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Celecoxib aggravates cardiac apoptosis in L-NAME-induced pressure overload model in rats: Immunohistochemical determination of cardiac caspase-3, Mcl-1, Bax and Bcl-2

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ABSTRACT

The mechanism of celecoxib cardiovascular adverse events was earlier investigated; yet in-depth investigations are needed to assess the involvement of its pro-apoptotic effect throughout this process. An *in-vivo* chronic rat model of pressure overload employing N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME) was tested at different time intervals to ensure the occurrence of persistent myocardial apoptosis along with pressure overload. Seven groups of male Wistar rats were assigned as (i) distilled water; (ii-iv) L-NAME (60 mg/kg) for 6, 12 or 16 weeks; (v-vii) L-NAME [16 weeks] + celecoxib (25, 50 or 100 mg/kg), from week 13 to week 16. Treatment with L-NAME for 6, 12 or 16 weeks increased systolic blood pressure, serum level of creatine kinase-MB and lactate dehydrogenase. Further, it induced cardiac hypertrophy, detected in terms of greater heart weight index and cardiomyocyte cross-sectional area and produced interstitial and perivascular fibrosis. Moreover, administration of L-NAME increased cardiac immunostaining for activated caspase-3 and Bax/Bcl-2 ratio whereas; immunostaining for Mcl-1 was decreased. Administration of celecoxib (25, 50 or 100 mg/kg) aggravated the L-NAME-induced toxicity. The work results shed the light on the putative pro-apoptotic effect of celecoxib at a risk state of pressure overload comparable to the clinical condition of essential hypertension.

1. Introduction

Celecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor is less harmful to the gastrointestinal tract compared to conventional NSAIDs [1,2]. It was demonstrated that celecoxib inhibit the growth of several types of cancers [3,4] and to improve the anti-neoplastic action of chemotherapeutic agents [5]. However, it was proven that COX-2 inhibitors lead to cardiovascular adverse events in patients with tendency to develop thrombosis or hypertension [6].

Celecoxib hazard was associated, at least partly, with the reduction of COX-2 vascular prostacyclin synthesis with unaltered thromboxane synthesis resulting in hypertension and atherosclerosis. The imbalance between the production of prostacyclin and thromboxane may result in an increased cardiovascular disease in patients [7–9]. Other suggested mechanisms include the down-regulation of thrombomodulin [10], adenylyl cyclase enzyme [11] in addition to upregulation of tissue factor [12]. Endothelial COX-2 enzyme mediates its effect in harmony with nitric oxide (NO), a fundamental vasodilator; therefore blocking the COX-2 action would probably reverse the beneficial effect of NO [13].

Several animal models were employed to mimic the condition of

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hypertensive patients with cardiovascular complications such as non-invasive rat models that were introduced to induce hypertension and consequently pressure overload using Nʷ-nitro-L-arginine methyl ester (L-NAME) [14–16].

In addition, treatment with L-NAME induced myocardial apoptosis in rodents [17,18]. A previous study concluded that treatment with L-NAME increased activity of caspase-3 within hearts of mice which was abolished in endogenous NO synthase-deficient mice [19] and increases TUNEL-positive nuclei and caspase-3 activity during myocardial ischemia reperfusion in rats [20].

Apoptosis is a regulated or programmed mode of cell death [21]. Moreover, maladaptive myocardial hypertrophy is associated with downstream signaling pathways that stimulate cardiac fibrosis and apoptosis [22]. In addition, celecoxib is suggested to stimulate tumor apoptosis by its action on intrinsic apoptotic pathway [23].

This work was designed to better understand the cardiotoxic effect of celecoxib in rats with experimental pressure overload induced by L-NAME. The harmful effect of celecoxib was investigated in terms of degree of hypertrophy, fibrosis and the level of myocardial expression of executive caspase-3 and intrinsic apoptotic proteins, Mcl-1, Bax and Bcl-2.

2. Materials and methods

2.1. Animals

Seventy male Wistar rats with body weights equal 200–220 g were used in the experiment. Rats were bought from the Modern Veterinary Office for Laboratory Animals (Cairo, Egypt) and adapted for 2 weeks before the experiment. Environmental conditions were adjusted (21–25 °C and normal dark/light cycle). Water and regular chow diet were given *ad libitum*. This work received the consent of the research ethics committee at the Faculty of Pharmacy, Suez Canal University (Ismailia, Egypt) (The license number 20146A18) and complies with the National Institutes of Health guide for the care and use of Laboratory animals.

2.2. Drugs and chemicals

Celecoxib was a kindly provided by Amoun pharmaceutical Co. (Egypt) and a suspension was prepared using 0.5% carboxymethyl cellulose (CMC) solution. L-NAME hydrochloride was bought from Cayman Chemical Co. (MI, USA) and dissolved in distilled water to prepare 3% solution. Solutions were prepared daily to guaranty maximum L-NAME stability. Active caspase-3 rabbit polyclonal antibodies, Bax polyclonal antibodies, Bcl-2 polyclonal antibodies and Mcl-1 rabbit monoclonal antibody were obtained from Abcam (Cambridge, UK). Immunohistochemistry analysis was performed using biotinylated anti-rabbit IgG, Vectastain ABC Elite kit and 3,3′-diaminobenzidine (DAB) kit (Vector Laboratories, CA, USA).

2.3. Rat model of pressure overload induced by chronic administration of L-NAME

Rats were administered L-NAME (60 mg/kg/day) [24] via gastric gavage for 6, 12 or 16 weeks to induce hypertension and consequently pressure overload characterized by cardiac hypertrophy, fibrosis and apoptosis. Previous studies reported that chronic administration of L-NAME to rats caused histopathological changes to the heart [25,26] and arterial blood vessels [27–29].

Following L-NAME treatment, the morphological abnormalities in rat heart included mainly ventricular hypertrophy, fibrosis and foci of necrosis [27,30,31]. L-NAME is an experimental model of hypertension that demonstrates a correlate of a cardiac disease characterized by the progress of hypertensive cardiomyopathy due to endothelial dysfunction as in patients suffering from diabetes mellitus and/or arterial hypertension [31]. This model was reported to be accompanied with decreased coronary flow due to disturbances in the myocardial microcirculation, including platelet activation [32] and vasoconstriction of blood vessels [33].

2.4. Experimental design

Rats were assigned into 7 groups as demonstrated in Fig. 1:

[1] Distilled water group.
[2] L-NAME (60 mg/kg/day, 6 weeks) [16,24].
[3] L-NAME (60 mg/kg/day, 12 weeks) [34].
[4] L-NAME (60 mg/kg/day, 16 weeks).
[5] L-NAME [16 weeks] + celecoxib (25 mg/kg, from week 13 to week 16).
[6] L-NAME [16 weeks] + celecoxib (50 mg/kg, from week 13 to week 16).
[7] L-NAME [16 weeks] + celecoxib (100 mg/kg, from week 13 to week 16 week) group.

The rational of using L-NAME for 6 and 12 weeks was to select the suitable duration that confirm the development of hypertrophy, fibrosis and cardiomyocyte apoptosis in rats. Administration of L-NAME (60 mg/kg) for 12 weeks fulfilled these targeted criteria and was chosen as a starting point for treatment with celecoxib.

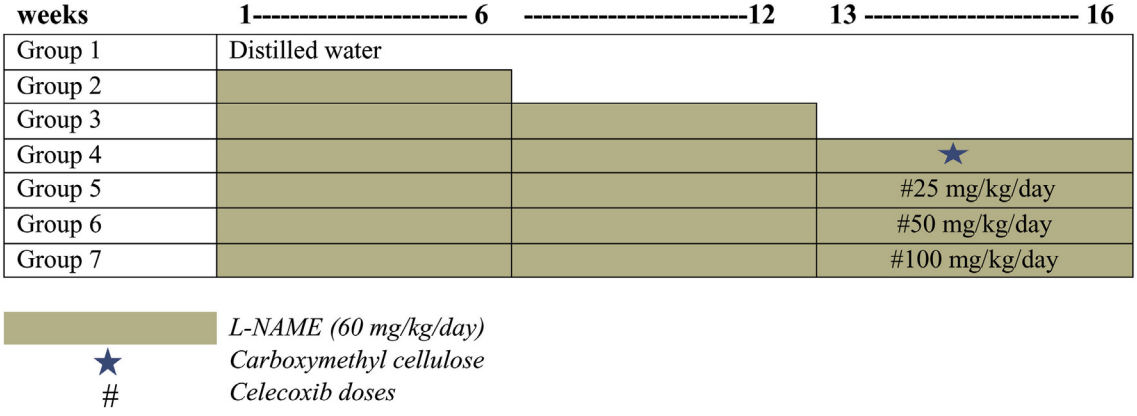

Fig. 1. Diagrammatic presentation for treatment regimens with L-NAME and celecoxib in experimental groups.