acceptability of the data included accuracy within ±15% relative error (R.E.) from the nominal values and a precision of within ±15% relative standard deviation (R.S.D.), except for LLOQ, whose accuracy and precision both within ±20%. The low, media and high concentrations for accuracy and precision assay were 4, 40, 400 ng·mL^{-1} for DNR and 0.8, 20, 300 ng·mL^{-1} for Tet.

Recovery and matrix effect

The extraction recovery (ER) of DNR, Tet and DOX in biological samples at three concentrations were determined as the peak area ratios of the biological sample matrix spiked after extraction with a standard solution to the blank matrix spiked with the same standard solution. The matrix effect (ME) of extraction on DNR, Tet and DOX analysis was calculated by comparing the peak areas of extracted blank samples spiked with DNR, Tet and DOX at three concentrations with those of DNR, Tet and DOX spiked in mobile phase at the equivalent concentration. The three concentrations for ER and ME assay were 4, 40, 400 ng·mL^{-1} for DNR and 0.8, 20, 300 ng·mL^{-1} for Tet, while the single concentration of DOX was 100 ng·mL^{-1}.

Stability

The stability of DNR, Tet in rat plasma or tissue samples was determined using low, media and high QC samples which stored at room temperature for 24 h, at 4 °C in auto sampler for 24 h, after three-thaw cycle and stored at -80 °C for 30 days.

Pharmacokinetics study

DNR and Tet were dissolved in mixture solution which consisted of Etoh, PEG400, Tween 80 and saline (5:5:2:88, v/v). Freeze-dried D/T-PPP-NPs and D/T-PPP-NPs-Tf were dissolved in saline. Rats received i.v. injection of D/T-PPP-NPs , D/T-PPP-NPs-Tf or D/T solution (DNR 3 mg/kg, Tet 2.5 mg/kg) in groups(n = 6) via the tail vein after an overnight fast (12 h) with free access to water. At predetermined time (5, 15, 30 min and 1, 2, 4, 6, 8, 12, 24, 48, 72 h), the rats were anaesthetized with diethyl ether. The blood samples were collected from the retro-orbital sinus of rats, and then were transferred into heparinized (10 µl, 500 IU·mL^{-1}) tubes and centrifuged at 3000× g for 10 min. The isolated plasma was stored at −80 ℃ for further analysis.

To determine the DNR and Tet concentration of blood samples, 10µl doxorubicin (100 ng·mL^{-1}) was added into 100 µl plasma. Then the samples were vortexed for 30 s (VORTEX-5 vortex mixer, QILINBEIER, China). Then 1 mL diethyl ether was added followed by vortexing for another 5 min. After centrifugation (Sigma Eppendorf Centrifuge 5810R, US) at 5000 rpm for 10 min, 800 µl of the supernatant was removed to clean test tubes and evaporated to dryness under vacuum (Speedvac concentrator, Thermo,USA) for 50 min. The Residue was reconstituted in 100 µl of mobile phase, and 8 µl aliquots were injected into an analytical column for analysis.

Tissue distribution study

DNR and Tet in tissue samples were determined after i.v. injection with D/T-PPP-NPs, D/T-PPP-NPs-Tf or D/T solution (DNR 3 mg/kg, Tet 2.5 mg/kg) in groups(n = 20) via the tail vein after an overnight fast (12 h) with free access to water. At predetermined time points (5 min and 1, 8, 24, 60 h), four rats from each group were euthanized by cervical dislocation after blood collecting from the femoral artery. Then 0.2 g of selected organs such as liver, kidney, spleen, heart, lung, brain, muscle, fat, intestine and gonad (testis or ovary, uterus) were collected, washed, weighed and stored at −80 ℃ for further analysis. All tissues samples were homogenized (Mixer Mill MM 400, Retsch, Germany) in 2 mL of saline, and then prepared using the method described above.

Statistical analysis

Pharmacokinetic analysis was estimated by DAS Version 2.1.1Software (Mathematical Pharmacology Professional Committee of China, Shanghai, China) using non-compartmental methods. The area under the plasma concentration time profiles (AUC), the mean residence time (MRT), total plasma clearance (CL), the elimination ($t_{1/2\beta}$) and distribution ($t_{1/2\alpha}$) half-life were calculated. Statistical significance on differences of pharmacokinetic parameters or tissue distribution samples was analyzed using SPSS (version 11.0 Student's t-test) and the statistical significance was defined by $P < 0.05$.

RESULTS AND DISCUSSION

Preparation of D/T-PPP-NPs-Tf

The average diameter, zeta potential and polydispersity index (PDI) of the D/T-PPP-NPs-Tf were, 197.6±22 nm, -20.54 ± 0.13 mV, 0.098 ± 0.054 respectively(n=3). The DL were 3.63±0.15% for DNR and 4.27±0.13% for Tet. The EE were 70.23±1.91% for DNR and 86.5±0.7% for Tet.

Validation of the Method

Specificity

Representative chromatographs of blank matrices, blank matrices spiked with DNR, Tet, IS, and biological samples collected after IV injection were shown in Figure 1, Figure 2 and Figure 3. The retention times of DNR, Tet, and IS were approximately 2.6, 3.1 and 2.2 min, respectively. The chromatograms showed that a clear and excellent separation between DNR, Tet, and IS without detectable endogenous interfering peaks from biological sample nearby.

Linearity and LLOQ

The regression equation and correlation coefficient of DNR and Tet in biological samples were listed in Table 1, exhibiting good linearity. The assay was linear at concentration of 2-500 $ng \cdot mL^{-1}$ for DNR and 0.5-400 $ng \cdot mL^{-1}$ for Tet in all biological matrices. The LLOQ was 2 $ng \cdot mL^{-1}$ (S / N > 10) for DNR and 0.5 $ng \cdot mL^{-1}$ (S / N > 10) for Tet 0.5 $ng \cdot mL^{-1}$. The accuracy and precision of LLOQ were less than 20% R.S.D. and R.E.

Accuracy and precision

The intra-day accuracies ranged from 2.60% to 4.51% for DNR, and from 3.44% to 6.59% for Tet in all biological matrices. The iner-day accuracies ranged from 2.85% to 3.88% for DNR, and from 2.08% to 5.37% for Tet. The results of precision for DNR and Tet were within 4.62%. These results were all within ±15% which was the acceptable range for biological samples analysis, which exhibited that this assay had good precision, reproducibility and accuracy.

Recovery and matrix effect

The results of extraction recovery and matrix effect for DNR and Tet in biological samples are summarized in Table 2. The extraction recoveries of DNR and Tet in biological samples were more than 70% at three QC concentrations, indicating reproducible and acceptable recoveries. The matrix effect of were DNR and Tet 93.2% to 105.7% in all biological matrices. These data indicated that there were no significant matrix effects for DNR and Tet in biological samples in the LC-MS / MS assay.

Stability

The accuracy ranged from 0.71 to 5.64% for DNR and -0.07 to 4.23% for Tet. These results showed that DNR and Tet demonstrated good stability in all biological matrices after being stored at room temperature for 24 h, at 4 °C in the autosampler for 24 h, after three-thaw cycle for 30 days.

Table 1: The regression equation and correlation coefficient of DNR and Tet in biological samples

Biological samples	The regression equation and correlation coefficient	
	DNR	Tet
Plasma	Y = 0.0863X-0.0223, R=0.9981	Y = 0.198X + 0.0369, R=0.9946
Heart	Y = 0.921 + 0.956X, R=0.9957	Y = 44.4 + 3.55 X, R=0.9937
Liver	Y = 0.805 + 1.63X, R=0.9908	Y =1.34+6.74 X, R=0.9929
Spleen	Y = 5.9 + 1.91 X, R=0.9932	Y = 343+16 X, R=0.9839
Lung	Y = -3.52 + 1.91 X, R=0.9874	Y = 107 + 7.43 X, R=0.9832
Kidney	Y = 0.981 + 1.63 X, R=0.9948	Y = 25.9 + 6.85 X, R=0.9979
Brain	Y = -0.125 +0.338 X, R=0.9951	Y = 0.0612 + 0.406 X, R=0.9889
Muscle	Y = 0.197 + 0.516 X, R=0.9962	Y = 6.62 + 1.25 X, R=0.9939
Fat	Y = 0.263 + 0.563 X, R=0.9956	Y = 1.81 + 0.602 X, R=0.9947
Intestine	Y = 3.98 + 0.561 X, R=0.9942	Y =57.7 + 1.65 X, R=0.9926
Testis	Y = 2.63 + 1.11X, R=0.9960	Y=15.2+1.34X, R=0.9963
Ovary	Y= -0.402+1.04X, R=0.9953	Y= 0.00634+1.58X, R=0.9854
Uterus	Y= 0.470+1.02X, R=0.9974	Y= -0.0985+1.40X, R=0.9882

Table 2: Extraction recovery and matrix effect of DNR and Tet in biological samples (n=3)				
Drug	**Biological sample**	**Drug concentration/ ng·mL^{-1}**	**Extraction Recovery %**	**Matrix effect %**
DNR	Plasma	4	86.35	98.66
		40	87.81	103.26
		400	84.37	102.03
	Heart	4	78.54	95.94
		40	81.91	93. 28
		400	72.62	101.78
	Liver	4	74.83	102.46
		40	85.96	97.87
		400	84.89	95.32
	Spleen	4	85.60	97. 69
		40	74.67	103.57
		400	71.03	104.11
	Lung	4	84.61	100.92
		40	72.38	101.61
		400	77.53	104. 85
	Kidney	4	78.18	101.41
		40	81.65	99.51
		400	81.54	98. 59
	Brain	4	71.78	101.06
		40	73.76	93.46
		400	84.15	96.87
	Muscle	4	81.69	93.43
		40	70.19	94.48
		400	75.87	96.55
	Fat	4	77.69	99.18
		40	71.78	103.18
		400	81.39	104.74
	Intestine	4	81.31	93.45
		40	79.57	104.52
		400	72.10	102.43
	Testis	4	78.32	98.33
		40	73.69	104. 76
		400	77.68	103.39
	Ovary	4	71.72	97.93
		40	84.62	95. 87
		400	70.46	101.41
	Uterus	4	82.78	105. 78
		40	79.58	94.54
		400	80.25	101. 59
Tet	Plasma	0.8	87.72	102.51
		20	88.73	102.35
		300	86.47	103.78
	Heart	0.8	83.27	104.38
		20	70.02	95.64
		300	76.51	96.82

Continued...

Table 2: Cont'd				
Drug	**Biological sample**	**Drug concentration/ ng·mL^{-1}**	**Extraction Recovery %**	**Matrix effect %**
	Liver	0.8	81.01	95.76
		20	72.81	96.41
		300	77.33	105.45
	Spleen	0.8	79.13	102.17
		20	84.91	95.27
		300	70.97	96.81
	Lung	0.8	81.19	104.34
		20	75.87	103.94
		300	70.31	102.47
	Kidney	0.8	80.72	94.43
		20	80.22	97.31
		300	77.29	97.63
	Brain	0.8	83.47	97. 71
		20	82.99	98.52
		300	78.52	96.68
	Muscle	0.8	72.98	102.16
		20	84.68	104.44
		300	79.71	98.59
	Fat	0.8	75.82	103.78
		20	73.57	104.56
		300	82.48	104.48
	Intestine	0.8	76.14	99.23
		20	81.76	103.32
		300	74.72	99.81
	Testis	0.8	82.30	101.29
		20	72.54	97.88
		300	74.47	101.94
	Ovary	0.8	71.84	105. 64
		20	83.03	102.92
		300	83.14	102.84
	Uterus	0.8	83.70	104.56
		20	85.10	94.78
		300	75.48	104.27

Pharmacokinetics

The plasma concentration–time profiles of DNR and Tet in different groups were shown in Figure 4 and the estimated pharmacokinetic parameters were shown in Table 3.

The data of plasma concentration time profile presented that the concentrations of DNR and Tet before 30min in groups of D/T-PPP-NPs and D/T-PPP-NPs-Tf NPs were significantly higher Compared with D/T solution. That was why the AUC of DNR and Tet in D/T-PPP-NPs and D/T-PPP-NPs-Tf groups were significantly higher than D/T solution. After 30 min, the concentrations of DNR and Tet decreased to the same level of D/T solution. As shown in Table 3, the parameters of DNR and Tet in D/T-PPP-NPs group presented no significant different compared with D/T solution. Compare to D/T solution, the parameters of DNR and Tet in D/T-PPP-NPs-Tf group presented a higher AUC and lower V ($p<0.05$). These results indicated D/T-PPP-NPs-Tf tended to stay in the blood after injection compared to D/T solution.

The *in vivo* characters of D/T-PPP-NPs-Tf were strongly depended on the nature of nanoparticle, such

Table 3: Pharmacokinetic parameters of DNR and Tet ($\bar{X}$±SD, n = 6)

Parameters	D/T solution		D/T-PPP-NPs		D/T-PPP-NPs-Tf	
	DNR	Tet	DNR	Tet	DNR	Tet
$T_{1/2}$ /h	9.04±5.53	31.26±13.67	5.62±2.16	34.84±12.38	6.93±3.75	39.68±21.82
AUC_{0-t} /ng·mL^{-1}·h^{-1}	693.34±203.87	932.48±538.49	768.16±125.04	1239.69±415.63*	1815.18±612.89**	1544.84±722.40 **
$AUC_{0-\infty}$ /ng·mL^{-1}·h^{-1}	727.46±143.12	1427.13±749.02	803.50±324.42	1963.11±1310.74*	1883.60±608.82**	1879.40±594.34*
$MRT_{(0-t)}$ /h	8.22±1.27	23.38±4.75	4.87±1.64	29.70±6.03	5.69±2.70	25.12±12.28
CL/L·h^{-1}·kg^{-1}	53.18±21.63	26.04±12.05	42.58±25.77	17.28±10.23	38.18±21.98	19.12±11.04
V /L·kg^{-1}	594. 74±56.59	905.38±68.27	476.61±47.49	818.28±191.72	329.35±46.46 *	463.90±48.52*

*p< 0.05, **p<0.01 vs D/T solution

Table 4: AUC values in various tissues of DNR and Tet

Drug	*Tissue*	AUC_{0-12h}/(ng·g^{-1}·h)			r_{e1}	r_{e2}
		D/T solution	(DNR/Tet)PLGA-PLL-PEG	(DNR/Tet)PLGA-PLL-PEG-Tf		
	Heart	29160.32	22273.93*	27747.84	0.76	0.95
	Liver	13936.78	320173.41**	349279.28**	22.97	25.06
	Spleen	170198.40	109210.14*	131789.33	0.64	0.77
	Lung	63614.89	94862.88*	532813.75**△	1.49	8.38
	Kidney	44920.42	29900.95**	28525.35**	0.67	0.64
	Brain	112.56	192.63*	130.13	1.71	1.16
DNR	Muscle	5251.82	5782.59	7834.56	1.10	1.49
	Fat	712.19	1677.86**	1717.73**	2.36	2.41
	Intestine	51151.50	3692.05**	3434.70**	0.07	0.07
	Testicle	139.66	1117.22**	875.25**	8.00	6.27
	Ovary	9251.83	4455.95*	14113.99*△△	0.48	1.53
	Uterus	8893.24	8655.83	10005.85	0.97	1.13
	Heart	118439.52	71059.67*	85003.30*	0.60	0.72
	Liver	145428.67	573772.81**	688867.5**	3.95	4.74
	Spleen	1109640.01	790657.50*	808787.94*	0.71	0.73
	Lung	1594564	364515.81**	550250.31**△	0.23	0.35
	Kidney	309396.16	88588.75**	65389.02**	0.29	0.21
	Brain	26.61	5455.66**	5653.54**	205.02	212.46
Tet	Muscle	34056.1875	25881.46*	28603.75*	0.76	0.84
	Fat	63475.83	21317.16**	21744.24**	0.34	0.34
	Intestine	72626.52	69265.58	102719.09	0.95	1.41
	Testicle	65139.84	61393.81	57942.11	0.94	0.89
	Ovary	1407354.88	1275525	1850362.63△	0.91	1.31
	Uterus	705667.94	1014357.5**	945235.06*	1.44	1.34

*p< 0.05, **p<0.01 vs D/T solution; △p< 0.05, △△p< 0.05vs D/T-PPP-NPs

r_{e1} =$AUC_{DNR/Tet-PLGA-PLL-PEG}$ / $AUC_{D/T\ solution}$

r_{e2} =$AUC_{DNR/Tet-PLGA-PLL-PEG-Tf}$ / $AUC_{D/T\ solution}$

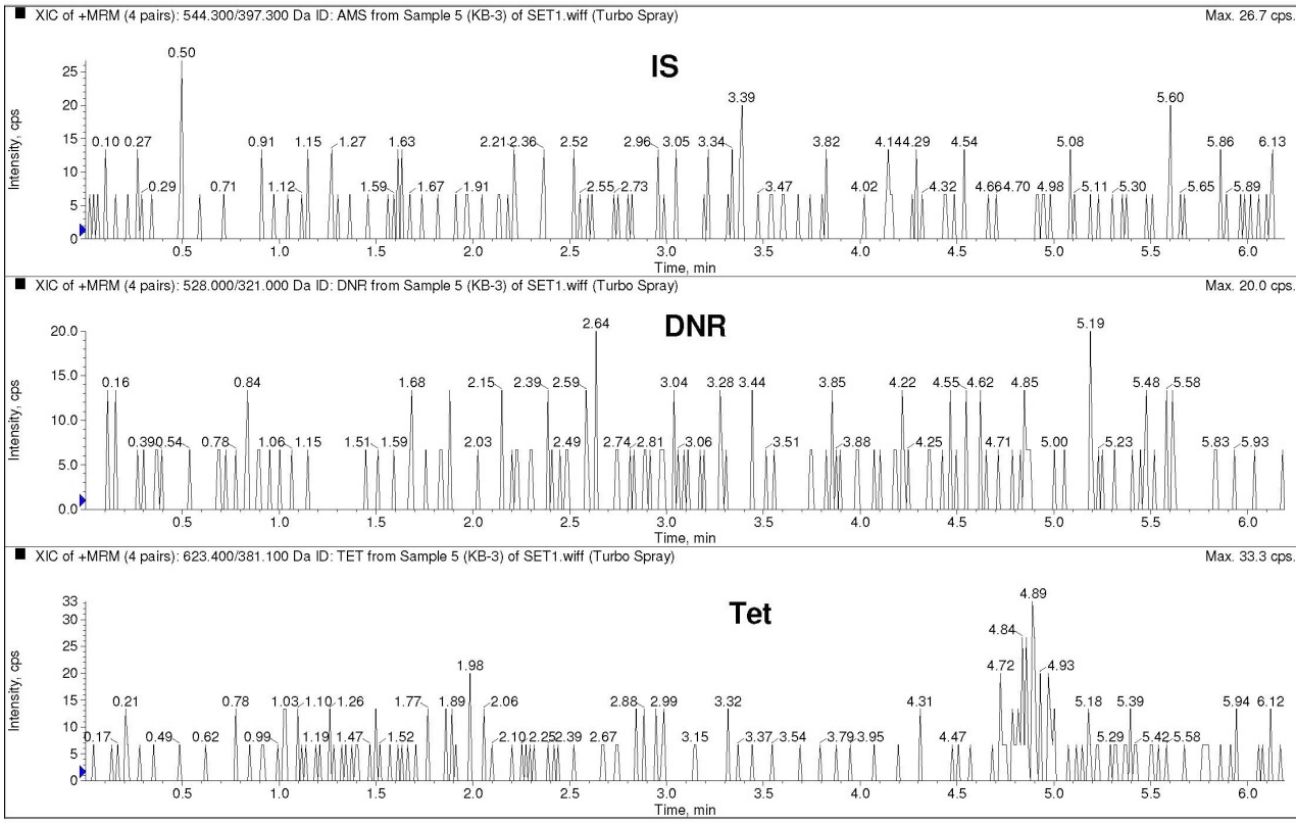

Figure 1: Representative MRM chromatograms of blank tissue (lung).

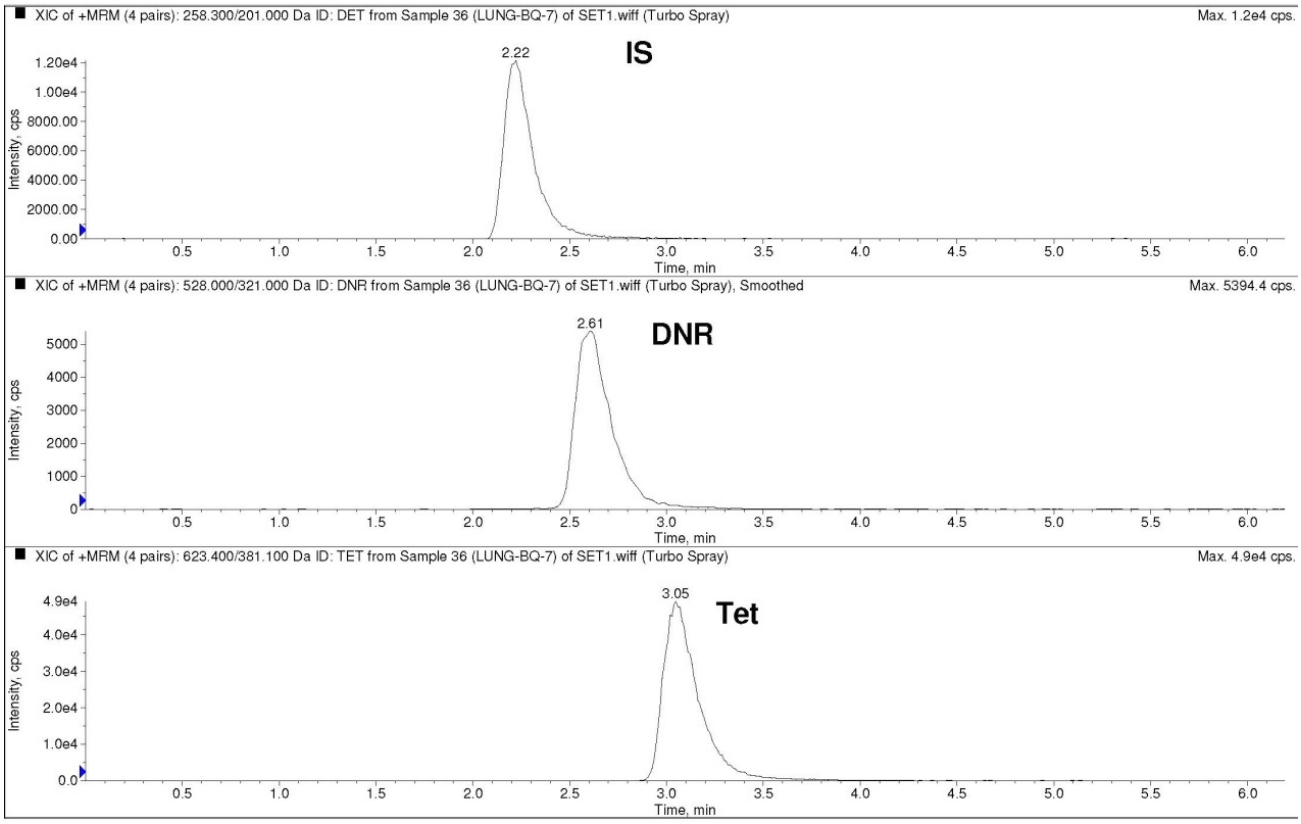

Figure 2: Representative MRM chromatograms of lung tissue spiked with DNR and Tet(DNR 206.4 ng·mL^{-1}, Tet 198.6ng·mL^{-1}).

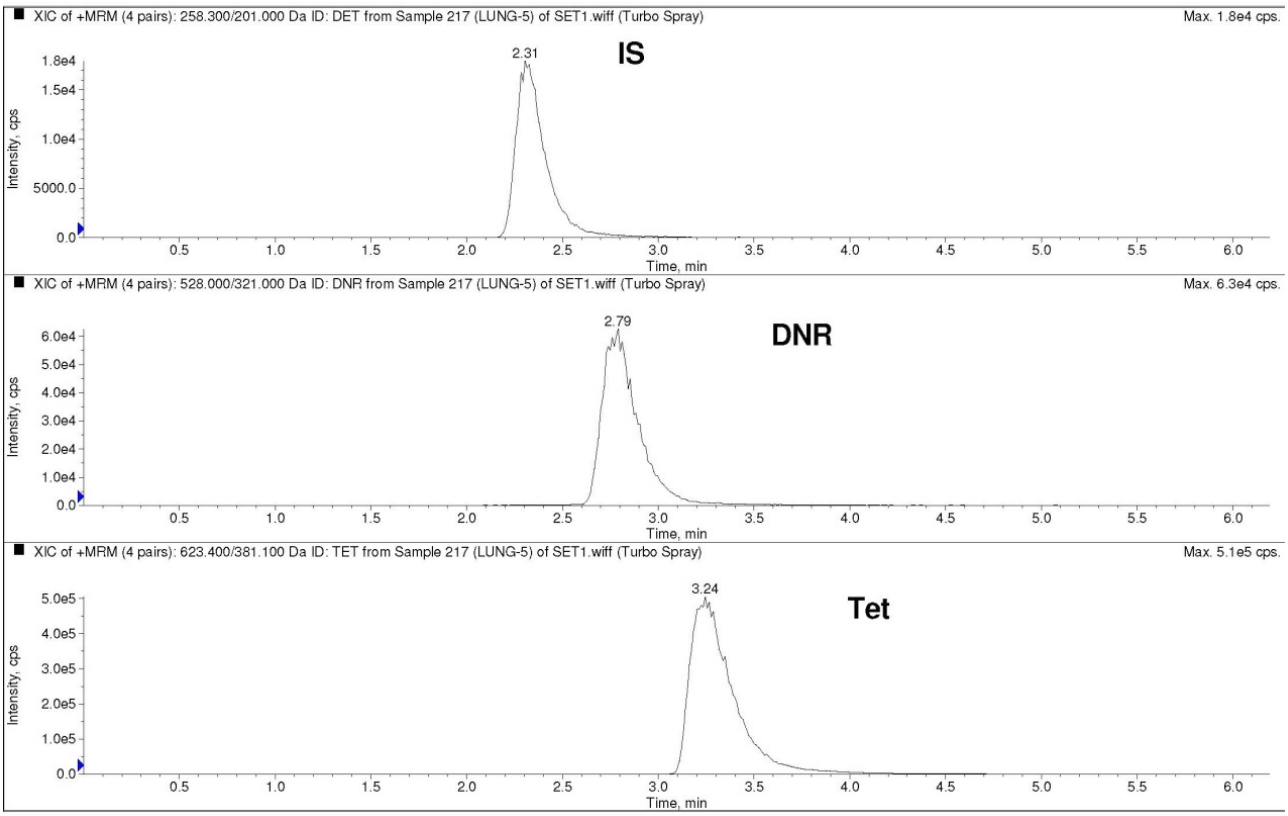

Figure 3: Representative MRM chromatograms of lung tissue of a rat lung tissue sample(3.275 ng·mL^{-1})obtained 60 h after an intravenous administration of DNR and Tet solution.

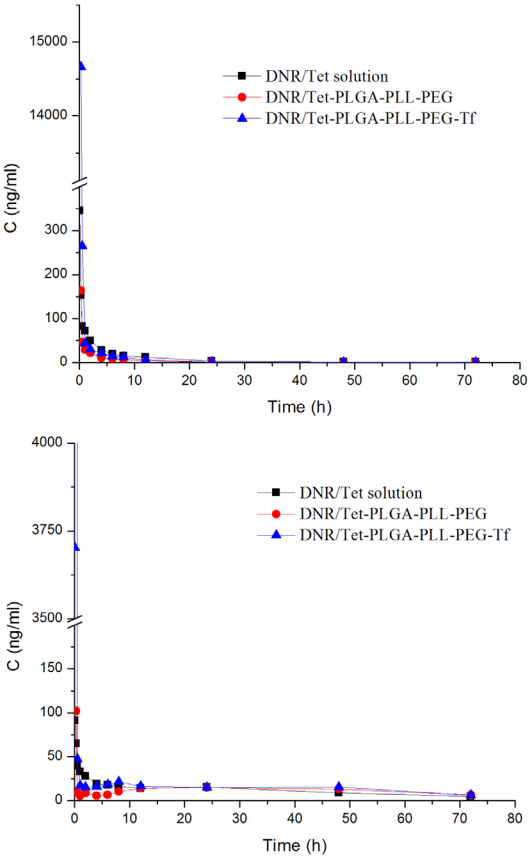

Figure 4: The Mean plasma concentration-time curves of DNR (A) and Tet (B) in D/T solution, D/T-PPP-NPs and D/T-PPP-NPs-Tf

as the MW of PLGA, the particle size and the length of PEG chains[22].

Based on our previous study, the results of *in vitro* release showed that 90% of free drugs were released in 3h, while only 50% to 60% drugs were released from the D/T-PPP-NPs-Tf in 24 h. The plasma concentration–time profiles (Figure 4) and the estimated pharmacokinetic parameters (Table 3) indicated that D/T-PPP-NPs-Tf did not have long term release effect *in vivo* compared with free drugs. The similar pharmacokinetic behavior suggested that D/T-PPP-NPs-Tf dissolved rapidly in the blood compared to free drugs. Usually PEG was regarded the main moiety in multi polymer nanoparticles which has the potential to enhance the circulation time and reduce the clearance of NPs by thereticuloendothelial system.[22] The clearance of the PEGylated moiety decreased significantly when one PEG chain with a molecular weight (MW) of 30 kDa or a branched PEG chain with two 20 kDa chains or several chains of 5 kDa.[22,23] In our study, the MW of PEG was only 4000 Da which was comparably small, so the pharmacokinetic parameters such of the NPs as $MRT_{(0\text{-}t)}$ and $T_{1/2}$ had no different compared with free drugs.

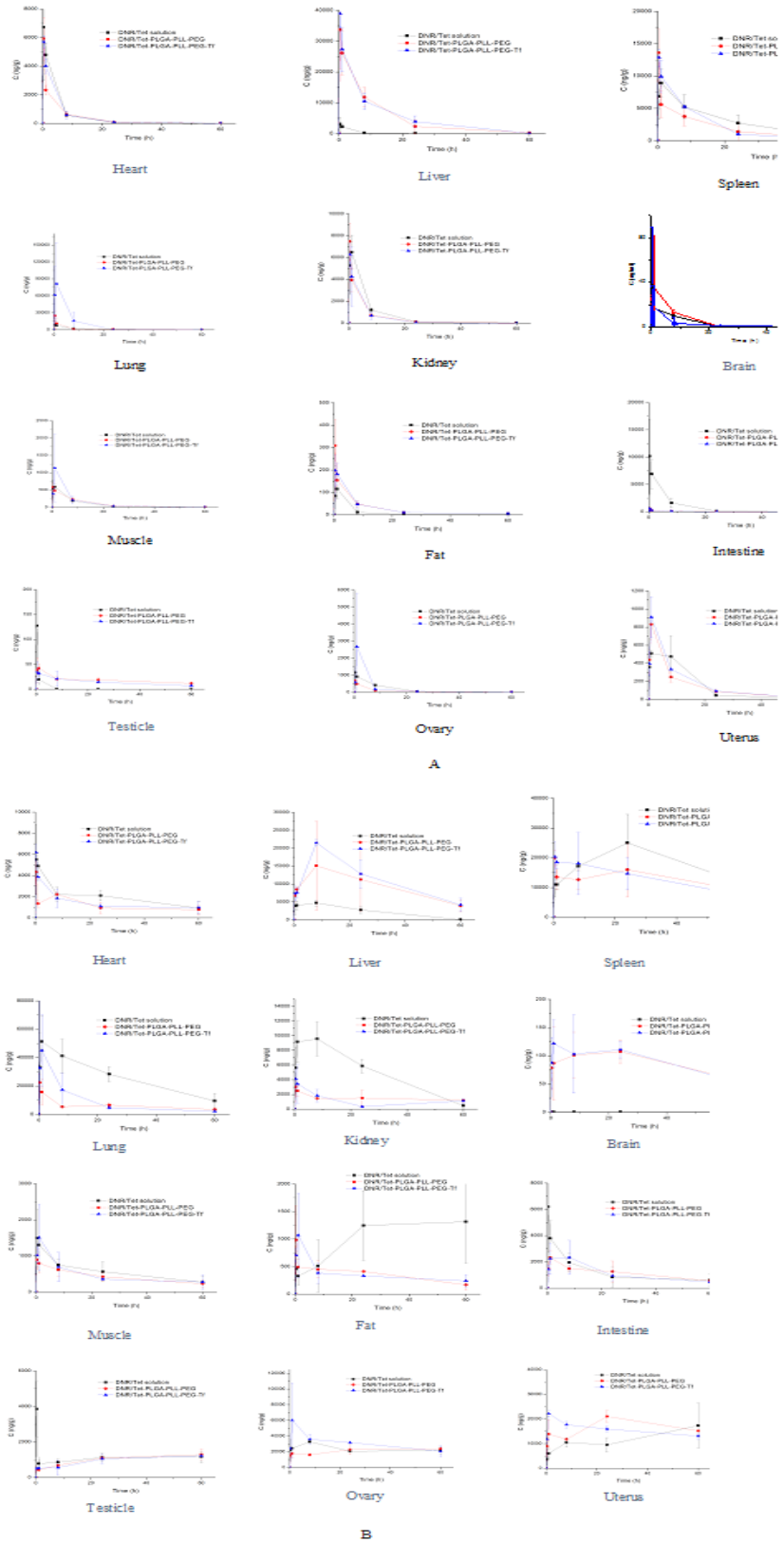

Figure 5: The Tissue distribution of DNR (A) and Tet (B) in D/T solution, D/T-PPP-NPs and D/T-PPP-NPs-Tf

Tissue distribution

The tissue distribution curves of DNR and Tet in different groups were shown in Figure 5 and the Re values in various tissues were shown in Table 4. There were very few reports about the pharmacokinetics and tissue distribution of Tet. Our results of Tet solution were familiar with Professor Cheng's report.[4] However, we gave more details about the tissue distribution of Tet in muscle, fat, intestine, and gonad.

Compared to D/T solution, the distribution of DNR in groups of D/T-PPP-NPs and D/T-PPP-NPs-Tf were higher in liver ($p<0.01$), lung ($p<0.05$), fat ($p<0.01$) and testicle ($p<0.01$), and were lower in heart, spleen, kidney ($p<0.05$) and intestine ($p<0.01$). The distribution of Tet in D/T-PPP-NPs and D/T-PPP-NPs-Tf groups were higher in liver ($p<0.01$), brain ($p<0.01$) and uterus ($p<0.05$), and were lower in heart ($p<0.05$), spleen ($p<0.05$), lung ($p<0.01$), kidney ($p<0.01$) and fat ($p<0.01$) compared with D/T solution. The distribution of DNR and Tet in D/T-PPP-NPs-Tf Group were similar with D/T-PPP-NPs, except the significantly higher concentration in lung($p<0.05$) and ovary($p<0.05$). D/T-PPP-NPs and DNR/Tet-PLGA-PLL-PEG-Tf presented targeting properties to liver. This result may be attributed to the uptake of nanoparticles by RES organs which have been widely reported.[18-24] Concerning the gastrointestinal reactions and high cardiac toxicity of DNR, the lower distribution of DNR in heart and intestine was a great advantage for D/T-PPP-NPs and D/T-PPP-NPs-Tf.

CONCLUSION

The LC-MS/MS method was specific, sensitive, rapid and reliable for the simultaneously determination of DNR and Tet in rat plasma and tissues. This LC-MS/MS method has been successfully used in the pharmacokinetic and tissue distribution research of DNR and Tet. D/T-PPP-NPs-Tf partly changed the pharmacokinetic parameter of DNR and Tet in rats. D/T-PPP-NPs-Tf tended to stay in the blood after injection compared to D/T solution. Compared with D/T solution, the distribution of D/T-PPP-NPs and D/T-PPP-NPs-Tf were significantly changed.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATION USED

DNR: Daunorubicin; **Tet:** Tetrandrine; **Tf:** Transferrin; **MDR:** Multidrug resistance; **PLGA:** Poly (lactic-co-glycolic acid); **PEG:** Polyethylene glycol; **MW:** Molecular Weight; **DCC:** dicyclohexyl carbodiimide; **DMF:** anhydrous dimethylformamide; **CDI:** N,N-carbonyldiimidazole; **DMAP:** 4-dimethylaminopyridine; **IS:** Doxrubicin; **SD:** Sprague Dawley; **PDI:** polydispersity index; **EE:** Entrapment efficiency; **DL:** Drug-loading efficiency; **DP:** Declustering potential; **CE:** collision energy; **LLOQ:** Lowest Limit of Quantitation; **QC:** Quality Control; **R.E.:** relative error; **R.S.D.:** Relative standard deviation; **ER:** Extraction recovery; **ME:** Matrix effect; **AUC:** Area under the plasma concentration time profiles; **MRT:** the mean residence time; **CL:** total plasma clearance; **t1/2β:** The elimination half-life; t1/2α:distribution half-life.

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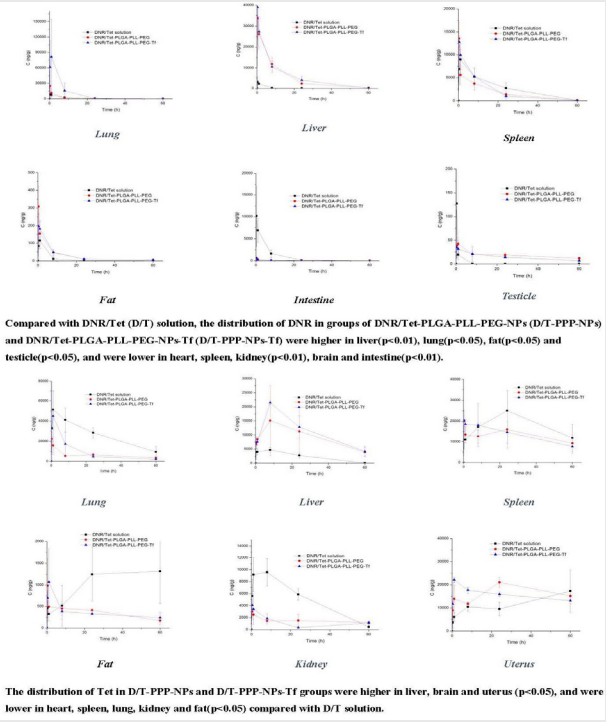
PICTORIAL ABSTRACT



SUMMARY

- A sensitive, rapid and reliable LC-MS/MS method was developed for simultaneously determination of daunorubicin (DNR) and tetrandrine (Tet) in rat plasma and tissues. The pharmacokinetics and tissue distribution of DNR/Tet-PLGA-PLL-PEG-NPs-Tf (D/T-PPP-NPs-Tf), DNR/Tet-PLGA-PLL-PEG-NPs (D/T-PPP-NPs) and DNR/Tet (D/T) solution were compared following intravenous injection in the rats. Compare to D/T solution, the parameters of DNR and Tet in D/T-PPP-NPs-Tf group presented significantly higher AUC($p<0.01$) and lower V($p<0.05$). These results indicated that D/T-PPP-NPs-Tf tended to stay in the blood after injection. Compared with D/T solution, the distribution of DNR in groups of D/T-PPP-NPs and D/T-PPP-NPs-Tf were higher in liver($p<0.01$), lung($p<0.05$), fat($p<0.05$) and testicle($p<0.05$), and were lower in heart, spleen, kidney($p<0.01$), brain and intestine($p<0.01$). The distribution of Tet in D/T-PPP-NPs and D/T-PPP-NPs-Tf groups were higher in liver, brain and uterus($p<0.05$), and were lower in heart, spleen, lung, kidney and fat($p<0.05$) compared with D/T solution.

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