Influence of colchicine on pulmonary silicotic fibrogenesis in rats*

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With an aim to evaluate the antifibrotic action of colchicine in experimental model of pulmonary silicosis, the effect of colchicine on developing and developed pulmonary silicosis induced by quartz was studied in rats in vivo and on alveolar macrophages exposed to quartz particulates in vitro. A progressive increase in wet and dry weight of lungs exposed to quartz dust alone, and quartz dust and colchicine injected orally was investigated. An increase in collagen contents, with lapse in time, in animals exposed intratracheally to quartz dust, or exposed similarly to quartz dust but receiving colchicine simultaneously through oral route was observed. A blindfold evaluation of histological sections of lungs of silicotic animals with or without colchicine administration during development of lesions did not reveal any difference between two groups of silicotic rats. Administration of colchicine for 4 weeks after the lesions were developed neither inhibited nor retarded the laying down of collagen. The studies were extended to investigate the effect of colchicine on quartz-induced alveolar macrophage cytotoxicity. The presence of varying concentrations of colchicine in the culture medium did not significantly alter cytotoxic potential of quartz. The results reveal that colchicine administration during the development of and on developed silicosis does not significantly alter pathogenesis of silicotic lesions. At the cellular level colchicine does not modulate quartz-induced alveolar macrophage cytotoxicity, believed to be a significant event for the onset of pulmonary silicotic fibrogenesis.

Keywords: Colchicine, Fibrogenesis, α-quartz, Silicosis

Silicosis is a chronic interstitial disease of lungs that develops after inhalation exposure to free crystalline silica. The disease is characterized by an increase in laying down of collagen with a decrease in respiratory area for gaseous exchange. Attempts have been made to retard/inhibit silicotic fibrogenesis by applying therapeutic intervention strategies involving use of metallic salts, corticosteroids, tetrandrine alkaloid isolated from Stephania tetrandra, free radical scavengers, glutamate, recombinant soluble tumour necrosis receptor, leucocyte chemotactic factor, inhibitors of phospholipases, and β amino propionitrile. Some of these agents have shown encouraging results in reducing the extent of damage and development of pulmonary fibrosis. However, the use of these agents in human silicotic patients has met with difficulties due to inherent toxic potential of the therapeutic agent itself over long period of usage to obtain meaningful results.

Colchicine is a known anti-inflammatory agent. The drug has a potential benefit in the treatment of idiopathic pulmonary fibrosis and a number of fibroproliferative conditions characterized by increased collagenesis. The aim of the present investigation is to evaluate the antifibrotic action of colchicine in an experimental model of pulmonary silicosis.

Materials and Methods

Dust — α-quartz. (Min-U-Sil, Pennsylvania Glass and Sand Corp. Pittsburgh, USA) of particle size less than 5 μ was used.

Animals — Wistar strain male albino rats weighing 150-200 g obtained from the animal colony of the Center were used. The animals were maintained under standard conditions of husbandry and acclimatized for 7 days prior to exposure. Before and throughout the period of experimentation the animals were fed ad libitum on pellet diet supplied by Hindustan Lever, Mumbai, India. The animals had free access to sufficient drinking water and were maintained in temperature-controlled rooms.

Production of silicotic lesions — The animals were lightly anaesthetised with ether. The trachea was exposed by blunt dissection and 50 mg quartz dust suspension in 1 ml physiological saline kept constantly agitated, to prevent sedimentation within the syringe, was directly injected into the trachea per os. Injecting

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forcibly and using 50% air usually helped to ease dispersion of the dust into the lung alveoli. Exposure of the trachea by blunt dissection was meant to be sure about the placement of the canula into the lumen of the trachea, which could be easily visualized from outside. The wound was closed by a single suture.

**In vivo studies**

Effect of colchicine administration on developing pulmonary silicosis — Following exposure to quartz dust, the animals were divided into 2 groups. The animals of group 1, consisting of 16 animals and representing the experimental series, were orally injected with 10 μg colchicine dissolved in 0.5 ml distilled water daily for 5 days/week over a period of 1, 2 or 4 weeks. The animals of group 2, comprising 17 animals and representing the control series, were similarly injected orally with 0.5 ml distilled water alone. Batches of animals from both groups were killed by administration of an overdose of pentobarbitone anaesthesia followed by exsanguination 1, 2 and 4 weeks after exposure to quartz dust.

Effect of colchicine on established pulmonary silicosis — Rats were divided into 2 groups. In group I (17 rats) pulmonary silicosis was produced by intratracheal injection of quartz as described previously. Rats of group II (5 rats) were similarly injected with 0.5 ml saline to serve as control. After 16 weeks, the silicotic animals of the 1st group were divided into 3 sub-groups. The 1st sub-group of 5 rats were killed 16 weeks after quartz treatment to determine the extent of silicotic fibrosis produced. The 2nd sub-group of 7 rats were injected orally with 10μg colchicine dissolved in 0.5 ml distilled water daily for 5 days/week for 4 weeks as described earlier. The 3rd sub-group of 5 animals were orally injected with 0.5 ml of distilled water alone. The animals of sub-groups II and III and group II were killed 20 weeks after the start of the experiment.

**Histological methods**

At different time intervals the animals were killed with intraperitoneal overdose of pentobarbitone 100 mg/kg (body weight). From the killed animals, lungs were removed and wet weight determined after detaching trachea. The lungs were gently distended by injecting 10% formal saline through a small remnant of major bronchi left intact. The lungs were put in 10% formal saline overnight. After preliminary fixation, blocks were selected along the long axis of both lungs at the level of hilum to include maximum representative area from each lung. Fixation was completed in fresh fixative. Blocks were embedded in paraffin and 5 μm thick sections were cut. Multiple sections from each block were stained with hematoxylin and eosin, silver impregnated for reticulin and Mason’s trichrome for collagen.

**Biochemical methods**

Collagen analysis — All the lung tissue except the portion used for histopathological studies was dried at 110°C. Paraffin embedded blocks and tissue shavings were added to it and the whole mixture was treated thrice with xylene at 37°C. The mixture was again dried till a constant weight was recorded. This represented the total dry weight of lungs minus a few sections of 5 μm thickness used for histological study. A sample of dried tissue was hydrolysed in 6N HCl in sealed tubes for 16 hr at 100°C in a hot air oven. The hydrolysate was neutralized with the theoretical amounts of NaOH and diluted as desired for analysis. In these samples hydroxyproline was estimated by the method of Stegemann. Collagen contents were determined by multiplying the hydroxyproline value by 7.46.

**In vitro studies**

Effect of colchicine on quartz-induced alveolar macrophage cytotoxicity in vitro — Alveolar macrophages were harvested from the lungs of 12 rats killed by an intraperitoneal overdose of pentobarbitone sodium. The lungs were lavaged thrice with 7 ml physiological saline. The lung washings were pooled and centrifuged at 200 g for 10 min. The supernatant was discarded and the pellet was resuspended in MEM containing penicillin 100 IU/ml, streptomycin 100 μg/ml, amphotericin B 2.5 μg/ml and gentamycin 200 μg/ml. Cell suspension (1ml; 1×10⁶ cells) was seeded in each well of a 24 well flat bottomed microtiter plate. The cells were incubated at 37°C in an atmosphere of 5% CO₂ for 1 hr. After incubation the nonadherent cells were removed and the monolayer of adherent cells rinsed with PBS and fed either with (a) fresh 1 ml MEM alone, (b) MEM containing 50 μg quartz dust in suspension or (c) MEM containing 50 μg quartz dust and concentrations of colchicine varying from 0.02 to 0.5 μg. The cells were incubated as before for 3 hr. A parallel plate containing 1.0×10⁶ cells in each well was run in an identical manner. The MEM medium in this case was fortified with 5% fetal calf serum (FCS) and the cells were incubated for 20 hr. The cultures
were incubated for 2 different periods to evaluate the effect of immediate and extended periods of interaction between the cells and quartz particulates. For extended period of cell survival, addition of 5% FCS in culture medium was essential. At the end of the incubation periods of 3 or 20 hr, the supernatant culture medium was removed from each well and assayed for LDH enzyme activity and protein content. Subtracting the corresponding values obtained in the cell-free medium with or without serum the extent of LDH and protein release from cells was calculated.

Statistical analysis — The data were analyzed for statistical significance by Student’s ‘t’ and by ‘F’ test. One way analysis of variance was used to determine if the values obtained in vitro studies for different concentrations of colchicine were statistically significant for 3 hr and 20 hr separately. Prior to the analysis, homogeneity of variance between the groups was ascertained. Inter-group comparisons were done by calculating least significant differences where F-values were found to be significant.

Results

With increase in exposure time, a progressive increase in the wet and dry weight of lungs exposed to quartz dust was observed. An increase in the collagen contents of silicotic lungs was also observed. The difference in the collagen contents of the two silicotic groups was nonsignificant (Table 1). Administration of colchicine for 4 weeks after the lesions were developed neither inhibited nor retarded the laying down of collagen in response to quartz exposure. In both groups of silicotic animals the collagen contents did not differ significantly (Table 2).

Histopathological examination of the lungs derived from quartz-exposed animals revealed the development of silicotic granulomata, diffusely distributed in the lung parenchyma during early stages of the development of silicotic lesions. The lesions were composed of macrophages, lymphocytes, fibroblasts and blood vessels. The stroma of the lesions consisted of an arborisation of reticulin fibres. With the lapse in time, the reticulin fibres were thickened, more entangled with one another and by 4 weeks the granulomatous lesions were composed predominantly of reticulin fibres with an admixture of few collagen fibres.

A blindfold evaluation of the histological sections of silicotic animals with and without colchicine administration during 4 weeks of the development of lesions did not reveal any difference in the histological picture of lungs of the two groups of silicotic rats.

<table>
<thead>
<tr>
<th>Exposure (weeks)</th>
<th>Wet weight (g)</th>
<th>Dry weight (g)</th>
<th>Total collagen (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Quartz alone</td>
<td>Quartz + colchicine</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.12 ± 0.74</td>
<td>1.53 ± 0.23 NS</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.64 ± 0.38</td>
<td>2.53 ± 0.71 NS</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4.43 ± 0.60</td>
<td>5.08 ± 0.60 NS</td>
<td></td>
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NS = Non significant, *P < 0.05

<table>
<thead>
<tr>
<th>Exposure period</th>
<th>Wet weight lung (g)</th>
<th>Dry weight lung (g)</th>
<th>Total collagen (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.02 ± 0.46</td>
<td>0.37 ± 0.07</td>
<td>11.41 ± 0.09</td>
</tr>
<tr>
<td>Silicotic 16 weeks</td>
<td>6.71 ± 1.71**</td>
<td>1.09 ± 0.29**</td>
<td>121.41 ± 31.33**</td>
</tr>
<tr>
<td>Silicotic 16 weeks + 4 weeks vehicle</td>
<td>8.55 ± 1.19***</td>
<td>1.30 ± 0.26***</td>
<td>141.89 ± 26.88***</td>
</tr>
<tr>
<td>Silicotic 16 weeks + 4 weeks colchicine</td>
<td>7.16 ± 0.96 NS</td>
<td>1.32 ± 0.18 NS</td>
<td>169.34 ± 13.37 NS</td>
</tr>
</tbody>
</table>

*Significantly different as compared to control
**Significantly different when compared between exposed groups
(quin t v/ quartz + colchicine)
* * *P < 0.01
NS = Non significant
The lesions were less cellular, hyalinised and predominantly collagenous after 16 weeks of quartz exposure. The lung sections of 20 week silicotic animals having administered colchicine for 4 weeks after 16 weeks of quartz exposure, presented a histological picture consisting of developed collagenous silicotic lesions. The extent of development of lesions and collagen matrix was similar to that present in the lung sections of 20 week silicotic animals not receiving colchicine.

In vitro studies—The results of in vitro studies demonstrate—that exposure to quartz particulates brings about an increase in the release of LDH enzyme activity in the supernatant culture medium. The increase was particularly marked in cultures in which the quartz- macrophage interaction took place over 20 hr in presence of serum. Leakage of proteins from cells followed a trend similar to that seen with LDH release. Addition of colchicine varying in concentration from 0.02 to 0.5 µg/ml in the test system did not significantly inhibit or retard quartz-induced release of LDH or protein into the cell supernatant (Table 3).

Discussion

The results of the present investigation reveal that colchicine administration does not significantly alter the development of silicotic lesions. The lung weight and collagen contents, measured biochemically and histologically, increased with lapse in time in groups of animals exposed intratracheally to quartz dust and animals similarly exposed but receiving simultaneously colchicine through oral route. Colchicine administration was also ineffective to cause resorption of already laid down collagen in response to quartz exposure.

The use of colchicine in the present investigation was dictated by a number of considerations. An initial inflammatory response, interaction between various cell types in which alveolar macrophages and fibroblasts play a key role and release of cytokines are critical for silica-induced pulmonary fibrosis. Colchicine is used as an anti-inflammatory agent to treat gouty arthritis. Besides, this antimitotic agent is known to inhibit synthesis of proteoglycans essential for collagen biosynthesis, inhibit release of fibronectin and alveolar macrophage-derived growth factor that stimulates fibroblast proliferation, interferes in transcellular movement of collagen, increases collagenase production and stabilizes plasma membrane. It has also been used as an antifibrotic agent in the management of pulmonary idiopathic fibrosis and hepatic cirrhosis. There are conflicting reports concerning the effect of colchicine on bleomycin-induced pulmonary fibrosis. Zhang et al. reported that colchicine administration to bleomycin-injected rats resulted in a slight drop of fibroblast proliferation as compared to the untreated group. The mechanism of protection by colchicine was shown to be due to its potential to diminish enzyme activity of collagenase, lysyl oxidase and prolyl hydroxylase, which play a key role in collagenesis. In contrast, Ben-Yehuda et al. have demonstrated that colchicine does not ameliorate the bleomycin induced lung inflammation and fibrosis. Release of proinflammatory mediators by the affected cells has been demonstrated to be essential event in the genesis of lung injury and fibrosis following silica dust exposure. Inability of colchicine to inhibit silicotic fibrogenesis may partly account for the slow turn over of collagen in lungs in response to silica

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Effect of colchicine on release of LDH and protein in culture supernatant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type of Exposure</strong></td>
<td><strong>3 hr</strong></td>
</tr>
<tr>
<td></td>
<td>LDH (mU/ml)</td>
</tr>
<tr>
<td>Control</td>
<td>3.13±1.77</td>
</tr>
<tr>
<td>Quartz 50 µg/ml</td>
<td>3.48±0.54</td>
</tr>
<tr>
<td>Quartz 50µg/ml+ colchicine 0.5 µg/ml</td>
<td>3.06±1.26</td>
</tr>
<tr>
<td>Quartz 50 µg/ml+ colchicine 0.1 µg/ml</td>
<td>3.49±0.39</td>
</tr>
<tr>
<td>Quartz 50 µg/ml+ colchicine 0.02 µg/ml</td>
<td>2.49±0.23</td>
</tr>
</tbody>
</table>

F-values 0.354NS 0.577NS 6.319* 1.358NS

Treatment df, Error df (4,9) (4,7) (4,9) (4,10)

*P < 0.05

During incubation for 20 hr the cultures were fortified with 5% serum.
exposure and developmental differences in the origin of cells involved in laying down collagen in lungs and elsewhere in the body. The results of the present study suggest that development and progression of pulmonary silicotic fibrogenesis is unlikely to be altered by colchicine administration and it would be unwise to advocate its use in the management of silica dust-induced pulmonary fibrosis.

Acknowledgement

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References


