REVIEW ARTICLE

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Investigating Pirfenidone and Vitamin D for Targeting Cardiac and Renal Fibrotic Pathways in Experimentally-Induced Animal Model

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Abstract

Breast cancer is considered as the most familiar cancer in females which represented 38.8 % in Egypt and 29% in the world. It is the second common cause of cancer-related death in women. Treatment of breast cancer with Doxorubicin may lead to many side effects, mainly cardiac and renal fibrosis. The present study aimed to investigate the underlying molecular mechanisms for the potential anti-fibrotic effect of pirfenidone (500mg/kg, P.O. once daily) and Vitamin D (0.5µg/kg I.P. once daily) against doxorubicin (15 mg/kg I.P.) induced cardio- and renal- fibrosis. Moreover, the anti-cancer potential of pirfenidone (PFD) and Vitamin D either alone or in combination with doxorubicin will be assessed in a xenograft experimental model of breast cancer. Then, tissue and blood samples will be collected after two weeks post-treatment to assess the toxicity of Doxorubicin.

Keywords: Breast cancer, Doxorubicin, Pirfenidone, Vitamin D.

1. Introduction

Breast cancer is one of the most common life-threatening diseases among women originating from breast tissue (Gaafer 2015). Treatment of breast cancer includes surgery followed by chemotherapy however; treatment with chemotherapeutic agents may lead to fibrosis in various organs. The development of fibrosis is a dynamic, multifactorial process. This process is initiated by healing after injury and by pathological conditions in which the normal balance of Genesis and breakdown of extracellular matrix (ECM) proteins is disturbed. Fibrogenesis involves the participation of various acute and chronic inflammatory cells that synthesize and secrete profibrotic growth factors. Myofibroblasts and macrophages are 2 key chronic inflammatory cell types that increase in number during fibrosis (Ricardo, Van Goor et al. 2008).

Excessive accumulation of ECM, mostly observed in kidney diseases may lead to renal fibrosis. The pathogenesis of fibrosis is a progressive process that ultimately lead to end-stage renal failure. Simply, renal fibrosis represents a failed wound healing process of the kidney tissue after chronic and sustained injury (Liu 2006). It is characterized by glomerulosclerosis and tubulointerstitial fibrosis expressed as final common manifestation of a wide variety of chronic kidney disease (CKD). The development of renal fibrosis is an unwanted side effect of treatment with chemotherapy (Eitner and Floege 2003).

Cardiac fibrosis is also a common adverse effect associated with cancer treatment, whether it

is systemic from chemotherapy or immunotherapy or local from radiotherapy. It limits the use of chemotherapeutic agents and is debilitating and life-threatening for patients(Carvalho, Burgeiro et al. 2014).

In this context, understanding cellular and molecular mechanisms of fibrosis became a necessity. This isn't only for illustrating the complicated pathogenesis of renal and cardiac fibrosis but also, for exploring and validating the efficient anti-fibrotic therapies.

Doxorubicin is anthracycline-based antibiotic that has a widespread application in cancer chemotherapy and used to treat several solid tumors, acute leukemia and malignant lymphoma. (López-Novoa, Martínez-Salgado et al. 2010). Pirfenidone is an anti-fibrotic drug which exhibits well-documented antifibrotic and anti-inflammatory activities in a variety of animal and cell-based models (Prud'Homme 2007). Furthermore, pirfenidone disrupts tumor–stromal interactions by suppressing the synthesis and secretion of factors involved in these interactions like Platelet derived growth factor-A ,collagen type I, and fibronectin (Kozono, Ohuchida et al. 2013). Vitamin D has been reported to have a role in the modulation of renal inflammation in addition to, anticancer activities against many cancer types, including breast cancer (Deeb, Trump et al. 2007).

Review of literature

Breast cancer is the most common disease that develops from breast tissue. Signs of breast cancer may include a lump in the breast, a change in breast shape, dimpling of the skin, fluid coming from the nipple, a newly inverted nipple, or a red or scaly patch of skin. In those with distant spread of the disease, there may be bone pain, swollen lymph nodes, shortness of breath, or yellow skin (Kabel and Baali 2015). Breast cancer is usually treated with surgery, which may be followed by chemotherapy or radiation therapy, or both. A multidisciplinary approach is the best (Bao and Rudek 2011).

Chemotherapy is predominantly used for cases of breast cancer in stages 2–4 and is particularly beneficial in estrogen receptor-negative (ER-) disease. Treatment with chemotherapeutic agents destroy the physiological homoeostasis and affects multiple organs during treatment process. Chemotherapy is usually administered in combinations, for periods of 3–6 months. Most chemotherapy medications work by eradicating fast-growing and/or fast-replicating cancer cells by causing DNA damage upon replication mechanisms. However, the medications also damage fast-growing normal cells, which may cause serious side effects like fibrosis (Kumral, Soluk-Tekkeşin et al. 2015).

Cardiac fibrosis may refer to an abnormal thickening of the heart valves due to inappropriate proliferation of cardiac fibroblasts but more commonly refers to the excess deposition of extracellular matrix in the cardiac muscle (Gourdie, Dimmeler et al. 2016). Fibrotic cardiac muscle is stiffer and less compliant and is seen in the progression to heart failure. Fibrocyte cells normally secrete collagen to provide structural support for the heart. The thickening and loss of flexibility eventually may lead to valvular dysfunction and heart failure (Berk, Fujiwara et al. 2007).

There are a variety of causes that trigger fibrotic remodeling of the myocardium as hemodynamic, toxic, metabolic, and immunologic disturbances. Among the causes leading to kidney fibrosis are diabetes and hypertension "two principal causes of CKD", infectious glomerulonephritis, renal vasculitis, ureteral obstruction, genetic alterations, autoimmune diseases and drugs like Doxorubicin (López-Novoa, Martínez-Salgado et al. 2010).

Doxorubicin is an anthracycline commonly used anticancer agent however, severe clinical side effects, such as cardiotoxicity and myelosuppression, are of major concern (Mady 2007). It exerts its antitumor activity through inhibiting the replication process of cells by crossing the tumor cellular membrane and intercalating into the base pairs of DNA. Doxorubicin chemotherapy serves as one of the routines in treating cancer clinically. Doxorubicin, is widely used in the treatment of malignant tumors, such as liver cancer, breast cancer, ovarian cancer, gastric cancer, non-small cell lung cancer, and prostate cancer (Al-Abbasi, Alghamdi et al. 2016). On the other hand, the severe toxic side effects of doxorubicin on human body have restricted its clinical application (Octavia, Tocchetti et al. 2012).

The increased risk of cardiac dysfunction from doxorubicin can manifest acutely during treatment or chronically weeks to years after treatment has

been ceased. Researches have shown that doxorubicin chemotherapy could generate severe tissue injury in heart and kidney (Kumral, Soluk-Tekkeşin et al. 2015) and the symptoms of renal damage like hematuresis and proteinuria are especially evident (Wang, Wang et al. 2000).

There are various signaling mechanisms involved in doxorubicin cardiotoxicity. First and foremost, the doxorubicin-induced cardiotoxicity is due to oxidative stress. Cardiac mitochondrial damage is supposed after few hours following the exposure to doxorubicin. Cardiac dysfunction may present across a broad spectrum of symptoms that may range from arrhythmias to overt heart failure. Doxorubicin also produced cardiomyopathy due to interstitial and perivascular fibrosis (Aerts, Velazquez et al. 2014). In addition, doxorubicin treatment significantly elevates expression of transforming growth factor-beta (TGF-β) and phosphor-SMAD3 along with increased collagen deposition, an increment of fibroblast to myofibroblast phenotypic transformation and pro-fibrotic signaling pathway(Diwan, Wansapura et al. 2008).

Renal fibrosis is mid to progressive renal failure and end stage renal disease (Liu 2006). One of the mechanisms of renal tissue injury caused by doxorubicin was the free radical production. This results in lipid peroxidation of glomerulus affecting normal physiological function of renal tissues, thus generating metabolic disorders (Heart, Karandrea et al. 2016). Therefore, one of the methods to reduce renal damage is to inhibit peroxidation.

The mechanisms of renal damage caused by doxorubicin chemotherapy mainly consists of two aspects. The first is direct damage in which general toxicity of doxorubicin on cells directly damaged renal cells producing inflammatory response in renal tissues. As a result, inflammatory mediators as TNF-α and interleukin are released, hence strengthening the growth of renal matrix and glomerular sclerosis (Donner, Yeh et al. 2015). The second is indirect damage. Under the action of multiple reductases, doxorubicin produced free radical, inducing lipid peroxidation in glomerular epithelial cell and causing abnormal glucose and protein metabolism. Moreover, elevated urinary albumin was detected due to altering and breaking the structure and filterability of renal filtration membrane (Rashikh, Pillai et al. 2014).

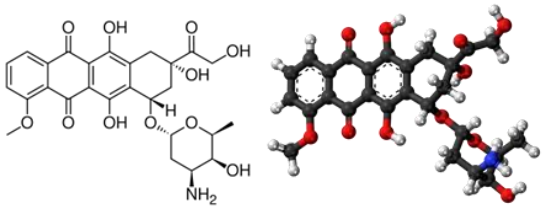

Figure 1: The structure of Doxorubicin.

Pirfenidone is an orally active small molecular comprising a modified phenyl pyridine. Pirfenidone is an anti-fibrotic drug, commonly used for the treatment of idiopathic pulmonary fibrosis which firstly approved in Japan under the trade name Pirespa® and some years later in Europe, United States and Canada under the trade name Esbriet® (Roche Pharmaceuticals) for the same cure. PFD has been reported to exert anti-inflammatory, antioxidant, and antifibrotic effects on the heart (Ma, Pan et al. 2014).

Pirfenidone has been shown to reduce the fibrosis of different organs (for example, lung, kidney, liver, heart and vascular remodeling. The compound exhibits well-documented antifibrotic and anti-inflammatory activities in a variety of animal and cell-based models (Schaefer, Ruhrmund et al. 2011). Intraperitoneal and oral administration of PFD reduced the tissue levels of inflammatory markers in both parietal and visceral peritoneum. Also, PFD is an effective agent on the prevention of postoperative fibrosis (Hasdemir, Ozkut et al. 2017)

Pirfenidone (PFD) has been shown to be effective in handling several fibrotic diseases (Macías-Barragán, Sandoval-Rodríguez et al. 2010) by reducing fibroblast proliferation and inhibiting TGF-β-stimulated collagen production. Furthermore, it can modulate multiple signaling pathways including tumor necrosis factor α (TNF-α), IL-1-β, and platelet derived growth factor (Kim, Choi et al. 2010). Also, pirfenidone disrupts tumor–stromal interactions by suppressing the synthesis and secretion of factors involved in these interactions like PDGF-A, collagen type I, and fibronectin. Pirfenidone showed antitumor activity (Kozono, Ohuchida et al. 2013). The compound also, exhibits well-documented anti-inflammatory activities in a variety of animal and cell-based models (Schaefer, Ruhrmund et al. 2011).

pirfenidone disrupts tumor–stromal interactions by suppressing the synthesis and secretion of factors involved in these interactions like PDGF-A, collagen type I, and fibronectin. Pirfenidone suppressed tumor growth, reduced the number of distributed nodules, and reduced the incidence of metastasis. Therefore, combining pirfenidone with traditional anticancer drugs such as gemcitabine may offer a promising treatment strategy for pancreatic cancer (Kozono, Ohuchida et al. 2013).

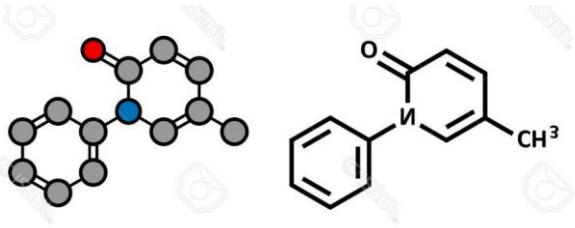

Figure 2. The structure of Pirfenidone

Also, vitamin D is used for treatment of fibrosis. It is a group of fat-soluble steroids responsible for increasing intestinal absorption of calcium, magnesium, and phosphate, and multiple other biological effects. In humans, the most important compounds in this group are vitamin D_3 (also known as cholecalciferol) and vitamin D_2 (ergocalciferol). Vitamin D has been reported to have anticancer activities against many cancer types, including breast cancer. In addition, it has been found to have several activities that might slow or prevent the development of cancer, including promoting cellular differentiation, decreasing cancer cell growth, stimulating cell death (apoptosis), and reducing tumor blood vessel formation (Deeb, Trump et al. 2007).

The kidney plays a central role in vitamin D metabolism and regulation of its circulating levels. Therefore, impaired renal function may lead to vitamin D deficiency, as has been observed in patients with CKD (Dusso and Tokumoto 2011). Several studies have shown the role of active vitamin D in the modulation of renal inflammation (Park, Cho et al. 2012). The anti-inflammatory properties of active vitamin D and its analogues may be attributed to their ability to suppress the NF-κB pathway, a key transcription factor that is thought to mediate acute and chronic inflammation and fibrogenesis by regulating gene expression of cytokines, chemokines, and adhesion molecules (including interleukin-6, MCP-1, and tumor necrosis factor-α) (Guijarro and Egido 2001).

Vitamin D plays a role in protecting against kidney injury by blocking NF-κB activity and reducing renal inflammation (Schwarz, Amann et al. 1998). In animal models of diabetic nephropathy, treatment with calcitriol and paricalcitol reduces infiltration of inflammatory cells and NF-κB activation in the glomerulus (Sanchez-Niño, Bozic et al. 2011). Similarly, administration of calcitriol attenuates glomerular hyper cellularity and inflammatory infiltration (Panichi, Migliori et al. 2001).

Studies showed that patients suffering from cardiovascular disease are frequently deficient in the steroid hormone vitamin D (Zittermann, Frisch et al. 2009). Approximately 90% of chronic HF patients have hypovitaminosis D (Kim, Sabour et al. 2008). Furthermore, vitamin D deficiency might contribute to cardiomyocyte hypertrophy, interstitial inflammation, and fibrosis (Chen, Rosner et al. 2011).Hence, vitamin D deficiency could contribute to a more rapid progression to HF following myocardial damage .

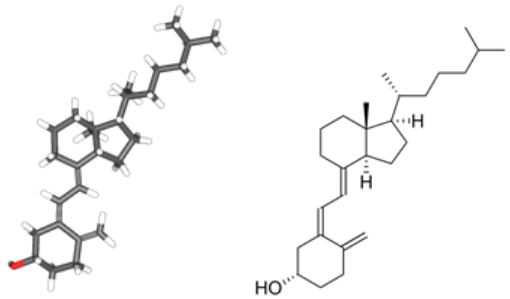

Figure 3. The structure of Vitamin D (Cholecalciferol (D_3)

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