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Original Research Article

Zileuton alleviates acute cisplatin nephrotoxicity: Inhibition of lipoxygenase pathway favorably modulates the renal oxidative/inflammatory/caspase-3 axis

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ABSTRACT

Objective: The current study investigated for the first time the possible beneficial effect of zileuton, a selective 5-lipoxygenase inhibitor (5-LOX), against cisplatin-induced acute renal failure.
Methodology: Male Sprague-Dawley rats (180–200 g) were administered cisplatin once (5 mg/kg, i.p.) alone or combined with oral zileuton (10 mg/kg, given twice; 1 h before and 12 h after cisplatin).
Results: Compared with control rats, acute cisplatin administration caused significant increases of BUN (33.76 ± 7.74 vs 61.88 ± 11.35 mg/dl), serum creatinine (0.61 ± 0.21 vs 1.56 ± 0.28 mg/dl), renal levels of MDA (6.40 ± 1.04 vs 20.52 ± 2.18 nmol/g tissue), NOx (3.45 ± 1.20 vs 17.70 ± 2.27 nmol/g tissue), TNF-α (6.71 ± 0.66 vs 23.71 ± 3.41 pg/g tissue), MPO (0.87 ± 0.09 vs 3.12 ± 0.41 U/mg tissue protein) and renal caspase-3 activity (2.81 ± 0.37 vs 12.70 ± 2.94 U/mg tissue protein). Whereas, total SOD activity (1.99 ± 0.53 vs 0.79 ± 0.06 U/mg tissue protein) and IL-10 (110.98 ± 19.70 vs 62.34 ± 14.42 pg/g tissue) were significantly decreased. Cisplatin-induced nephrotoxicity was further confirmed histopathologically (tubular necrosis, cystic dilatation of renal tubules, vacuolar degeneration of renal tubular epithelium with perivascular oedema, and interstitial fibrosis). These changes were accompanied by alteration in 5-LOX pathway manifested as elevated renal levels of 5-LOX, LTD4 and LTB4. Simultaneous administration of zileuton to the cisplatin-treated rats reversed the deleterious renal insults and restored the measured parameters near to control values.
Conclusions: These data establish the first experimental evidence that zileuton abrogates cisplatin nephrotoxicity in rats probably via the inhibition of detrimental actions of 5-LOX products, thus favorably affecting renal oxidative/inflammatory/caspase-3 axis. Based on current findings, the therapeutic prospect of zileuton for this purpose is recommended.

1. Introduction

Cisplatin, chemically known as *cis*-diamine-dichloroplatinum II, is an anti-cancer drug widely used against multiple solid tumors [1]. It targets DNA, bivalently reacting with purine bases (mainly guanidine), generating inter-/intra-strand crosslinks that disrupt DNA helical structure, and activate DNA damage response, thus obstructing transcription, replication [2], and cell division [1]. Cisplatin generates reactive oxygen species (ROS) that activate the pro-apoptotic family members, leading to release of cytochrome c, which finally activates caspase-3 and induces apoptosis [3].

Nephrotoxicity is an important complication of cisplatin chemotherapy. Cisplatin primarily induces proximal tubular injury through multiple mechanisms, including oxidative stress, inflammatory [4], immunological reactions, and tubular cell apoptosis [1].

Arachidonic acid released from membrane diacylglycerols and/or phospholipids by the action of phospholipase-A_2 & C, is enzymatically transformed (Major pathways); to prostaglandins [5] via the cyclooxygenase (COX) pathway and to leukotrienes (LTs), 5-hydroxyeicosatetraenoic acid, and 5-oxo-eicosatetraenoic acid from arachidonic acid via the 5-lipoxygenase (5-LOX) pathway. LTs exert potent vasoactive and pro-inflammatory effects. The activation of 5-LOX is calcium-dependent, and it acts together with the 5-LOX activating protein to form LTA4. LTA4 is unstable and is rapidly converted to

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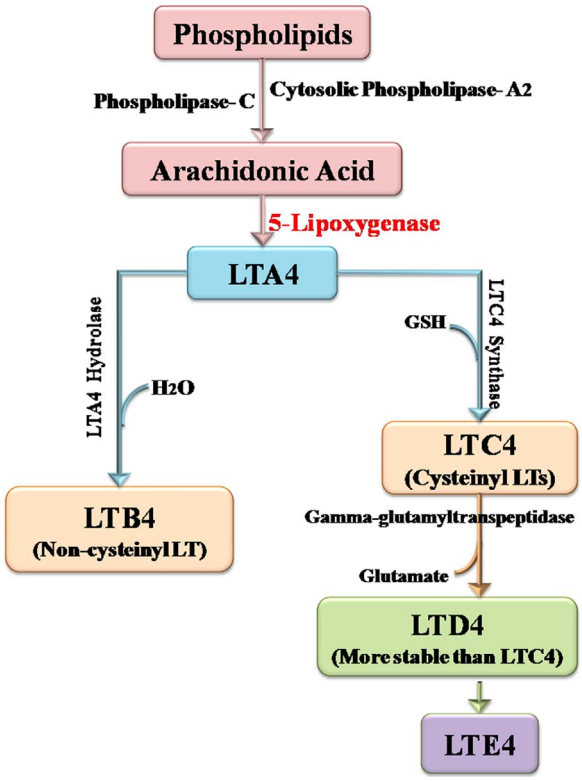

Fig. 1. Schematic diagram of arachidonic acid metabolism.

either non-cysteinyl LTB4 by LTA4 hydrolase or cysteinyl LTs; LTC4, LTD4, and LTE4 [6] as shown in Fig. 1 below.

LTB4 is one of the most potent chemoattractant mediators of inflammation, exerting its actions through a seven transmembrane-spanning G protein receptors, LTB4 R-1 & LTB4 R-2 [7], and the former is the high affinity receptor for LTB4 that is expressed in inflammatory and immune cells including leukocytes and mediates chemotaxis [8,9]. LTB4 induces neutrophils aggregation and expression of cell-adhesion molecules promoting their binding to endothelium and increases vascular permeability. The activated neutrophils transmigrate into ischemic tissues and release ROS, cytokines and various other mediators, all of which exacerbate inflammation and contribute to tissue injury [6,10,11] as in case of asthma [12], ischemia-reperfusion (I/R) of skin [13], brain [14], and kidney [15–17].

The peptidoleukotrienes (LTC4, LTD4, and LTE4) are potent vasoactive substances [18], which induce their actions through G protein coupled receptors, CysLT R-1 and CysLT R-2 [7]. LTC4 and LTD4 have been shown to increase the tone of mesenteric arteries. LTD4-evoked pressor responses were found higher in spontaneously hypertensive rats [19]. Recently, the role of cysteinyl LTs particularly LTD4 in mediating inflammation had been documented [20,21].

Although several studies have investigated the role of COX pathway [22,23] in cisplatin-induced nephrotoxicity, little is known about the effect of cisplatin on the other pathways of arachidonate metabolism. For example, recent studies [24,25] demonstrating the reno-protective effect of montelukast, a selective cysteinyl LT receptor antagonist, against cisplatin-induced acute renal failure did not elaborate a causal relationship between altered LTs metabolism and cisplatin nephrotoxicity. These studies attributed the protective effect of montelukast to its antioxidant and/or anti-inflammatory effects [24,25]. However, a recent study conducted by Alkhamees et al. demonstated the involvement of enhanced 5-LOX pathway and its products, namely LTB4 and cysteinyl LTs, in mediating cisplatin-induced nephrotoxicity in rats and concluded that combining LOX inhibitors with cisplatin may decrease the burden of nephrotoxicity associated with this frequently used antineoplastic agent [26].

Zileuton (benzothiophene N-hydroxyurea), a selective 5-LOX inhibitor, not only by chelating the iron at the enzyme active site but also possesses weak reducing properties, thus highly effective in preventing LTs formation [16]. It is currently in clinical use for the treatment of patients with asthma. Various studies reported the ability of zileuton to decrease the severity of injury (I/R) in various organs [27]. Zileuton has also been shown to decrease hydrogen peroxide-induced cytotoxicity via protein kinase C activation [28,29].

Here, we evaluated for the first time the possible beneficial effect of zileuton, the selective 5-LOX inhibitor, against cisplatin-induced acute renal failure. In addition, the experimentally selected dose of zileuton (10 mg/kg/orally, given twice; 1 h before and 12 h after the cisplatin dose) was smaller than those employed in previous studies [30,31] which might help in minimizing its potential adverse effects.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Laboratory Animal Colony, Helwan, Cairo, Egypt) weighing 180–200 g were used in this study. Animals were housed individually in stainless steel cages with wood shaving bedding and kept on a light–dark cycle of equal duration, under constant environmental conditions (temperature and humidity). Rats were fed with a commercially available rat normal pellet diet and water ad libitum. All efforts were made to minimize animal suffering and experiments were performed under thiopental sodium anesthesia. All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals [32,33]. The research experimental protocol was approved by the Animal Care & Use Committee (ACUC) at Faculty of Veterinary Medicine, Cairo University, Egypt.

2.2. Drugs and chemicals

Cisplatin (Oncotec Pharma production, Germany), zileuton (Sigma-Aldrich, St. Louis, MO, USA), and thiopental sodium (Biochemie GmbH, Vienna, Austria) were purchased from commercial vendors. Drugs were dissolved/dispersed in physiological saline, pH 7.4, immediately before use.

2.3. Experimental protocols

Three doses of zileuton (5, 10 and 20 mg/kg/day, orally) were examined in an exploratory pilot study to choose the dose offering a proper protective effect against cisplatin-induced renal damage. The dose 10 mg/kg was the minimal one offering this reno-protection. It is worth mentioning that several studies administered zileuton orally in a single daily basis even though its plasma half-life is about 2.5 h [30,31]. For instance, Chen et al. used zileuton in three doses (10, 30 and 50 mg/kg/day, given once orally) for three successive days and demonstrated its ability to alleviate cerebral ischemia [30]. It seems that the effect of 5-LOX inhibition lasts longer than the half-life of the drug itself which may explain the evident protective effect against cisplatin-induced nephrotoxicity seen in the current study as well as previous ones [30,31].

A total of 4 groups of rats (n = 10 each) were employed in the main study: (i) control, given physiological saline (1 ml/kg), (ii) zileuton (10 mg/kg, orally), (iii) cisplatin, given a single dose of cisplatin (5 mg/kg, intraperitoneally; i.p.) [34], and (iv) cisplatin + zileuton. Zileuton was given in two doses; one hour before and 12 h after cisplatin dose. The dosing schedule was selected based on the previous study of Patel et al. demonstrating that giving an intravenous dose of zileuton prior I/R and 12 h after reperfusion was suffient to reverse I/R renal injury [6]. The validity of this dosing schedule was further confirmed during our exploratory pilot study, where the renal function parameters were