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Original Article

Carvacrol Protects Against Paclitaxel-Induced Retinal and Optic Nerve Cytotoxicity: A Histopathological Study

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Abstract

Objectives: Carvacrol (CV) is a phenolic monoterpenoid found in the essential oil of a number of aromatic plants and herbs. The present study was an investigation of the potential protective effect of CV against paclitaxel (PTX)-induced retinal and optic nerve cytotoxicity in rats.

Methods: A total of 18 adult male Wistar albino rats (250-400g) were randomized into 3 equal groups comprising 6 animals each. Group 1 (control group) received intraperitoneal (IP) saline solution (0.5 mL/200 g) weekly for 4 weeks. Group 2 received an IP dose of PTX (5 mg/kg), and Group 3 received CV (25 mg/kg) 30 minutes after an IP dose of PTX (5 mg/kg) weekly for 4 weeks. At the conclusion of the experimental period, the retinal and optic nerve tissues of the subjects were evaluated histopathologically.

Results: All of the retinal specimens in Group 1 (control) were histopathologically normal. In Group 2 (PTX), all of the eyes (6/6) revealed increased retinal vascularity and rosette-like structures in the outer nuclear layer, and in Group 3 (PTX-CV), all of the eyes (6/6) demonstrated normal retinal vascularity and the absence of rosette-like structures. All of the optic nerve specimens in Group 1 (control) were histopathologically normal. In Group 2 (PTX), all of the eyes (6/6) demonstrated severe vacuolization and a decreased number of astrocytes and oligodendrocytes in the optic nerve specimens, while 3 eyes (3/6) showed marked single cell necrosis. None of the eyes in Group 3 (PTX-CV) demonstrated either vacuolization or a reduction in the number of astrocytes and oligodendrocytes. No remarkable single cell necrosis was observed in the optic nerve specimens of Group 3 (PTX-CV).

Conclusion: The histopathological findings indicated that CV played a protective role against PTX-induced cytotoxicity. CV might be a promising resource to counteract oxidative stress-based cytotoxicity in the field of retinal and optic nerve disorders.

Keywords: Antioxidant, carvacrol, oxidative stress, paclitaxel, paclitaxel-induced cytotoxicity.

Introduction

Paclitaxel (PTX), one of the most commonly prescribed chemotherapeutic agents, is used to treat a wide variety of human cancers (1). PTX promotes the stable assembly of microtubules from alpha- and beta-tubulin heterodimers and inhibits their depolymerization (1, 2). The anti-tumor effect is primarily a result of interference with the normal function of microtubules and from blocking cell cycle progression

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in the late mitotic gap 2 (G2) phases (1). Clinically evident, PTX-induced ocular toxicity is rare. Ophthalmic adverse effects include decreased vision, scintillating scotomas, abnormal visual evoked potentials, and cystoid macular edema (3). One of the possible mechanisms underlying PTX-induced cytotoxicity in normal cells, particularly in non-proliferative tissues, is disruption of the microtubule system, which is critical for cell structure, motility, and cytoplasmic trafficking (4, 5). Others include induction of apoptosis and oxidative stress (OS) (4, 5). PTX has been reported to induce the formation of reactive oxygen species (ROS)-altering mitochondrial membrane permeability (6). OS induced by ROS can be described as a dynamic imbalance between the quantity of free radicals generated in the cell and the levels of antioxidants to quench and or/scavenge them and protect the cell against their deleterious effects (7). OS generated by PTX in non-targeted tissues may explain PTX-induced cytotoxicity without an antimitotic effect.

Bioactive compounds from plants continue to be studied for their capacity to protect cells from damage induced by OS. Carvacrol (CV) is a phenolic monoterpenoid found in the essential oil of oregano (Origanum vulgare), thyme (Thymus vulgaris), pepperweed (Lepidium flavum), wild bergamot (Citrus aurantium bergamia) and other plants (8). CV has been reported to have a number of pharmacological properties, including antioxidant, antitumor and anti-inflammatory effects (8). To our knowledge, there is no previous research regarding the potentially protective effect of CV on retinal and/or optic nerve tissues. The aim of this study was to histopathologically evaluate whether CV exhibited any protective effect against OS-based cytotoxicity induced by PTX in retinal and optic nerve tissues.

Methods

A total of 18 adult male Wistar albino rats (250-400g) were obtained from the research animal center of Atatürk University (11.01.2017, 42190979-000-E.1700010216). The rats were kept in ventilated cages at a temperature 22±2°C with a 12-hour light/dark cycle. All of the rats were fed with standard laboratory feed and had free access to water. The rats were randomized into 3 groups comprising 6 animals. Group 1 (control) received intraperitoneal (IP) saline solution (0.5 mL/200 g) weekly for 4 weeks. Group 2 received IP PTX (Taxol; Bristol-Myers Squibb Co., New York, NY, USA) at a dose of 5 mg/kg weekly for 4 weeks. Group 3 received IP CV (25 mg/kg, 98%; Sigma-Aldrich, St. Louis, MO, USA) 30 minutes after IP PTX (5 mg/kg) weekly for 4 weeks. At the end of the fourth week, the rats were euthanized using ketamine anesthesia. The enucleation material was fixed in 10% formaldehyde and samples were taken. The tissues were embedded in paraffin wax and sections of 4-µm thickness were mounted on slides and stained with hematoxylin-eosin. The observations were made by 2 independent observers under a light microscope (Eclipse 80i; Nikon Corp., Tokyo, Japan). The experimental protocol conformed to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research and was approved by the ethics committee of Ataturk University.

Results

All of the retinal specimens in Group 1 (control) were histopathologically normal (Fig. 1A). In Group 2 (PTX), all of the eyes (6/6) demonstrated increased retinal vascularity and rosette-like structures in the outer nuclear layer (Fig. 1B), while in Group 3 (PTX-CV), all of the eyes (6/6) revealed normal retinal vascularity and no rosette-like structures (Fig. 1C). All of the optic nerve specimens in Group 1 (control) were histopathologically normal (Fig. 2A). In Group 2 (PTX), all of the eyes (6/6) revealed severe vacuolization and a decrease in the number of astrocytes and oligodendrocytes, while 3 eyes (3/6) revealed marked single cell necrosis (Fig. 2B). In Group 3 (PTX-CV) none of the eyes demonstrated vacuolization or decreased glial cells. No significant single cell necrosis was observed in the optic nerve tissues of Group 3 (Fig. 2C).

Discussion

In mammals, although retinal progenitor cells persist throughout life, retinal neurogenesis ceases by the early postnatal period (9). The optic nerve, like the adult mammalian central nervous system, shows no evidence of cell proliferation (10). Since PTX targets highly proliferative malignant cells and quickly dividing non-neoplastic cells, (11) it is unlikely that PTX-induced cytotoxicity in a mature retina or optic nerve could be attributed to PTX's antimitotic effect. The molecular mechanisms underlying PTX-induced cytotoxicity remain controversial; however, OS is thought to be a critical mechanism leading to PTX-induced cytotoxicity. It has been reported that PTX increases the generation of ROS, down-regulates the activity of several antioxidative enzymes and increases hydrogen peroxide (H_2O_2) production by enhancing the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which contributes to OS (4). Ramanathan et al. (1) reported that PTX may exert its toxicity and DNA damage via elevation of intracellular oxygen (O_2), H_2O_2, and nitric oxide (NO) levels and cellular total antioxidant capacity is a critical determinant of cellular sensitivity to PTX. The combination of PTX with inhibitors of H_2O_2 metabolism has been found to enhance the killing of breast cancer cells via H_2O_2-induced metabolic OS (12). In an in vitro rat model, McCormick et al. (13) demonstrated that MitoVitE, a mitochondria-targeted antioxidant, limited

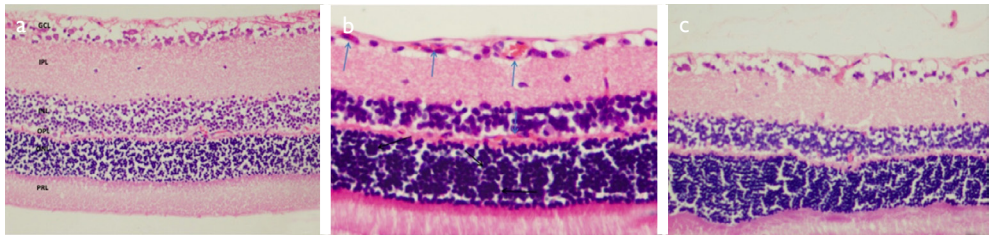

Figure 1. (a) Normal histological appearance of control group's (Group-1) retinas(H&E). (GCL: Ganglion cell layer, IPL: Inner plexiform layer, INL: Inner nuclear layer, OPL: Outer plexiform layer, ONL: Outer nuclear layer, PRL: Photoreceptor layer) **(b)** Paclitaxel applied group's (Group-2) retinas; rosette like structures (black arrows) in the outer nuclear layer and increased retinal vascularity (blue arrows) **(c)** Paclitaxel-carvacrol applied group's (Group-3) retinas; normal histomorphological appearance.

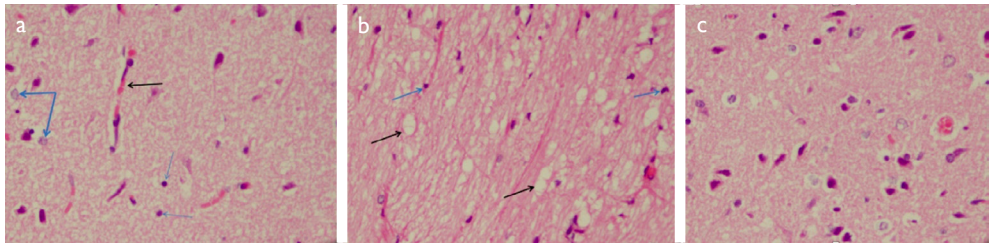

Figure 2. (a) Normal histological appearance of the control group's (Group-1) optic nerve (H&E). Oligodendrocytes (thin blue arrows), astrocytes (thick blue arrows) and vascular structures (black arrow) **(b)** Paclitaxel applied group's (Group-2) optic nerve; marked vacuolization (black arrows), decrease in numbers of astrocytes and oligodentrocytes and single cell necrosis (blue arrows) **(c)** Paclitaxel-carvacrol applied group's (Group-3) optic nerve; no marked vacuolization, usual appearance of astrocytes and oligodendrocytes.

PTX-induced OS, mitochondrial damage, and related mechanical hypersensitivity. Mitochondrial dysfunction and OS are strongly associated with some of the PTX-induced side effects like peripheral neuropathy. PTX affects mitochondria in the neurons by opening mitochondrial permeability transition pores and increasing ROS production, (14) which leads to peripheral nerve neuropathy (15). Pharmacological scavenging of ROS inhibited development and maintenance of the PTX-induced mechanical hypersensitivity and neuropathic pain in vivo (13). Ghrelin has been shown to alleviate PTX-induced peripheral neuropathy by reducing OS and enhancing mitochondrial anti-oxidant functions in rats (16). The oxidative mechanism underlying PTX-induced peripheral neuropathy may also cause PTX-induced cytotoxicity in retinal and optic nerve tissues. Based on the notion that OS is a key pathogenic mechanism underlying PTX-induced cytotoxicity in non-proliferative tissues, antioxidant molecules could, in theory, alleviate some adverse effects of PTX in these tissues.

The potential toxicity of synthetic antioxidants has stimulated great interest on antioxidants from natural sources, such as plants. The antioxidant potential of CV has been investigated in a few studies. Ozkan et al. (17) reported that CV exhibited antioxidant activity at lower concentrations and anticancer activity at higher concentrations in the hepatoma G2 cell line. Bagul et al. (18) synthesized CV sulfonate derivatives and demonstrated in vitro antioxidant properties through 2,2-diphenyl-1-picrylhydrazyl free radical scavenging. Another study reported a CV protective effect against 6-OHDA neurotoxicity in a rat model, partly through attenuating OS (19). Using a chromatographic method, Aybastıer et al. (20) demonstrated the free radical scavenging activity of CV in a Fenton reaction. In our study, we used PTX to induce OS-based cytotoxicity in an adult rat model. All of the eyes in Group 2 (PTX) demonstrated retinal rosette-like structures in the outer nuclear layer as well as an increase in retinal vascularity. It has been suggested that PTX-induced vascular endothelial growth factor expression could be mediated by PTX-induced ROS production through nuclear factor-κB activation and hypoxia-inducible factor 1-alpha stabilization (21). In our study, decreased retinal vascularity in Group 3 (PTX-CV) compared with Group 2 (PTX) might be attributable to the antioxidant effect of CV reversing this angiogenic process. PTX-induced histopathological changes in the optic nerve specimens of Group 2 (PTX) (vacuolization, reduction in the number of astrocytes and oligodendrocytes, single cell necrosis) were diminished in Group 3 (PTX-CV). Jana et al. (22) suggested OS-induced apoptosis of human primary oligodendrocytes, (22) while Juknat et al. (23) reported that OS promoted apoptotic death of cultured rat

astrocytes. CV may have prevented the OS-associated apoptotic process and loss of glial cells in Group 3 (PTX-CV).

The search for natural antioxidants from aromatic plants is a growing trend. In this study, to the best of our knowledge, we were the first to investigate whether CV exhibited protective effects in retinal and optic nerve tissues experimentally. We demonstrated histopathologically that CV protected against cytotoxicity induced by PTX. This effect might be due to the antioxidant activity of CV. CV might be a promising molecule to counteract OS-induced cytotoxicity in the field of retinal and optic nerve disorders. Further investigations, including immunohistochemical and biochemical analyses, are needed before implementing changes to clinical practice.

Disclosures

Ethics Committee Approval: Atatürk University Medical Experimental Application and Research Center Laboratory,11.01.2017, 42190979-000-E.1700010216.

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Conflict of Interest: None declared.

Authorship Contributions: Involved in design and conduct of the study (EC, ZK, BD, OC, HB, IC); preparation and review of the study (EC, ND, OC); data collection (EC, ZK, BD); and statistical analysis (EC, ND).

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