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Ameliorating Effect of Platelet-Rich Plasma on Lung Fibrosis Induced by Amiodarone in Albino Rats

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Abstract

Objectives: This study aimed to evaluate the histopathological impact of platelet rich plasma (PRP) in treatment of lung fibrosis induced by amiodarone drug.

Materials and Methods: The rats were divided into three groups (n=15). The first group used as control group. The rats in group 2 were injected (i.p.) daily with amiodarone drug at (80 mg/ kg. bwt) for three weeks, then injected (24 hour after last dose of amiodarone) (i.p.) with phosphate buffer saline (PBS) (0.5 ml/ kg.bwt) two times weekly for three weeks. Group 3 were injected (i.p.) daily with amiodarone drug at (80 mg/ kg. bwt) for three weeks, plus (i.p.) injection with platelet rich plasma (PRP) at dose (0.5ml/kg.bwt) (24 hour after last dose of amiodarone injection) two time for three weeks. The animals were examined during the experiment and sacrificed at 4th, 5th, and 6th week of the experiment.

Results: amiodarone resulted in significant lung tissues alteration as interstitial pneumonia and fibroblast proliferation. Group 3 revealed significant reduction in inflammatory lesion and tissues fibrosis.

Conclusions: This study concluded that platelet rich plasma has remodeling effects against damaged effect of amiodarone in albino rats.

Keywords

Amiodarone; Platelet rich plasma; Lung fibrosis; Rats

Introduction

Pulmonary fibrosis is a progressive and fatal lungs disease characterized by fibroblasts proliferation and deposition of extracellular matrix in lungs tissues. It consider the end stage of a wide range of lung inflammatory conditions. It initiated after collagen diseases, scleroderma [1], viral infection, rheumatoid arthritis, radiotherapy, inorganic substances as (silica, asbestos) and developed as an adverse effect of some drugs as bleomycin and amiodarone [2]. Pulmonary fibrosis is the most common interstitial lungs disease affecting over five million individuals worldwide with limited therapeutic options and mean survival time about three years [3]. It induced through different factors contributing the development and persistence of the disease including genetic factors, chronic lungs injury, aging, oxidative stress, and impaired healing process [4]. There is no effective treatment reported for pulmonary fibrosis except lung transplantation [5].

Amiodarone is one of the most common iodine-containing compound used for treatment of a wide variety of disease as cardiac arrhythmias [6], ventricular tachycardia, ventricular fibrillation [7], left ventricular dysfunction and heart failure [8]; In otherwise, it causes many adverse effects in lungs as pulmonary toxicity, chronic interstitial pneumonia, diffuse alveolar damage [9] and life-threating pulmonary fibrosis [10].

The use of biologic therapy in treatment of various diseases has increased significantly over the last ten years specifically, platelet-rich plasma (PRP). PRP is a modern treatment strategy with worldwide recognition. It was introduced in the 1950s and is currently used in many branches of medicine [11]. PRP is an autologous blood derivatives with high platelets concentration in a small volume of plasma and considered an alternative treatment for several diseases as, it is low-cost human by product. It decreases the chances of adverse effects and rejection [12]. PRP was used in cardiac surgery, pediatric surgery, gynecology, urology, plastic surgery and ophthalmology [13]. In addition to, oral, maxillofacial surgery and dermatology [14]. It also used in wound healing process as it accelerate repairing of the damaged tissues [15].

Materials and Methods

Drug

Amiodarone produced by Alexandria Company for Arab company for pharmaceuticals

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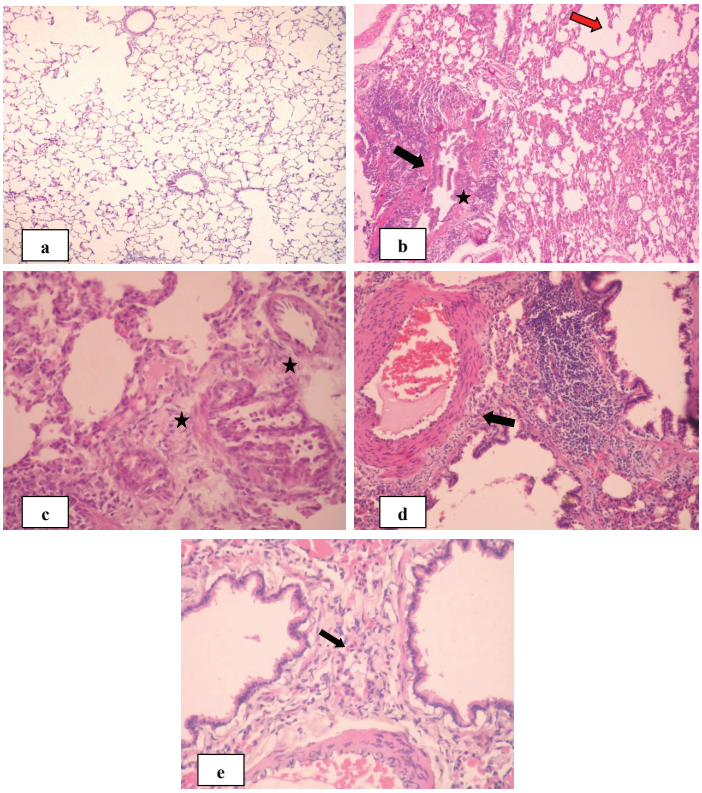

Figure 1: Lung stained with hematoxylin and eosin, (a) control group showing normal lung tissue, (b) amiodarone injected group (gp.2) at 4th week of experiment showing obstructed bronchioles with desquamated epithelial cells and neutrophilic debris inside the lumen (star) emphysematous areas (red arrow) beside, mononuclear cells infiltration around bronchiole (black arrow) (x200), (c,d) amiodarone injected group at 5th week of experiment showing fibroblast proliferation(stars) and periartirial fibrillation (arrow) (x200), (e) group (2) at 6th week of experiment showing fibrosing tissue (x400).

and medicinal plant (MEPACO – MEDIFOOD), Enshas El Raml, Sharkeya, Egypt. The drug was stored in dry place at temperature below 30°C. Each 3ml ampoule containing 150 mg amiodarone hydrochloride, benzyl alcohol, polysorbate 80 and water for injection [16].

Animals and experimental design

Animals: Experimental design and animal handling procedures were approved by the Research Ethical Committee of the Faculty of Veterinary Medicine, South Valley University, Qena, Egypt. Fifty five male Wistar albino rats (each weighing 180-200 gm and two months old) were purchased from the Animal House Facility of the Egyptian Organization for Biological Products and Vaccines (VACSERA), Helwan, Cairo, Egypt, and maintained in clean environment. All animals were allowed to acclimatize in plastic cages (5 animals/cage) inside a well-ventilated room for one week prior to the experiment. The animals were maintained under standard conditions (temperature of 23 ± 3ºC, relative humidity of 60–70%, and a 12 hour light/dark cycle), fed a diet of standard commercial pellets, and given water ad libitum.

Experimental design: Forty five adult male Wistar albino rats were divided into 3 groups (n=15). Group (1) the rats were injected intraperitoneal (i.p.) daily with phosphate buffered saline (PBS) at 0.5 ml/rat for 6 weeks and used as a control group. Group (2) the rats were injected (i.p.) daily with amiodarone drug at (80 mg/ kg. bwt) (0.5 ml /rat) for three weeks, then injected (24 hour after last dose of amiodarone) (i.p.) with (PBS) (0.5ml/kg.bwt) twice weekly until the end of the experiment [17]. Group (3) the rats were injected (i.p.) daily with amiodarone drug at (80 mg/ kg. bwt) (0.5 ml drug/rat) for three weeks, plus (i.p.) injection with platelet rich plasma at dose (0.5ml/kg.bwt) (24 hour after last dose of amiodarone injection) twice weekly until the end of the experiment [18].

Preparation of platelet rich plasma (PRP)

Ten age-matched healthy male Wistar rats were used as PRP donors. The whole blood of rats was drawn through median eye canthus veins and mixed with 3.2% sodium citrate at a blood/citrate ratio of 9/1, centrifuged at 1000 r.p.m for 10 minutes at room temperature and the supernatant was separated and centrifuged again at 800 r.p.m for 10 minutes at room temperature then the above two third was discard and the remaining third (sediment) was used as PRP. The average platelet in PRP was evaluated using a Sysmex XT-1600i system. The platelet count was 800×103 platelets/µL [19].

Tissues samples and histopathological examination

All animals in all groups were examined daily along the experiment and clinical signs and mortalities were recorded. The rats in all groups were sacrificed at 4th ,5th and 6th week from the beginning of the experiment. Lungs were collected from all sacrificed animals and fixed directly in 10% neutral buffered formalin for histopathology examinations. Sections about 5µm thickness were prepared and stained with Harries hematoxylin and eosin stain for histopathological examination [20] and Masson's trichrome stain for detection of collagen fiber in lungs tissues [21].

Results

Histological and histochemical results

Hematoxylin and eosin stain

Group (1) (control group): Examination of lung sections of control albino rats revealed normal typical histological architecture (Figure 1a).

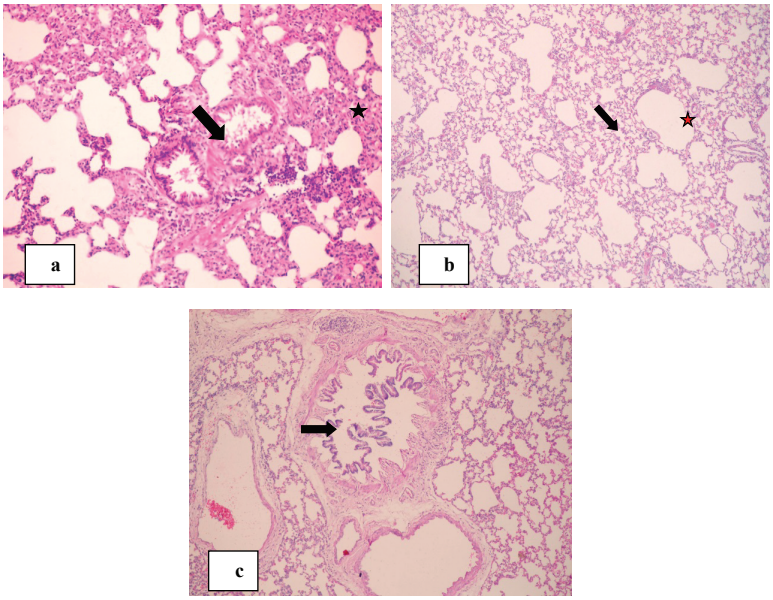

Figure 2: Lung stained with hematoxylin and eosin, (a) platelet rich plasma treated group (gp.3) at 4th week of experiment showing interstitial pneumonia with inflammatory cells infiltration (star) in addition, fibroblast proliferation (arrow) (x200), (b) group (2) at 5th week of experiment showing emphysematous areas (stars) with perialveolar inflammatory cells infiltration (arrow) (x200), (c) group (2) at 6th week of experiment showing fibrosing desquamated epithelium inside bronchial lumen (x200).

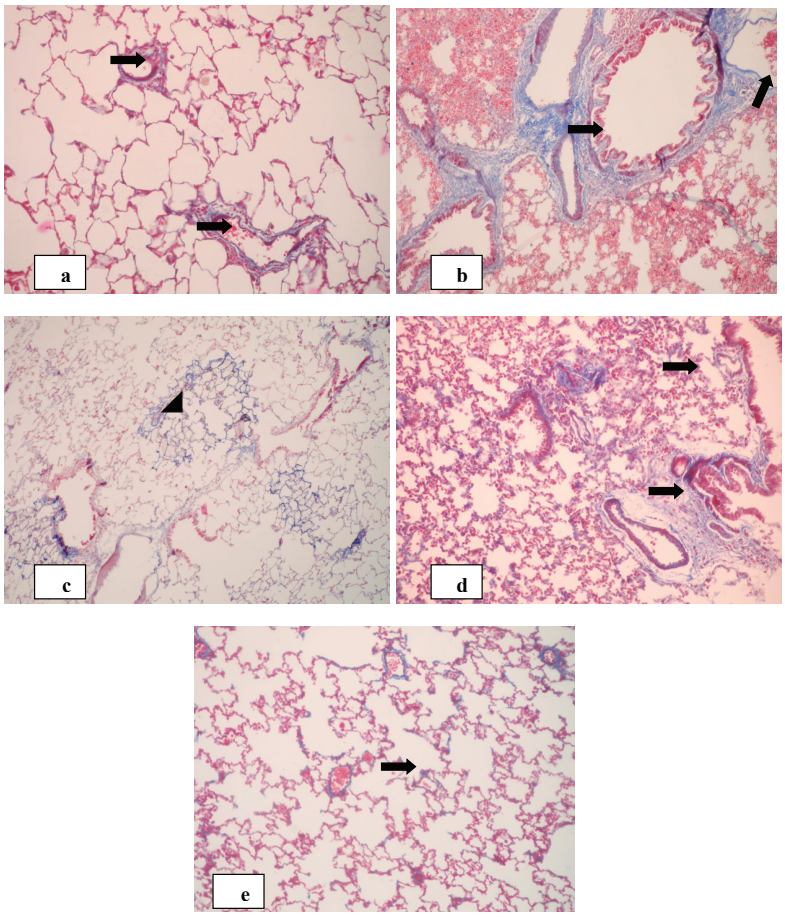

Figure 3: Lung stained with Masson's trichrome (a) control group showing few amount of collagen fibers stained blue peribronchiolar and perivascular tissues in normal lungs (arrows) (x200), (b,c) group (2) at 4th, 5th and 6th week of the experiment showing collagen fibers detected in perivascular and peribronchiolar tissues stained blue (arrows) besides, collagen fiber in perialveolar tissue (arrow head)(x200), (d,e) group (3) at 5th and 6th week of experiment showing few amount of collagen fibers stained blue peribronchiolar and perialveolar tissues (x200).

Group (2) (amiodarone treated group): Lungs at 4th week of the experiment revealed desquamated epithelium and cell debris inside bronchiole lumen with peribronchiolar mononuclear inflammatory cells infiltration (Figure 1b) alveoli showed emphysematous parts and disruption in their wall. Figure (1c, d and e) showed lungs at 5th and 6th week of the experiment massive fibrous tissue proliferation scattered in lung tissue, in addition to, periartirial fibrillation in pulmonary vasculature.

Group (3) (platelet rich plasma treated group): After 4th week of (PRP) injection lungs sections showed interalveolar septa thickening with fibrous tissue proliferation in between bronchioles (Figure 2a). Partial regaining of the normal lung structure, patent alveoli, alveolar sacs, and thin interalveolar septa but with mildly congested blood capillaries and less inflammatory cells infiltration was found. Bronchioles appeared with patent lumen and mildly thickened surrounding layer with less peribronchiolar cellular infiltrations were seen but some bronchioles revealed intraluminal desquamated epithelial cells at 5th and 6th week of the experiment respectively (Figure 2b,c).

Masson's trichrome stain

Minimal collagen fibers were found around bronchioles, and blood vessels of lung sections of control rats (Figure 3a). However, groups 2 showed extensive collagen fibers deposition appeared blue in color in the interalveolar septa, peribronchial and around blood vessels (Figure 3b,c). After PRP injection, moderate to mild collagen fibers deposition was observed (Figure 3 d,e).

Discussion

Amiodarone is an iodinated class III antiarrhythmic drug widely used in treatment of many forms of life-threatening cardiac arrhythmia and sudden cardiac death [22]. However, its use related to many side effects involving different organs as lungs causing pulmonary complications [23]. Lung fibrosis is a lethal pathological process worldwide and has limited therapeutic options [24]. Platelet rich plasma widely used nowadays in various medicinal fields as bone defects [25] and tissue regeneration [26]. As it contains growth factors it plays role in angiogenesis and tissue regeneration [27].

In our work, amiodarone administrated group showed obliteration of the lumen of bronchioles, detachment of the bronchiolar lining epithelium this result was agreed with [28] who referred that to direct toxic effect of amiodarone on the bronchioles mucosa and injury of bronchiolar epithelium as well as its inflammatory reactions. The highly significant increase in collagen fibers percent reported by [29,30] that found increased collagen deposition in amiodarone injected rats was attributed to amiodarone immune and inflammatory mechanisms that lead to fibroblasts stimulation and collagen deposition.

Other others suggested that to amiodarone oxidative stress contributes overexpression of fibrogenic cytokines such as connective tissue growth factor these factors stimulate the differentiation and proliferation of fibroblasts from epithelial and endothelial cells with increased synthesis and deposition of collagen [31].

After PRP treatment (group 3), there was moderate improvement with return of the normal lung structure. Thin interalveolar septa with few inflammatory cellular infiltrations were observed with a highly significant decrease in collagen fibers in comparison with amiodarone-injected group. These results could be explained by the ability of PRP to secrete many cytokines and growth factors that have essential role in tissue regeneration, healing, reducing inflammation and fibrosis reduction (Villela and Santos 2010).

Conclusion

It could be concluded that amiodarone has adverse effects on lung tissue. Platelet rich plasma proved moderate improvement in lung injury and decrease pulmonary fibrosis caused by amiodarone drug and further biochemical and immunohistochimical studies should be evaluated.

References

1. Rafii R, Juarez MM, Albertson TE, Chan AL. A review of current and novel therapies for idiopathic pulmonary fibrosis. J Thorac Dis. 2013 Feb;5(1):48-73.
2. Gross TJ, Hunninghake GW. Idiopathic pulmonary fibrosis. N Engl J Med. 2001 Aug;345(7):517-525.
3. Cottin V. Changing the idiopathic pulmonary fibrosis treatment approach and improving patient outcomes. Eur Respir Rev. 2012 Jun 1;21(124):161-167.
4. Chanda D, Otoupalova E, Smith SR, Volckaert T, De Langhe SP, et al. Developmental pathways in the pathogenesis of lung fibrosis. Mol Aspects Med. 2019 Feb;65:56-69.
5. O'Brien K, Troendle J, Gochuico B. R, Markello TC, Salas J, et al. Pirfenidone for the treatment of Hermansky–Pudlak syndrome pulmonary fibrosis. Mol Genet Metab. 2011 Jun;103(2):128–134.
6. Lapenna D, Ciofani G, Bruno C, Pierdomenico SD, Cuccurullo F. Antioxidant activity of amiodarone on human lipoprotein oxidation. Br J Pharmacol. 2001 Jul;133(5):739–745.
7. Dorian P, Cass D, Schwartz B, Cooper R, Gelaznikas R, et al. Amiodarone as compared with lidocaine for shock-resistant ventricular fibrillation. N Engl J Med. 2002 Mar;346:884-890.
8. Chevalier P, Durand-Dubief A, Burri H, Cucherat M, Kirkorian G, et al. Amiodarone versus placebo and class Ic drugs for cardioversion of recent-onset atrial fibrillation: a meta-analysis. J Am Coll Cardiol. 2003 Jan;41(2):255-262.
9. Chung WH, Bennett BM, Racz WJ, Brien JF, Massey TE. Induction of c-jun and TGF-β1 in Fischer 344 rats during amiodarone-induced pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol. 2001 Nov;281(5):L1180-1188.
10. Wolkove N, Baltzan M. Amiodarone pulmonary toxicity. Can Respir J. Mar-Apr 2009;16(2):43-48.
11. Lana JD, Santana M, Belangero W, Luzo A. Platelet-rich plasma. Lecture Notes in Bioengineering.
12. Marx RE. Platelet-rich plasma: evidence to support its use. J Oral Maxillofac Surg. 2004 Apr;62(4):489-496.
13. Andia I, Rubio-Azpeitia E, Martin JI, Abate M. Current concepts and translational uses of platelet rich plasma biotechnology. Biotechnology: Intech. 2015 Apr;1-31.
14. Del Fabbro, M, Corbella, S, Ceresoli V, Ceci C, Taschieri S. Plasma rich in growth factors improves patients' postoperative quality of life in maxillary sinus floor augmentation: preliminary results of a randomized clinical study. Clin Implant Dent Relat Res. 2015 Aug;17(4):708-716.
15. Villela DL, Santos VLC. Evidence on the use of platelet-rich plasma for diabetic ulcer: a systematic review. Growth factors. Growth Factors. 2010 Apr;28(2):111-116.
16. Waldhauser KM, Török M, Ha HR, Thomet U, Konrad D, et al. Hepatocellular toxicity and pharmacological effect of amiodarone and amiodarone derivatives. J Pharmacol Exp Ther. 2006 Dec;319(3):1413-1423.
17. Al-Shammari B, Khalifa M, Bakheet SA, Yasser M. A mechanistic study on the amiodarone-induced pulmonary toxicity. Oxidative medicine and cellular longevity. 2016 Jan;1-10.
18. Hesami Z, Jamshidzadeh A, Ayatollahi M, Geramizadeh B, Farshad O, et al. Effect of platelet-rich plasma on CCl4-induced chronic liver injury in male rats. Int J Hepatol. 2014;2014:932930.
19. Pazzini JM, Nardi AD, Huppes RR, Gering AP, Ferreira MG, et al. Method to obtain platelet-rich plasma from rabbits (Oryctolagus cuniculus). Pesquisa Veterinária Brasileira. 2016 Jan;36(1):39-44.
20. Drury RB, Wallington EA. Carleton's Histological Technique, 5th edt. Oxford University Press. New York. 1980.

21. Bancroft JD, Gamble M. (Eds.). Theory and practice of histological techniques. Elsevier health sciences. 2008.
22. Saad A, Falciglia M, Steward DL, Nikiforov YE. Amiodarone-induced thyrotoxicosis and thyroid cancer: clinical, immunohistochemical, and molecular genetic studies of a case and review of the literature. Arch Pathol Lab Med. 2004 Jul;128(7):807-810.
23. Uhal BD, Wang R, Laukka J, Zhuang J, Soledad-Conrad V, et al. Inhibition of amiodarone-induced lung fibrosis but not alveolitis by angiotensin system antagonists. Pharmacol Toxicol. 2003 Feb;92(2):81-87.
24. Cooper JA. Jr. Pulmonary fibrosis: pathways are slowly coming into light. Am J Respir Cell Mol Biol. 2000 May;22(5):520-523.
25. Mehta V. Platelet-rich plasma: a review of the science and possible clinical applications. Orthopedics. 2010 Feb;33(2):111.
26. Okamoto SI, Ikeda T, Sawamura K, Nagae M, Hase H, et al. Positive effect on bone fusion by the combination of platelet-rich plasma and a gelatin β-tricalcium phosphate sponge: a study using a posterolateral fusion model of lumbar vertebrae in rats. Tissue Eng Part A. 2012 Jan;18(1-2):157-166.
27. Fortier LA, Barker JU, Strauss EJ, McCarrel TM, Cole BJ. The role of growth factors in cartilage repair. Clin Orthop Relat Res. 2011 Oct;469(10):2706–2715.
28. Barker AF, Bergeron A, Rom WN, Hertz MI Obliterative bronchiolitis. N Engl J Med. 2014 May;370:1820-1828.
29. King TE, Schwarz MI, Brown K, Tooze JA, Colby TV, et al. Idiopathic pulmonary fibrosis: relationship between histopathologic features and mortality. Am J Respir Crit Care Med. 2001 Sep;164:1025-1032.
30. Zidan RA. Effect of long-term administration of amiodarone on rat lung and the possible protective role of vitamin E: a histological and immunohistochemical study. Egypt J Histol. 2011 Mar;4:117–128.
31. Shroff A, Mamalis A, Jagdeo J. Oxidative stress and skin fibrosis. Curr Pathobiol Rep. 2014;2(4):257-267.