The appropriate time for stem cell transplantation in albino rat with amiodarone induced lung fibrosis: histological and immunohistochemical study

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ABSTRACT

Background: The fibrosing form of lung injury had bad progressive course and lethal outcome.

Aim of the work: This study throws light on the possible protective and/or therapeutic consequence of mesenchymal stem cell therapy in lung fibrosis induced by amiodarone in albino rat. In addition to study the possible recovery of damaged lung after amiodarone withdrawal.

Material & Methods: Forty five adult male albino rats were divided into five equal groups. Group I (control); group II (amiodarone) received amiodarone 30 mg/kg orally for 6 days/week for 6 weeks, group III (withdrawal); group IV (early stem cell) mesenchymal stem cell was administrated after 1st dose of amiodarone therapy; group VI (late stem cell) mesenchymal stem cell was administrated immediately after amiodarone suspension. Lung samples were processed and examined using histological and immunohistochemical techniques and the obtained data was subjected to statistical analysis.

Results: Amiodarone treated group displayed obstructed bronchioles, collapsed alveoli, inflammatory cell infiltration, thickened interalveolar septa and pulmonary vessels wall. Increased collagen fibers deposition between alveoli and around blood vessels in addition to highly expressed caspase-3. Group III (withdrawal) recorded only mild improvement. Group IV (early stem cell therapy) showed advanced improvement of pulmonary alveolar architecture, on other side group V (late stem cell therapy) revealed moderate improvement of lung injury.

Conclusion: The majority of amiodarone lung toxicity manifestations and lung fibrosis were regressed by bone marrow derived mesenchymal stem cell (BMSCs) therapy and the best result obtained when stem cell initiated early in the development of pathogenesis.

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Key Words: Amiodarone; Bone marrow derived mesenchymal stem cell; Idiopathic pulmonary fibrosis; Interalveolar septa; Stem cell.

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INTRODUCTION

Idiopathic Pulmonary Fibrosis is a chronic progressive (IPF) and gradually fibrotic lung disease[1]. IPF is now generally regarded as a consequence of multiple interacting genetic and environmental risk factors with repetitive local micro injuries to aging alveolar epithelium. These micro injuries stimulate fibroblasts proliferation, produces extra cellular matrix expansions and altered matrix composition and biomechanics, induces matrix produces myofibroblast and aberrant remodeling of lung interstitium[2]. Clinically, these changes can result in decreased oxygenation, respiratory failure, and eventually death[3].

The precise pathologic mechanisms of pulmonary fibrosis are not fully understood, the ideal therapy for pulmonary fibrosis is still controversial[4]. Therapeutic options remain limited despite an increased and recent interest for new antifibrotic and anti-inflammatory[5]. Lung transplantation is considered the only effective therapy for pulmonary fibrosis[6]. However, lung transplantation has many obstacles due to organ shortages and complications resulted from long-term immunosuppression[7]. Therefore, it is necessary to uncover new effective therapies to reduce or reverse pulmonary fibrosis for reducing the morbidity and mortality associated with pulmonary fibrosis and the urgent for lung transplantation.

Amiodarone is an iodinated benzofuran derivative is the best effective agent used for the treatment of various cardiac arrhythmias especially to suppress ventricular and supraventricular tachyarrhythmias also for long-term management of atrial fibrillation and even prophylactic of its recurrence[8]. Amiodarone pulmonary toxicity is considered the most serious adverse effects of amiodarone and limits its use[9]. Interstitial pneumonitis is the most common presentation of amiodarone-induced pulmonary disease[10].

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Bone marrow mesenchymal stem cells (BMSCs) are multipotent stem cells which are capable of self-renewal and can differentiate into a variety of lineages under suitable conditions. BMSCs are now extensively used in several fields help in regenerative medicine, gene therapy and tissue repair and represent an promising field research for the future[11].

The ability to regenerate human lung tissue, if successful, would constitute a breakthrough in modern medicine. however, currently there is no effective strategy to regenerate lost bronchioles or alveoli in humans. Stem cell-based regenerative medicine holds great potential for combating tissue damage in lung diseases and other disorders by providing unlimited materials for transplant[12,13,14]. Ma et al identified a previously unknown population of adult human lung stem cells, which were successfully used for generating functional human lung air exchanging units following their transplantation to a mouse model and also in a pilot human clinical trial[15].

The present study aimed to study if the time factor is important in BMSCs therapy in interstitial pulmonary fibrosis and detection the possible regression of fibrosis after recovery period.

MATERIAL AND METHODS

Animals

Forty five adult male albino rats weighing 150–200 were divided into equal five groups. The animals were obtained from the animal house, Moshtohor Faculty of Veterinary Medicine, Benha University.

Each group was kept in a separate cage under good hygienic conditions and normal balanced diet and tap water. All ethical protocols for animal treatment were followed.

Drug

Amiodarone was given in the form of tablet (Cordarone, 200 mg) that was obtained from Global Napi Pharmaceuticals, Egypt. The requested daily dose (30 mg/kg body weight) was dissolved in 3 ml of 0.6% methylcellulose (vehicle of amiodarone)[16], so rats received 4.5-6 mg of AMD dissolved in 3 ml of 0.6% methylcellulose. Methylcellulose was obtained from El-Gomhoria, the company for chemical and medical trading, Benha, Egypt. Systemically injected 5 x106 MSCs/100 ul in PBS (Phosphate Buffered Saline) was administered by intravenous injection in the tail vein immediately after the 1st dose of amiodarone in group II and in the 1st day of amiodarone therapy 5 x106 MSCs/100 ul in PBS was administered by intravenous in the tail vein.

Experimental procedure

Forty five adult albino male rats (Sprague-Dawley strain) were divided into five groups of nine animals in each group as following:

(1) Group I (control group) this group included 9 rats, divided into three rats for each subgroup:

a. Subgroup Ia (n=3) were not received any treatment and served as negative control.

b. Subgroup Ib (n=3) received equivalent dose of methylcellulose through an orogastric tube daily for 6 weeks.

c. Subgroup Ic (n=3) received 0.5 ml of buffer phosphate saline (PBS) by IV injection in the tail vein and then they were left for 6 weeks.

(2) Group II (amiodarone-treated group) included 9 rats that received the prescribed dose of amiodarone orally for 6 weeks daily and administrated by orogastric tube[17].

(3) Group III (withdrawal group) included 9 rats that received the same dose of amiodarone for 6 weeks and then left for recovery for another 6 weeks[18].

(4) Group IV (early SC group) included 9 rats that received AMD therapy as before in the same dose, by the same route and for the same duration (6 weeks) as in group II in the 1st day of amiodarone therapy 5 x106 MSCs/100 ul in PBS was administered by intravenous in the tail vein.

(5) Group V (late SC group) included 9 rats that received amiodarone as before for 6 weeks, after the end of amiodarone therapy then animals were injected with bone marrow derived stem cell (BMSCs) by the same dose as in group IV in the tail vein, then rats were left for another 6 weeks following MSC therapy.

At the end of experiment, the animals Anaesthetized with an intraperitoneal injection of 75 mg/kg ketamine (intraperitoneal), sacrificed and the chest was opened and specimens of the left lung were fixed in 10% formal saline for 24 hours. Paraffin blocks were prepared and 5 μm thick sections mounted on canda balsm coated slides in case of ordinary and special staining and poly-L-lysine coated and charged slides in case of immunostaining. Subjected to the following stains.

Histological stains

Hematoxylin and eosin[9].

Masson’s trichrome stains for collagen fibers[20].

Immunohistochemical study

Caspase stain section detection of caspase-3 using a standard avidin-biotin peroxidase complex system according to the kit used (Neomarkers) followed by diaminobenzidine (DAB) visualization[21].

CD105 immunostaining[22] the marker for or human mesenchymal stem cells. 0.1 ml prediluted primary antibody (CD105) rabbit polyclonal Ab (ab27422) and incubated at room temperature in moist chamber for 30–60 minutes. Tonsil used as positive control specimens. Cellular localization is the cell membrane. On the other hand, one of the lung sections was used as a negative control by passing the step of applying the primary antibody.

Labeling of MSCs[23]

MSCs were marked using 50 μm iron oxide in 4 ml
alveoli are apparent normal (Fig. 4). Compensatory dilatation of neighboring ones, some other blood vessels wall, focal areas of collapsed alveoli with thickened septa, around alveoli and bronchioles, thickened moderate inflammatory cell infiltration in the moderately The lung sections revealed distended bronchioles, moderate inflammatory cell infiltration in the moderately thickened septa, around alveoli and bronchioles, thickened blood vessels wall, focal areas of collapsed alveoli with compensatory dilatation of neighboring ones, some other alveoli are apparent normal (Fig. 4).

Morphometric and quantitative study

Fibrosis fraction the degree of fibrosis was quantified by analyzing slides that were stained with Masson’s trichrome stain. The image analysis program was configured to detect areas of blue stained collagen within each field. At a magnification of 400, ten randomly selected constant fields per slide were selected. The fraction of blue-stained collagen areas for each field was averaged for each animal. The area fraction of fibrosis is presented as a percentage.

Caspase-3 Labeling Index (caspase 3-LI) as brown cytoplasmic staining (index for the degree of nuclear apoptosis) was quantified in ten images from ten non-overlapping fields of each rat using Image-Pro Plus program version 6.0 (Media Cybernetics Inc., Bethesda, Maryland, USA), by the total number of evaluated cells at a magnification of 400 then multiplying by 100. Statistical analysis

All the data collected from the experiment were recorded and analyzed using Statistical Package for Social Sciences software (SPSS) Statistics software for Windows, Version 20 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) with Pošť Hoc LSD test was used to compare differences among the groups. In each test, the data was expressed as the mean (M) value, standard deviation (SD) and differences were considered to be statistically significant at P<0.01.

RESULTS

Haematoxylin and eosin (H&E) stained sections

Group I (control) lung sections revealed numerous alveolar sacs, the alveoli appeared patent with thin interalveolar septa, the bronchiolone lined by simple cilicated coelamer epithelium (Fig. 1). The lining epithelium of the alveoli was composed of squamous cells (type I pneumocytes) and large cuboidal cells (type II pneumocytes) (Fig. 2).

Group II (AMD) revealed distorted bronchiole with partial obliteration of the lumen by detached epithelial cells, inflammatory cellular infiltration around alveoli and in the adventitia of bronchioles were observed, also cellular aggregates detected in marked thickened septa, collapsed alveoli and thickened pulmonary vessel, with extravasations of red blood cells in the septa (Fig. 3).

Group III (withdrawal) showed relative improvement of lung architecture but not complete histological recovery. The lung sections revealed distended bronchioles, moderate inflammatory cell infiltration in the moderately thickened septa, around alveoli and bronchioles, thickened blood vessels wall, focal areas of collapsed alveoli with compensatory dilatation of neighboring ones, some other alveoli are apparent normal (Fig. 4).

Group IV (early stem cell) showed that the destructive effects of AMD on the lung are deteriorated except for relative thickening of blood vessels, mild thickened interalveolar septa with mild inflammatory cell infiltration around alveoli and around the bronchiole (Fig. 5).

Group V (late stem cell) showed moderate improvement of lung tissue, moderate thickened IAS with moderate inflammatory cell infiltration, scattered extravasations of RBCs in the septa and thickened pulmonary blood vessels (Figs. 6).

Masson’s trichrome stained sections

Group I showed minimal collagen fibers present around alveoli and the blood vessels (Fig. 7). Group II showed heavy collagen deposition around the blood vessels, bronchioles and the alveoli (Fig. 8). Group III showed moderate collagen fibers deposition around bronchioles, alveoli and blood vessels (Fig. 9). Group IV showed minimal collagen fibers around alveoli, bronchioles and blood vessels (Fig. 10). Group V showed moderate collagen fibers deposition around bronchioles, alveoli and blood vessels (Fig. 11).

CD105 immunostaining sections

The control group showed negative immunostaining for CD105 in the alveoli, bronchioles and interalveolar septa (Fig. 12). Group IV (early SC) showed squamous shaped and cuboidal positive CD105 cytoplasmic reaction in alveoli and septa (Fig. 13). Group V (late SC) showed fewer positive CD105 cells were detected in the septa and among the lining of alveoli (Fig. 14).

Caspase-3 immunostaining

Positive immunohisto-chemical staining of caspase-3 demonstrated as brown cytoplasmic staining (index for the degree of nuclear apoptosis). Group I showed very minimal cytoplasmic staining of caspase-3 in the cytoplasm of alveolar and septa cells (Fig. 15). Group II showed highly expressed caspase-3 reaction in the cytoplasm of pneumocyte type 1 and pneumocyte type II of alveolar and septal cells (Fig. 16). Moderate caspase-3 expression was detected group III in the cytoplasm of pneumocyte type 1 and pneumocyte type II of alveolar and septa cells (Fig. 17). Group IV showed mildly expressed caspase-3 reaction (Fig. 18) in the cytoplasm of pneumocyte type 1 and pneumocyte type II of alveolar and septa cells, while in group V was detected in limited areas in the cytoplasm of pneumocyte type 1 and pneumocyte type II of alveolar and septa cells (Fig. 19).

Morphometric results

The mean area % of collagen fibers deposition and of caspase-3 labeled index for all groups was represented in (Tables 1, 2) and (Histograms 1, 2). The collagen fiber area percentages and caspase-3 labeled index of group II rats revealed significant increase (P< 0.01) compared to that of the control group. There was significant decrease
(P< 0.01) in collagen fibers accumulation and in caspase-3 expression in groups IV and V compared to that of group II (AMD). Group III (withdrawal) showed insignificant decrease in collagen fibers accumulation and caspase-3 labeled index compared to that of group II.

Fig. 1: A photomicrograph of a section in the lung of rat of group I (control) showing alveoli (a), thin septa (I), alveolar sacs (s) and bronchiole(b). (H&E x 100)

Fig. 2: A photomicrograph of a section in the lung of rat of group I (control) showing lung alveoli (a) lined with type I pneumocyte squamous cells (P1), type II pneumocyte cuboidal cells (P2), alveolar sacs (s), thin septa(I). (H&E x 400).

Fig. 3: A photomicrograph of a section in the lung of rat of group II (amiodarone) showing collapsed alveoli (ca) wall, heavy cellular infiltration (arrow) in IAS and around bronchiole, markedly thickened septa (I), partially obliterated bronchiole (b) by detached epithelial cells (c), congested and thickened pulmonary vessel wall (V). Note many extravasations of RBCs (*) in the septa and around alveoli. (H&E x100)

Fig. 4: A photomicrograph of a section in the lung rat of group III (withdrawal) showing focal areas of collapsed alveoli (ca), some patent alveoli (a), moderate cellular infiltration (arrow) in the septa and the adventitia of distended bronchiole, moderate thickened septa (I) and thickened wall of pulmonary vessels(V). (H&E x 100).

Fig. 5: A photomicrograph of a section in the lung of rat of group IV (early stem cell) showing collapsed alveoli (ca), some patent alveoli (a), moderate cellular infiltration (arrow) in the septa and the adventitia of distended bronchiole (b). Moderate thickened septa (I) and thickened wall of pulmonary vessels (V). Note little extravasations of RBCs (*) in the septa. (H&E x100).

Fig. 6: A photomicrograph of a section in the lung of rat of group V (late stem cell) showing alveoli (a) with mild thickened septa(I) in between, moderate inflammatory cellular infiltration (arrow) in the septa and around adventitia of bronchiole(b) and around congested moderate thickened blood vessels(V). Note little extravasations of RBCs (*) in the septa. (H&E x100).
Fig. 7: A photomicrograph of a section in the lung of rat of group I (control) showing little collagen fibers (arrow) around alveoli, bronchiole and blood vessel. (Masson x100).

Fig. 8: A photomicrograph of a section in the lung of rat of group II (amiodarone) showing marked collagen fibers deposition (arrow) around alveoli, bronchiole and blood vessel. (Masson x100)

Fig. 9: A photomicrograph of a section in the lung of rat of group III (withdrawal) showing moderate collagen fibers (arrow) around bronchioles, alveoli and around blood vessel. (Masson x100)

Fig. 10: A photomicrograph of a section in the lung of rat of group IV (early stem cell) showing little collagen fibers (arrow) present around alveoli, bronchiole and blood vessel. (Masson x100).

Fig. 11: A photomicrograph of a section in the lung of rat of group V (late stem cells) showing moderate collagen fibers deposition (arrow) around alveoli, bronchiole and around blood vessel. (Masson x100)

Fig. 12: A photomicrograph of a section in the lung of rat of group I (control) showing negative cytoplasmic CD105 reaction in the alveoli (a), bronchi and interalveolar septa (I). (CD105 x 400).
Fig. 13: A photomicrograph of a section in the lung of rat of group IV (early stem cell) showing squamous shaped (black arrow) and cuboidal cells (green arrow) positive CD105 cytoplasmic reaction in alveolar lining and septal cells. (CD105 x 400).

Fig. 14: A photomicrograph of a section in the lung of rat of group V (late stem cell) showing squamous shaped (black arrow) and cuboidal cells (green arrow) positive CD105 cytoplasmic reaction in alveolar lining and septal cells (CD105 x 400).

Fig. 15: A photomicrograph of a section in the lung of rat of group I (control) showing slight caspase-3 expression of the cytoplasm of pneumocyte type 1 (black arrow) and pneumocyte type II (green arrow) in the alveolar lining and septal cells. [Caspase-3 x 400].

Fig. 16: A photomicrograph of a section in the lung rat of group II (amiodarone) showing highly caspase-3 expression of the cytoplasm of pneumocyte type 1 (black arrow) and pneumocyte type II (green arrow) in the alveolar lining and septal cells. [Caspase-3 x 400].

Fig. 17: A photomicrograph of a section in the lung of rat of group III (withdrawal) showing moderate caspase-3 expression of the cytoplasm of pneumocyte type 1 (black arrow) and pneumocyte type II (green arrow) in the alveolar lining and septal cells. [Caspase-3 x 400].

Fig. 18: A photomicrograph of a section in rat Lung of the group IV (early stem cell) showing limited caspase-3 expression (arrow) of the cytoplasm of pneumocyte type 1 (black arrow) and pneumocyte type II (green arrow) in the alveolar lining and septal cells. [Caspase-3 x 400].
**Fig. 19:** A photomicrograph of a section in the lung of rat of group V (late stem cell) showing mild caspase-3 expression (arrow) of the cytoplasm of pneumocyte type I (black arrow) and pneumocyte type II (green arrow) in the alveolar lining and septal cells. [Caspase-3 x 400].

**Table 1:** showing the mean area %, SD of collagen fibers deposition in groups I, II, III, IV and V with comparison between all groups by Post Hoc LSD test.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean area %</th>
<th>SD</th>
<th>Significance at P &lt; 0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0.18%</td>
<td>0.0343</td>
<td>b,c,d,e</td>
</tr>
<tr>
<td>Group II</td>
<td>4.26%</td>
<td>0.2978</td>
<td>a,d,e</td>
</tr>
<tr>
<td>Group III</td>
<td>3.89%</td>
<td>0.4017</td>
<td>a,d,e</td>
</tr>
<tr>
<td>Group IV</td>
<td>1.63%</td>
<td>0.0804</td>
<td>a,b,c,e</td>
</tr>
<tr>
<td>Group V</td>
<td>2.41%</td>
<td>0.3342</td>
<td>a,b,c,d</td>
</tr>
</tbody>
</table>

**Table 2:** showing the mean area %, SD of caspase-3 expression in groups I, II, III, IV and V with comparison between all groups by Post Hoc LSD test.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean area %</th>
<th>SD</th>
<th>Significance at P &lt; 0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0.12%</td>
<td>0.0476</td>
<td>b,c,d,e</td>
</tr>
<tr>
<td>Group II</td>
<td>7.13%</td>
<td>0.4651</td>
<td>a,d,e</td>
</tr>
<tr>
<td>Group III</td>
<td>6.89%</td>
<td>0.4017</td>
<td>a,d,e</td>
</tr>
<tr>
<td>Group IV</td>
<td>0.96%</td>
<td>0.0804</td>
<td>a,b,c,e</td>
</tr>
<tr>
<td>Group V</td>
<td>1.91%</td>
<td>0.4558</td>
<td>a,b,c,d</td>
</tr>
</tbody>
</table>

**DISCUSSION**

IPF is an intractable respiratory disease with poor prognosis. It has been associated with a histopathologic and/or radiologic pattern of usual interstitial pneumonia [27]. Chronic interstitial pneumonitis with pulmonary fibrosis is the most common serious pulmonary complications often associated with AMD therapy [28].

The current study showed that administration of amiodarone altered the histological architecture of the lung and appeared in the form of damaged alveoli, focal areas of collapsed alveoli, marked thickening of septa which infiltrated by inflammatory cellular aggregates. The inflammatory cells aggregates also detected around alveoli and adventitia of bronchioles. Closer observation showed many mononuclear inflammatory cell infiltration most probably fibroblasts and fibrocytes in interalveolar pta. these destructive effects induced by AMD confirmed in the study of [29] who study the therapeutic effect of stem cell on amiodarone induced lung fibrosis in rats. In another study on human cardiac case treated with amiodarone his pathologic examination revealed organizing pneumonia or diffuse alveolar damage with hyaline membrane formation caused by amiodarone lung toxicity [30].

Several mechanisms of amiodarone lung toxicity have been postulated, including direct cellular damage, induction of phospholipidosis and indirectly by an immunomlogic mechanisms by the activation of natural killer cell activity [31]. Activation of alveolar macrophages and cytokine release [32,33]. It was reported that cell death in the lungs in advanced lung disease was followed by inflammation and fibrosis [34].

The alveolar collapse might be attributed to the imbalance between output and degradation of the surfactant. Excessive production of surfactant by hyperplastic pneumocytes type II appeared to exceed the ability of alveolar macrophages to degrade it [35].

The present work showed administration of amiodarone led to distortion in the bronchioles and partial obliteration of the lumen by detached cells and this agreed with the...
study of[36] on rats received amidarone daily for one month. The occurrence of obliterative bronchiolitis explained by[37] who stated that the injury of bronchiolar epithelium and inflammation of subepithelial structures lead to excessive fibrocytes and fibroblast proliferation, which is due to improper tissue repair and ineffective epithelial regeneration.

In the current study, the blood vessels appeared sclerotic with marked thickened wall. Thickening of the wall of pulmonary arterioles can lead to the development of pulmonary hypertension[38]. The pulmonary blood vessels were congested with extravasations of red blood cells between alveolar cells and septa. These results were in line with[39] who added the presence of intra-alveolar hemorrhage and formation of hyaline material and cellular aggregates of fibroblasts and macrophages in his study on rats received AMD therapy for six weeks.

The pathologic pattern of pulmonary capillaritis, is characterized by neutrophil infiltration of the alveolar septa which sequentially leads to necrosis of these structures, loss of capillary structural integrity, and spilling of red blood cells into the alveolar space and interstitium[40,41,42]. However, diffuse alveolar hemorrhage was uncommon in AMD toxicity[43].

The current study stated that most of the pathological changes induced by amidarone relieved in withdrawal group but not reach to the level of complete recovery, lung tissues improved except for the presence of scattered focal areas of collapsed alveoli, moderate thickened IAS with compensatory dilatation of neighboring ones, other alveoli are normal and moderate inflammatory cellular infiltration, thickening of blood vessel wall. These data confirmed by an insignificant decrease of collagen fiber accumulation and caspase-3 expression reaction in the recovery group when compared to AMD group.

The incomplete reversibility of the histological changes of lung tissue after amidarone withdrawal reported in the study of[44] and might be explained by the short time of stoppage of the amidarone and its tendency to accumulate in the lung therapy[45].

The incomplete reversibility of the histological changes of lung tissue after amidarone withdrawal might be explained by the short time of stoppage of the amidarone and its tendency to accumulate in the lung tissue[46].

MSCs are multipotent cells capable of differentiating into a number of different cell lines and exerting antiproliferative, immunomodulatory, and anti-inflammatory effects. Because of their combination of multipotency, migratory ability, and immunoprivileged state (MSCs do not express major histocompatibility factor II, making allogeneic transplant possible)[47].

In the current work, it was reported that SC therapy is very effective when initiated in the start of amidarone treatment, histopathologically the early administration of stem cell led to definite improvement of destructed lung tissue and even regaining of normal alveolar architecture except for mildly thickened septa, mild inflammatory cellular infiltration and moderately thickened blood vessels wall and this may be due to the direct relieving effect of early stem cell therapy on lung fibrosis.

On the other side late SC administration after the end of amidarone suspension led to relative improvement but not effective like early SC administration, histopathologically lung tissue section showed moderately thickened septa with moderate inflammatory cell infiltrations and moderate thickened blood vessels walls. These results were supported by a significant decrease in the mean area percentage of both collagen fibers and caspase-3 expression in both stem cell groups compared to group II and group III.

These important data in accordance with the study of[48] who proved that the early administration of bone marrow-derived mesenchymal stem cells (BMSCs) were more effective on relieving of lung. However, in his study a significant reduction in the fibrotic changes with a significant decrease in mean area % of collagen fibers detected only in early SC group but not in the late SC group.

These data explained in another study who reported that delaying MSC administration eliminated the ability of the cells to alter the course of disease progression, and further added that early treatment with MSCs may produce antagonists cytokines that disrupt signal pathways reducing the extent of inflammation within the lungs[49].

The study of[50] it was documented that the timing of cell administration after lung injury can influence phenotypic conversion of donor-derived cells. Early administration of MSCs resulted in engraftment of cells as epithelial and vascular endothelial cell while administration of cells at later time points resulted in engraftment as interstitial cells.

This idea is confirmed by another study discussed the effect of SC therapy on bleomycin-induced lung fibrosis and reported that the exogenous administration of MSCs was proved to attenuate bleomycin-induced lung injury by downmodulating the inflammatory responses and ameliorating fibrotic effects[51].

In another study on the role of mesenchymal stem cell on lung fibrosis due to post exposure to irradiation stated that the infusion of BMSCs at a later stage of lung injury can be harmful and even may augment fibrosis and even further added that MSCs injected into the lung immediately after lung injury could differentiate into functional lung cells, while those injected at later stage would be involved in fibrosis.

Development and differentiation of BMSCs occurred in response to mediators produced in the injured tissue[52], so unwise to give stem cell late to those patients.

These results point to take care for the selection the optimal time for administration of stem cell and ensured that the timing factor has an important role in altering the course of fibrosis progression so it was recommended to be
given at the beginning of pathogenesis and consequently increase the survival chances for those suffering from lung fibrosis.

CONFLICTS OF INTEREST

None declared.

REFERENCES


الملخص العربي

الوقت المناسب لزرع الخلايا الجذعية في الجرذان البيضاء مع التليف الرئوي المستحث بعقار الأميودارون: دراسة نسيجية ومناعة كيميائية

المقدمة:
الإصابة الليفية في الرئة لها مسار تدريجي سيئ ونتائج مميتة.

الهدف من العمل:
تلقى هذه الدراسة الضوء على التأثير الوقائي و/أو العلاجي المحتمل للعلاج بالخلايا الجذعية الميزنشيمية في تليف الرئة الناجم عن الأميودارون في الجرذان البيضاء. بالإضافة إلى دراسة إمكانية استعاد الرئة التالفة بعد انسحاب الأميودارون.

المواد والطرق:
تم تقسيم خمسة وأربعين من ذكور الجرذان البيضاء إلى خمس مجموعات متساوية. المجموعة الأولى (الضابطة)؛ المجموعة الثانية (الأميودارون) تلقى الأميودارون 30 مجم/كج عن طريق الفم لمدة 6 أيام/أسبوع لسنتين. المجموعة الثالثة (الانسحاب)؛ المجموعة الرابعة (الخلايا الجذعية المبكرة) تم إعطاء الخلايا الجذعية الميزنشيمية في بداية العلاج بالأميودارون؛ المجموعة الخامسة (الخلايا الجذعية المتأخرة) تم إعطاء الخلايا الجذعية الميزنشيمية مباشرة بعد تعليق الأميودارون.

النتائج:
المجموعة المعالجة أميودارون أظهرت انسداد القصيبات الهوائية، وانغلاق الحوصلات الهوائية، زيادة سبائك الكولاجن بين الحوصلات الهوائية، تورمها، زيادة عد مظاهر التسمم الرئوي، وتشكل التليف الرئوي، وزيادة عدد الخلايا الالتهابية في الجرذان. لم يظهر أي تأثير في المجموعة_without_العلاج. المجموعة III (الخلايا الجذعية المبكرة) أظهرت تحسنًا في النسيج الرئوي في حين لم يظهر أي تحسن في المجموعة د (العلاج بالخلايا الجذعية المتأخرة).

الاستنتاج:
تم تراجع معظم مظاهر التليف الرئوي المصاحبة بالنمط الأمريكي وأفضل نتيجة تم الحصول عليها عندما بدأت الخلايا الجذعية في مرحلة مبكرة من تطور المرض.