Clinicopathological Studies on the Remodeling Effect of Platelet-Rich Plasma on Lung Fibrosis Induced by Amiodarone in Albino Rats

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Results: The PRP treatment (gp. 3) noticed increase in the level of WBCs and RBCs count in comparison with group 2. In addition to, significant increase in glutathione reductase together with decrease in malondialdehyde level in group 3, when compared with group 2. The histopathological finding showed improvement in the fibroed lungs compared to gp (2).

Conclusions: This study concluded the remodeling effects of PRP, which observed clinically and pathologically against damaged effect of amiodarone in albino rats.

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I. Background

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 (Cottin, 2012). 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 (Chanda et al; 2019). There is no effective treatment reported for pulmonary fibrosis except lung transplantation (O’Brien et al; 2011).

Amiodarone is one of the most common iodine-containing compound used for treatment of a wide variety of disease as cardiac arrhythmias (Lapenna et al; 2001), ventricular tachycardia, ventricular fibrillation (Dorian et al; 2002) left ventricular dysfunction and heart failure (Chevalier et al; 2003). In otherwise, it causes many adverse effects in lungs as pulmonary toxicity, chronic interstitial pneumonia, diffuse alveolar damage (Chung et al; 2001) and life-threatening pulmonary fibrosis (Wolkove and Baltzan, 2009).

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 introduced in the 1950s and currently used in many branches of medicine (Lana et al; 2014). PRP 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 (Marx, 2004). PRP used in cardiac surgery, pediatric surgery, gynecology, urology, plastic surgery, and ophthalmology (Andia et al; 2015), in addition to the oral maxillofacial surgery and dermatology (Del Fabbro et al; 2015). It also used in wound healing process as it accelerate repairing of the damaged tissues (Villela and Santos 2010).

II. Materials and Methods

a) Experimental animals

Fifteen male Wistar albino rats (each weighing 180-200 gm and two months old), purchased from the Animal House Facility of the Egyptian Organization for...
III. MATERIALS AND METHODS

1. Hematological analysis
The blood samples were collected in EDTA containing tubes from all animals. This blood was used for determination of white blood cells count (WBCs) red blood cells (RBCs) count and platelet count, which done by automated hematology analyzer (Diff3) Mek-6410.

2. Bronchoalveolar lavage (BAL) collection
Bronchoalveolar lavage was done for detection of differential count of leukocytes in BAL. The BAL was collected from the animals by intratracheal injection of saline (3ml/rat) and then collected and centrifuged, the sediments used for making smear slides then stained with Giemsa (Henderson, 2005).

3. Antioxidant and lipid peroxidase determination in lung homogenates
A. Determination of antioxidant biomarker glutathione reductase
It was purchased from (Bio diagnostic, Co, Egypt) for detection of oxidative stress in lungs homogenate and determined according to (Goldberg and Spooner, 1983).

B. Determination of lipid peroxides malondialdehyde (MDA)
It was purchased from (Bio diagnostic, Co, Egypt) for detection of lipid peroxidase in lungs homogenate and determined according to (Ohkawa et al; 1979).

4. Histopathological studies
Specimens from the lungs of the dead and sacrificed animals were collected, then fixed in 10% neutral buffered formalin. Sections about 5µm thickness were prepared and stained with Harries hematoxylin and eosin for histopathological examination (Drudy and Willington, 1980) and Masson's trichrome stain for detection of collagen fiber in lungs tissues (Bancroft and Gamble, 2008).

5. Statistical analysis
Statistical analysis induced using a one-way analysis of variance (ANOVA). It was done to compare the control and all other treated groups and was followed by a post-hoc analysis (Dunnett’s test) using the Statistical Package for the Social Sciences (SPSS), version 17 according to (Borenstein et al; 1997). The data presented as the mean ± standard deviation. The difference was considered statistically significant when p < 0.05.

IV. RESULTS

1. Hematological Findings
Blood parameters
Fig. (1 a, b and c) showed significant decrease in total leucocytic count (leucocytopenia) in group 2 along the experiment when compared to control while,
group 3 showed significant increase at 4th, 5th and 6th weeks when compared to group 2 and reached to control one when P>0.05%.

**Figure (1a, b, c):** The effect of amiodarone on RBCs count, WBCs count and platelet in rat. Amiodarone was given (80mg/kg/day i.p.) for three weeks and PRP treated group for three weeks. Each group was compared with its respective group. The data represent the mean ± SE. (∗) significant difference from control group when (P < 0.05).

Red blood cells count (RBCs) showed significant decrease in rats of group 2 (amiodarone group), at 3rd, 4th, 5th and 6th week of the beginning of experiment when compared to control group. While group 3 (platelet rich plasma treated group) showed significant decrease in RBCs count at 4th week in comparison with control and, significant increase in RBCs recorded at 5th and 6th weeks when compared to group 2 when P>0.05%.

Platelets count showed significant decrease (thrombocytopenia) in amiodarone treated group (gp.2) along the experiment when compared to control rats. However, PRP treated group (gp.3) showed significant thrombocytopenia at 4th week of the experiment when compared to control rats and significant increase at 5th and 6th weeks when compared to group 2 when P>0.05%.

2. Bronchoalveolar lavage (BAL) collection results

**Fig. (2 a, b, c and d)** showed significant increase in leucocytes count in BAL with neutrophilia, lymphocytosis, monocytosis and eosinophilia in amiodarone group at along the experiment when compared to control group and gp. 3. Furthermore, group 3 showed significant decrease in neutrophils, lymphocytes, monocytes and eosinophils count at 5th and 6th week of the experiment when compared to gp. 2 when P>0.05%.
Figure (2): The effect of amiodarone on differential leukocytic count in BAL in rats. Amiodarone given (80mg/kg/day i.p.) for three weeks and PRP treated group for three weeks. Each group compared with its respective group. The data represent the mean ± SE. (∗) significant difference from control group when \( P < 0.05 \).

3. Determination of antioxidant biomarker and lipid peroxidation in lungs homogenates

1- Determination of antioxidant biomarker glutathione reductase

Fig. (3) showed significant decrease in glutathione reductase level in rats of group 2 along the time of experiment when compared to control rats. While, group 3 which treated with platelet rich plasma showed significant decrease in glutathione reductase level at 4th week in comparison with control rats but, significant increase recorded at 4\(^\text{th}\), 5\(^\text{th}\) and 6\(^\text{th}\) week of the experiment when compared to group 2 when \( P > 0.05\% \).

2- Determination of lipid peroxidation malondialdehyde (MDA)

Fig. (4) exhibited significant increase in lungs tissue MDA level in group 2 in comparison to control group till the end of experiment. Meanwhile, group 3 showed significant decrease in MDA level at 5\(^\text{th}\) and 6\(^\text{th}\) weeks of the experiment when compared to group 2 and significantly increased at 4\(^\text{th}\) week of the experiment when compared to control rats when \( P > 0.05\% \).

Figure (3, 4): The effect of amiodarone on glutathione reductase and malondialdehyde activity in rat lungs homogenate. Amiodarone given (80mg/kg/day i.p.) for three week and PRP treated group for three week. Each group compared with its respective group. The data represent the mean ± SE. (∗) significant difference from control group (a) significant difference from gp.2 when \( P < 0.05 \).
4. Pathological findings

The rats of group 2 lungs displayed interstitial pneumonia with emphysematous areas and congestion, besides bronchoectasia. Bronchioles exhibited bronchiolitis characterized by hyperplasia in the lining epithelial cells forming finger like projection with cells debris obstructed the lumen (Fig. 5a, b). While, the lungs of the rats (gp. 3) appeared apparently normal alveoli and bronchus with slightly congested blood vessels and few mononuclear inflammatory cells infiltration (Fig. 6).

**Figure 5 (a, b):** The lung of the rats which received amiodarone at 3th day showing interstitial pneumonia with emphysematous areas and congestion, besides bronchoectasia (a). Bronchioles exhibited bronchiolitis characterized by hyperplasia in the lining epithelial cells forming finger like projection with cells debris obstructed the lumen (b). (H&E., x 60, 120)

**Figure 6:** The lung of the rats PRP treated group at 5th day showing alveoli and bronchus apparently normal in appearance with slightly congested blood vessels and few mononuclear inflammatory cells infiltration. (H&E., x 60)

V. 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 (Bargout et al; 2000 and Saad et al; 2004). However, its use related to many side effects involving different organs as lungs causing pulmonary complications (Uhal et al; 2003). Lung fibrosis is a lethal pathological process with gradual increasing incidence worldwide and limited therapeutic options. It characterised by abnormal deposition of collagen following tissues damage (Cooper, 2000). Platelet rich plasma widely used nowadays in various medicinal fields as bone defects (Mehta, 2010) oral and maxillofacial surgery (Albanese et al; 2013) aesthetic plastic surgery (Cervelli et al., 2009), spinal surgery and later its applications extended to wound healing and tissue regeneration (Okamoto et al; 2012). As it contains growth factors it plays role in angiogenesis and tissue regeneration (Fortier et al; 2011).

In our work amiodarone treated group recorded leucocytocytopenia, characterized by significant decrease in total leukocytic count (WBCs) similar to that reported by (Erie et al; 2010). Mohamed et al; (2007) referred that signs to bone marrow granuloma formed by amiodarone due to accumulation of iodine in tissues or accumulation of phospholipid-like substance due to inhibition of phospholipase enzymes causing pancytopenia.

In our experiment, blood samples were collected from all groups to detect effect of amiodarone
(gp.2), which, recorded anemia characterised by significant decrease in RBCs count at 3rd, 4th, 5th and 6th week when compared to control gp. and gp. 3. This was attributed to amiodarone induced bone marrow granuloma through inhibition of phospholipases, leading to accumulation of phospholipids in bone marrow (Lossos and Matzner 1992). Mukhopadhyay et al; (2014) suggested that granuloma formation occur due to immunological reaction. Other author (Chang and Ng, 2008) suggested that amiodarone initiate (erythropoietin) EPO-resistant anemia after treatment for arrhythmia which consequently cause decrease in RBCS synthesis and anemia. Amiodarone using also caused hemolytic anemia (Arpin et al; 1991). PRP treated group showed significant increase in RBCS count at 5th and 6th week when compared to gp.2. As platelets stimulate the mitogenic activity of human bone cells to increase the proliferation of stem cells, thus lead to regeneration of tissues (Marx et al; 1999).

The present study showed that amiodarone administration cause significant thrombocytopenia in gp.2 along the experiment when compared to control one. On the other hand, group 3 showed significant increase in platelet count at 5th and 6th week when compared to gp.2 this result caused by drug-dependent antibodies specific for platelet glycoproteins GPIa/IIa and/or GPIIb/IIIa which, produced by amiodarone in patient serum (Sahud et al; 2013). Slow return of platelet levels occur even, after discontinuation of amiodarone related to slow clearance of this lipophilic drug from body tissues (Aster & Bougie, 2007). Aster et al; (2009) found that amiodarone bound to plasma protein and localized in glycoproteins of megakaryocytes and platelets producing structural changes which, are immunogenic in some individuals enabling amiodarone-induced antibodies to be detected and destruction of platelets occurred.

In our work, group (2) showed leukocytosis in bronchoalveolar lavage (BAL) fluid along the time of the experiment characterised by lymphocytosis and abundant macrophages with and eosinophilia same result reported by (Ohar et al; 1992 and Kaushik et al; 2001). On the other hand, rats treated with PRP showed a significant decrease in (BAL) leukocyte count at 5th and 6th week of the experiment. This was attributed to platelet rich plasma has a 5 to 10 fold higher concentration of growth factors than whole blood. These growth factors promote natural healing processes (Lai et al; 2015) as the first response of the body to tissue injury is to deliver platelets to injured area and attract stem cells to the site of the injury (Wang et al; 2015). PRP suppress cytokine release and limit inflammation (Marx, 2004).

VI. Conclusion

In conclusion, amiodarone caused lungs injury in rats following daily i.p. administration resulted in inflammatory reactions, and imbalance between antioxidant oxidative stress ending ultimately in severe lung toxicity. platelet rich plasma can cause moderate regeneration in lung damage.

References Références Referencias


