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The Protective Effects of Fucoidan on Cisplatin Induced Testicular Cytotoxicity in Rats †

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† Presented at the 2nd International Cell Death Research Congress, Izmir, Turkey, 1–4 November 2018.

Published: 6 December 2018

Abstract: Cisplatin is prevalently used as a chemotherapeutic agent for testis cancer, ovarian cancer, bladder cancer, brain tumors and many cancers such as those. Infertility and sterility may emerge, after treatment with chemotherapeutic agents, depending on patient characteristics and the dose of chemotherapeutic agent. Fucoidan is a sulfated polysaccharide, which is derived from brown seaweeds. It is first isolated in 1913 from marine brown algae by Henrik Kylin. Fucoidan is especially found in the cell-wall of marine brown algae. Many effects of fucoidan such as anti-inflammatory, anti-viral, anti-complement, anti-cancer, anti-oxidant and anti-coagulant effects have been revealed by many studies in the literature. Furthermore, some studies suggest that fucoidan may enhance the antineoplastic effects of anticancer drugs. Besides beneficial effects of fucoidan, no toxic or adverse effect has been shown to date. In this study, we aimed to investigate the effects of fucoidan on testicular tissue and its effects on cisplatin induced testicular damage when used concomitantly with cisplatin.

Keywords: cisplatin; fucoidan; rat; testis

1. Introduction

Cisplatin is prevalently used as a chemotherapeutic agent for testis cancer, ovarian cancer, bladder cancer, brain tumors and many cancers such as those. Infertility and sterility may emerge, after treatment with chemotherapeutic agents, depending on patient characteristics and the dose of chemotherapeutic agent [1,2]. Fucoidan is a sulfated polysaccharide, which is derived from brown seaweeds. It is first isolated in 1913 from marine brown algae by Henrik Kylin. Fucoidan is especially found in the cell-wall of marine brown algae. Many effects of fucoidan such as anti-inflammatory, anti-viral, anti-complement, anti-cancer, anti-oxidant and anti-coagulant effects have been revealed by many studies in the literature [3]. Furthermore, some studies suggest that fucoidan may enhance the antineoplastic effects of anticancer drugs. Besides beneficial effects of fucoidan, no toxic or adverse effect has been shown to date. In this study, we aimed to investigate the effects of fucoidan on testicular tissue and its effects on cisplatin induced testicular damage when used concomitantly with cisplatin.

2. Materials and Methods

28 Wistar Albino male rats, randomly divided in four groups, including control group (n = 7), cisplatin group (n = 7), fucoidan group (n = 7) and cisplatin-fucoidan group (n = 7), were supplied by Experiment Laboratory in Dokuz Eylul University Faculty of Medicine Animal. Dokuz Eylul University Multidisciplinary Animal Ethical Committee in Izmir approved all of the experimental protocols (protocol number: 93/2013). In the first day, both the control and fucoidan groups were injected with only saline whereas cisplatin and cisplatin-fucoidan groups were injected with cisplatin (7 mg/mL) [2]. Cisplatin was given as 7 mg/kg acute dose dissolved in 1 mL normal saline, through intraperitoneal injection, to cisplatin group and cisplatin and fucoidan combined group, the other groups was given just normal saline 1 mL to make optimization toward the damage of injections. For the following six days control and cisplatin groups were given 1 mL distilled water through oral gavage whereas fucoidan and cisplatin-fucoidan groups were given through oral gavage fucoidan (150 mg/kg) dissolved in 1 mL distilled water [3]. At the end of the experiment, all animals were sacrificed and their testes were removed and examined by hematoxylin-eosin staining and immunohistochemical staining in terms of histomorphological aspects. Tissue Glutathione (GSH), Glutathione Peroxidase (GPx) and Malondialdehyde (MDA) levels were also measured. Apoptotic cells of the testicular tissues were detected by TUNEL staining and active caspase-3 antibody, using In Situ Cell Death Detection kit (ROCHE) protocol and AB3623 Anti-Caspase 3 Antibody active (cleaved) form (Millipore), respectively.

Statistical Analysis: Data analysis were done by SPSS 20 statistic software package programme. All results were compared using Mann Whitney U test. A p value <0.05 was considered as statistically significant.

3. Results

In the Cisplatin group, there were a reduction in the body weight ($p < 0.05$) and histomorphologically basement membrane thickening ($p < 0.002$). Basal lamina degradation, lipid vacuolization, decreased amount of spermatids due to injury of germ line cells and the lumen expansion were detected ($p < 0.001$). Vascular congestion and degenerative Leydig cells were observed in the interstitial area. By contrast, histomorphological findings in the groups treated with fucoidan (fucoidan and cisplatin-fucoidan groups) were similar with the control group ($p > 0.05$). For Immunohistochemically examinations tissues were stained with TUNEL, anti-active caspase-3. A significant increase was observed in the number of apoptotic cells in the cisplatin group ($p < 0.001$). The group treated with fucoidan after cisplatin treatment showed decreased number of apoptotic cells, but this result was not statistically significant.

Biochemical examinations indicated significantly increased MDA levels ($p < 0.001$) and significantly reduced GSH levels ($p < 0.05$) and GPx activity ($p < 0.05$) in the Cisplatin group compared to control group. In cisplatin-fucoidan group, in which cisplatin treatment were followed by fucoidan injections, MDA levels ($p < 0.001$) were significantly reduced and GSH levels ($p < 0.001$) and GPx activity ($p < 0.05$) were significantly increased compared to cisplatin group.

Ultrastructure examination indicated that fucoidan regulated the permability of mitochondrian membrane and the adhesion abilities of the cells. When the sections of the control group were exanimated; structural formation was evaluated as normal morphology. When Cisplatin group was exanimated, structural deformations and thickening of the basement membrane were observed. Spermatogenic cells were deteriorated in their connections with each other and with basal lamina. Sections from Fucoidan group were evaluated and structural formation was similar to sections from control groups. Also, basal membrane structure was smooth morphology. The cells in semiferous ducts, located on the basement membrane, arranged regularly and their organelles revealed normal morphology. The last group cisplatin- fucoidan were examined; the results were similar to the sections from control group. When the results compared to cisplatin groups, a significant reduction in intracellular vacuolations and damaging organelles especially mitochondria.

4. Discussion

Cisplatin, is a chemotherapeutic agent, it has been widely used for solid tumors' treatments in adults and in children cancers. Due to its' adverse effects, such as ototoxicity, gonadal toxicity and nephrotoxicity, it has limited application. Besides, patients who used cisplatin could be infertile or sterile [1,4]. Fucoidan is a sulfated polysaccharide and it has some protective effects such as anti-oxidant, anti-viral and anti-inflammatory effects. The aim of this study was to evaluate the role of possible protective effects of Fucoidan against cisplatin induced testicular toxicity in rats [5].

In this study, cisplatin was induced testicular toxicity whereas fucoidan was repaired testicular damage in rats. Cisplatin group and cisplatin-fucoidan combination group showed significantly reduction of body weight, atrophy in parenchymal tissue and disorder of cells located in seminiferous ducts because of cisplatin treatment. According to the study of Mohammadnejad et al., which is similar to this study, testis parenchymal tissue showed atrophy after cisplatin treatment. Regarding to this atrophy, cisplatin group showed significantly decrease body weight, epithelium disorder and inflammation in interstitial area [6].

The findings of this study have shown that fucoidan significantly reduces the damage of testicular tissue regarding to oxidative stress. Moreover, fucoidan reduced the number of apoptotic cells in response to cisplatin treatment. Han et al. have showed that fucoidan reduces oxidative stress in ischemic tissue, which is occurred by transplantation mesenchymal stem cells (MSCs), by modulating reactive oxygen species (ROS) levels. Besides, Han et al. showed that fucoidan induces vascular regeneration [7].

This study showed that fucoidan makes firm mitochondrial permeability investigating ultrastructural observation by use of electron microscope (ZEISS, Sigma 500). The result is like Hyun et al.'s findings. Hyun et al. discussed that fucoidan enhances mitochondrial permeability in colon cancer cell line. Hyun et al. showed the pro-apoptotic effect of fucoidan has mediated through the activation of ERK, p38 and the blocking of the PI3K/Akt signal pathway in HCT-15 cells [8].

5. Conclusions

In this study, we have shown the favorable effects of fucoidan treatment seemingly by balancing oxidative stress and fixing membrane permeability, which is due to experimental testicular damage induced by cisplatin. In light of these findings, treatment with fucoidan following cisplatin administration might be considered as a treatment option against secondary infertility induced by cisplatin.

Acknowledgments: This work was supported by Dokuz Eylul University, Department of Scientific Research Projects. This research used the facilities at Electron Microscopy Core Facility in İzmir Biomedicine and Genome Center (İBG) İzmir, Turkey**.** We thank Zekiye ALTUN (Department of Basic Oncology, Institute of Oncology, Dokuz Eylul University for suggestions) and Ozcan USTUN (Department of Histology and Embryology, Faculty of Medicine, Dokuz Eylul University for technical assistance).

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