

# The Safety and Exploration of the Pharmacokinetics of Intrapleural Liposomal Curcumin

This article was published in the following Dove Press journal:  
*International Journal of Nanomedicine*

Ashleigh Hocking<sup>1</sup>

Sara Tommasi<sup>2</sup>

Peter Sordillo<sup>3</sup>

Sonja Klebe<sup>1,4</sup>

<sup>1</sup>Department of Anatomical Pathology, Flinders University, Adelaide, SA, Australia; <sup>2</sup>Department of Clinical Pharmacology, Flinders University, Adelaide, SA, Australia; <sup>3</sup>SignPath Pharma Inc, New York, NY, USA; <sup>4</sup>Department of Surgical Pathology, SA Health, Flinders Medical Centre, Bedford Park, SA, Australia

**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.<sup>1</sup> 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.<sup>2</sup>

The use of anti-cancer agents, in conjunction with MPE management, could help alleviate patient's symptoms and reduce their cancer burden.<sup>3</sup> Curcumin—a polyphenol

Correspondence: Ashleigh Hocking  
Department of Anatomical Pathology,  
Flinders University, Flinders Medical  
Centre, Flinders Drive, Bedford Park, SA  
5041, Australia  
Email ash.hocking@flinders.edu.au

derived from turmeric— can modulate numerous pathways involved in carcinogenesis, including those controlling inflammation, cell cycle progression, and angiogenesis and cell survival.<sup>4</sup> 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.<sup>1,5–7</sup> 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.<sup>4</sup>

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.<sup>8,9</sup> 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.<sup>10,11</sup> 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.<sup>12–15</sup> 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.<sup>12,15–25</sup> 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.<sup>20,21</sup> 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-phosphorylglycerol) (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.<sup>11,26–30</sup> Liposomal curcumin was administered into the pleural cavity of Fischer 344 rats (n=12, equal proportions of males and females) using an anterior sub-diaphragmatic 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 3/4 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<sup>30–39</sup> 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.<sup>31,32</sup> 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.<sup>32,33</sup> 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 ( $[M-H]^- = 367.13$  amu) and for curcumin-d6 as internal standard ( $[M-H]^- = 373.16$  amu). System suitability testing and quality control assessments were conducted according to quality management guidelines.<sup>34</sup>

## Statistics

All results are expressed as mean  $\pm$  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

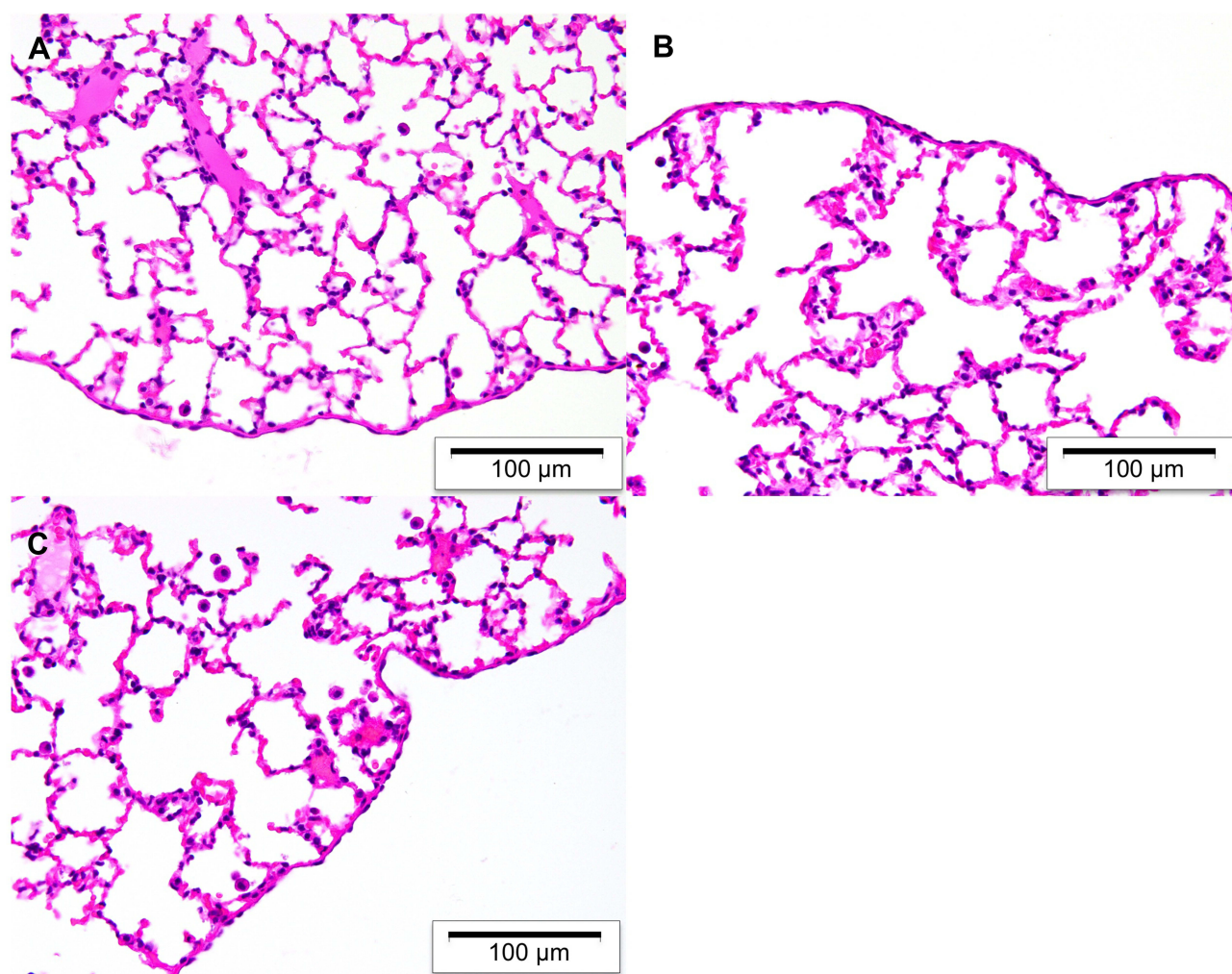
### Macroscopic and Histological Observations

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%).<sup>34</sup> 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  $\beta$ -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 ( $0.235 \pm 0.0762 \mu\text{g/mL}$ ). 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 ( $1.276 \pm 0.505 \mu\text{g/mL}$ ); however, they were considerably reduced at 1.5 h ( $0.192 \pm 0.06 \mu\text{g/mL}$ ) (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  $\pm$  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  $\mu\text{g/mL}$ . 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)

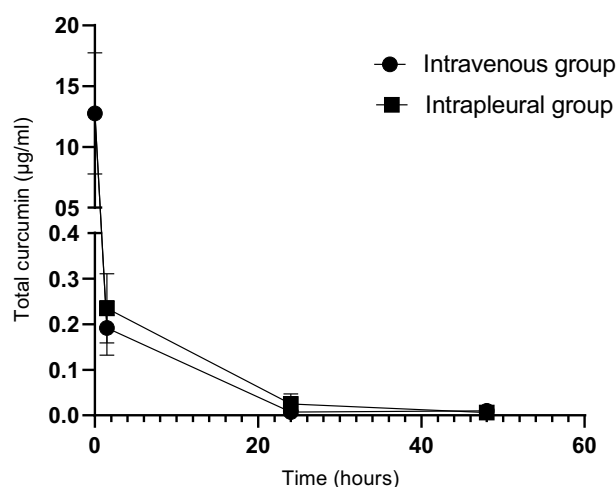
Time (Hours)	Intrapleural Administration ( $\mu\text{g/mL}$ ) <sup>a</sup>	Intravenous Infusion ( $\mu\text{g/mL}$ ) <sup>a</sup>
0 h	Not measured	1.276 $\pm$ 0.505 (n=4) <sup>b</sup>
1.5 h	0.235 $\pm$ 0.0762 (n=10) <sup>b</sup>	0.192 $\pm$ 0.06 (n=3) <sup>b</sup>
24 h	0.025 $\pm$ 0.022 (n=9) <sup>b</sup>	0.007 $\pm$ 0.01 (n=3) <sup>b</sup>
48 h	0.006 $\pm$ 0.009 (n=9) <sup>b</sup>	0.011 $\pm$ 0.03 (n=4) <sup>b</sup>
168 h (1-week)	Not detected (n=6) <sup>b</sup>	Not measured
336 h (2-weeks)	Not detected (n=3) <sup>b</sup>	Not measured
504 h (3-weeks)	Not detected (n=3) <sup>b</sup>	Not measured

**Notes:** <sup>a</sup>Values are presented as the mean  $\pm$  standard deviation of at least 3 separate animals. <sup>b</sup>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  $\mu\text{g/mL}$ .

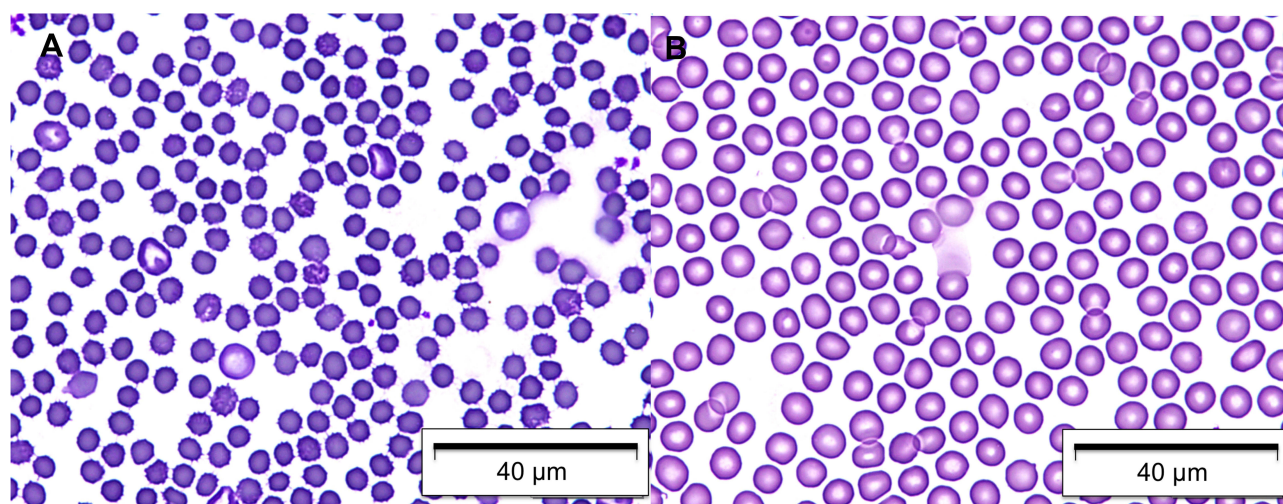
**Table 2** Curcumin tissue Concentrations (Mean  $\pm$  Standard Deviation) Following Intrapleural and Intravenous Administration of Liposomal Curcumin (16 mg/kg). No Significant Difference in Tissue Concentrations Was Detected Between the Rats in the Intrapleural Injection and Intravenous Infusion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

Delivery Method	Concentration of Curcumin ( $\mu\text{g/g}$ ) <sup>a</sup>		
	Diaphragm	Lung	Liver
Intrapleural	0.1281 $\pm$ 0.076	0.17585 $\pm$ 0.193	0.02995 $\pm$ 0.029
Intravenous	0.1737 $\pm$ 0.	0.06515 $\pm$ 0.017	0.03487 $\pm$ 0.012

**Notes:** <sup>a</sup>Values are presented as the mean  $\pm$  standard deviation of 4 separate animals.

stimulate tumour cell death and decrease tumour cell proliferation including phosphatidylinositol-3-kinase (PI3K)/Akt signaling,<sup>35–39</sup> Nuclear Factor (NF)- $\kappa$ B, and JAK/STAT3 signaling.<sup>40</sup> It has also been shown to reduce chemotherapy-induced toxic side effects.<sup>41,42</sup> 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.<sup>43</sup> 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  $\pm$  0.505  $\mu\text{g/mL}$ ). Total curcumin plasma concentration rapidly dropped 1.5 h after cessation of the infusion (0.192  $\pm$  0.06  $\mu\text{g/mL}$ ), which was consistent with other studies conducted in animals and humans that assessed the pharmacokinetics of intravenous liposomal curcumin.<sup>11,26</sup> 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.<sup>11</sup> 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.<sup>44,45</sup>

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.<sup>10,11,26,27,46</sup> 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.<sup>46</sup> In advanced cancer patients, researchers observed a significant increase in hematological adverse events in patients receiving intravenous liposomal curcumin (300 mg/m<sup>2</sup>), including one case of dose-limiting hemolysis.<sup>10</sup> 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.<sup>47</sup>

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 predominantly 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.<sup>48</sup> 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.<sup>49,50</sup> 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.<sup>51</sup> Nevertheless, Marazioti and colleagues recently demonstrated that there was no difference in liposome retention time between healthy mice and mice with pleural adenocarcinoma.<sup>52</sup>

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.<sup>12–14,50,53</sup> 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 loculated pleural effusion, chest wall invasion and the mediastinum showed no tumor response towards intrapleural liposomal-entrapped chemotherapy.<sup>25</sup> 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.

## Acknowledgments

This research was funded by the Tour de Cure Pioneering Cancer Research Grant. Liposomal curcumin was a kind gift from SignPath Pharma Inc.

## 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.

## References

1. Psallidas I, Kalomenidis I, Porcel JM, Robinson BW, Stathopoulos GT. Malignant pleural effusion: from bench to bedside. *Eur Respir Rev*. 2016;25(140):189–198. doi:10.1183/16000617.0019-2016
2. Thomas R, Fysh ETH, Smith NA, et al. Effect of an indwelling pleural catheter vs talc pleurodesis on hospitalization days in patients with malignant pleural effusion: the AMPLE randomized clinical trial. *JAMA*. 2017;318(19):1903–1912. doi:10.1001/jama.2017.17426



3. Bibby AC, Dorn P, Psallidas I, et al. ERS/EACTS statement on the management of malignant pleural effusions. *Eur Respir J*. 2018;52(1):1800349. doi:10.1183/13993003.00349-2018
4. Heger M, van Golen RF, Broekgaarden M, Michel MC. The molecular basis for the pharmacokinetics and pharmacodynamics of curcumin and its metabolites in relation to cancer. *Pharmacol Rev*. 2014;66(1):222–307.
5. Yeh HH, Lai WW, Chen HH, Liu HS, Su WC. Autocrine IL-6-induced Stat3 activation contributes to the pathogenesis of lung adenocarcinoma and malignant pleural effusion. *Oncogene*. 2006;25(31):4300–4309. doi:10.1038/sj.onc.1209464
6. Stathopoulos GT, Kollintza A, Moschos C, et al. Tumor necrosis factor- $\alpha$  promotes malignant pleural effusion. *Cancer Res*. 2007;67(20):9825–9834. doi:10.1158/0008-5472.CAN-07-1064
7. Shanmugam MK, Rane G, Kanchi MM, et al. The multifaceted role of curcumin in cancer prevention and treatment. *Molecules*. 2015;20(2):2728–2769. doi:10.3390/molecules20022728
8. Feng T, Wei Y, Lee RJ, Zhao L. Liposomal curcumin and its application in cancer. *Int J Nanomedicine*. 2017;12:6027–6044. doi:10.2147/IJN
9. Sercombe L, Veerati T, Moheimani F, Wu SY, Sood AK, Hua S. Advances and challenges of liposome assisted drug delivery. *Front Pharmacol*. 2015;6:286. doi:10.3389/fphar.2015.00286
10. Greil R, Greil-Ressler S, Weiss L, et al. A Phase I dose-escalation study on the safety, tolerability and activity of liposomal curcumin (Lipocur<sup>TM</sup>) in patients with locally advanced or metastatic cancer. *Cancer Chemother Pharmacol*. 2018;82(4):695–706. doi:10.1007/s00280-018-3654-0
11. Storka A, Vcelar B, Klickovic U, et al. Safety, tolerability and pharmacokinetics of liposomal curcumin in healthy humans. *Int J Clin Pharmacol Ther*. 2015;53(1):54–65. doi:10.5414/CP202076
12. Sakaguchi H, Ishida H, Nitanda H, Yamazaki N, Kaneko K, Kobayashi K. Pharmacokinetic evaluation of intrapleural perfusion with hyperthermic chemotherapy using cisplatin in patients with malignant pleural effusion. *Lung Cancer*. 2017;104:70–74. doi:10.1016/j.lungcan.2016.12.015
13. Froudarakis ME, Greillier L, Monjanel-Mouterde S, et al. Intrapleural administration of lipoplatin in an animal model. *Lung Cancer*. 2011;72(1):78–83. doi:10.1016/j.lungcan.2010.07.010
14. Li J, Tang J, Li Y, Yu J, Zhang B, Yu C. Pharmacokinetic profile of paclitaxel in the plasma, lung, and diaphragm following intravenous or intrapleural administration in rats. *Thorac Cancer*. 2015;6(1):43–48. doi:10.1111/1759-7714.12139
15. Perng RP, Chen YM, Wu MF, et al. Phase II trial of intrapleural paclitaxel injection for non-small-cell lung cancer patients with malignant pleural effusions. *Respir Med*. 1998;92(3):473–479. doi:10.1016/S0954-6111(98)90294-3
16. Biao Xue R, Hui P, Wenlong G, Shuanying Y. Evaluation of efficacy and safety for recombinant human adenovirus-p53 in the control of the malignant pleural effusions via thoracic perfusion. *Sci Rep*. 2016;6:39355. doi:10.1038/srep39355
17. Biao Xue R, Xiguang C, Hua L, Wenlong G, Shuanying Y. Thoracic perfusion of recombinant human endostatin (Endostar) combined with chemotherapeutic agents versus chemotherapeutic agents alone for treating malignant pleural effusions: a systematic evaluation and meta-analysis. *BMC Cancer*. 2016;16(1):888. doi:10.1186/s12885-016-2935-4
18. Hu R, Jiang H, Li H, Wei D, Wang G, Ma S. Intrapleural perfusion thermo-chemotherapy for pleural effusion caused by lung carcinoma under VATS. *J Thorac Dis*. 2017;9(5):1317–1321. doi:10.21037/jtd
19. Ishida A, Miyazawa T, Miyazu Y, et al. Intrapleural cisplatin and OK432 therapy for malignant pleural effusion caused by non-small cell lung cancer. *Respirology*. 2006;11(1):90–97. doi:10.1111/res.2006.11.issue-1
20. Lombardi G, Nicoletto MO, Gusella M, et al. Intrapleural paclitaxel for malignant pleural effusion from ovarian and breast cancer: a phase II study with pharmacokinetic analysis. *Cancer Chemother Pharmacol*. 2012;69(3):781–787. doi:10.1007/s00280-011-1765-y
21. Serman DH, Alley E, Stevenson JP, et al. Pilot and feasibility trial evaluating immuno-gene therapy of malignant mesothelioma using intrapleural delivery of adenovirus-IFN $\alpha$  combined with chemotherapy. *Clin Cancer Res*. 2016;22(15):3791–3800. doi:10.1158/1078-0432.CCR-15-2133
22. Serman DH, Recio A, Carroll RG, et al. A Phase I clinical trial of single-dose intrapleural IFN- $\beta$  gene transfer for malignant pleural mesothelioma and metastatic pleural effusions: high rate of antitumor immune responses. *Clin Cancer Res*. 2007;13(15 Pt 1):4456–4466. doi:10.1158/1078-0432.CCR-07-0403
23. Perez-Soler R, Shin DM, Siddik ZH, et al. Phase I clinical and pharmacological study of liposome-entrapped NDDP administered intrapleurally in patients with malignant pleural effusions. *Clin Cancer Res*. 1997;3(3):373–379.
24. Perng RP, Wu MF, Lin SY, Chen YM, Lin JY, Whang-Peng J. A phase I feasibility and pharmacokinetic study of intrapleural paclitaxel in patients with malignant pleural effusions. *Anticancer Drugs*. 1997;8(6):565–573. doi:10.1097/00001813-199707000-00003
25. Lu C, Perez-Soler R, Piperdi B, et al. Phase II study of a liposome-entrapped cisplatin analog (L-NDDP) administered intrapleurally and pathologic response rates in patients with malignant pleural mesothelioma. *J Clin Oncol*. 2005;23(15):3495–3501. doi:10.1200/JCO.2005.00.802
26. Helson L, Bolger G, Majeed M, Vcelar B, Pucak K, Matabudul D. Infusion pharmacokinetics of Lipocurc (liposomal curcumin) and its metabolite tetrahydrocurcumin in beagle dogs. *Anticancer Res*. 2012;32(10):4365–4370.
27. Matabudul D, Pucak 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(10):4359–4364.
28. Kanai M, Yoshimura K, Asada M, et al. A phase I/II study of gemcitabine-based chemotherapy plus curcumin for patients with gemcitabine-resistant pancreatic cancer. *Cancer Chemother Pharmacol*. 2011;68(1):157–164. doi:10.1007/s00280-010-1470-2
29. Bayet-Robert M, Kwiatkowski F, Leheutier M, et al. Phase I dose escalation trial of docetaxel plus curcumin in patients with advanced and metastatic breast cancer. *Cancer Biol Ther*. 2010;9(1):8–14. doi:10.4161/cbt.9.1.10392
30. Dhillon N, Aggarwal BB, Newman RA, et al. Phase II trial of curcumin in patients with advanced pancreatic cancer. *Clin Cancer Res*. 2008;14(14):4491–4499. doi:10.1158/1078-0432.CCR-08-0024
31. Asai A, Miyazawa T. Occurrence of orally administered curcuminoid as glucuronide and glucuronide/sulfate conjugates in rat plasma. *Life Sci*. 2000;67(23):2785–2793. doi:10.1016/S0024-3205(00)00868-7
32. Vareed SK, Kakarala M, Ruffin MT, et al. Pharmacokinetics of curcumin conjugate metabolites in healthy human subjects. *Cancer Epidemiol Biomarkers Prev*. 2008;17(6):1411–1417. doi:10.1158/1055-9965.EPI-07-2693
33. Heath DD, Pruitt MA, Brenner DE, Rock CL. Curcumin in plasma and urine: quantitation by high-performance liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2003;783(1):287–295. doi:10.1016/S1570-0232(02)00714-6
34. U.S Food and Drug Administration. Bioanalytical method validation. Guidance for industry. 2018. Available from: <https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf>. Accessed February 03, 2020.
35. Xu X, Qin J, Liu W. Curcumin inhibits the invasion of thyroid cancer cells via down-regulation of PI3K/Akt signaling pathway. *Gene*. 2014;546(2):226–232. doi:10.1016/j.gene.2014.06.006
36. Yu Z, Wan Y, Liu Y, Yang J, Li L, Zhang W. Curcumin induced apoptosis via PI3K/Akt-signalling pathways in SKOV3 cells. *Pharm Biol*. 2016;54(10):2026–2032. doi:10.3109/13880209.2016.1139601

37. Qiao Q, Jiang Y, Li G. Inhibition of the PI3K/AKT-NF-kappaB pathway with curcumin enhanced radiation-induced apoptosis in human burkitt's lymphoma. *J Pharmacol Sci.* **2013**;121(4):247–256. doi:10.1254/jphs.12149FP
38. Akkoc Y, Berrak O, Arisan ED, Obakan P, Coker-Gurkan A, Palavan-Unsal N. Inhibition of PI3K signaling triggered apoptotic potential of curcumin which is hindered by Bcl-2 through activation of autophagy in MCF-7 cells. *Biomed Pharmacother.* **2015**;71:161–171. doi:10.1016/j.biopha.2015.02.029
39. Fu H, Wang C, Yang D, et al. Curcumin regulates proliferation, autophagy and apoptosis in gastric cancer cells by affecting PI3K and P53 signaling. *J Cell Physiol.* **2017**.
40. Zhang C, Li B, Zhang X, Hazarika P, Aggarwal BB, Duvic M. Curcumin selectively induces apoptosis in cutaneous T-cell lymphoma cell lines and patients' PBMCs: potential role for STAT-3 and NF-kappaB signaling. *J Invest Dermatol.* **2010**;130(8):2110–2119. doi:10.1038/jid.2010.86
41. Liu Z, Huang P, Law S, Tian H, Leung W, Xu C. Preventive effect of curcumin against chemotherapy-induced side-effects. *Front Pharmacol.* **2018**;9:1374. doi:10.3389/fphar.2018.01374
42. Onen HI, Yilmaz A, Alp E, et al. EF24 and RAD001 potentiates the anticancer effect of platinum-based agents in human malignant pleural mesothelioma (MSTO-211H) cells and protects nonmalignant mesothelial (MET-5A) cells. *Hum Exp Toxicol.* **2015**;34(2):117–126. doi:10.1177/0960327114542965
43. Hocking AJ, Elliot D, Hua J, Klebe S. Administering fixed oral doses of curcumin to rats through voluntary consumption. *J Am Assoc Lab Anim Sci.* **2018**;57(5):508–512. doi:10.30802/AALAS-JAALAS-17-000143
44. Bolger GT, Licollari A, Tan A, et al. Distribution of curcumin and THC in peripheral blood mononuclear cells isolated from healthy individuals and patients with chronic lymphocytic leukemia. *Anticancer Res.* **2018**;38(1):121–130. doi:10.21873/anticancer.12199
45. Bolger GT, Licollari A, Bagshaw R, et al. Intense uptake of liposomal curcumin by multiple myeloma cell lines: comparison to normal lymphocytes, red blood cells and chronic lymphocytic leukemia cells. *Anticancer Res.* **2019**;39(3):1161–1168. doi:10.21873/anticancer.13225
46. Storka A, Vcelar B, Klickovic U, et al. Effect of liposomal curcumin on red blood cells in vitro. *Anticancer Res.* **2013**;33(9):3629–3634.
47. Guidelines for best practice methodology for the use of animals for scientific purposes 2017. **2018**. Contract No.: ISBN: 9781925129977.
48. Ando H, Kobayashi S, Abu Lila AS, Eldin NE, Kato C, Shimizu T, et al. Advanced therapeutic approach for the treatment of malignant pleural mesothelioma via the intrapleural administration of liposomal pemetrexed. *J Control Release.* **2015**;220(Pt A):29–36. doi:10.1016/j.jconrel.2015.10.019
49. Popowicz N, Sparling B. Pleural pharmacokinetics. In: Lee YCG, Light RW, editors. *Textbook of Pleural Diseases*. 3rd ed. London, UK: CRC Press; **2016**:104–121.
50. Tada Y, Hiroshima K, Shimada H, et al. An intrapleural administration of zoledronic acid for inoperable malignant mesothelioma patients: a phase I clinical study protocol. *Springerplus.* **2016**;5:195. doi:10.1186/s40064-016-1893-2
51. Penz E, Watt KN, Hergott CA, Rahman NM, Psallidas I. Management of malignant pleural effusion: challenges and solutions. *Cancer Manag Res.* **2017**;9:229–241. doi:10.2147/CMAR
52. Marazioti A, Papadia K, Giannou A, Stathopoulos GT, Antimisariis SG. Prolonged retention of liposomes in the pleural cavity of normal mice and high tumor distribution in mice with malignant pleural effusion, after intrapleural injection. *Int J Nanomedicine.* **2019**;14:3773–3784. doi:10.2147/IJN.S202568
53. Bogliolo GV, Lerza R, Bottino GB, et al. Regional pharmacokinetic selectivity of intrapleural cisplatin. *Eur J Cancer.* **1991**;27(7):839–842. doi:10.1016/0277-5379(91)90129-2

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