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Research paper

Tissue localization of nanoparticles is altered due to hypoxia resulting in poor efficacy of curcumin nanoparticles in pulmonary hypertension

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ABSTRACT

The present study is an attempt to leverage therapeutic benefits of curcumin in pulmonary hypertension by encapsulating it in biodegradable poly(lactide-co-glycolic) acid nanoparticles. Pulmonary hypertension is induced in experimental animals by subjecting them to chronic hypoxic conditions. The ability of curcumin encapsulated nanoparticles to manage pulmonary hypertension is measured by right ventricular hypertrophy, haematocrit, vascular remodelling and target tissue levels of curcumin. Further, single oral dose tissue distribution of the nanoparticulate curcumin was also assessed under normoxic and hypoxic conditions. Orally administered nanoparticulate curcumin failed to offer any protection against hypoxia induced pulmonary hypertension as indicated by insignificant changes in right ventricular hypertrophy and vascular remodelling that are similar to untreated groups. A significant difference in the target tissue levels was observed between normoxic vs. hypoxic rats. The study suggests that hypoxia has a major role in the particle localization in lungs probably due to the altered blood flow, increased barrier properties of the lung vasculature and decreased endocytosis. The target tissue levels of curcumin under hypoxia are much lower to that achieved in normoxic rats probably due to difference in particle dynamics, resulting in the failure of treatment.

1. Introduction

The studies identifying the role of hypoxia in pulmonary hypertension (PH) are performed utilizing isolated cells [1], isolated pulmonary arterial rings [2,3], perfused lung studies [3], hypoxic animal models [4–6] or humans [7]. All the studies in a broader sense report the altered physiology of the pulmonary arteries. Pulmonary arterioles <70 μm in diameter normally contain only a single elastic lamina without any smooth muscle. However, with alveolar hypoxia, smooth muscles are extended into these small pulmonary arterioles, and the elastic lamina is split into inner and outer layers surrounding the smooth muscles. The extent of muscularization also increases in the arterioles with smooth muscle cells.

Chronic hypoxia increases reactive oxygen species (ROS) and impairs endothelial nitric oxide (NO)-dependent relaxation [2]. Reduced antioxidant status in the endothelial cells is believed to be involved in the pathogenesis of PH from the evidence that superoxide dismutase (SOD) and glutathione peroxidise activities were decreased in idiopathic pulmonary arterial hypertension [8]. Superoxide generation was enhanced when pulmonary arterial pressure was elevated. The development of PH in lambs, with surgically created heart defect and increased pulmonary blood flow (Shunt), is associated with increases in oxidative stress that are not explained by decreases in antioxidant expression or activity. The observed increase in oxidative stress is due, at least in part, to increased expression and activity of NADPH oxidase complex [9]. Superoxide in the presence of nitric oxide forms peroxynitrite, which reacts avidly with tyrosine residues in proteins to form nitrotyrosine. Nitrotyrosine is up-regulated in pulmonary arteries of rats correlating with the development of PH in hypoxic environment [10]. Nitrotyrosine is also raised in patients with severe and long-standing PH [11]. Peroxynitrite besides causing mitochondrial dysfunction leading to excessive ROS production [12] can also cause pulmonary arterial smooth muscle and endothelial cell proliferation [13]. ROS mediates vascular responses, at least in part, by stimulating thromboxane production [14] and enthelin-1 secretion [15].

There are mixed results with the treatment of antioxidants in PH. Concomitant β-carotene treatment protected the lung parenchyma from the inflammatory reaction and the septal fibrosis, but did not prevent right ventricular hypertrophy and only slightly reduced the thickening of the wall of small arteries and arterioles in monocrotaline (MCT) induced pulmonary hypertensive rats [16]. Vitamin E has a protective role on the development of MCT

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induced PH in rats. A modest reduction of the right ventricular hypertrophy in these rats was observed with high-dose vitamin E treatment. However, this treatment could not prevent the vascular hypertrophy caused by MCT [17]. Studies with N-acetyl cystein (NAC) suggest that treatment during the initial stages of hypoxia can prevent PH [5,6]. Other antioxidants tempol [4] and erdostein [18] are also shown to have beneficial roles in PH. Antioxidants prevent the cellular infiltration by altering the increased vascular permeability under hypoxia [19].

Pharmacological activities of curcumin are reported in ayurveda, and curcumin is being studied for its antioxidant, anti-inflammatory and immunomodulatory effects [20–23]. As the pathogenesis and progression of PH involve oxidative pathways, it is hypothesized that curcumin would be beneficial in the treatment of PH; however, its use is limited because of the poor biopharmaceutical properties *in vivo* [24]. In our previous study, advanced delivery approach using nanoparticle formulations for curcumin has proven to increase the oral bioavailability by ninefold when compared to that of plain curcumin [25]. Considering these facts, the aim of the present study is to determine the effect of orally administered curcumin encapsulated poly(lactide-co-glycolic) acid nanoparticles on right ventricular hypertrophy and vascular remodelling in chronic hypoxic rats.

2. Materials and methods

2.1. Materials

Poly(lactide-co-glycolic) acid (PLGA) (Resomer R503H; MW 35–40 kDa) was purchased from Boehringer Ingelheim, (Ingelheim, Germany). Polyvinyl alcohol (PVA) (MW 30–70 kDa) and ethyl acetate were purchased from Sigma–Aldrich (Poole, UK). Curcumin was purchased from Indsaff, Punjab, India.

2.2. Curcumin encapsulated nanoparticles

Nanoparticles encapsulated with curcumin were prepared by a modified emulsion–diffusion–evaporation method, previously reported [23,25]. Briefly, curcumin (7.5 mg) and PLGA (50 mg) were dissolved in 2.5 ml of ethyl acetate and stirred at 1000 rpm for 30 min under room temperature to obtain a homogeneous solution. Stabilizer, PVA (50 mg) was dissolved in 5 ml distilled water. The organic phase containing curcumin and PLGA was then added in a drop-wise manner to the stabilizer solution during homogenization. Homogenization was continued for 5 min at 15,000 rpm. After this step, the emulsion was transferred to 20 ml water to facilitate diffusion of ethyl acetate and was stirred overnight to ensure the complete evaporation of the solvent. The nanoparticle solution was then centrifuged at 15,000*g* for 15 min to separate free active ingredient and any unbound stabilizer in the solution. The supernatant was separated, and the pellet was redispersed in 20 ml water for further use.

2.3. Experimental animals

All procedures on animals were performed according to project licence under the Animals (Scientific Procedures) Act 1986 (UK). Animals were housed in the Biological Procedures Unit (BPU), University of Strathclyde, allowed free access to food and water, and maintained on a 12 h light and dark cycle.

2.4. Chronic hypoxic rats

Male Sprague–Dawley rats (180–200 g) were placed in a hypobaric chamber at 600 mbar for two weeks. Age matched controls were housed at atmospheric pressure. The rats were acclimatized for 2 days in the hypoxic chamber during which the pressure was brought down to 600 mbar by reducing 70–75 mbar pressure in each step, and the duration between each step was at least 6 h. After the acclimatization, the chambers were opened everyday to treat the animals. Whenever the chamber is repressurized, the pressure was brought up in steps of 70–75 mbar having 10-min interval between them. The chamber was never opened for more than half an hour in a day. After treatment, the chamber was depressurized in 70–75 mbar steps with 10-min interval between each step until 600 mbar is reached. The bedding was changed every three days; food and water were supplied whenever required.

2.5. Treatment with formulations

The fresh resuspended nanoparticles at concentration of 20 mg/ml of curcumin were used. The plain curcumin suspension (20 mg/ml) was prepared in 0.5% w/v sodium carboxymethyl cellulose. The hypoxic rats were divided into three groups. First group (H) received water daily, the second group (HCS) received curcumin suspension (100 mg/kg) daily and the third group (HCN) received curcumin nanoparticles (100 mg/kg) daily. Normoxic animals (N) received water daily. All the formulations were given orally using a gavage needle, and the treatment was carried out for 2 weeks simultaneously with hypoxia.

2.6. Measurement of right ventricular hypertrophy

After the treatment with different formulations, rats were euthanized in a CO_2 chamber. Heart was isolated, and the auricles were separated out. The right ventricle was then separated from the left ventricle leaving the septum. The weights of the right ventricle and the left ventricle + septum were then recorded. The ratio between right ventricular weight and left ventricle + septum weights, RV/(LV + S), was used as the measure to compare the right ventricular hypertrophy among the groups.

2.7. Measurement of haematocrit

Blood was collected immediately from the rats after euthanasia by cardiac puncture in heparinized syringes. The blood was then transferred into the heparinized capillary tubes. These tubes were spun in a centrifuge at 12,000 rpm for 10 min. Then, the lengths of the packed cells and the total length including the plasma were recorded. The haematocrit was expressed as the percentage of the ratio between packed cells to total length of blood in the capillary tubes.

2.8. Histology

After euthanasia, lungs from the rats were isolated and different lobes were separated. The left lobe was used for histological examination. The lobes were inflated with 10% neutral buffered formalin solution by injecting through the bronchus. Then, they were kept in the same solution. These tissues were fixed for at least one day before dehydration. Then, the tissues were paraffin embedded, and thin (3 μm) sections were cut and mounted onto slides. The slides were rehydrated and stained using haematoxylin and eosin (H&E).

2.9. Immunohistology

The lungs were fixed, dehydrated and embedded in paraffin wax, and 3 μm sections were cut and mounted onto silanated slides. Then, the slides were de-paraffinized, rehydrated and

treated with 0.3% H_2O_2 for 10 min to block the endogenous peroxidise activity. This was followed by microwave pressure cooker treatment in order to expose the antigen. The sections were blocked with 20% normal goat serum (NGS) and incubated with the anti-smooth muscle α-actin mouse monoclonal (1:1000) for 1 h. Horse radish peroxidise-labelled secondary antibody (goat anti-mouse antibody 1:400) was added to the sections and incubated for 30 min. Then, the sections were incubated for 10 min with 3,3-diaminobenzidine tetrahydrochloride, which was activated with 30% H_2O_2, followed by the treatment with 1% copper sulphate solution and counterstaining with haematoxylin.

Smooth muscle α-actin staining was used as a measure to investigate the extent of muscularization of arteries. For quantitation of the extent of muscularization, the identity of the slides was blinded to the investigator during scoring; 50 consecutive small pulmonary arteries less than 100 μm were photographed from each slide using 800× magnification. Vessels were counted as thickened if they possessed both of the following criteria. 1. The artery wall thickness was at least one-third the radius on any given side of artery wall and 2. At least 50% of the total wall area was thickened. Any vessels, which were on the borderline, were not taken into account, and number of uncounted vessels was negligible. The number of thickened arteries was expressed as percentage of the total arteries less than 100 μm taken into consideration.

2.10. Curcumin tissue distribution studies

PH rat groups, HCS and HCN, were euthanized at the end of the treatment period, blood was collected and brain, heart, kidneys, liver, lungs and spleen were isolated and stored at −80 °C until analysis.

To study the tissue distribution of a single dose of curcumin under hypoxia, male Sprague–Dawley rats weighing 180–200 g were housed in hypoxic chamber and acclimatized for 2 days, during which the pressure was brought down to 600 mbar by reducing 70–75 mbar pressure in each step with 6 h duration between each step. Six rats were divided into two groups, one group was administered 100 mg/kg curcumin suspension and the other was administered 100 mg/kg curcumin nanoparticles. After 24 h, rats were euthanized, blood was collected, and brain, heart, kidneys, liver, lungs, spleen, stomach, small intestine, caecum and large intestine were isolated and stored at −80 °C until analysis.

To study the tissue distribution of a single dose of curcumin under normoxic conditions after 2 h and 24 h, twelve male Sprague–Dawley rats weighing 180–200 g were divided into four groups. Two groups received curcumin suspension, and the other two groups received curcumin nanoparticles each 100 mg/kg. After 2 h and 24 h, one group receiving suspension and one group receiving nanoparticles were euthanized, blood was collected and brain, heart, kidneys, liver, lungs, spleen, stomach, small intestine, caecum and large intestine were isolated and stored at −80 °C until analysis.

Plasma was separated from the blood, and the tissues were homogenized in pH 7.4 buffer followed by curcumin extraction and analysis using HPLC with slight modifications in the internal standard and the mobile phase composition from a previous method [25]. Estradiol was used as the internal standard, and the mobile phase was 30:35:35 acetonitrile:methanol:2% acetic acid using a C18 column (Hypersil GOLD 15 cm × 4.6 mm, 5 μm).

2.11. Statistical analysis

Statistical analysis was carried out by one-way analysis of variance followed by Tukey's test for multiple comparisons using Sigma Stat (Systat Software, Inc., San Jose, CA).

3. Results

Curcumin encapsulated nanoparticles have been prepared successfully using emulsion diffusion evaporation method. The particles were of 237 ± 6 nm with 66 ± 3% entrapment efficiency [23].

Significant weight loss was observed in rats with the hypoxic treatment for 2 weeks (Fig. 1A). Hypoxic exposure resulted in increased haematocrit and right ventricular hypertrophy (Fig. 1B and C). The histological examination of H&E stained lung sections (Fig. 2) and smooth muscle α-actin immunostained lung sections of the rats revealed that the small pulmonary arterial walls were thickened and muscularized with chronic hypoxia for 2 weeks (Fig. 3).

All the treatments have been ineffective in preventing the loss of body weight, lowering the haematocrit percentage and alleviating the right ventricular hypertrophy (Fig. 1). The right ventricular hypertrophy results, in hypoxic rats treated with curcumin formulations, were corroborated with the histological examination of the pulmonary arterial wall thickness (Figs. 2 and 3). Percentage of thickened arteries in the smooth muscle α-actin immunostained lung sections of normoxic, hypoxic, curcumin suspension treated and curcumin nanoparticles treated rats were 6 ± 4, 39 ± 16, 60 ± 10 and 32 ± 10, respectively. Fig. 4 shows the presence of

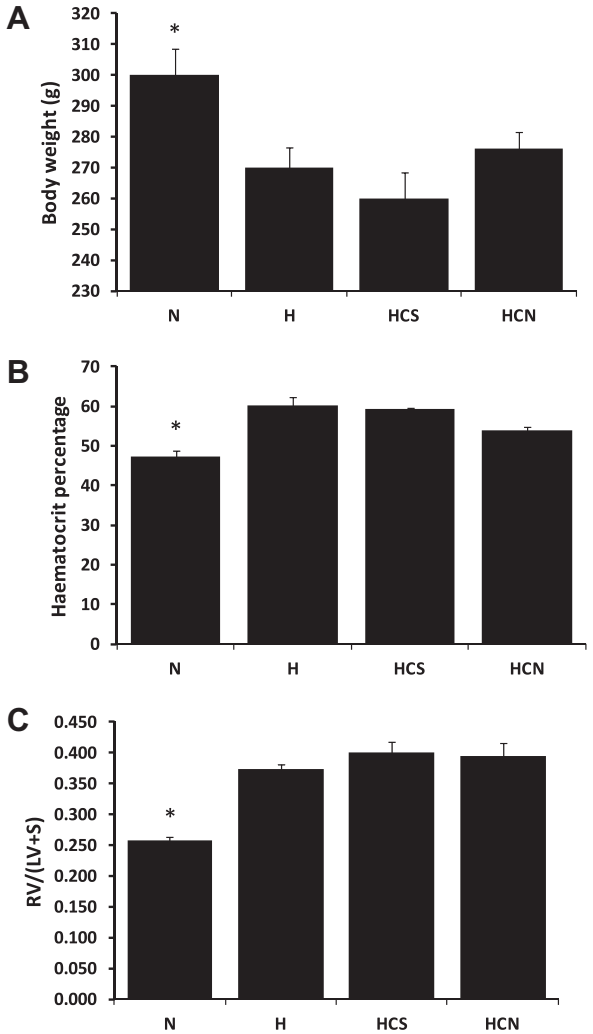

Fig. 1. Final body weights (A), haematocrit percentage (B) and right ventricular hypertrophy (C) in chronic hypoxic rats. N – Normoxic, H – hypoxic, HCS – hypoxic curcumin suspension treated and HCN – hypoxic curcumin nanoparticles treated groups. Values are expressed as mean ± SEM (n = 6–10). *p < 0.05 vs. H.

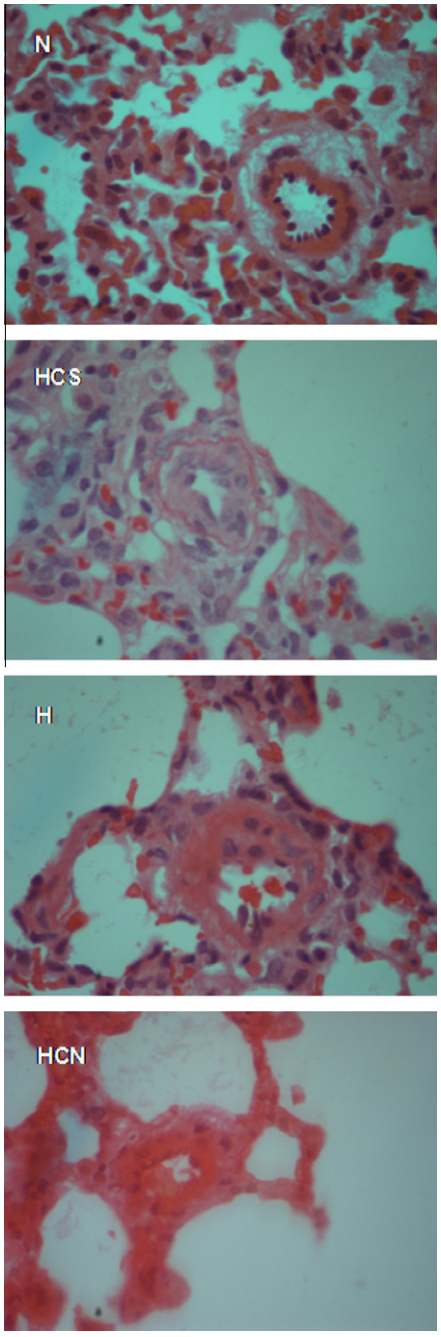

Fig. 2. Haematoxylin and eosin (H&E) stained histological photographs of lung sections obtained after different treatments. The small pulmonary arterioles are thickened in case of hypoxic treatment in comparison with normoxic rats, and the different antioxidant treatments have no effects on the hypoxia induced vascular hypertrophy. (All the pictures were taken at $40 \times 2 \times 10$ magnification). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

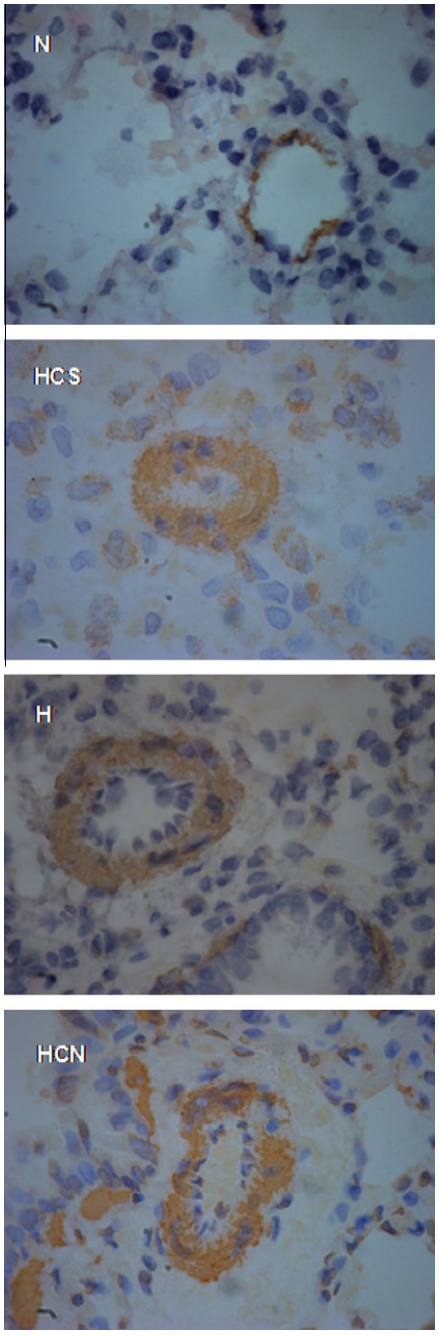

Fig. 3. Immunohistological photographs of lung sections, for smooth muscle α-actin, obtained after different treatments. Brown colouration depicts the presence of smooth muscle α-actin and was very thick in the hypoxic lungs in comparison with normoxic lungs (All the pictures were taken at $40 \times 2 \times 10$ magnification). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

curcumin nanoparticles as clusters in the cells of the lung sections of HCN group rats stained with H&E at the end of the treatment period.

Curcumin suspension or the nanoparticle form showed almost similar tissue levels of curcumin in lung and spleen of chronically hypoxic rats. Additionally, curcumin nanoparticles resulted in measurable levels in heart and kidney but not with curcumin suspension treatment (Table 1).

At 2 h, curcumin nanoparticles demonstrated significant improvement in oral bioavailability with plasma levels of ~182 ng/ml in normoxic rats in comparison with the curcumin suspension, and at 24 h, curcumin was not seen in plasma of both suspension or nanoparticles treated groups (Table 2). At 2 h, only the small intestine, among the four intestinal parts analysed, showed the presence of curcumin with oral curcumin suspension. Curcumin levels were present in the stomach and small intestine after 2 h with curcumin nanoparticles treatment. At 24 h, significantly lower levels of curcumin were detected in stomach, small intestine, caecum and large intestines with nanoparticles treatment in comparison with the suspension group in normoxic rats. Among the internal organs, curcumin levels were observed only

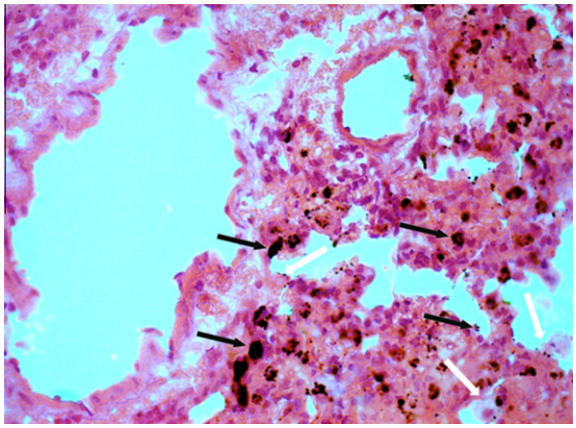

Fig. 4. Haematoxylin and eosin stained lung section of the rat treated with curcumin nanoparticles daily. The section shows accumulation of nanoparticles in the lung due to the inherent yellow colour of curcumin as yellowish dark spots (black arrows). White arrows show the alveolar macrophages (400×). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Tissue distribution of curcumin in PH rats ($n = 6$).

Group	Plasma	Brain	Heart	Kidneys	Liver	Lungs	Spleen
HCS	ND	ND	ND	ND	ND	48 ± 12	60 ± 4
HCN	ND	ND	26 ± 2	47 ± 11	ND	42 ± 10	72 ± 13

Units for plasma levels are ng/ml and for tissues ng/gm.
ND = not detectable; limit of detection = 20 ng/ml.
HCS – Curcumin suspension treated chronic hypoxic rats.
HCN – Curcumin nanoparticles treated chronic hypoxic rats.

in the spleen at 2 h and in lungs at 24 h with suspension treatment in normoxic rats, and in heart, lungs and spleen at 2 h and in brain and lungs at 24 h with curcumin nanoparticles treatment in normoxic rats.

Curcumin was not detected in the livers of all the rats administered suspension or nanoparticles in both normoxic and hypoxic conditions. At 24 h, nanoparticles treated normoxic rats showed presence of curcumin in brain but not hypoxic rats. Only hypoxic rats showed the presence of curcumin levels in kidneys. The curcumin kidney levels were significantly higher in the nanoparticles treated group in comparison with the suspension group. Lung levels of curcumin were detected in all the nanoparticles administered groups. At 24 h, curcumin was detected (191 ± 13 ng/gm) in the lungs of suspension treated normoxic rats. Curcumin levels were below the detection limit at 24 h, in the lungs of hypoxic rats treated with curcumin suspension. Lung levels of curcumin in rats treated with curcumin nanoparticles decreased significantly in hypoxic rats in comparison with the normoxic rats (Table 2).

4. Discussion

In rats, PH develops during the first two weeks of exposure to chronic hypoxia, and then, it stabilizes and does not increase in severity [6]. There has been growing literature on the free radical injury to pulmonary vascular wall in the early days of the hypoxic exposure [4–6,18]. Antioxidant treatment just before and at the beginning of hypoxia is thought to be more effective in reducing PH [6].

The body weight gain in hypoxic rats is decreased at the acclimatization phase and later on started to follow the same trend as normoxic rats; however, at the end of the experiment, the hypoxic rats were less in weight in comparison with normoxic rats. The possible reason for the reduction of body weights under hypoxia is related to the increased basal metabolic rate [26]. There was no significant effect of curcumin treatments on the body weights of the rats either in the form of suspension or in the form of nanoparticles in comparison with the untreated hypoxic rats (Fig. 1).

Many of the antioxidants have shown positive effects on right ventricular hypertrophy, which include vitamin E [17], NAC [6], tempol [4] and allopurinol [5]. These antioxidants except vitamin E are hydrophilic and have no known bioavailability problems associated with them, on the other hand curcumin is poorly bioavailable [24]. The bioavailability of curcumin is very poor, which is contributing to its under utilization. Apart from poor absorption of curcumin, rapid clearance rate contributes to the poor efficacy of curcumin [27]. Although curcumin nanoparticle shown to increase the bioavailability of nanoparticles in normal rats, these nanoparticles in chronic hypoxic rats failed to show the reduction in right ventricular hypertrophy. The lung levels of curcumin were not improved with HCN treatment in comparison with the HCS treatment. However, curcumin levels in the heart and kidney were higher for nanoparticles treated chronic hypoxic rats in comparison with the suspension treated chronic hypoxic rats (below detection limit), suggesting an improvement of tissue levels in tissues other than lungs and spleen (Table 1). Nanoparticles are known to localize more in spleen due to the rapid clearance from the blood circulation by mononuclear phagocytic system; however, it is also known that this process is influenced by the charge, particle size and surface characteristics of the nanoparticles [28].

Table 2
Plasma and tissue concentrations of curcumin in normoxic and hypoxic rats ($n = 3$).

Formulation	Normoxia/ Hypoxia	Plasma	Brain	Heart	Kidneys	Liver	Lungs	Spleen	Stomach	Caecum	Small intestine	Large intestine
After 2 h												
Suspension	Normoxia	ND	ND	ND	ND	ND	ND	1519 ± 992	ND	ND	1198 ± 225	ND
Nanoparticles	Normoxia	182 ± 44	ND	43 ± 11	ND	ND	46 ± 5	137 ± 20	188 ± 57	ND	841 ± 27	ND
After 24 h												
Suspension	Normoxia	ND	ND	ND	ND	ND	191 ± 13	ND	3607 ± 61	7487 ± 1184	2290 ± 741	1796 ± 585
Nanoparticles	Normoxia	ND	274 ± 45	ND	ND	ND	252 ± 11[a]	ND	439 ± 199[a]	750 ± 16[a]	72 ± 44[a]	433 ± 52[a]
Suspension	Hypoxia	ND	ND	ND	77 ± 6	ND	ND	ND	ND	ND	222 ± 15[a]	301 ± 82[a]
Nanoparticles	Hypoxia	ND	ND	ND	255 ± 35[c]	ND	57 ± 14[a,b]	25 ± 2	93 ± 11[a,b]	ND	92 ± 11[a]	51 ± 16[a]

Both the formulations were administered orally at 100 mg/kg dose.
Units for plasma levels are ng/ml and for tissues ng/gm.
ND = not detectable; limit of detection = 20 ng/ml.
[a] vs. 24 h suspension treated normoxic group.
[b] vs. 24 h nanoparticles treated normoxic group.
[c] vs. 24 h suspension treated hypoxic group.

The inactivity of the curcumin in PH and lack of improvement in curcumin lung levels with nanoparticles prompted us to investigate the differences in distribution of curcumin delivered in suspension and nanoparticulate form under hypoxic and normoxic conditions. Hypoxia causes increase in aortic blood flow and alters tissue blood flows [29]. Hypoxia increases vascular permeability in acute hypoxic conditions [19] and is responsible for decreased endocytosis in cells [30]. Differences in blood flow due to hypoxia have been reported to alter the pharmacokinetics of some pharmaceutical compounds [31].

In the present experiment, we observed no increase in the lung levels of curcumin and increased levels in the kidneys of pulmonary hypertensive rats receiving nanoparticles when compared to suspension treatment (Table 1). Increased levels in kidneys could be an indication of increased excretion of curcumin under hypoxia when compared to normoxia. The altered lung and kidney levels of curcumin under hypoxia can be correlated with altered tissue blood flows under hypoxia. Although nanoparticles treatment in hypoxic rats improved the lung levels of curcumin in comparison with suspension treated hypoxic rats, they were significantly lower than the lung levels of suspension and nanoparticles treated normoxic rats (Table 2). The poor localization of curcumin in lungs under hypoxia resulted in the inefficacy of curcumin in PH. The reason for the poor localization of nanoparticles in the lungs under hypoxia is vascular hypertrophy and probable reduction in endocytosis. Vascular hypertrophy is a result of proliferation of smooth muscle cells and to some extent endothelial cells. This causes the extravasation of nanoparticles difficult from the blood vessels in the lungs. In the hypoxic rat lungs the vascular wall is at least 5–10 times thicker than the normoxic lungs (Fig. 3). As the number of cell layers is increased under hypoxia, the penetration of nanoparticles becomes much difficult (Fig. 5). Literature reports suggest that the acute hypoxia (less than 4 h) increases the vascular permeability, which can be reverted back with antioxidant treatment. The present study utilizes chronic hypoxic exposure and treatment with antioxidant curcumin. Both antioxidant treatment and chronic hypoxia increase the barrier properties of the lung vasculature. Increased barrier property hinders the possible paracellular pathway for the nanoparticles under these conditions. Transcellular transport of nanoparticles might have also been hindered under hypoxia due to reduced endocytosis by vascular cells. Our report is first in identifying the role of systemic hypoxia in particle localization. Hypoxia alters the tissue distribution (penetration) of nanoparticles in chronic hypoxic lungs by one or more of the following effects that include (i) decreased extravasation of nanoparticles from the vascular wall because of the vascular hypertrophy, (ii) altered blood flow to the major organs and (iii) decrease in endocytosis due to hypoxia.

Other findings from the tissue distribution studies provide insights in the absorption of nanoparticles from the gastrointestinal tract. Curcumin is found mainly in the intestinal tract after oral administration, which is evident from considerable high levels in stomach, caecum, small intestine and large intestinal tissue (Table 2). The levels in the lower parts of the intestine, caecum and large intestine increase with time from 2 h to 24 h. This suggests that the nanoparticles move down along the gastrointestinal tract similar to other formulations. Curcumin in nanoparticles resulted in lesser accumulation of curcumin than the suspension form in the intestinal tissues suggesting improved absorption than the suspension formulation. The nanoparticles seem to have increased the distribution of curcumin into major organs when compared to the suspension of curcumin. Under hypoxia, the levels of curcumin in different parts of the intestine were decreased with the suspension and nanoparticles of curcumin suggesting the reduced amount of curcumin present in the body in comparison with the normoxic rats. As a whole, hypoxia reduced the amount of curcumin present in different tissues except kidneys; however, higher levels in kidneys might suggest rapid elimination of curcumin through kidneys under hypoxic conditions further strengthening the notion of reduced bioavailability under hypoxia.

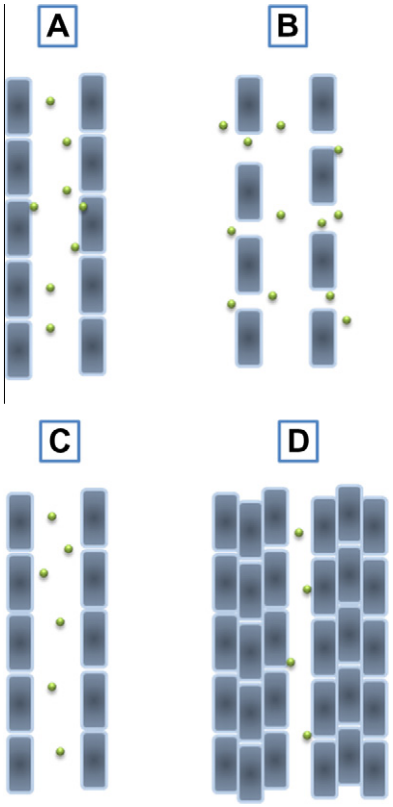

Fig. 5. In normoxic rats, the vascular wall is tight and does not allow easy paracellular transport to take place (A). With hypoxia, initially the vascular permeability increases which can facilitate the nanoparticles penetration (B). The vascular permeability is minimized by the antioxidants (C), and during the chronic hypoxia, vascular wall thickens drastically which will hinder the extravasation of nanoparticles from the vascular wall (D). For the ease of portraying, endothelial cells and smooth muscle cells are depicted as rectangular cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Curcumin nanoparticles were observed in the lung histological sections of HCN treatment (Fig. 4). Curcumin is a yellow coloured natural product sometimes used as stain in histology, due to this virtue nanoparticles containing curcumin were seen in the lung sections as dark yellow clusters. This observation suggests that the curcumin encapsulated in nanoparticles reached the lungs intact as nanoparticles when administered through the oral route. From the pathological results, tissue distribution and the histological observation after 2 weeks of chronic hypoxia, it can be understood that the levels reaching the lung are insufficient to treat PH in chronic hypoxic rats.

The data strongly suggest the difference in target tissue (lung) distribution under hypoxic conditions, and as a result, nanoparticulate curcumin was ineffective in PH. Curcumin as such has severe bioavailability problems due to poor physicochemical/biopharmaceutical properties; therefore, a simple suspension is ineffective as well.

5. Conclusions

The analytical and histological evidence suggests that orally delivered nanoparticles can reach the lung; however, this can be altered significantly under hypoxia. From the data, it is clear that

apart from dosage form design the disease model used, how the pathology is achieved also plays a major role in the therapeutic outcome.

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