European Journal of Pharmacology 738 (2014) 222–229

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Pulmonary, gastrointestinal and urogenital pharmacology

Tropisetron attenuates cisplatin-induced nephrotoxicity in mice

Mohammad Reza Zirak [a,b], Reza Rahimian [a,c,*], Mahmoud Ghazi-Khansari [a], Ata Abbasi [d], Ali Razmi [e], Shahram Ejtemaei Mehr [a], Kazem Mousavizadeh [f], Ahmad Reza Dehpour [a,g,*]

[a] Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, PO Box 13145-784, Tehran, Iran
[b] Department of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[c] Brain and Spinal Cord Injury Research Center, Tehran University of Medical Sciences, Tehran, Iran
[d] Department of Pathology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[e] Pharmacology and Applied Medicine Department of Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran
[f] Cellular and Molecular Research Center and Department of Molecular Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran
[g] Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 29 January 2014
Received in revised form
26 May 2014
Accepted 26 May 2014
Available online 4 June 2014

Chemical compounds studied in this article:
Cisplatin (PubChem CID: 441203)
Granisetron (PubChem CID: 5284566)
Methyllycaconitine citrate (PubChem CID: 163135989)
Ondansetron (PubChem CID: 4595)
Tropisetron (PubChem CID: 72165)

Keywords:
Tropisetron
Nephrotoxicity
Cisplatin

ABSTRACT

Nephrotoxicity is one of the most important complications of cisplatin, a potent chemotherapeutic agent used in the treatment of various malignancies. 5-HT_3 antagonists are widely used to counteract chemotherapy-induced emesis and new studies reveal that they posses notable anti-inflammatory properties. In current study, we investigated the effects of 5-HT_3 antagonists on cisplatin induced nephrotoxicity in mice. To identify the underlying mechanism of renal protection by tropisetron, we investigated the probable involvement of alpha7 nicotinic acetylcholine receptor (α7nAChR). A single injection of cisplatin (20 mg/kg; i.p) induced nephrotoxicity, 5-HT_3 antagonists (tropisetron, granisetron and ondansetron,) were given twice daily for 3 day (3 mg/kg; i.p). Finally animals were euthanized and blood sample was collected to measure urea and creatinin level. Also kidneys were removed for histopatological examination and biochemical measurements including glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD) activity, inducible nitric oxide synthase (iNOS) expression and inflammatory cytokines. Tropisetron decreased the expression of inflammatory molecules including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-$1_β$) and iNOS and improved histopathalogical damage and renal dysfunction. However other 5-HT_3 antagonists, granisetron or ondansetron do not have any elicit effects on biochemical markers and histological damages. Since methyllycaconitine, antagonist of α7nAChR, was unable to reverse the beneficial effect of tropisetron, we concluded that this effect of tropisetron is not mediated by α7nAChR.Our results showed that tropisetron treatment markedly ameliorated the experimental cisplatin induced-nephrotoxicity and this effect might be 5-HT_3 receptor and α7nAChR independent.

1. Introduction

Cisplatin (cis-diamminedichloroplatinum II) is one of the most potent antitumor drugs used against a wide spectrum of malignancies; however, its application in counteracting cancer is restricted due to the development of nephrotoxicity. Despite intensive care measures, one-third of cisplatin-treated patients experience irreversible renal damage (Taguchi et al., 2005). Cisplatin is accumulated in the renal tissue more than other organs, thereby resulting in necrosis of the proximal renal tubules and apoptosis in the distal nephron. The molecular mechanisms underlying cisplatin-induced cell death in renal tubular cells are not fully understood yet but it has been shown that oxidative stress and inflammatory mediators such as tumor necrosis

Abbreviations: Aβ, amyloid beta; COX-2, cyclooxygenase-2; ELISA, enzyme-linked immunosorbent assay; Grani, granisetron; GSH, glutathione; IL1β, interleukin-1 beta; iNOS, inducible nitric oxide synthase; MAPK, mitogen activated kinase; MDA, Malondialdehyde; MLA, Methyllycaconitine citrate; NF-κB, nuclear factor kappa B; NO, nitric oxide; Ondan, ondansetron; SDS, sodium dodecylsulphate; S.E.M, standard error of the mean; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-alpha; Tropi, tropisetron; α7nAChR, alpha7 nicotinic acetylcholine receptor

* Corresponding authors at: Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, PO Box 13145-784, Tehran, Iran.
Tel.: +98 21 8897 3652; fax: +98 21 6640 2569.
E-mail addresses: rahimian78@gmail.com (R. Rahimian), dehpour@sina.tums.ac.ir (A.R. Dehpour).

http://dx.doi.org/10.1016/j.ejphar.2014.05.050
0014-2999/

factor-alpha (TNF-α) and interleukin-1 beta ($IL_{1\beta}$) are involved in the pathogenesis of cisplatin induced nephrotoxicity (Miller et al., 2010; Zhang et al., 2007). Functional alterations are characterized by changes in urine volume, increase in blood urea nitrogen and serum creatinine (Ferguson et al., 2008). Prevention of cisplatin-induced nephrotoxicity would reduce morbidity and complications, decrease hospitalization costs and may allow administration of higher doses of this effective antitumor drug. Recently, numerous studies investigated the efficacy of different pharmacological agents such as antioxidants, modulators of nitric oxide, diuretics, and cytoprotective agents on cisplatin nephrotoxicity, but unfortunately none of them are universally effective in clinical settings. Therefore, there is a pressing need for research that leads to the development of new therapeutic approaches for overcoming cisplatin dose limiting nephrotoxicity.

5-HT_3 receptor antagonists including tropisetron, graniseron and ondansetron are safe drugs with large therapeutic index and are widely used to counteract chemotherapy-induced emesis. Currently in clinical and experimental studies new pharmacological indications have been reported with 5-HT_3 receptor antagonists (Fakhfouri et al., 2012b). It had been shown that 5-HT_3 receptor antagonists elicit notable antiphlogistic properties, *in vivo* and *in vitro*. In rat model of colitis, it had been shown that tropiseron could attenuate elevated level of pro-inflammatory cytokines (TNF-α, $IL\text{-}1_\beta$) and lipid peroxidation (Mousavizadeh et al., 2009). In another interesting study, in an embolic model of stroke, it has been reported that tropisetron could ameliorate the ischemic brain injury via decreasing myeloperoxidase activity and TNF-α level (Rahimian et al., 2011).

Since 5-HT_3 receptor antagonists have anti-inflammatory properties and both cisplatin and 5-HT_3 antagonists are pivotal medications in chemotherapy, it would reasonable to investigate the potential of 5-HT_3 receptor antagonists in amelioration of cisplatin nephrotoxicity.

Also, some studies declare that anti-inflammatory effects of 5-HT_3 receptor antagonists especially tropisetron are exerted via alpha7 nicotinic acetylcholine receptor (α7nAChR) (Kohnomi et al., 2010; Stegemann et al., 2013). Furthermore, in different studies, α7nAChR agonists have shown anti-inflammatory effects (Kox et al., 2011; Rosas-Ballina et al., 2009). This study, aimed to investigate the protective aspects of different 5-HT_3 receptor antagonists on cisplatin-induced nephrotoxicity and possible involvement of α7nAChR in probable protective properties of tropisetron.

2. Materials and method

2.1. Animals

Male albino mice weighing 30 ± 2 g were randomly divided in 7 experimental groups. The animals were housed for 1 week before the experiments to acclimatize to new condition. The experimental procedures were approved by the Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran in accordance with the Standards for the Care and Use of Laboratory Animals.

2.2. Experimental protocol

Cisplatin 20 mg/kg was injected to all groups except for control group (group 1) that received normal saline alone. Group 2 received cisplatin only. In addition to cisplatin groups 3, 4 and 5 received tropisetron (≥ 98%, HPLC grade, Sigma), granisetron or ondansetron, respectively at the dose of 3 mg/kg twice a day for 3 days until the animals were killed ($N=6$ for each group). Group 6 received methyllycaconitine citrate (MLA) (3 mg/kg twice a day for all 3 days) in addition to cisplatin ($N=4$). Group 7 received tropisetron and MLA (both, 3 mg/kg twice a day for 3 days until the animals were killed) in addition to cisplatin ($N=4$). All drugs were administrated Intraperitoneally (IP). The doses of drugs and time courses were selected based on previous pathological study (Hung et al., 2007; Jia et al., 2010; Rahimian et al., 2011; Ramesh and Reeves, 2004). 72 h after cisplatin injection, animals were euthanized, blood sample was collected and kidney was removed and snap frozen in liquid nitrogen and then kept at −80 °C for later biochemical measurements.

2.3. Tissue homogenization

In order to determine the inflammatory cytokines, tissues were homogenized as follows; first, we prepared a lysis buffer containing 200 mM NaCl, 5 mM EDTA, 10 mM tris, 10% glycerin, 1 mM PMSF. Then, 1 tablet of protease inhibitor cocktail for 10 ml of lysis buffer was added. Finally, pH was set at 7.4. Also, 200 μl lysis buffer was added to 10 mg tissue before homogenization. Samples were centrifuged twice (201*g* for 15 min, at 4 °C) to avoid contamination of cell debris. Supernatants were used for the measurement of cytokines level (Fakhfouri et al., 2010).

To measure superoxide dismutase (SOD) activity, tissues were homogenized according to the kit protocol. For determination of glutathione (GSH) and malondialdehyde (MDA) level, 1000 μl normal saline was added to 100 mg tissue and homogenized. Finally, centrifuged at 13,835*g* for 5 min at 4 °C and the supernatants were used for determination of GSH and MDA level.

2.4. Determination of blood urea and creatinine

After collecting blood sample, specimens were centrifuged at 906*g* for 10 min for serum separation. Using an AutoAnalyzer 7070 (Hitachi, Japan), blood urea and creatinine were measured.

2.5. Determination of lipid peroxidation

Lipid peroxidation was measured through determination of MDA level. MDA level was measured using a thiobarbituric acid reactive substances method as described by Avila et al. (2005). Concisely, 180 μl of a reaction mixture containing 0.1 M HCl, 0.67% TBA, 10% phosphotungstic acid and 7% sodium dodecylsulphate (SDS) was added to 100 μl of tissue supernatant and heated in boiling water at 95 °C for 1 h. Then150 μl n-butanol was added and stirred. Finally, 100 μl of n-butanol was taken and read in duplicates in a 96-well plate with a fluorometer (Biotec 5, USA) set at excitation at 530/25 and emission at 575/15. Samples were read against MDA standards (Sigma) prepared at different concentrations.

2.6. Determination of GSH level

Renal GSH level was measured by the method described by Kuo et al. (1983) with little modifications. The method is based on the reduction of 5,5-dithiobis-2-nitrobenzoic acid by GSH to yield a yellow component. The absorbance of this yellow component was measured at 412 nm and compared with the standard curve to determine GSH concentration.

2.7. Determination of total SOD activity

SOD activity was determined using a commercial kit (Biovision, USA). SOD measurement was based on the reduction of WST-1 with a superoxide anion that produces a water-soluble formazan dye. Superoxide anions are produced by addition of xanthine