Chemico-Biological Interactions 294 (2018) 56–64

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Curcumin alleviates colistin-induced nephrotoxicity and neurotoxicity in rats via attenuation of oxidative stress, inflammation and apoptosis

Nagah E. Edrees[a], Azza A.A. Galal[a,*], Aliaa R. Abdel Monaem[a], Rasha R. Beheiry[b], Mohamed M.M. Metwally[c]

[a] Department of Pharmacology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, 44511, Egypt
[b] Department of Histology and Cytology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, 44511, Egypt
[c] Department of Pathology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, 44511, Egypt

ARTICLE INFO

Keywords:
Colistin
Curcumin
Oxidative stress
Pro-inflammatory cytokines
Immunohistochemistry

ABSTRACT

Colistin is an effective antibiotic against multidrug-resistant (MDR) gram-negative bacterial infections; however, nephrotoxic and neurotoxic effects are fundamental dose-limiting factors for this treatment. This study was conducted to assess the potential protective effects of curcumin, a phenolic constituent of turmeric, against colistin-induced nephrotoxicity and neurotoxicity, and the possible mechanisms underlying any effect. Twenty-four adult male albino rats were randomly classified into 4 equal groups; the control group (orally received saline solution), the curcumin-treated group (orally administered 200 mg curcumin/kg/day), the colistin-treated group (IP administered 300,000 IU colistin/kg/day) and the concurrent group (orally received 200 mg curcumin/kg/day concurrently with colistin injection); all rats were treated for 6 successive days. Colistin administration significantly increased serum creatinine, urea and uric acid levels as well as brain gamma butyric acid (GABA) concentrations. In renal and brain tissues, colistin significantly increased malondialdehyde (MDA), nitric oxide (NO), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and caspase-3 expression levels. In addition, colistin significantly decreased catalase (CAT), glutathione (GSH), and B-cell lymphoma 2 (Bcl-2) expressions. Curcumin administration in colistin-treated rats partially restored each of these altered biochemical, antioxidant, inflammatory and apoptotic markers. Histopathological changes in renal and brain tissues were also alleviated by curcumin co-treatment. Our study reveals a critical role of oxidative damage, inflammation and apoptosis in colistin-induced nephrotoxicity and neurotoxicity and showed that they were markedly ameliorated by curcumin co-administration. Therefore, curcumin could represent a promising agent for prevention of colistin-induced nephrotoxicity and neurotoxicity.

1. Introduction

Colistin, also known as polymyxin E, is a glycopeptide antibiotic produced by *Bacillus polymixa* var *colistinus* [1]. It was discovered by Koyama in 1947, and since 1959, it has been utilized in the treatment of infections caused by multidrug-resistant (MDR) gram-negative bacteria [2,3]. Colistin induces bactericidal effects via the interaction of its cationic polypeptides with the anionic lipopolysaccharide (LPS) molecule of the gram-negative bacterial membrane, leading to displacement of Ca^{2+} and Mg^{+} of LPS; these changes in turn disturb membrane stability and increase membrane permeability, causing leakage of cell contents and ultimately cell death. It also binds to the endotoxin of gram-negative bacteria, the lipid A portion of the LPS molecule, and neutralizes it [1,2,4,5].

Colistin was efficacious and had good results in the treatment of infections caused by *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Acinetobacter baumannii, Salmonella sp., Enterobacter sp., Haemophilus influenza, and Shigella sp*. The resistance of these bacteria against the drug was extremely low. In 1970, it was reported that colistin had nephrotoxic and neurotoxic side effects; thus, its use was temporarily stopped [6,7]. Renal toxicity is the most common side effect associated with colistin administration, because colistin is excreted primarily via the kidneys, and elevated blood levels may deteriorate renal function (Lewis and Lewis, 2004). Furthermore, neurological symptoms, such as confusion, dizziness, vertigo, seizures, and facial/peripheral paresthesia, and less common fatal effects, including respiratory muscle weakness, apnea, and ataxia, were recorded in colistin-treated patients [8,9]. Clearly, identification of nephroprotective

* Corresponding author.
E-mail addresses: azzagalal@zu.edu.eg, azzapharma@yahoo.com (A.A.A. Galal).

https://doi.org/10.1016/j.cbi.2018.08.012
Received 30 May 2018; Received in revised form 13 July 2018; Accepted 15 August 2018
Available online 20 August 2018

and neuroprotective agents that can be co-administered with colistin has the potential to allow the clinical application of this essential drug.

A potential nephroprotective and neuroprotective candidate is curcumin, a compound found within the bright yellow spice turmeric, obtained from the rhizome of *Curcuma longa Linn* [10]. It has an outstanding safety profile and a number of pleiotropic actions, including anti-inflammatory [11–13], antioxidant and radical scavenging [14], cytotoxic and anti-apoptotic activities [15–17]. Additionally, it has hepatoprotective [18–20] and nephroprotective effects [21–23]. Notably, curcumin can cross the blood-brain barrier, suggesting a possible usage as a neuroprotective substance [24–27].

The spread of infections caused by MDR gram-negative bacteria and the lack of new antibiotics to fight them have led to a revival in colistin use [1]. Therefore, there is an urgent need to alleviate colistin-induced nephrotoxicity and neurotoxicity, as this would increase the therapeutic index of colistin, and thereby permit the administration of higher doses. Natural ingredients have been used to ameliorate the side effects of colistin [28–30]. Thus, the main aim of this work was to assess the mechanism by which colistin induces nephrotoxic and neurotoxic effects, and to determine whether curcumin can protect against colistin side effects via its antioxidant, anti-inflammatory and anti-apoptotic properties.

2. Materials and methods

Colistin (Colomycin®) vials were produced by Forest Laboratories UK, Ltd. Each vial contains 1 million IU colistimethate sodium powder for injection. Crystalline 99% extra pure curcumin was purchased from Loba Chemie Pvt Ltd-India. Colistin and curcumin were dissolved in sterile normal saline solution.

2.1. Experimental animals

Experiments were conducted on 24 adult male albino rats weighing 150–180 g obtained from the animal house at the Faculty of Veterinary Medicine, Zagazig University. The rats were housed in metal cages at 23 ± 2 °C and 40–60% relative humidity with a 12 h light cycle. Food and water was provided *ad libitum* throughout the experimental period. The rats were adapted to the experimental location for two weeks prior to testing. Animal housing and care and the experimental protocols were conducted as stipulated in the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health (NIH) and as approved by the local authorities of Zagazig University, Zagazig, Egypt. All efforts were made to minimize animal suffering.

2.2. Experimental design

Rats were randomly classified into four equal groups (n = 6 each) as follows: Group 1, control group: each rat received 1/2 ml sterile saline solution orally as well as intraperitoneally once daily for 6 successive days. Group 2, curcumin-treated group (Curcumin): each rat was gavaged with curcumin (200 mg/kg/day) [31]. Group 3, colistin-treated group (Colistin): each rat received colistin (300,000 IU/kg/day) intraperitoneally for 6 successive days [32]. Group 4, (Concurrent): each rat received curcumin (200 mg/kg/day) 1 h before colistin (300,000 IU/kg/day) administration at for 6 days.

2.3. Evaluation of biochemical parameters

At the end of the 6-day experimental period, rats were fasted overnight and then sacrificed. Blood samples were collected from each rat in a glass tube without EDTA, left for 20 min to coagulate at room temperature and then centrifuged at 3000 rpm for 20 min to obtain serum. Serum samples were preserved at −20 °C until use for colorimetric evaluation of serum urea, creatinine and uric acid concentrations using a CE1020 spectrophotometer, according to previously described protocols [33–35]. Gamma amino butyric acid (GABA) concentration was determined in brain tissue homogenate using a specific rat ELISA kit in accordance with the manufacturer's protocol.

2.4. Evaluation of oxidative stress markers

The brain and both kidneys were removed immediately after sacrifice and washed in physiological saline. One kidney and half of the brain were preserved at −80 °C until preparation of tissue homogenates, which were used for colorimetric assessment of glutathione (GSH), malondialdehyde (MDA) and catalase (CAT) levels using a CE1020 spectrophotometer [36–38].

2.5. Evaluation of inflammatory markers

Levels of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were measured from brain and kidney homogenates using a specific rat ELISA kit following the manufacturer's manual. Additionally, nitric oxide (NO) level was colorimetrically evaluated [39].

2.6. Histopathological and immunohistochemical investigations

The other kidney and the second half of the brain were fixed in 10% neutral buffered formalin solution immediately after sacrifice. Paraffin sections of 5–7 μm thickness were cut and stained with hematoxylin and eosin (H&E) and then examined microscopically [40]. Another group of embedded-paraffin sections was also prepared for immunodetection of caspase-3 using a rabbit polyclonal antibody (cat no: RB-1197-R7 Thermo Fisher Scientific, Waltham, MA, USA) and Bcl-2-positive cells using a mouse monoclonal antibody (cat no: MS-123-R7, ready-to-use Neomarkers, Thermo Fisher Scientific, Waltham, MA, USA) using an avidin-biotin-peroxidase (ABC) method [41,42]. Negative control sections were prepared by incubating with phosphate buffer saline (PBS) as an alternative to the primary antibodies. All stained sections were examined with a standard light microscope, and photographs were taken using AmScope Digital Imaging System.

The reported histopathological lesions in the kidneys, and cerebral and cerebellar cortices in all groups were scored according to the following scoring system: (−) absence of the lesion in all animals of the group, (+) the lesion was rare within the group, (++) the lesion not so often observed in all animals of the group, (+++), the lesion observed in almost all animals of the group, (++++) the lesion often found in all animals of the group.

Quantitative assessment of the Bcl-2 and caspase-3 expression in the kidneys, cerebral and cerebellar cortices was calculated based on the percentage of positive cells per five non-overlapping randomly selected high-power microscopic fields (400X)/section as following: 0 (negative to weak) = less than 10%, + (mild) = 10–25%, ++ (moderate) = 26–50%, +++ (strong) = 51–75%, and ++++ (severe) = more than 75% (severe).

2.7. Statistical analysis

The data are presented as the mean ± SE for each group. The variation between groups was statistically analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range post hoc test for pairwise comparisons. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Effects of curcumin on several biochemical parameters of colistin-treated rats

Table 1 shows that rats treated only with colistin had significantly higher levels of serum creatinine (0.86 mg/dl), uric acid (4.32 mg/dl)