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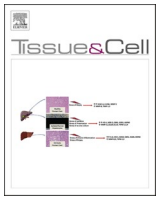

Modulation of the Sirtuin-1 signaling pathway in doxorubicin-induced nephrotoxicity (synergistic amelioration by resveratrol and pirfenidone)

Samia Mahmoud Manawy [a], Eman Mohamed Faruk [b,c,*], Rabab Fawzy Hindawy [d], Mahmoud M. Hassan [e], Diaa M.G. Farrag [f], Mansour A.E. Bashar [f], Hanan Fouad [g,h], Rania Abubaker Bagabir [i], Dina Allam Abdelmaksoud Hassan [j], Ahmed Mohammed Zaazaa [k], Mohamed Ghazy Attia Hablas [l], K Mostafa Kamal [a]

[a] Department of Anatomy and Embryology, Faculty of Medicine, Benha University, Benha, Egypt
[b] Anatomy Department, College of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia
[c] Department of Histology and Cell Biology, Faculty of Medicine, Benha University, Benha, Egypt
[d] Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Benha University, Benha, Egypt
[e] Department of Physiology, Faculty of Medicine, Benha University, Benha, Egypt
[f] Marine Biology Branch, Zoology Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo 11884, Egypt
[g] Basic Medical Sciences, Faculty of Medicine, Galala University, Galala City, POB 43711, ATTAKA, Suez Governorate, Egypt
[h] Department of Medical Biochemistry, Faculty of Medicine, Cairo University, Cairo POB 12613, Egypt
[i] College of Medicine, Hematology and Immunology Department, Umm Al-Qura University, Makkah, Saudi Arabia
[j] Histology and Cell Biology Department, Faculty of Medicine for Girls, Al-Azhar University, Egypt
[k] Students at Faculty of Medicine, Benha National University, Benha Colleges in Cairo, Egypt
[l] Department of Histology and Cell Biology, Faculty of Medicine, Suez University, Suez, Egypt

A R T I C L E I N F O

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A B S T R A C T

The current study was conducted to determine the precise mechanisms of Sirtuin-1 (Sirt-1), TGF- β (Transforming Growth Factor-β), and long non-coding RNA Metastasis Associated Lung Adenocarcinoma Transcript 1 (LncRNA MALAT-1) in signaling pathways in doxorubicin (DOX)-induced nephrotoxicity. The potential therapeutic effect of Resveratrol and Pirfenidone in DOX toxicity was also assessed. Thirty-six male adult rats were evenly distributed into four groups: Group 1: control rats. Group 2: DOX exposed rats' group, each animal received 7.5 mg/kg DOX as a single intravenous dose, Group 3: DOX exposed group subjected to oral resveratrol (20 mg/kg/daily for two weeks), Group 4: DOX exposed group subjected to oral Pirfenidone (200 mg/kg once daily for 10 days). At the planned time, animals were sacrificed. Renal tissue was collected to assess matrix metalloproteinase-9 (MMP9), inflammatory and apoptotic markers: tumor necrosis factor-alpha (TNF- β, caspase-3, cyclo-oxygenase-2 (COX-2), and oxidative stress markers: nitric oxide (NO), Glutathione (GSH), malondialdehyde (MDA), and superoxide dismutase (SOD). Sirtuin-1 (Sirt-1), TGF-β, and LncRNA MALAT-1 were quantitatively assessed by real-time RT-PCR in the whole blood. Results showed that the DOX group exhibited a significant increase in oxidative stress markers, and inflammatory, and apoptotic markers in the renal tissue. Histologically, the renal tubule lining cells exhibited vacuolar alterations in the cytoplasm, glomerular atrophy, and vascular congestion. Furthermore, renal degeneration was evident, as confirmed by the heightened immunoexpression of MMP9. Exposure to DOX resulted in a significant decrease in Sirtuin-1 (Sirt-1) with a significant increase in the TGFβ, and LncRNA MALAT-1 gene expression. However, pre-treatment with either resveratrol/or Pirefenidone ameliorated the histological renal alterations, regulated the pathways of Sirt-1, TGFβ, and LncRNA MALAT-1, and decreased all oxidative stress, inflammatory and apoptotic markers. In conclusion, DOX exposure leads to renal toxicity by inducing renal degeneration, oxidative stress, and apoptosis. Administration of either resveratrol or Pirfenidone counteracted these changes and protected the kidney against DOX-induced renal damage.

* Corresponding author at: Anatomy Department, College of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia.
E-mail addresses: samia.manawy@fmed.bu.edu.eg (S.M. Manawy), emkandel@uqu.edu.sa (E.M. Faruk), dr-rababhidawi@yahoo.com (R.F. Hindawy), diaa.magdy@azhar.edu.eg (D.M.G. Farrag), dr_mb2020682@azhar.edu.eg (M.A.E. Bashar), hanan.fouad@kasralainy.edu.eg (H. Fouad).

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1. Introduction

Doxorubicin (DOX, also known as Adriamycin) is a potent antitumor antibiotic used to treat various malignant tumors. DOX has many hazards and side effects involving several organs (Shao et al., 2019). One of these dangerous side effects is its adverse effect on the kidney (Ikewuchi et al., 2021). It is one of the drugs that cause acute kidney injury, and nephrotoxicity that leads to a life-threatening impact (Afsar et al., 2020).

DOX (Adriamycin) mediates nephrotoxicity via induction of oxidative stress, inflammatory changes, and induction of apoptosis (El-Sayed et al., 2017; Songbo et al., 2019). Its oxidative stress action has a deleterious impact affecting several organs such as the liver, heart, and kidney (Songbo et al., 2019). Accumulation of DOX inside renal tubules contributes to its direct degenerative mechanism in the kidney (Yalcın et al., 2023).

Resveratrol (RVS) is a phytoalexin present in a minimum of 72 plant species, several of which humans consume, such as mulberries, grapes, and peanuts. Resveratrol acts as an antioxidant and it is part of a group of members known as polyphenols (Singh et al., 2019). Resveratrol has several health benefits as it can act as an anti-inflammatory, cardioprotective, and antioxidant. Studies have proven its potential therapeutic effects on heart and kidney diseases (Singh et al., 2019; Den Hartogh and Tsiani, 2019; Shahbazi et al., 2020).

Pirfenidone, which is orally effective, is a modified phenyl pyridine with the ability to traverse cell membranes independently of receptor assistance. It is easily taken up from the gastrointestinal tract following oral intake. A recent study proved that pirfenidone suppresses DOX-induced renal damage through inhibition of MCP-1 and JNK1 signaling pathways. Although it is unclear in detail how pirfenidone modulates fibrogenesis, its effects are likely to possess multiple targets, pirfenidone exhibits antioxidant properties as well as anti-transforming growth factor (anti-TGF) and anti-platelet-derived growth factor effects. It appears to be generally safe for use in chronic fibrotic disorders, multiple sclerosis, chronic hepatitis C, and chronic allograft rejection (Lopez et al., 2015; Hazem et al., 2022a).

Pirfenidone does not have a direct antihypertensive effect, but it was reported to decrease the expression of mineralocorticoid receptors and prevent angiotensin II-induced cardiac hypertrophy in hypertensive mice (Hazem et al., 2022a). As regards resveratrol, the antihypertensive or vasorelaxant effects of resveratrol have been attributed to increased expression of endothelial nitric synthase, improved nitric oxide (NO) release, and endothelium-dependent vasorelaxation, 2 decreased endothelin-1 and angiotensin II production,7 decreased sympathetic activity,8 and decreased oxidative stress (Mozafari et al., 2015).

Sirtuins exist in seven variations, designated as SIRT1–SIRT7, each distinguished by their specific locations. SIRT1, the most well-known variant, exerts its influence on proteins through NAD+ coenzymes, associating it with cellular energy metabolism and the 'redox' state. Deficiencies in SIRT1 are implicated in various medical conditions, including diabetes mellitus, cardiovascular diseases, neurodegenerative syndromes, and kidney diseases, especially during stressful situations (Dou and Zhao, 2022). In kidney disorders, SIRT1 promotes cell survival in affected kidneys by modulating responses to diverse stress stimuli. It also participates in arterial blood pressure control, guards against cellular apoptosis in renal tubules through catalase induction, and initiates autophagy. Growing in vitro and in vivo evidence suggests that SIRT1 activity is directed, among other functions, towards nephroprotection. Consequently, SIRT1 is potentially a novel therapeutic element in addressing age-related renal diseases, including diabetic nephropathy (Stojanović et al., 2022).

The current study was conducted to evaluate the potential therapeutic effects of resveratrol and pirfenidone on DOX-induced nephrotoxicity through assessment of their effects on Sirtuin-1 (Sirt-1), TGF-β, LncRNA MALAT-1 signaling pathway, apoptotic and inflammatory markers.

2. Material and method

2.1. Experimental animals

Ethical approval No.12–6–2023 was obtained from Banha University Faculty of Medicine, Egypt, for all animal procedures. The study was conducted on 36 adult male Sprague Dawley rats, 6–8 weeks old (200–220 g). Animal experiments were conducted at the Experimental Animal Unit of the Faculty of Medicine at Banha University. During the experiment, animals were kept at a suitable temperature (21±2 °C) and humidity (60%), with 12 hours of dark light alternated with unrestricted access to food and water. The animals were fed balanced diets. We conducted all animal procedures under the Declaration of Helsinki (2008) and the guidelines of Cairo University's Ethical Committee for the care and use of laboratory animals. Before starting the experiments, the animals were acclimatized to laboratory conditions for 1 week.

2.2. Chemicals and drugs

The DOX was purchased from Sigma Chemical Company (St. Louis, MO, USA) as DOX hydrochloride (2 mg/ml). Resveratrol (99% purity) was delivered from Sigma Chemicals Company (St. Louis, MO, USA) (CAS Number: 501–36–0). Pirfenidone (200 mg tab) was delivered from Sigma Chemical Company (St. Louis, Mo.) with CAS number 53179–13–8. Most chemicals and reagents were sourced from Sigma Chemical Company (St. Louis, MO, USA).

2.3. Preparation and dosage of resveratrol, and pirfenidone

Resveratrol was prepared in a normal saline solution and given to rats at a dose of (20 mg/kg body weight/day) for 14 successive days (Yalcın et al., 2024).

pirfenidone (PFD) tablets were ground and suspended in 1% carboxymethyl cellulose. One pirfenidone tablet was dissolved in 4 ml CMC to obtain a final concentration of 50 mg/ml and was given in a dose of 200 mg/kg/d oral gavage for two weeks [16&17].

2.4. Experimental design and animal grouping

Thirty-six adult male rats (200–220 gm) were divided equally into four groups (nine rats per group): Group 1(G1) represents control and received saline. Group 2(G2) represents pathological control and received a single dose of DOX (7.5 mg/kg) in the tail vein (El-Sayed et al., 2017). Group 3(G3) included DOX-exposed rats and they were given resveratrol (20 mg/kg B wt/day) orally for two weeks (Yalcın et al., 2024). Group 4(G4) included the DOX-exposed group and received pirfenidone (200 mg/kg of body weight once daily for 10 days) orally by a gastric gavage (Hazem et al., 2022b; Morsi et al., 2023; Fouad et al., 2021). (Diagram 1.).

2.5. Assessment of blood pressure

The blood pressure was measured as previously reported (Chen et al., 2012). A 2 mg/kg intraperitoneal injection of ethyl carbamate was used to sedate the animals. Systolic blood pressure was evaluated using tail-cuff plethysmography on three occasions (Letica LE 5100, pain lab, Barcelona, Spain), and the average of these three measurements was documented.

2.6. Serum and tissue preparation

At the end of the experiment (two weeks after the DOX injection), blood samples were obtained from the retro-orbital venous plexus using nonheparinized tubes after anesthetizing the rats with light ether. The serum samples underwent centrifugation at 4000x g for 20 minutes and were subsequently stored at –20 °C. After that, all animals were