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The protective effect of astaxanthin against cisplatin-induced nephrotoxicity in rats

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ABSTRACT

Purpose: The aim of this experimental study was to investigate the antioxidant effects of astaxanthin against cisplatin-induced nephrotoxicity in rats.

Methods: Forty-eight male Sprague-Dawley rats weighing 264.83 ± 7.39 g were randomly divided into six groups of eight animals each. These were constituted as control, olive oil control, astaxanthin control, cisplatin control, 16 mg/kg cisplatin & 25 mg/kg astaxanthin and 16 mg/kg cisplatin & 75 mg/kg astaxanthin groups. Biochemical evaluation was performed by measuring blood urea nitrogen, serum creatinine, total oxidant status and total antioxidant status. Renal corpuscle, proximal and distal tubules areas (μm^2) were calculated for histopathological evaluation, and Caspase-3 staining was performed for immunohistochemical evaluation.

Results: Cisplatin reduced total antioxidant status levels and increased blood urea nitrogen, serum creatinine, total oxidant status, and Caspase-3 levels. It also caused dilatation, vacuolization, and loss of tubular epithelial cells in the proximal and distal tubules, and glomerular degeneration and edema were determined in kidney tissue ($p < 0.05$). Administration of 25 mg and 75 mg astaxanthin increased total antioxidant status levels, reduced blood urea nitrogen, serum creatinine, total oxidant status, and Caspase-3, and ameliorated degenerative distal and proximal tubules, glomerular degeneration and edema in kidney tissue ($p < 0.05$).

Conclusions: The nephrotoxic effect of cisplatin was diminished by the antioxidant effect of astaxanthin.

1. Introduction

Antineoplastic chemotherapeutic agents have been used for many years, but because these agents disrupt the cell cycle, both normal and neoplastic cells are affected by them [1]. Cisplatin is a platinum-based drug and a useful chemotherapeutic agent in various malignancies, including solid malignant tumours of the head, neck, bladder, testis, ovary, prostate, cervix, oesophagus and lung [2]. Cisplatin has several side effects, such as nausea and vomiting, nephrotoxicity, neurotoxicity, ototoxicity and, rarely, ocular toxicity [3]. Nephrotoxicity is the most frequent and dose-limiting side effect of cisplatin therapy, and irreversible kidney damage occurs in one-third of patients despite protective measures [1,2]. Nephrotoxic effects occur in several ways, including via oxidative stress, inflammation, fibrogenesis, mitochondrial damage, apoptosis and necrosis [4,5]. Cisplatin accumulates most in the S3 segment of the proximal tubules, followed by the distal collecting tubules and the S1 segment of the proximal tubules [6].

Exposure to oxidant radicals is increasing steadily in industrialised societies. Oxidative stress plays an active role in acute kidney injury induced by cisplatin administration. Cisplatin specifically increases the production of hydroxyl radicals, potent free radicals that consume intracellular antioxidant stores and affect directly cell components, such as lipids, proteins and DNA, thus impairing the cellular structure [7,8]. Reactive oxygen species (ROS) involved in cisplatin nephrotoxicity include superoxide anion, hydrogen peroxide, hydroxyl radical and reactive nitrogen species, such as peroxynitrite and nitric oxide [9–11]. Cisplatin also inhibits antioxidant enzymes; hence, the renal activities of superoxide dismutase (SOD), glutathione peroxidase (GPx),

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glutathione reductase, total antioxidant status (TAS) and catalase decrease markedly [12,13]. Malondialdehyde (MDA) and total oxidant status (TOS) levels also increase as a result of damage to antioxidant systems [13–15]. TAS is an effective assay in both human and animal models for the measurement of serum antioxidative stress [16,17]. Its use as an antioxidant in the literature is increasing steadily [18]. TAS and TOS are also easy-to-perform, stable, reliable, sensitive and inexpensive methods for measuring total antioxidant capacity [19].

Carotenoids, an antioxidant group, include over 700 organic, soluble substances produced by phytoplankton, algae, plants and limited numbers of fungi and bacteria [20]. Astaxanthin is a highly antioxidant, anti-inflammatory, carotenoid pigment, and it is considered the strongest and safest known antioxidant in nature. It is obtained from the microalgae *Haematococcus pluvialis* [21]. Astaxanthin exhibits antioxidant activities through two pathways: it scavenges free radicals and protects against chain reactions that may be caused by free radicals or it terminates chain reactions that may already have taken place [22]. Due to its beneficial effects on human health, astaxanthin is widely used as a nutritional supplement and antioxidant [23,24]. The U.S. Food and Drug Administration (FDA) approves the use of astaxanthin as a human dietary supplement.

This study employed biochemical, histopathological and immunohistochemical methods to investigate the antioxidant effects of astaxanthin against cisplatin nephrotoxicity under experimental conditions.

2. Materials and methods

Forty-eight male Sprague Dawley rats aged 3–5 months and weighing 264.83 ± 7.39 g were procured from the Recep Tayyip Erdogan University Animal Care and Research Unit (Rize, Turkey). All animals received care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Sciences and published by the National Institutes of Health. Approval for the study was granted by the Recep Tayyip Erdogan University (Rize, Turkey) Animal Ethical Committee (No. 2016/32).

2.1. Study design

Animals were kept in Recep Tayyip Erdogan University Animal Care and Research Unit (Rize, Turkey). All animals were maintained and fed in a sterile experimental animal unit environment having 55–60% humidity, at a temperature of 22 ± 3 °C, and 12-h light:12-h light-dark cycle. Rats were allowed ad libitum access to commercially available standard rat chow (Bayramoğlu Feed and Flour Industry Trading Corporation Erzurum, Turkey) and tap water throughout the experiment. After sufficient time had been allowed for adaptation to the laboratory conditions, the experimental animals were divided randomly into six groups.

Anesthesia was administered to all groups with 50 mg/kg intraperitoneal ketamine hydrochloride (Ketalar ®, Eczacibası Parke-Davis, Istanbul, Turkey) and 10 mg/kg intraperitoneal Xylazine HCl (Alfazyne ®, Alfasan International BV Woerden, Holland).

All groups received oral distilled water daily for eight days. Group 1, the control group (n = 8), received no drug injections except for anaesthetics. Group 2 acted as the olive oil control group (n = 8). Olive oil was used for dissolving astaxanthin in Groups 3–6. Group 2 received intra-peritoneal olive oil only for eight days [25]. Group 3, the astaxanthin control group (n = 8), received only intra-peritoneal astaxanthin 75 mg/kg dissolved in olive oil [26]. Group 4 acted as the cisplatin only group (n = 8) and received a single 16 mg/kg dose of cisplatin (Cisplatin DBL 100 mg/100 ml vial, Orna Ilac, Istanbul) intraperitoneally on the fifth day of the study [27]. Group 5 represented the 16 mg/kg cisplatin and 25 mg/kg astaxanthin group (n = 8), having received 25 mg/kg astaxanthin intraperitoneally daily for eight days and a single 16 mg/kg dose of cisplatin intraperitoneally on the fifth day. Group 6 was the 16 mg/kg cisplatin and 75 mg/kg astaxanthin group (n = 8), having received 75 mg/kg astaxanthin intraperitoneally daily for eight days and a single 16 mg/kg dose of cisplatin intraperitoneally on the fifth day (Fig. 1).

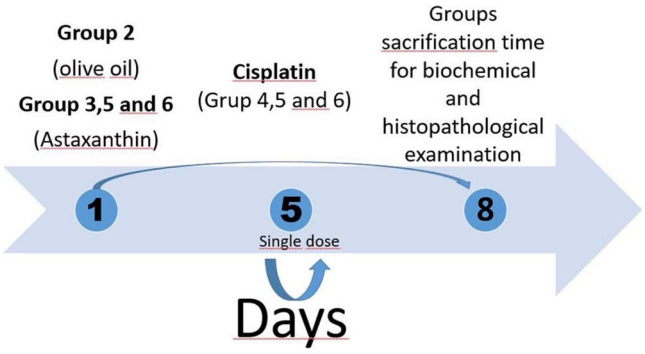

Fig. 1. Schematic experimental timeline.

2.2. Biochemical analysis procedure

The kidneys were removed from the abdominal cavities. Phosphate buffered saline PBS), a water-based salt solution containing sodium phosphate dibasic, sodium chloride and sodium phosphate monobasic, was then prepared for kidney tissues (pH: 7.4). The specimens were washed in ice-cold PBS and weighed. The ratio of tissue weight to homogenisation buffer was 1:10. Specimens were homogenised in PBS for one min. The homogenates were centrifuged at 2717 g for 20 min. TAS and TOS levels were measured using a commercial kit (RL0024, Rel Assay Diagnostics, Turkey). The TAS results were expressed as Trolox Equivalent/L and the TOS results as μmol H_2O_2 Equivalent/L.

Blood samples were collected from all rats for biochemical evaluation. Blood was separated by centrifugation at 1739 g for 15 min at 4 °C. Levels of blood urea nitrogen (BUN) and serum creatinine were determined using a standard AutoAnalyzer (Architect c16000 AutoAnalyzer, Abbott Diagnostics, Waltham, Massachusetts, USA). The results were expressed as mg/dL [28–30].

2.3. Histopathological analysis procedure

Kidneys were fixed in 10% neutral formaldehyde. Specimens were then dehydrated in an ascending alcohol series. Next, all sections were cleared in xylene and embedded in paraffin using routine laboratory methods. They were stained with hematoxylin and eosin (H&E) and analysed by two histologists blinded to the study groups under a light microscope (Leica DM6200-Germany). Photographs were taken with an Olympus DP20 camera.

2.4. Quantitative analysis

The renal corpuscle, proximal and distal tubule areas (μm^2) were measured using the Olympus DP2-BSW (Ver.2.1 to Ver.2.2, Build 6212, Olympus Corporation, Tokyo, Japan) software system. This consists of a camera (Olympus DP20, Olympus Corporation, Tokyo, Japan) attached to a light microscope (Leica DM6200, Germany) and a computer with a software system. H&E (Darmstadt, Germany)-stained sections were placed onto the microscope tray, and their sectional boundaries were determined using this programme (40x objective lens). Once the area was identified, distinct and separate frames were assessed by two blinded histopathologists (Fig. 2).

2.5. Immunohistochemistry (IHC) analysis procedure

Sections were deparaffinised and treated with proteinase K solution