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Safety, tolerability and pharmacokinetics of liposomal curcumin (Lipocurc™) in healthy humans

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Key words
liposomal curcumin – human – pharmacokinetics – red blood cells

Abstract. Introduction: Experimental studies have shown that liposomal curcumin can exert a reduction in tumor growth in pancreatic and colorectal cancer. In this phase I clinical trial we investigated the pharmacokinetics, safety, and tolerability of intravenously administered liposomal curcumin in healthy subjects. Material and methods: 50 male and female participants were included in this randomized, placebo-controlled double-blind phase I dose escalation study. Subjects received a single dose of liposomal curcumin (10 – 400 mg/m^2; n = 2 – 6 per group) or placebo over 2 hours intravenously. Results: Dose-dependent increases in the plasma concentrations of curcumin and its metabolite tetrahydrocurcumin (THC) were detected. After the end of drug infusion, curcumin and THC plasma concentrations decreased within 6 – 60 minutes below the limit of quantification. Mean urinary excretion was ~ 0.1% of total systemic clearance. Liposomal curcumin was tolerated well, but a transient red blood cell echinocyte formation with concomitant increase in mean cellular volume was observed at dosages ≥ 120 mg/m^2. Conclusion: Short-term intravenous dosing of liposomal curcumin appears to be safe up to a dose of 120 mg/m^2. Changes in red blood cell morphology may represent a dose limiting sign of toxicity.

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Introduction

Dried turmeric root has been traditionally used as a homeopathic remedy [1]. Curcumin (diferuloylmethane) constitutes 2 – 5% of the root and modulates cell signaling pathways including cell cycle, apoptosis, proliferation, survival, invasion, angiogenesis, metastasis, and inflammation [2, 3]. The anticancer activity of turmeric was first demonstrated in vitro and in animal studies by Kuttan et al. [4] in 1985. Given its ability to affect multiple cellular targets, curcumin may be effective in the treatment of cancers, as well as in inflammatory and parasitic diseases.

Liposomal curcumin produced a concentration-dependent inhibition of cell growth at concentrations of 2.5 to 100 μM in pancreatic and colorectal cancer cell lines in vitro and in xenograft mouse models with a minimal effective dose of 20 mg/kg. No toxicity was observed in the dosages up to 40 mg/kg [5, 6]. However, preclinical toxicological studies of liposomal curcumin in dogs showed dose-dependent hemolysis following infusion of ≥ 20 mg/kg [7].

Curcumin is nearly insoluble in water [8] and is stable in the acidic pH of the stomach [8]. After oral administration it has a poor bioavailability being rapidly metabolized by glucuronidation and sulfation in the intestinal wall and the liver and is excreted in feces [9]. Data on pharmacokinetics and systemic bioavailability of orally administered curcumin in humans are inconclusive. Sharma et al. [10] observed in patients with cancer a mean plasma concentration of ~ 11 nmol/L after an oral dose of 3.6 g curcumin. In another clinical trial, 45 times higher plasma levels were reached after administration of 4 g curcumin [11]. Vareed et al. [12] applied 10 or 12 g curcumin per os to 12 healthy volunteers and free curcumin was detectable in only one subject. The reason for these discrepancies is unknown but various influencing factors could

be responsible, such as different sampling schemes, analytical methods, metabolic conjugation in the gut wall and liver, and intestinal enzymatic reduction by *Escherichia coli* to tetrahydrocurcumin. To overcome the pharmacokinetic and bioavailability limitations of oral administration, a liposomal formulation of curcumin has been developed for intravenous administration and represents a promising drug delivery system.

In an effort to address the safety and tolerability of liposomal curcumin in humans we conducted a phase I clinical study employing the recently developed liposomal drug formulation. A single intravenous dose of liposomal curcumin was administered and the pharmacokinetics investigated in healthy humans.

Material and methods

Medicinal product description

Curcumin, (1E6E) -1,7- bis(4 hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5 dione, molecular weight 368.38 g/mol, was synthesized at Sami Labs Limited (Bangalore, India) under Good Manufacturing Practice (GMP) with a purity of 99.2%. Liposomal curcumin was manufactured, tested, packaged, and labeled by Polymun Scientific, Klosterneuburg, Austria, in compliance with GMP. The entire production process is performed under aseptic conditions and the last step of the production process includes 0.2 µm filtration.

To ensure the quality of liposomal curcumin, product specifications were set and tested for various parameters at the level of drug substance (free curcumin) and at the level of the drug product (liposomal curcumin). For the drug product, the following parameters were tested: visual appearance, content of curcumin, DMPC, and DMPG, liposome size and size distribution, surface charge, residual EtOH, particulate matter, pH, osmolality, endotoxin, and sterility.

The curcumin concentration was 6.9 mg/mL embedded into liposomal membranes composed of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dimyristoyl-sn-glycero-3-phosphoric-1-glycerol sodium salt (DMPG) with a molar ratio of 9 : 1.

Subjects

94 subjects were screened, but only 50 qualified for treatment. The other subjects had to be excluded because of abnormalities in screening exams. Thus, 50 healthy male or female Caucasian volunteers (21 females and 28 males), aged 18 – 45 (age 27 ± 5, mean ± SD) years were enrolled in this study. All participants were healthy volunteers and recruited using a database maintained by our Department. They were compensated for their participation. The compensation scheme was reviewed and approved by the Ethics Committee and was included in the informed consent document. Subjects had a body mass index of 18 – 27 kg/m^2 (BMI 22.3 ± 2.6 kg/m^2). All subjects were non-smokers except for 2 subjects. Subjects were excluded if they had participated in another clinical trial or donated blood during the preceding 3 months, had a medical disorder, condition, or history that could interfere with the study results or impair their ability to participate in or complete the study. These included coagulation disorders, history or evidence of disease (e.g., hemolytic diathesis, anemia requiring substitution therapy, hemochromatosis) that could be exacerbated by administration of liposomal curcumin. Other key exclusion criteria included regular use of therapeutic or recreational drugs; use of medication within the 2 weeks preceding the study that could interfere with any of the study drugs; relevant deviation from the normal range in clinical chemistry, hematology, blood pressure, electrocardiogram or urinalysis; a positive test for HIV or hepatitis B or C; and pregnancy or lactation in women of child-bearing potential.

All subjects gave written informed consent to participate in the study before undergoing any study-specific procedures. The study was conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Clinical Practice (ICH GCP) Guideline and local regulations. The clinical trial was registered with EUDRACT (2011-001861-41) and with clinicaltrials.gov (NCT 01403545).

Study design

In this phase I, single-center, randomized, double-blind and placebo-controlled dose es-

Table 1. Pharmacokinetic parameters of curcumin administered intravenously in a liposomal formulation.

Curcumin dose (mg/m^2)	n[1]		C_{max} (ng/mL)	t_{max} (h)	AUC_{last} (ng×h/mL)	$AUC_{0–2h}$ (ng×h/mL)	$AUC_{2–6h}$ (ng×h/mL)	C_{last} (ng/mL)	t_{last} (h)	MRT_{last} (min)
10	3	**Mean**	**42**	**0.8**	**43**	**41**	**0**	**41**	**0.9**	**7[2]**
		SD	22	1.0	62	23	0	23	0.9	n.c.
		Median	31	0.3	11	28	0	28	0.5	7
		Geomean	38	0.5	16	37	n.c.	37	0.6	n.c.
		Range	40	1.8	111	40	0	40	1.8	7
20	3	**Mean**	**97**	**1.8**	**133**	**130**	**3**	**40**	**2.0**	**10**
		SD	61	0.3	78	73	5	14	0.0	2
		Median	69	2.0	101	101	0	40	2.0	10
		Geomean	85	1.8	119	118	n.c.	39	2.0	9
		Range	112	0.5	145	137	8	27	0.1	4
40	3	**Mean**	**317**	**1.7**	**466**	**466**	**0**	**237**	**2.0**	**11**
		SD	13	0.3	81	81	0	75	0.0	6
		Median	319	1.5	445	445	0	252	2.0	14
		Geomean	317	1.7	461	461	n.c.	228	2.0	10
		Range	25	0.5	157	157	0	148	0.0	10
80	3	**Mean**	**775**	**1.3**	**990**	**976**	**15**	**174**	**2.1**	**14[2]**
		SD	360	0.8	439	453	14	217	0.1	4
		Median	585	1.5	811	796	15	62	2.1	14
		Geomean	726	1.1	932	912	n.c.	98	2.1	13
		Range	640	1.5	822	851	29	388	0.2	5
120	8	**Mean**	**714**	**1.4**	**1,002**	**981**	**21**	**73**	**2.1**	**10**
		SD	633	0.3	871	856	17	64	0.1	4
		Median	518	1.5	756	733	23	43	2.1	10
		Geomean	548	1.4	773	758	n.c.	56	2.1	9
		Range	1,949	1.0	2,629	2,581	47	169	0.3	10
180	7	**Mean**	**1,709**	**1.4**	**2,533**	**2,457**	**76**	**55**	**2.2**	**11**
		SD	661	0.3	894	870	28	30	0.0	5
		Median	1,782	1.5	2,944	2,847	74	51	2.3	12
		Geomean	1,591	1.4	2,384	2,311	72	49	2.2	10
		Range	1,750	1.0	2,372	2,329	78	76	0.1	14
240	6	**Mean**	**1,446**	**1.7**	**1,981**	**1,907**	**74**	**52**	**2.3**	**12**
		SD	382	0.4	434	414	48	27	0.0	3
		Median	1,374	1.8	2,122	2,060	67	42	2.3	12
		Geomean	1,404	1.6	1,938	1,867	76	46	2.3	12
		Range	864	1.0	1,038	976	130	64	0.0	9
320	4	**Mean**	**2,575**	**1.5**	**3,607**	**3,455**	**152**	**101**	**2.5**	**15**
		SD	453	0.4	1,056	1,040	37	109	0.4	7
		Median	2,444	1.5	3,399	3,276	157	55	2.4	13
		Geomean	2,547	1.5	3,498	3,343	148	71	2.5	14
		Range	1,018	1.0	2,471	2,455	84	233	0.8	15
400	2	**Mean**	**2,358**	**0.9**	**3,886**	**3,684**	**202**	**40**	**2.8**	**6**
		SD	412	0.9	397	442	45	5	0.4	5
		Median	2,358	0.9	3,886	3,684	202	40	2.8	6
		Geomean	2,340	0.6	3,876	3,670	200	40	2.7	5
		Range	582	1.3	561	625	63	7	0.5	6

Maximum plasma curcumin concentration (C_{max}), time to peak concentration (t_{max}), AUC levels during ($AUC_{0–2h}$) and after ($AUC_{0–6h}$) infusion, and mean residence time from the time of dosing to the time of last quantifiable concentration (MRT_{last}) are shown. The following descriptive statistics, mean, standard deviation (SD), median, geometric mean (Geomean) and the range are also provided. Mean values are bolded; n.c. = could not be calculated; [1]Number of determinations; [2]MRTlast values represent 1 and the mean of 2 values at 10 and 80 mg/m^2, respectively.

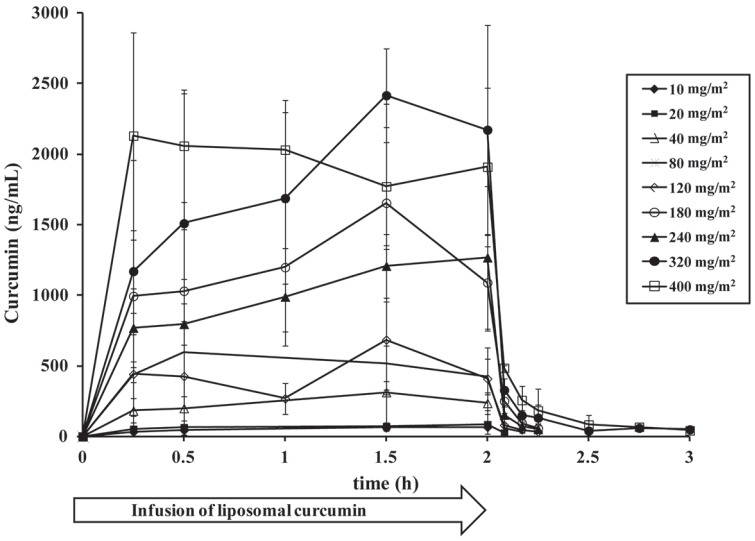

Figure 1. Plasma concentrations of curcumin following infusion of liposomal curcumin. The data is presented as the mean ± SD of the plasma concentrations that were above the limit of detection. The number of subjects at each dose is shown in Table 1.

calating study, different doses of intravenously administered liposomal curcumin (10, 20, 40, 80, 120, 180, 240, 320, and 400 mg/m^2) were investigated. Each dose group for the first 24 subjects comprised 3 subjects with curcumin and 1 subject receiving placebo (10 – 180 mg/m^2) and for subjects 25 – 50 the groups were composed of 4 subjects with curcumin and 1 subject receiving placebo (120 – 400 mg/m^2). Subjects were allocated to treatment groups starting with the lowest dose and randomized to liposomal curcumin or placebo. A validated software (RANCODE; idv-Datenanalyse und Versuchsplanung, Gauting, Germany) was used to generate the randomization list. Randomization and preparation of study medication was done by study personnel otherwise not involved in the study conduct. The blinding for study participants and staff was maintained using covered infusion bags and tubing. Because of adverse reactions including mean MCV red blood cell volume increase and echinocyte formation in the highest dosage group, only 2 subjects received 400 mg/m^2 liposomal curcumin and a further down-titration was performed to evaluate the threshold dosage leading to morphological alterations. Thus a total of 8 subjects received 120 mg/m^2, 7 subjects 180 mg/m^2, 6 subjects 240 mg/m^2, and 4 subjects 320 mg/m^2.

On the study day, subjects randomly received a single intravenous dose of study medication (liposomal curcumin or placebo) over 2 hours after pre-treatment with 4 mg dexamethasone and 30 mg diphenhydramine (subject 1 – 24) or 30 mg diphenhydramine alone (subject 25 – 50) to prevent hypersensitivity reactions. The change of the pre-treatment was amended to the study protocol during the trial because of transient glucosuria in 1 subject, which may have been induced by the administration of dexamethasone. Following infusion of the study medication, the participants were observed for 24 hours in the hospital and attended a subsequent follow-up 48 hours after administration of the study drug and an end-of-study visit 7 – 9 days after administration. Each subject had only 1 study day. Venous blood was drawn for safety blood count before the treatment period and 30 and 90 minutes after the start of the 2-hour drug infusion and 15 minutes and 4 hours after the end of the infusion. Additional chemistry for safety issues was determined before intervention and 15 minutes after the end of drug infusion. During the interventional phase, blood pressure and heart rate were monitored non-invasively and a 12-lead ECG was recorded at timed intervals. Venous blood pharmacokinetic samples were obtained from a venous catheter before, and at 15, 30, and 90 minutes of drug infusion and 5, 10, 15, 30, 45 minutes, as well as 1 hour, 2, 4, and 6 hours after the end of treatment. The clinical staff who obtained blood samples was masked to treatment allocation to maintain the blind.

Plasma sample processing

A liquid chromatographic-tandem mass spectrometric method was developed for the determination of curcumin and its metabolite tetrahydrocurcumin (THC) in human plasma [13]. Quantification with K_3-EDTA as the anticoagulant was developed and validated according to GLP requirements at Nucro-Technics (Scarborough, Ontario, Canada). Naproxen was used as the internal standard. The method involved a solid phase extraction. The analytical concentration range was 25.00 – 20,000.00 ng/mL for curcumin and THC. The same method was used for the determination of curcumin and THC in urine samples, but was not validated in this matrix. Reference standard for curcumin was

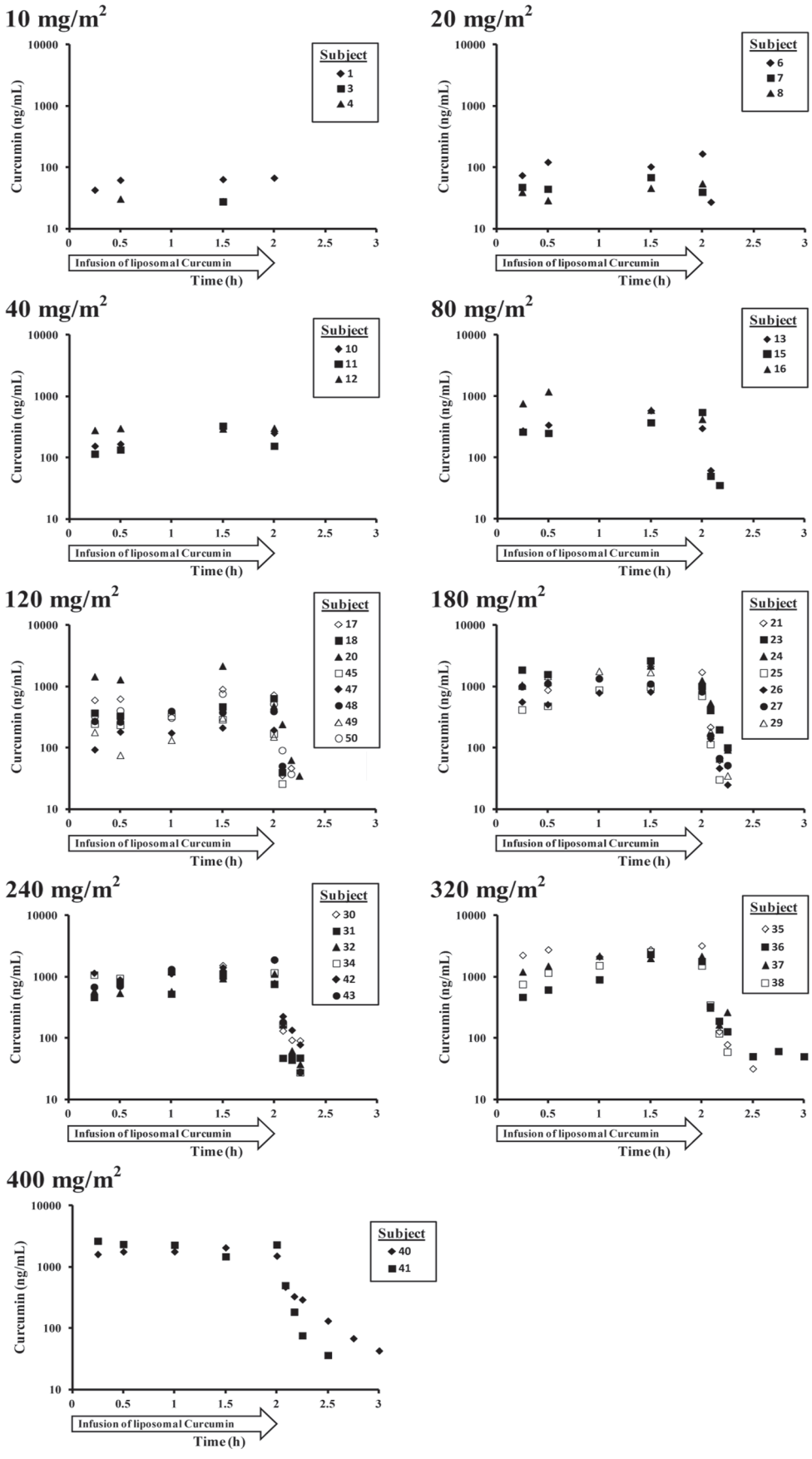

Figure 2. Individual plasma concentration time profiles for curcumin. Plasma concentrations of curcumin above the limit of detection were not measured at times greater than 1 hour post-infusion.

Table 2. Pharmacokinetic parameters of THC.

Curcumin dose (mg/m^2)	n[1]		C_{max} (ng/mL)	t_{max} (h)	AUC_{last} (ng×h/mL)	$AUC_{0–2h}$ (ng×h/mL)	$AUC_{2–6h}$ (ng×h/mL)	C_{last} (ng/mL)	t_{last} (h)	MRT_{last} (min)
80	2	**Mean**	**41**	**1.2**	**28**	**28**	**0**	**37**	**1.3**	**38**
		SD	7	0.9	22	22	0	13	1.1	n.c.
		Median	41	1.1	43	43	0	37	1.3	38
		Geomean	40	0.7	23	23	n.c.	36	1.0	38
		Range	10	1.8	31	31	0	18	1.5	n.c.
120	8	**Mean**	**96**	**0.5**	**117**	**131**	**3**	**50**	**1.8**	**10**
		SD	71	0.2	88	84	6	19	0.5	2
		Median	74	1.5	105	113	0	44	2.0	10
		Geomean	81	1.5	89	113	n.c.	48	1.7	10
		Range	213	1.5	288	254	16	51	1.6	8
180	7	**Mean**	**109**	**0.5**	**159**	**153**	**5**	**45**	**2.1**	**12**
		SD	32	0.2	42	41	2	25	0.0	3
		Median	101	1.5	137	131	5	37	2.1	11
		Geomean	106	1.2	154	159	5	40	2.1	11
		Range	77	1.3	106	102	5	59	0.1	7
240	6	**Mean**	**110**	**0.4**	**174**	**165**	**9**	**42**	**2.1**	**12**
		SD	37	0.2	56	52	15	20	0.1	4
		Median	102	1.5	156	154	10	34	2.1	12
		Geomean	106	1.5	168	159	n.c.	39	2.1	11
		Range	102	1.0	152	140	42	48	0.3	12
320	4	**Mean**	**159**	**0.5**	**261**	**243**	**18**	**30**	**2.2**	**13**
		SD	39	0.3	61	55	7	4	0.0	2
		Median	157	2.0	249	230	18	29	2.2	12
		Geomean	155	1.7	256	239	17	30	2.2	13
		Range	81	1.0	134	122	12	10	0.1	5
400	2	**Mean**	**265**	**0.7**	**385**	**346**	**39**	**28**	**2.4**	**16**
		SD	101	0.5	54	50	5	3	0.2	4
		Median	265	1.5	385	346	202	28	2.4	16
		Geomean	255	1.4	383	344	39	28	2.4	16
		Range	143	1.0	77	70	63	4	0.3	6

Maximum plasma curcumin concentration (C_{max}), time to peak concentration (t_{max}), AUC levels during ($AUC_{0–2h}$) and after ($AUC_{0–6h}$) infusion, and mean residence time from the time of dosing to the time of last quantifiable concentration (MRT_{last}) are shown. The following descriptive statistics, mean, standard deviation (SD), median, geometric mean (Geomean) and the range are also provided. Mean values are bolded; n.c. = could not be calculated; [1]Number of determinations; [2]MRT_{last} values represent 1 and the mean of 7 and 6 values at 80 and 120 and 180 mg/m^2, respectively.

supplied by Sigma Aldrich (■■ city, country?) and THC was supplied by Sign Path Pharma Inc. (Quakertown, PA, USA).

Pharmacokinetic analysis

The maximum plasma concentration (C_{max}) and time at which the maximum plasma concentration was reached (t_{max}) were observed values. Calculated parameters were: terminal half-life of elimination (HL λz), area under the plasma concentration vs. time curve from time 0 (baseline) to 2 h ($AUC_{0–2}$; ng×h/mL), 2 hours to 6 hours ($AUC_{0–2}$; ng×h/mL), to the last measurable plasma concentration time point (AUC_{last}; ng×h/mL), or extrapolated area under the observed curve ($AUC_{INF_}$ob; µg×h/mL). The value for HL λz was calculated as 0.693/Kel. Kel was calculated by log-linear regression analysis of the terminal portion of the plasma concentration versus time curves. AUC_{last} was calculated using the linear trapezoidal rule. MRT_{last}, mean residence time for plasma concentration time profiles up to 6 hours, was calculated from the ratio of $AUMC_{last}$/AUC_{last}. $AUMC_{last}$ was the calculation of the

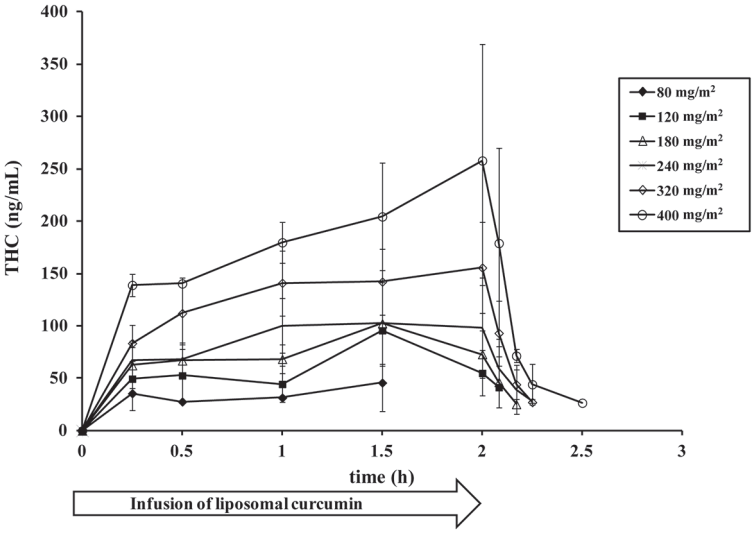

Figure 3. Plasma concentrations of THC following infusion of liposomal curcumin. The data is presented as the mean ± SD of the plasma concentrations that were above the limit of detection. The number of subjects at each dose is shown in Table 2.

area under the first-moment curve (plasma concentration × time vs. time).

Results

Pharmacokinetics

Intravenous administration of liposomal curcumin resulted in a rapid and dose-dependent increase in the plasma concentration of curcumin with mean t_{max} values ranging from 0.8 to 1.8 hours and mean C_{max} values ranging from 42 to 2,575 ng/mL in the dose range of 10 – 400 mg/m^2 (Table 1) (Figures 1, 2). Individual plasma concentrations of the metabolite, THC were below the limit of detection in the dose range of 10 – 40 mg/m^2. In the dose range of 80 – 400, the mean plasma exposure of THC was 9 – 35 times lower than that of curcumin

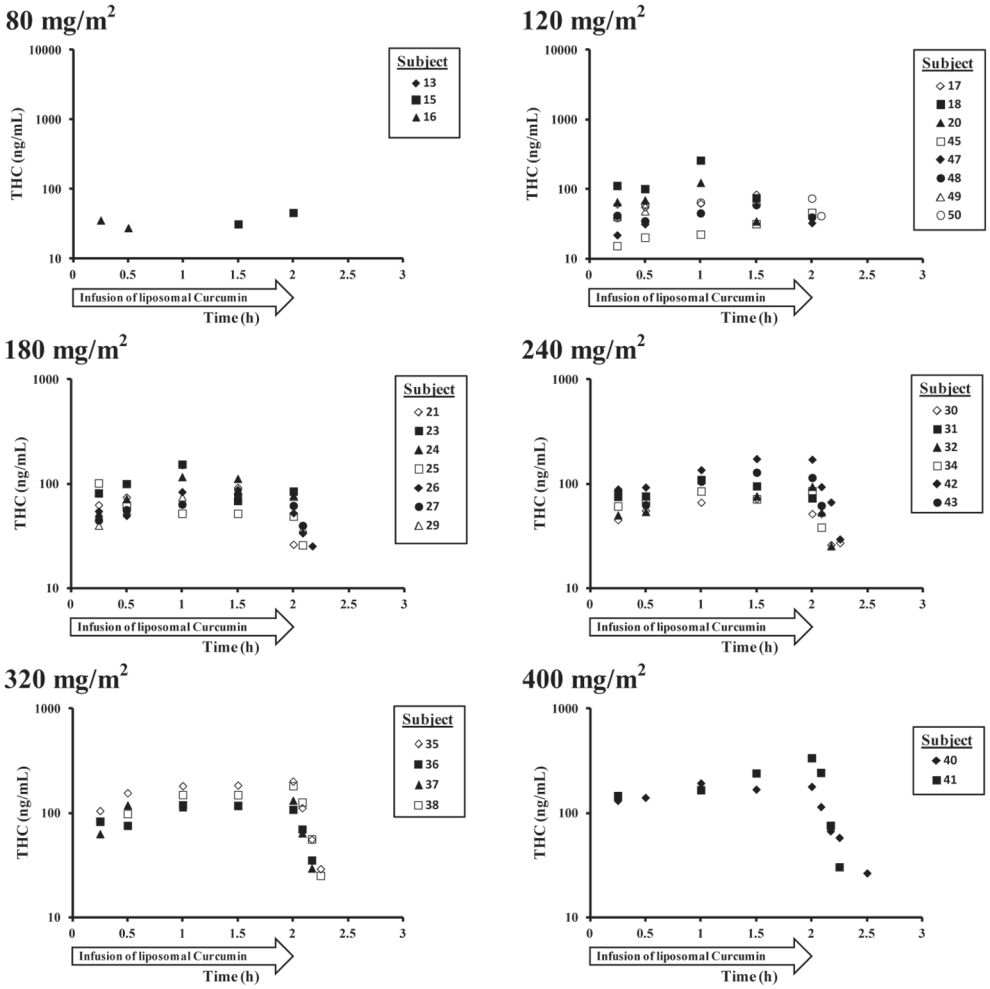

Figure 4. Individual plasma concentration time profiles for THC. Plasma concentrations of curcumin above the limit of quantification were not measured at times greater than 0.5 hours post-infusion. Plasma levels of THC at curcumin doses of 10, 20, and 40 mg/m^2 were all below the limit of quantification.

Table 3. Dose proportionality data.

Curcumin dose (mg/m^2)	n[1]	Dose normalized AUC_{last} ($ng \times h \times m^2/mL \times mg$)	Dose normalized C_{max} ($ng \times m^2/mL \times mg$)
20	3	6.6 ± 3.9	4.8 ± 3.1
40	3	11.7 ± 2.0	7.9 ± 0.3
80	3	12.4 ± 5.5	9.7 ± 4.5
120	8	8.4 ± 7.3	6.0 ± 5.3
180	7	14.1 ± 5.0	9.5 ± 3.7
240	6	8.3 ± 1.8	6.0 ± 1.6
320	4	11.3 ± 3.3	8.0 ± 1.4
400	2	9.7 ± 1.0	5.9 ± 1.0

Values are presented as the mean ± SD. Dose normalization data is not presented for the 10 mg/m^2 dose, due to the number of limited plasma concentration data points. There was no significant difference between doses for either the dose normalized AUC_{last} or dose normalized C_{max} values, one way ANOVA $p > 0.05$. [1]Number of determinations.

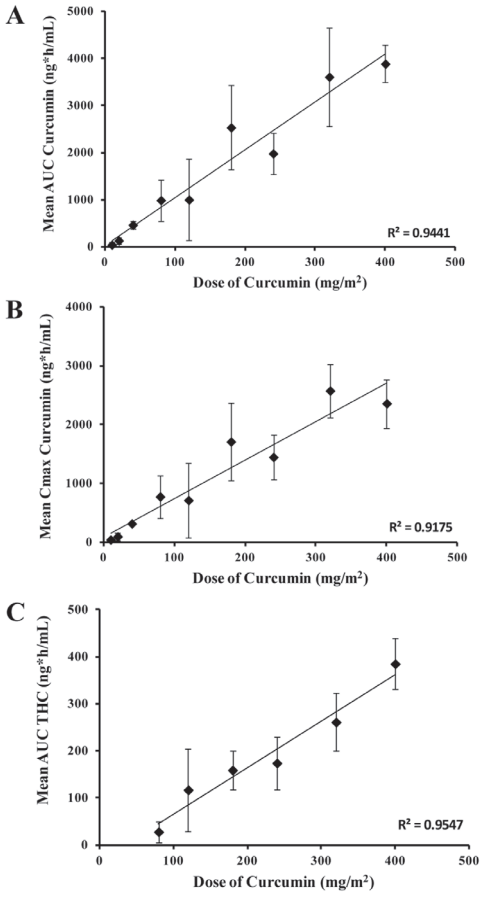

Figure 5. Relationship between plasma exposure and C_{max} for curcumin and plasma exposure for THC with dose. The relationship between (A) plasma exposure of curcumin and dose, dose range 10 – 400 mg/m^2, (B) C_{max} of curcumin and dose, dose range 10 – 400 mg/m^2, and (C) plasma exposure of THC and dose, dose range 80 – 400 mg/m^2 are presented with the values reported as the mean ± SD for the number of subjects shown in Table 2. R^2 is the correlation coefficient of linear regression.

with mean t_{max} and C_{max} values ranging from 0.4 to 1.2 hours and 41 to 265 ng/mL, respectively (Table 2) (Figures 3, 4). At the end of infusion, plasma concentrations of curcumin and THC decreased within 6 – 60 minutes to below the limit of quantitation. This is reflected by mean MRT_{last} values that ranged from 7 to 15 minutes for curcumin and 10 to 38 minutes for THC, the higher range of MRT_{last} values for THC consistent with the metabolic conversion of curcumin to THC. The rapid plasma decreases were also reflected by the times measured (t_{last}) for the last measured concentration above the limit of quantification, which for curcumin ranged from 0.9 to 2.8 hours (during ■■**word missing!** to 0.4 hours following infusion) and for THC ranged from 1.3 to 2.4 hours (during ■■**word missing!** to 0.4 hours following infusion). The last measurable concentrations (C_{last}) for curcumin ranged from 40 to 237 ng/mL and for THC ranged from 28 to 50 ng/mL.

The plasma exposures as represented by AUC for curcumin and THC during infusion ($AUC_{0–2h}$) and following infusion ($AUC_{2–6h}$) are presented in Tables 1 and 2, respectively. For curcumin the ratio of mean $AUC_{0–2h}$ to mean $AUC_{0–6h}$ ranged from 18 to 65 with mean AUC_{last} values ranging from 43 to 3,886 $ng \times h/mL$ in the dose range of 10 – 400 mg/m^2. For THC, the ratio of $AUC_{0–2h}$ to $AUC_{2–6h}$ was lower ranging from 2.7 to 3.9 with AUC_{last} values ranging from 28 to 385 $ng \times h/mL$ in the concentration range of 80 – 400 mg/m^2. Based on both the mean AUC_{last} and mean C_{max} values, the plasma exposure and levels of curcumin were found to be dose-proportional based on the linearity of mean AUC_{last} and mean C_{max} vs. dose (linear correlation coefficients, R^2 of 0.9441 and 0.9175, respectively) and no significant differences between dose groups for dose-normalized AUC_{last} and C_{max} (Table 3) (Figure 5). Furthermore, in the dose range of 80 – 400 mg/m^2 of liposomal curcumin, the plasma exposure to THC displayed a linear relationship with dose (linear correlation coefficient, R^2 of 0.9547).

Urine levels of curcumin or THC were measured from urine samples collected from start of infusion to 1 hour during infusion, 1 hour during infusion to 0.25 hours post-infusion, and 0.25 hours post-infusion to 4 hours post-infusion. Urinary curcumin concentrations were highest at 0 – 1 hour during

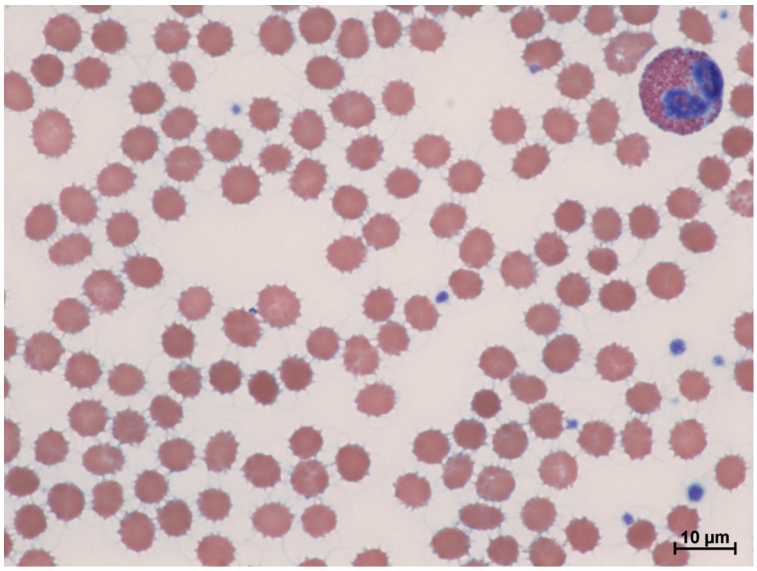

Figure 6. Echinocytes in blood smear. Blood smear (H & E staining) 15 minutes after administration of 400 mg/m^2 liposomal curcumin. Formation of echinocytes was transient and fully reversible.

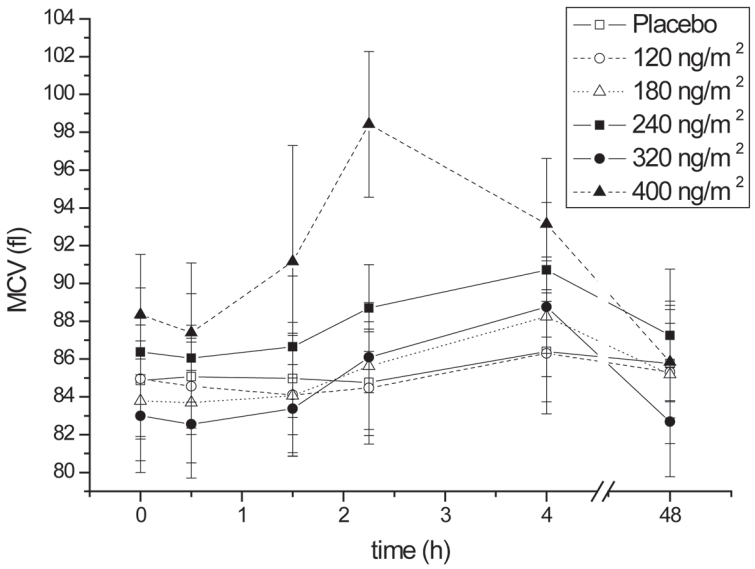

Figure 7. Mean red blood cellular volume following infusion of liposomal curcumin. Mean red blood cellular volume (MCV) following infusion with either different concentrations of liposomal curcumin or placebo. Values are expressed as means ± SD of 2 subjects (400 mg/m^2), 4 subjects (180 mg/m^2 and 320 mg/m^2), 5 subjects (120 mg/m^2), and 6 subjects (240 mg/m^2) per dose group, respectively.

infusion. The urinary excretion rate during infusion was ~ 25 µg/h and correlated only weakly with the dose of curcumin administered (correlation coefficient $r^2 = 0.17$). No THC levels were detectable in 56% of subjects in the dosage groups 120 – 400 mg/m^2.

Adverse events

In 3 subjects, glucosuria was detectable ~ 4 hours after end of the study drug infusion. This adverse event was not related to the dose of liposomal curcumin. Further investigations, showed that glucosuria was also measurable in these subjects when 4 mg dexamethasone without or with co-administration of glucose was administered (data not shown). Therefore, from subject 25 to 50 the premedication was reduced to diphenhydramine alone.

Red blood cell echinocyte formation was dose-related and detectable at a threshold dose of 120 mg/m^2 (Figure 6). The earliest onset of echinocyte formation was observed in blood smears 90 minutes after start of drug infusion. This morphological change was without clinical symptoms, was transient until 4 hours after the end of infusion, and was fully recovered 6 hours after the end of infusion. At a dose of 400 mg/m^2 an increase of mean cellular volume (MCV) was detected in the 2 subjects under study (Figure 7). Markers of hemolysis (HBDH, potassium, haptoglobin, LDH, erythrocytes, and hemoglobin) remained unchanged and in the reference range. In these 2 subjects, a high dose of intravenous corticosteroids was administered to prevent hemolysis.

25 subjects experienced at least 1 adverse event (AE). Overall, 49 AEs occurred of which 38 AEs were mild, 11 AEs were moderate and none was severe. The relationship to the drug was classified as probable or possible in 67% and as unlikely or unrelated in 33% of events, respectively. 9 AEs needed medical treatment and all events were resolved without sequelae. An AE summary is provided in Table 4.

Discussion

In-vitro as well as in-vivo studies have shown that curcumin influences cell signaling pathways and has anticancer, antiinflammatory, antioxidant, and antimicrobial properties [2, 3, 14]. The present liposomal formulation was developed to enhance the solubility and bioavailability of curcumin for use in humans.

Table 4. Adverse events summary.

MedDRA term	n
Gastrointestinal disorders	2
Diarrhea	
Nausea	
General disorders and administration site conditions	5
Asthenia	
Chest discomfort	
Feeling hot	
Pyrexia	
Infections and infestations	8
Nasopharyngitis	
Rhinitis	
Investigations	19
Aspartate aminotransferase increased	
Blood lactic acid increased	
Electrocardiogram QT prolonged	
Red blood cell burr cells present*	
Mean cell volume increased*	
Musculoskeletal and connective tissue disorders	2
Pain in extremity	
Nervous system disorders	6
Dizziness	
Headache	
Renal and urinary disorders	3
Glucosuria	
Respiratory, thoracic and mediastinal disorders	3
Epistaxis	
Oropharyngeal pain	
Vascular disorders	1
Circulatory collapse	

Events were categorized according to the Medical Dictionary for Regulatory Activities (MedDRA). *were dose-related.

In this first-in-human study, 10 – 400 mg/m^2 of liposomal curcumin were infused as a single intravenous dose over 2 hours. This regimen resulted in dose-dependent and dose-proportional increases of plasma curcumin (C_{max} 42 – 2,575 ng/mL) and its metabolite THC (C_{max} 41 – 265 ng/mL). The concentrations increased rapidly in the first 15 minutes and remained stable during the infusion time. In some dose groups, the maximum plasma concentration was observed prior to the end of infusion. This finding was not consistent across the study and may be explained either by the variability of the data or a "dose-loading" phenomenon (Figures 2, 4). After the termination of infusion, plasma levels of both curcumin and THC dropped rapidly such that either there was no plasma exposure above the limit of quantification post-infusion (at low doses) or the plasma exposure was 18 – 65 times lower for curcumin and 2.7 – 3.9 times lower for THC. The mean residence times (MRT) for doses of 120 – 400 mg/m^2 ranged from 10 to 15 minutes for curcumin and 10 to 16 minutes for THC. The observation that the plasma exposure to THC post-infusion compared to during infusion was not decreased as much as for curcumin, suggests that curcumin cleared rapidly from the plasma into tissues is still being metabolized to THC post-infusion. The high ratio of $AUC_{0–2h}$/$AUC_{2–6h}$ argues that continuous intravenous administration may be required if prolonged exposures to curcumin and THC are required for clinical efficacy.

Urinary excretion of curcumin and THC were trivial in the subjects under study and averaged 0.12% of total systemic clearance. These results are consistent with preclinical studies [7, 15].

While infusions were tolerated without symptoms and no local reaction was noted, reversible changes in red blood cell morphology occurred at the dose of 120 mg/m^2 or greater. These structural changes were paralleled by an increase in mean cellular volume of red blood cells (MCV) from 4 to 13 fl at doses of 180 – 400 mg/m^2. In the 2 subjects who received 400 mg/m^2 liposomal curcumin, venous serum lactate concentrations increased to a maximum of 3.7 mmol/L (normal range $\leq$ 2.2 mmol/L). Of note, all adverse events were transient and reversible, suggesting that close surveillance and monitoring of subjects may prevent hazardous experiences such as hemolysis. Furthermore, the rapid clearance of curcumin from the plasma via redistribution into tissues and metabolism may present a safety and therapeutic advantage.

The underlying mechanism(s) for the changes in red blood cell morphology is unclear. Similar red blood cell volumetric or spherical effects have been described for anticancer drugs with different pharmacodynamic actions. For example, echinocyte formation is observed with adriamycin, mesna, gamma globulins and 5-fluoruracil [16, 17, 18]. Diverse mechanisms can cause echinocyte formation, such as changes in cytoskeletal components, inositol phospholipids,

calcium-calmodulin-controlled kinase activity and membrane potential [19, 20, 21, 22, 23, 24]. One possible mechanism mediating the changes of red blood cell morphology involves an increase in intracellular Ca^{2+} by stimulation of Ca^{2+} entry through formation of ceramide as described by Banerjee et al. [25]. Previous in-vitro experiments have shown that not only curcumin, but liposomes containing predominantly DMPC caused echinocyte formation [26]. This effect may be due to an interaction and incorporation of liposomal bilayers into the red blood cell membrane as described by Cosimati et al. [27]. One potential limitation for the interpretation of this data was that the number of subjects per dose was low and the plasma concentrations varied within the groups.

In summary, a single intravenous dose of liposomal curcumin is considered safe up to a dose of 120 mg/m^2 when infused over a period of 2 hours. Liposomal curcumin-induced transient changes in red blood cell morphology, characterized by an increase in cellular volume and echinocyte formation were reversible at all doses administered.

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Conflict of interest

Lawrence Helson is CEO and President of SignPath Pharma Inc., which supported this study.

References

[1] *Aggarwal BB, Sundaram C, Malani N, Ichikawa H*. Curcumin: the Indian solid gold. Adv Exp Med Biol. 2007; *595:* 1-75.

[2] *Goel A, Jhurani S, Aggarwal BB*. Multi-targeted therapy by curcumin: how spicy is it? Mol Nutr Food Res. 2008; *52:* 1010-1030.

[3] *Ammon HP, Wahl MA*. Pharmacology of Curcuma longa. Planta Med. 1991; *57:* 1-7.

[4] *Kuttan R, Bhanumathy P, Nirmala K, George MC*. Potential anticancer activity of turmeric (Curcuma longa). Cancer Lett. 1985; *29:* 197-202.

[5] *Li L, Braiteh FS, Kurzrock R*. Liposome-encapsulated curcumin: in vitro and in vivo effects on proliferation, apoptosis, signaling, and angiogenesis. Cancer. 2005; *104:* 1322-1331.

[6] *Li L, Ahmed B, Mehta K, Kurzrock R*. Liposomal curcumin with and without oxaliplatin: effects on cell growth, apoptosis, and angiogenesis in colorectal cancer. Mol Cancer Ther. 2007; *6:* 1276-1282.

[7] *Helson L, Bolger G, Majeed M, Vcelar B, Pucaj K, Matabudul D*. Infusion pharmacokinetics of Lipocurc™ (liposomal curcumin) and its metabolite tetrahydrocurcumin in Beagle dogs. Anticancer Res. 2012; *32:* 4365-4370.

[8] *Wang YJ, Pan MH, Cheng AL, Lin LI, Ho YS, Hsieh CY, Lin JK*. Stability of curcumin in buffer solutions and characterization of its degradation products. J Pharm Biomed Anal. 1997; *15:* 1867-1876.

[9] *Sharma RA, Steward WP, Gescher AJ*. Pharmacokinetics and pharmacodynamics of curcumin. Adv Exp Med Biol. 2007; *595:* 453-470.

[10] *Sharma RA, Euden SA, Platton SL, Cooke DN, Shafayat A, Hewitt HR, Marczylo TH, Morgan B, Hemingway D, Plummer SM, Pirmohamed M, Gescher AJ, Steward WP*. Phase I clinical trial of oral curcumin: biomarkers of systemic activity and compliance. Clin Cancer Res. 2004; *10:* 6847-6854.

[11] *Cheng AL, Hsu CH, Lin JK, Hsu MM, Ho YF, Shen TS, Ko JY, Lin JT, Lin BR, Ming-Shiang W, Yu HS, Jee SH, Chen GS, Chen TM, Chen CA, Lai MK, Pu YS, Pan MH, Wang YJ, Tsai CC, et al*. Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. Anticancer Res. 2001; *21* (*4B*)*:* 2895-2900.

[12] *Vareed SK, Kakarala M, Ruffin MT, Crowell JA, Normolle DP, Djuric Z, Brenner DE*. Pharmacokinetics of curcumin conjugate metabolites in healthy human subjects. Cancer Epidemiol Biomarkers Prev. 2008; *17:* 1411-1417.

[13] report N-TV. Development and validation of an LC/MS/MS method for the measurement of Curcumin and Tetrahydrocurcumin (THC) in human plasma and its use in support of pharmacokinetic studies.2012. Report No.: NUCRO-TECHNICS PROJECT NO. 246975.

[14] *Aggarwal BB, Kumar A, Bharti AC*. Anticancer potential of curcumin: preclinical and clinical studies. Anticancer Res. 2003; *23* (*1A*)*:* 363-398.

[15] *Matabudul D, Pucaj K, Bolger G, Vcelar B, Majeed M, Helson L*. Tissue distribution of (Lipocurc™) liposomal curcumin and tetrahydrocurcumin following two- and eight-hour infusions in Beagle dogs. Anticancer Res. 2012; *32:* 4359-4364.

[16] *Baerlocher GM, Beer JH, Owen GR, Meiselman HJ, Reinhart WH*. The anti-neoplastic drug 5-fluorouracil produces echinocytosis and affects blood rheology. Br J Haematol. 1997; *99:* 426-432.

[17] *Reinhart WH, Baerlocher GM, Cerny T, Owen GR, Meiselman HJ, Beer JH*. Ifosfamide-induced stomatocytosis and mesna-induced echinocytosis: influence on biorheological properties of blood. Eur J Haematol. 1999; *62:* 223-230.

[18] *Suwalsky M, Hernández P, Villena F, Aguilar F, Sotomayor CP*. The anticancer drug adriamycin interacts with the human erythrocyte membrane. Z Naturforsch C. 1999; *54:* 271-277.
[19] *Nelson GA, Andrews ML, Karnovsky MJ*. Control of erythrocyte shape by calmodulin. J Cell Biol. 1983; *96:* 730-735.
[20] *Glaser R*. Echinocyte formation induced by potential changes of human red blood cells. J Membr Biol. 1982; *66:* 79-85.
[21] *Sheetz MP, Singer SJ*. Biological membranes as bilayer couples. A molecular mechanism of drug-erythrocyte interactions. Proc Natl Acad Sci USA. 1974; *71:* 4457-4461.
[22] *Anderson RA, Lovrien RE*. Erythrocyte membrane sidedness in lectin control of the Ca2+-A23187-mediated diskocyte goes to and comes from echinocyte conversion. Nature. 1981; *292:* 158-161.
[23] *Lovrien RE, Anderson RA*. Stoichiometry of wheat germ agglutinin as a morphology controlling agent and as a morphology controlling agent and as a morphology protective agent for the human erythrocyte. J Cell Biol. 1980; *85:* 534-548.
[24] *Ferrell JE Jr, Huestis WH*. Phosphoinositide metabolism and the morphology of human erythrocytes. J Cell Biol. 1984; *98:* 1992-1998.
[25] *Banerjee A, Kunwar A, Mishra B, Priyadarsini KI*. Concentration dependent antioxidant/pro-oxidant activity of curcumin studies from AAPH induced hemolysis of RBCs. Chem Biol Interact. 2008; *174:* 134-139.
[26] *Storka A, Vcelar B, Klickovic U, Gouya G, Weisshaar S, Aschauer S, Helson L, Wolzt M*. Effect of liposomal curcumin on red blood cells in vitro. Anticancer Res. 2013; *33:* 3629-3634.
[27] *Cosimati R, Milardi GL, Bombelli C, Bonincontro A, Bordi F, Mancini G, Risuleo G*. Interactions of DMPC and DMPC/gemini liposomes with the cell membrane investigated by electrorotation. Biochim Biophys Acta. 2013; *1828:* 352-356.