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ORIGINAL RESEARCH

The Safety and Exploration of the Pharmacokinetics of Intrapleural Liposomal Curcumin

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Background: Malignant pleural effusion (MPE) is the accumulation of fluid in the pleural cavity as a result of malignancies affecting the lung, pleura and mediastinal lymph nodes. Curcumin, a compound found in turmeric, has anti-cancer properties that could not only treat MPE accumulation but also reduce cancer burden. To our knowledge, direct administration of curcumin into the pleural cavity has never been reported, neither in animals nor in humans.

Purpose: To explore the compartmental distribution, targeted pharmacokinetics and the safety profile of liposomal curcumin following intrapleural and intravenous administration.

Methods: Liposomal curcumin (16 mg/kg) was administered into Fischer 344 rats by either intrapleural injection or intravenous infusion. The concentration of curcumin in plasma and tissues (lung, liver and diaphragm) were measured using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS). Blood and tissues were examined for pathological changes.

Results: No pleural or lung pathologies were observed following intrapleural liposomal curcumin administration. Total curcumin concentration peaked 1.5 hrs after the administration of intrapleural liposomal curcumin and red blood cell morphology appeared normal. A red blood cells abnormality (echinocytosis) was observed immediately and at 1.5 hrs after intravenous infusion of liposomal curcumin.

Conclusion: These results indicate that liposomal curcumin is safe when administered directly into the pleural cavity and may represent a viable alternative to intravenous infusion in patients with pleural-based tumors.

Keywords: malignant pleural effusion, liposomal, curcumin, intrapleural, local administration

Introduction

A malignant pleural effusion (MPE) is the accumulation of fluid in the pleural cavity as a result of malignancy. The most common causes of MPE are malignancies that have metastasized to the pleural or mediastinal lymph nodes; breast and lung cancer are the most prevalent causes in women and men, respectively. Malignant pleural mesothelioma, a tumor arising in the mesothelial cells lining the pleural cavity, also commonly results in MPE. Not only does MPE produce significant discomfort and breathing difficulties in these patients, but it is also a frequent cause of mortality.[1] Controlling recurrent MPE is an integral part of palliative care for these patients, which is achieved by either pleurodesis or insertion of an indwelling pleural catheter for ongoing drainage.[2]

The use of anti-cancer agents, in conjunction with MPE management, could help alleviate patient's symptoms and reduce their cancer burden.[3] Curcumin −a polyphenol

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derived from turmeric− can modulate numerous pathways involved in carcinogenesis, including those controlling inflammation, cell cycle progression, and angiogenesis and cell survival.[4] Curcumin also has the potential to help to reduce MPE since it can moderate numerous factors involved in fluid accumulation, including vascular endothelial growth factor-A (VEGF-A), interleukin-6 (IL-6) and tumor necrosis factor-alpha, although this has not been verified.[1,5–7] However, difficulties with clinical translation exist because of curcumin's low solubility in aqueous solution and oils, instability at physiological pH, low bioavailability and rapid molecular transformation and degradation.[4]

Liposomes are phospholipid vesicles that act as delivery systems for both hydrophobic and hydrophilic drugs. They are utilized to reduce early degradation and improve stability, biodistribution and cellular uptake.[8,9] In an effort to overcome curcumin's poor oral bioavailability and water solubility, researchers have developed a liposomal curcumin formulation, which has been safely administered in humans via intravenous infusion.[10,11] Liposomal curcumin could be administered directly into the pleural cavity of patients with MPE through an existing intrapleural catheter or at the time of pleurodesis. Intrapleural drug delivery is an attractive alternative to intravenous therapies for pleural cancers because i) drugs reach higher concentrations at the site of the tumor ii) concentrations are sustained for longer periods due to a slower clearance rate and iii) there are reduced systemic toxicities.[12–15] Numerous drugs including paclitaxel, bevacizumab and cisplatin have been administered into the pleural space in clinical trial settings to control malignant pleural effusion, alleviate symptoms, or slow disease progression.[12,15–25] To the best of our knowledge, the direct administration of curcumin into the pleural cavity has never been reported. The purpose of this study was to evaluate the safety and bio-distribution of a pharmaceutical-grade liposomal curcumin formulation after intrapleural administration in healthy rats.

Materials and Methods

Chemicals and Reagents

Liposomal curcumin (Lipocurc™) was a kind gift from SignPath Pharma Inc. (Sandy, Utah, United States). Liposomal curcumin was synthesized at Polymun Scientific GmbH, Vienna, Austria, according to the encapsulation protocol previously described.[20,21] The formulation was comprised of curcumin (6.0 mg/mL), DMPC (14:0–-1,2-dimyristoyl-sn-glycero-3-phosphocholine) (72 mg/mL) and DMPG (14:0–1,2-dimyristoyl-sn-glycero-3- phosphoryl-glycerol) (8.0 mg/mL). Liposomal curcumin exhibited a zeta potential of −36 mV at pH 5.0 and mean particle diameter of 117 nm. Aliquots were stored at −20°C in storage boxes that were protected from light and aliquots were thawed immediately before use to avoid degradation.

Animals

Male and female Fischer 344 rats (aged 12-weeks, Flinders University School of Medicine Animal Facility) were used for in vivo experiments. Rats were housed 3 per cage with Back-2-Nature Animal Bedding (Fibrecycle Pty Ltd, Queensland, Australia) in temperature-controlled (22±1°C), and humidity-controlled (60±5%) environment on a 12:12 light-dark cycle. Rats had free access to food (Gordon's Premium Rat and Mouse Pellets, Gordon's Specialty Stock Feed, New South Wales, Australia) and water. Approval for the use of animals was obtained from the Flinders University and Southern Adelaide Local Health Network Animal Welfare Committee (approval number 892/15) in accordance with the State Government of South Australia Animal Welfare Act, 1985 and the National Health and Medical Research Council Australian Code for the Care and Use of Animals for Scientific Purposes, 2013.

Liposomal Curcumin Administration Protocol

Intrapleural and Intravenous Administration of Liposomal Curcumin

Liposomal curcumin (16 mg/kg) was administered by intrapleural or intravenous delivery. The dose of liposomal curcumin that was used in this study was based on doses previously administered intravenously in human and animal studies.[11,26–30] Liposomal curcumin was administered into the pleural cavity of Fischer 344 rats (n=12, equal proportions of males and females) using an anterior subdiaphragmatic approach, which has been validating in our laboratory using a talc model of pleurodesis. Rats were anaesthetized before intrapleural curcumin injections using isoflurane (Veterinary Companies of Australia Pty Ltd, New South Wales, Australia) in an isoflurane induction chamber (Flinders University Biomedical Engineering, Adelaide, Australia) set at 4% isoflurane and 2% oxygen. Once fully anaesthetized rats were transferred to a nose mask with 2% isoflurane and 2% oxygen for ongoing anesthesia. Rats were given 0.3 mg/kg of buprenorphine for pain relief via a subcutaneous injection. A small section of the rat's chest

was shaved using an electric shaver to expose the bottom of the rib cage and xiphoid process. The injection point was positioned under the bottom of the right rib cage approximately 0.5 cm away from the xiphoid process. Liposomal curcumin was slowly administered into the right lateral side of the pleural cavity using a 25-gauge 16 mm needle. Rats were then taken off the isoflurane mask and were transferred to a recovery cage for post-procedural monitoring of respiratory rate, righting reflex and temperature. Blood was taken from the tail vein following intrapleural liposomal curcumin administration at 1.5 h, 24 h and 48 h, 1-week, 2-weeks and 3-weeks or until euthanasia (48 h (n=4), 1-week (n=4) and 3-weeks (n=4)). A separate group of male Fischer 344 rats (n=4) received liposomal curcumin via intravenous infusion. Prior to the infusion, rats were left in an incubator set to 35° C for at least 15 mins to allow vasodilation of the tail vein. Rats were anaesthetized before intravenous curcumin infusions using isoflurane in an isoflurane induction chamber with 3% isoflurane and 2% oxygen. Once fully anaesthetized, rats were transferred to a nose mask (1–2% isoflurane and 2% oxygen) for ongoing anesthesia on an insulated heat pad. Cannulation of the lateral tail vein was achieved using a 24G ¾ inch SURFLO I.V catheter set (Terumo Corporation, Tokyo, Japan). The cannula was flushed with 200 µL 10 IU of heparinized saline before it was immobilized. Liposomal curcumin was administered intravenously over 2 h at a dose rate of 3.4 mL/kg/h via a compact infusion pump (Harvard Apparatus, Holliston, Massachusetts, United States of America). Blood was taken immediately following cessation of the infusion and then at 1.5 h, 24 h and 48 h after the infusion. All rats in the intravenous infusion group were euthanized 48 h after the cessation of the infusion.

Tissue Collection

At euthanasia, approximately 100 mg each of lung, diaphragm and liver tissue was washed in saline and then snap-frozen in liquid nitrogen and stored at −80 °C until curcumin concentrations could be measured. Sections of lungs, diaphragm, small intestine, chest wall, brain, heart, liver and kidney were fixed in 4% formalin for histological analysis. Sections were labeled with CONFIRM Rabbit Anti-Human Ki-67[30–39] monoclonal antibody on a BenchMark ULTRA, automated immunohistochemistry slide staining system (Ventana Medical Systems, Oro Valley, Arizona, United States) using validated clinical procedures.

Blood Collection

Blood smears were performed at 0 h, 1.5 h, 24 h and 48 h, to evaluate the morphology of red blood cells after intrapleural and intravenous liposomal curcumin administration. Slides were then air-dried and Romanowsky-stained (Diff-Quik). Approximately, 200 µL of blood was collected into Lithium Heparin Microvette® (Sarstedt AG & Co. Nümbrecht, Germany) and centrifuged for 5 mins at 2000 g. Plasma was transferred into a fresh tube and stored at −80°C until ultra-performance liquid chromatography mass-spectrometry analysis (UPLC-MS) was performed.

Quantification of Curcumin Concentrations in Plasma via UPLC-MS

Sample Preparation

Enzymatic hydrolysis of curcumin conjugates was performed using β-glucuronidase, and sulfatase as previously described.[31,32] Briefly, plasma samples (200 µL) were diluted in 70 µL of water, 50 µL of β-glucuronidase (446 units) in 0.1 M-phosphate buffer (pH 6.8) and 45 µL of sulfatase (52 units) in 0.1 M sodium acetate buffer (pH 5.0) and incubated for 3.5 h at 37°C. Tissue samples were weighed and homogenized in 1 mL of human plasma. Calibrators and quality controls (QCs) were prepared using pooled human plasma from 5 healthy volunteers with no detectable curcumin. Plasma aliquots (190 µL) were spiked with 10 µL of a stock solution of curcumin in DMSO to yield final curcumin concentrations of 0, 10, 20, 100, 200, 500, 900, and 1000 ng/mL for calibration standards and 40, 160, and 800 ng/mL for QCs. The spiked plasma samples (200 µL), or homogenized tissue (200µL) were diluted in 70 µL of water, 50 µL of 0.1M-phosphate buffer (pH 6.8), and 45 µL 0.1 M sodium acetate buffer (pH 5.0). Rat plasma samples were diluted using pooled human plasma from 5 healthy volunteers to make up a final volume of 200 µL when blood volumes collected yielded less than 200 µL. Curcumin-d6 (Toronto Research Chemicals, C838502) was used as the internal standard and 10 µL of an 8 µg/mL stock solution was added to each sample, calibrator or QC prior to the curcumin extraction.

Extraction of Curcumin from Plasma Samples

The extraction method was carried out as previously described.[32,33] Briefly, samples were mixed with 1 mL of extraction buffer (ethyl acetate: methanol, 95:5; v/v) and vortex mixed for 30 seconds. The upper solvent and lower

aqueous phases were left to separate for 10 mins at room temperature. The lower aqueous layer was frozen in an ethanol/dry-ice bath, and then the upper solvent layer was decanted into a clean 5 mL tube. The extraction was repeated twice more on the lower aqueous phase for a total of three extractions. The pooled solvent extracts were evaporated to dryness using a miVac Duo concentrator for 30 mins at 40°C and the extracts were reconstituted in 100 µL of methanol. A 5 µL aliquot was analyzed by UPLC-mass spectrometry.

Quantitation of Curcumin

Analysis was performed on a Waters Acquity ultra-performance liquid chromatography (UPLC) system coupled to a Waters Premier quadrupole time of flight mass spectrometer (MS) with an electrospray ionization source operated in negative ionization mode. Time-of-flight data were collected in MS mode between 100 and 1000 Da with an instrument scan time of 1 second and inter-scan delay of 0.02 second. The experimental parameters were set as follows: capillary voltage 3.0 kV, source temperature 100°C, desolvation temperature 300°C, sampling and extraction cone voltages were 30 and 5 eV respectively. The collision gas flow was 0.5 mL per minute. Instrument control, data acquisition, and data processing were performed using Waters MassLynx version 4.1 software. The ultraviolet-visible chromatogram was recorded at 420 nm. Chromatographic separation was performed at a flow rate of 0.3 mL per minute on a Waters Acquity UPLC BEH C18 column (1.7 µm, 2.1 mm x 100 mm) held at 35 °C. The mobile phase composition was 10% v/v acetonitrile in water (mobile phase A) and acetonitrile (mobile phase B). Initial conditions were 70% mobile phase A and 30% mobile phase B. The proportion of mobile phase B was increased linearly to 60% over 5 mins and then returned to 30% for 2 mins to re-establish equilibrium before injection of the samples for analysis. Extracted ion chromatograms were obtained with a mass window of 0.02 Da from total ion chromatograms employing the m/z corresponding to the monoisotopic mass of curcumin ($[M\text{-}H]^-$ = 367.13 amu) and for curcumin-d6 as internal standard ($[M\text{-}H]^-$ = 373.16 amu). System suitability testing and quality control assessments were conducted according to quality management guidelines.[34]

Statistics

All results are expressed as mean ± standard deviation from at least three separate animals. A Mann–Whitney *t*-test was used to determine the significance of variability of curcumin concentrations between intrapleural and intravenous routes of administration.

Results

Macroscopic and Histological Observations

The visceral and parietal pleura appeared macroscopically normal following intrapleural administration of liposomal curcumin at all time points. There was no macroscopic or histological evidence of pleural adhesions following intrapleural liposomal curcumin administration. Normal pleural fluid volumes were observed at post-mortem in rats at all time points. No lung or pleural pathologies were observed in the histology sections 48 h, 1-week and 3-weeks following instillation of liposomal curcumin (Figure 1). We did not observe hemosiderin-laden macrophages, signs of hemorrhage or lung injury indicating that liposomal curcumin was not erroneously injected into the lung tissue. Ki-67 (a protein present during the active stages of the cell cycle) immunolabelling revealed no active proliferation in these cells. Similarly, morphologically unremarkable mesothelial and lung histology was observed in all rats after the administration of intravenous liposomal curcumin and Ki-67 immunolabelling revealed that there was no active proliferation in mesothelial cells. Heart, liver, kidney and chest wall from all time points displayed morphologically normal histological appearances (Supplementary Figure 1).

Analytical Assessment, System Suitability Testing and Quality Control

Curcumin and internal standards were resolved by UPLC-MS. The retention time of both curcumin and internal standard was 4.14 mins. The lower limit of quantification (LLOQ) of curcumin based on this method was 10 ng/mL. A system suitability assessment was used as part of the assay validation protocol. The assessment confirmed that: analyte peak area accuracy at the LLOQ remained between 80–120% and blank analyte peak area was less than 20% of LLOQ analyte peak area following detector saturation with six consecutive upper limit of quantification (ULOQ, 1000 ng/mL) samples. Additionally, variation in analyte and internal standard peak area at ULOQ was determined to be within the range recommended by quality management guidelines (% coefficient of variation (CV) <15%).[34] Four replicate quality control samples (40, 160, 800 ng/mL) were used to confirm that the assay precision (%CV <15%) and accuracy (within 85–115%) were compliant with quality management guidelines.

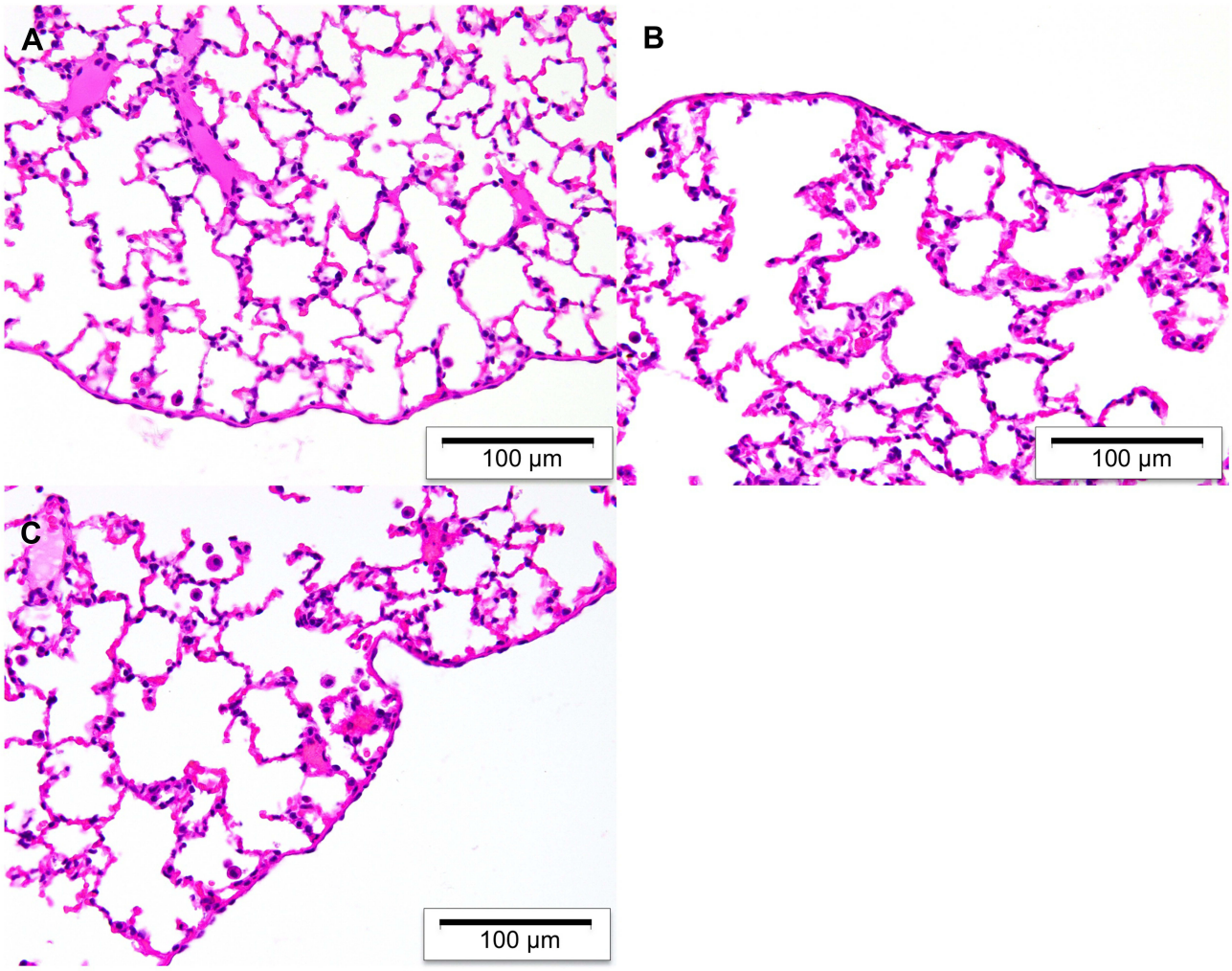

Figure 1 Representative H&E stained sections of rat visceral pleura and underlying lung parenchyma after intrapleural administration of liposomal curcumin. Morphologically normal histology was observed at (**A**) 48 h (**B**) 1-week and (**C**) 3-weeks. A total of four rats were assessed at each time point.

The Concentration of Curcumin in the Blood Following Liposomal Curcumin Injections

The concentration of total curcumin (free curcumin and the β-glucuronidase and sulfatase de-conjugation portion) was measured at various time points following administration of either intrapleural or intravenous liposomal curcumin (16 mg/kg). Total curcumin was detected in the plasma of rats up to 48 h after administration of intrapleural liposomal curcumin with concentrations peaking at 1.5 h (0.235 ± 0.0762 µg/mL). Curcumin was not detected in any sample at 1-week, 2-weeks and 3-weeks following intrapleural injections. High total curcumin concentrations were measured in plasma samples immediately after cessation of the intravenous infusion (1.276 ± 0.505 µg/mL); however, they were considerably reduced at 1.5 h (0.192 ± 0.06 µg/mL) (Table 1). Comparable concentrations of total curcumin were observed in the plasma of rats at 1.5 h, 24 h and 48 h irrespective of the delivery method (Figure 2).

The Concentration of Curcumin in Tissues Following Liposomal Curcumin Injections

Free curcumin was detected at similar concentrations in the lung, diaphragm and liver tissues of rats 48 h following intrapleural and intravenous liposomal curcumin administration (Table 2). No significant difference in tissue concentrations was detected amongst the rats in the intrapleural injection and intravenous infusion groups.

Red Blood Cell Morphology

We observed changes in red blood cell morphology immediately and 1.5 h after intravenous liposomal curcumin

Table 1 Total Curcumin Plasma Concentrations (Mean ± Standard Deviation) Following Intrapleural and Intravenous Administration of Liposomal Curcumin (16 mg/kg). Values That Were Below the Detection Limit of the Assay Were Assigned a Value of 0 μg/Ml. No Significant Difference in Plasma Concentrations Was Detected Between the Rats in the Intrapleural Injection and Intravenous Infusion Group (p=0.287, p=0.2545, p=0.6476, for 1.5 h, 24 h and 48 h Respectively)

Time (Hours)	Intrapleural Administration (μg/mL) [a]	Intravenous Infusion (μg/mL) [a]
0 h	Not measured	1.276 ± 0.505 (n=4) [b]
1.5 h	0.235 ± 0.0762 (n=10) [b]	0.192 ± 0.06 (n=3) [b]
24 h	0.025 ± 0.022 (n=9) [b]	0.007 ± 0.01 (n=3) [b]
48 h	0.006 ± 0.009 (n=9) [b]	0.011 ± 0.03 (n=4) [b]
168 h (1-week)	Not detected (n=6) [b]	Not measured
336 h (2-weeks)	Not detected (n=3) [b]	Not measured
504 h (3-weeks)	Not detected (n=3) [b]	Not measured

Notes: [a]Values are presented as the mean ± standard deviation of at least 3 separate animals. [b]Number of animals.

infusion. Red blood cells showed marked echinocytosis, an abnormality wherein numerous, spikey projections are present on the cell membrane, indicating that red blood cells are at risk of rupturing (Figure 3). Echinocytes were absent from blood samples at 24 h and 48 h. Red blood cells displayed normal cell morphology at all time points following intrapleural liposomal curcumin administration (Figure 3).

Discussion

Curcumin is an attractive potential anti-cancer agent as it can act on a wide range of molecular pathways to

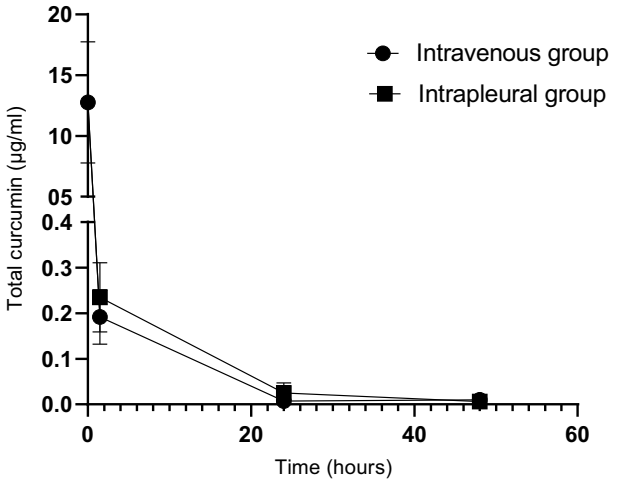

Figure 2 The concentration of total curcumin in the plasma of rats following intravenous and intrapleural administration of liposomal curcumin (16 mg/kg). Each data point represents the mean total curcumin concentration in at least three separate animals and error bars represent the standard deviation. Values that were below the detection limit of the assay were assigned a value of 0 μg/mL.

Table 2 Curcumin tissue Concentrations (Mean ± Standard Deviation) Following Intrapleural and Intravenous Administration of Liposomal Curcumin (16 mg/kg). No Significant Difference in Tissue Concentrations Was Detected Between the Rats in the Intrapleural Injection and Intravenous Infusion Group (p=0.4857, p=0.3429, p=0.6857, for Diaphragm, Lung and Liver Respectively)

Delivery Method	Concentration of Curcumin (μg/g)[a]		
	Diaphragm	Lung	Liver
Intrapleural	0.1281 ± 0.076	0.17585 ± 0.193	0.02995 ± 0.029
Intravenous	0.1737 ± 0.	0.06515 ± 0.017	0.03487 ± 0.012

Notes: [a]Values are presented as the mean ± standard deviation of 4 separate animals.

stimulate tumour cell death and decrease tumour cell proliferation including phosphatidylinositol-3-kinase (PI3K)/Akt signaling,[35–39] Nuclear Factor (NF)-κB, and JAK/STAT3 signaling.[40] It has also been shown to reduce chemotherapy-induced toxic side effects.[41,42] To the best of our knowledge, curcumin has never been administered directly into the pleural cavity of animals, or humans. Therefore, the safety and compartmental distribution of intrapleural liposomal curcumin following intrapleural needs to be evaluated.

We detected peak total curcumin plasma concentrations in the plasma 1.5 h after intrapleural delivery of liposomal curcumin, indicating that a proportion of curcumin had entered the systemic circulation. These peak plasma concentrations are comparable to the systemic levels of total curcumin that we have previously measured in rats after consumption of an oral, bioavailable curcumin formulation, which can be purchased over-the-counter for human use and therefore is considered safe.[43] We detected little to no total curcumin in the plasma of rats at 24 h, and 48 h after intrapleural administration of liposomal curcumin, suggesting that liposomal curcumin is mostly metabolized or distributed to blood cells or tissues within the first 24 h after administration. We detected high levels of total curcumin in the plasma immediately following intravenous infusion of liposomal curcumin (1.276 ± 0.505 μg/mL). Total curcumin plasma concentration rapidly dropped 1.5 h after cessation of the infusion (0.192 ± 0.06 μg/mL), which was consistent with other studies conducted in animals and humans that assessed the pharmacokinetics of intravenous liposomal curcumin.[11,26] In humans, plasma concentrations of free curcumin were not detected above the limit of detection (25 ng/mL) at times greater than 1 hr post-infusion.[11] Bolger and colleagues recently established that liposomal curcumin rapidly diffuses into peripheral blood mononuclear cells and red blood cells; therefore, curcumin may be present

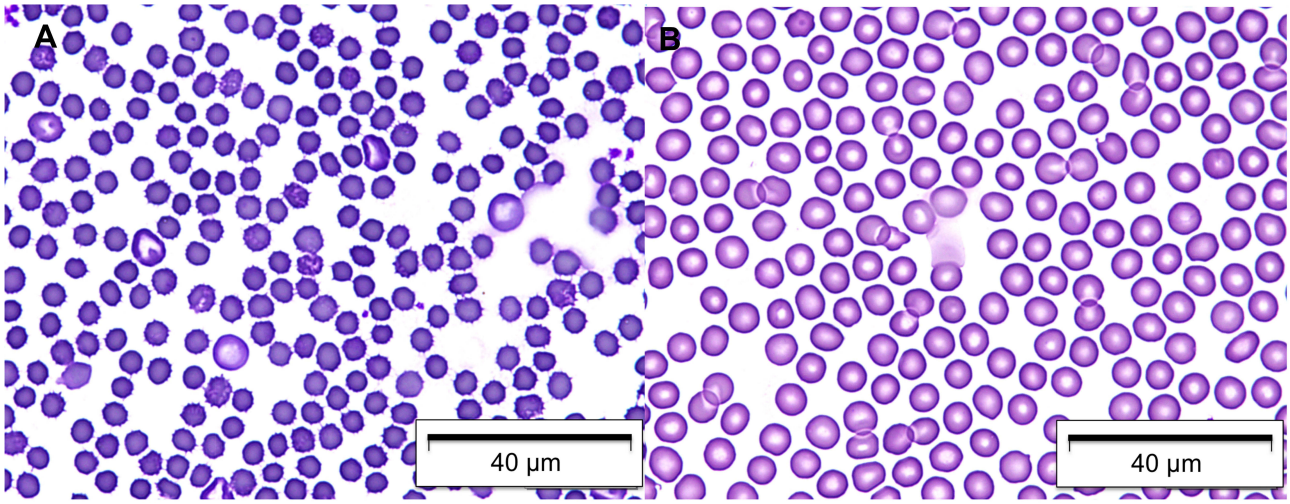

Figure 3 Representative Romanowski stained blood smears collected following intravenous infusion and intrapleural injection of liposomal curcumin (16 mg/kg) (**A**) Echinocyte formation was observed in the blood 1.5 h after intravenous liposomal curcumin infusions (**B**) Normal erythrocyte morphology was observed at 1.5 h following the administration of intrapleural liposomal curcumin. A total of four rats were assessed in each group.

with circulating blood cells and subsequently distributed to tissues.[44,45]

We observed transient echinocytosis and possible hemolysis in the blood of rats following a 2 h intravenous infusion of liposomal curcumin. Our results are in agreement with data from other studies investigating the safety of intravenous liposomal curcumin administration.[10,11,26,27,46] Several factors can trigger echinocytosis, which include, but are not limited to, uremia, chronic renal disease, liver disease and hyperlipidemia. To confirm that the observed echinocytosis was real and not an artifact of the processes of drying or staining of the blood sample on the slide, control and test blood smears were run alongside and the former showed normal morphology. Storka and colleagues demonstrated that both empty liposomes and curcumin itself could contribute to echinocytosis in vivo, and may indicate dose-limiting toxicity.[46] In advanced cancer patients, researchers observed a significant increase in hematological adverse events in patients receiving intravenous liposomal curcumin (300 mg/m^2), including one case of dose-limiting hemolysis.[10] Here, we observed normal red blood cell morphology in rats after the administration of intrapleural liposomal curcumin at all time points. This was not surprising since we observed lower peak concentrations in the systemic circulation after intrapleural delivery. From these data, we conclude that liposomal curcumin can be administered at higher concentrations in the pleural cavity without causing red blood cell abnormalities. Importantly, we also showed that intrapleural delivery of liposomal curcumin was not associated with pleural or lung toxicity in healthy rats, indicating that this mode of delivery is a feasible alternative to intravenous infusion, which may achieve higher drug concentrations within a pleural-based tumour. We utilized both male and female rats to investigate the effects of intrapleural liposomal curcumin in accordance with The National Health and Medical Research Council (NHMRC) 'guidelines for best practice methodology for the use of animals for scientific purposes' as sex-specific variation in angiogenesis, inflammation and wound healing exist.[47]

We measured free curcumin in diaphragm and lungs to estimate the amount of curcumin that diffused from the pleural cavity into surrounding tissues after intrapleural administration and compared the values to those found after intravenous administration. We also measured the concentration of free curcumin in the liver, as this is where curcumin is predominately metabolized. We detected free curcumin in the diaphragm, lungs and liver at similar concentrations in both the intrapleural and intravenous administration groups. This was expected since little to no total curcumin was detected in the plasma at the 48 h time point. Measuring curcumin tissue concentrations at earlier time points in an MPE tumor model, would be valuable to ascertain if intrapleural delivery is, in fact, superior to intravenous delivery when targeting pleural tumors.

Liposomal-drug release rates will impact a drug's ability to elicit a therapeutic response. Ando and colleagues investigated intrapleural delivery of two liposomal formulations of pemetrexed: cholesterol-containing, and cholesterol-free liposomes, in an orthotopic mouse model of mesothelioma.[48] The authors found that only the cholesterol-free liposomes reduced tumor growth. This was thought to be dependent on the

higher release rate of pemetrexed from the cholesterol-free liposomes since the incorporation of cholesterol in liposomes can increase membrane rigidity, thereby delaying the drug-release. The liposomal curcumin used in this study does not contain cholesterol and has an average particle size of 117 nm, (a comparable size to both liposomes utilized in these studies (cholesterol-liposomes; 117.8 nm, cholesterol-free liposomes; 103.8 nm)). Additionally, our results indicate that liposomal curcumin is not retained within the pleural cavity past 48 h, suggesting that liposomal curcumin is a suitable liposome formulation to deliver high doses of curcumin to a pleural tumor by intrapleural administration.

These experiments were conducted in healthy animals and therefore, may not reflect the situation in patients suffering from MPE. It is important to note that pleural pharmacokinetics may be altered in patients with an MPE; For example, the propensity of a drug to enter systemic circulation may be limited if a tumor obstructs the lymphatic stomata or if the lymphatic vessels become saturated due to the presence of an MPE. Consequently, drugs are more likely to diffuse into the visceral and parietal pleura, thereby maximizing drug exposure to the tumor. An obstructing tumor may slow the redistribution of drugs into blood, reducing systemic toxicities, but as a consequence, may also increase the risk of local toxicities, such as pleural adhesions.[49,50] Pleural adhesions are induced in patients undergoing talc pleurodesis as a way to prevent recurrent pleural effusion and are not considered life-threatening, and are indeed desired in this circumstance.[51] Nevertheless, Marazioti and colleagues recently demonstrated that there was no difference in liposome retention time between healthy mice and mice with pleural adenocarcinoma.[52]

Intrapleural liposomal curcumin therapy offers several potential advantages over intravenous therapy in patients with primary and secondary malignancies of the pleura. Few studies have directly compared intrapleural and intravenous delivery of drugs; but these studies have consistently shown that intrapleural administration reduces peak plasma levels, reduces systemic toxicity and yields a higher drug concentration at the pleura compared with intravenous administration.[12–14,50,53] The position of the tumour cells adjacent to the pleural cavity provides a unique opportunity to administer therapeutics directly to the tumour site. Therapeutic delivery via an existing pleura catheter or at pleurodesis means patients could be given tumour-site targeted therapies while also avoiding any additional needling of the pleura. The efficacy of intrapleural liposomal curcumin may be restricted in areas of the tumor that does not have a direct connection to the pleural cavity. Areas of locculated pleural effusion, chest wall invasion and the mediastinum showed no tumor response towards intrapleural liposomal-entrapped chemotherapy.[25] Intravenous liposomal curcumin could be used in combination with intrapleural administration as these may target regions of tumors that are not in direct contact with the pleural cavity.

Conclusion

No local or systemic toxicity was observed following intrapleural administration liposomal curcumin, indicating that it is a safe alternative to intravenous administration. We hypothesize that intrapleural liposomal curcumin could provide patients with MPE an alternative approach to chemotherapy, which could help to alleviate their symptoms, and reduce their cancer burden. Additionally, curcumin could be used as an adjunct therapy to improve outcomes and reduce toxic side effects. From a practice standpoint, liposomal curcumin could be delivered via patients existing indwelling pleural catheter, which is placed to manage pleural fluid drainage, or at the time of pleurodesis. Further investigations are required to determine the efficacy of intrapleural liposomal curcumin in patients with MPE.

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Disclosure

Dr. Sordillo is the Chief Scientific Officer at SignPath Pharma, Inc. Dr Sordillo has a patent “Numerous” issued to SignPath Pharma, Inc. Professor Sonja Klebe prepares medicolegal reports for the courts of Australia on the diagnosis of lung disease, outside the submitted work. The authors report no other conflicts of interest in this work.

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