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Autumn 2019, Volume 4, Issue15 (10-12)

The Role of Curcumin Supplementation on the Side Effects of Busulfan and the Process of Apoptosis

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DOI: 10.21859/pmj01034

Submitted: 2019/07/13
Accepted: 2019/08/17

Keywords:
Cancer
Busulfan
Curcumin
Antioxidant
Apoptosis

Abstract

One of the most well-known side effects of anticancer drugs is the disruption of spermatogenesis. One of the most important causes of infertility in men is impaired spermatogenesis. Busulfan is a cytotoxic alkylating agent and belongs to the alkyl sulfonate group. This agent causes DNA damage by cross-linking with DNA. It kills spermatogonial stem cells. The use of healthy antioxidants can reduce the side effects of alkylating agents. In this study, we measured the protective effect of curcumin on testicular tissue of mice treated with busulfan by measuring BAX expression as an apoptotic inducer and Bcl2 as an apoptotic inhibitor. The expression levels of the two apoptotic regulatory genes Bax and Bcl-2 were evaluated by realtime PCR in four groups of mice (control, treatment with busulfan, treatment with curcumin, and treatment with busulfan + curcumin). It was observed in the busulfan group that bcl2 expression decreased, and Bax increased significantly, and the busulfan + curcumin group showed the opposite effect. Curcumin can be beneficial as a dietary supplement in cancer patients treated with drugs such as busulfan.

INTRODUCTION

According to the World Cancer Report released by the International Agency for Research on Cancer (IARC) in 2014, about 24 million new people develop the disease each year (1). It is expected in the next two decades, that number will reach 22 million (2). Most cancer patients are treated with chemotherapy during their illness. Surgery, chemotherapy, radiation therapy, hormone therapy, immunotherapy, and gene therapy (bone marrow transplant) are the primary options for cancer treatment. Since cancer cells can invade surrounding tissues and migrate to other parts of the body, chemotherapy is used by physicians as systemic therapy (3). During this treatment, anticancer drugs are used to treat metastatic cancers. These drugs inhibit the division of cells that are rapidly growing and dividing. But unfortunately, it also affects high-growth natural cells such as hair follicles, bone marrow, and gastrointestinal cells. These drugs have many side effects (4). One of the most well-known side effects of anticancer drugs is the disruption of spermatogenesis. One of the most important causes of infertility in men is impaired spermatogenesis (5). Spermatogenesis is the process of producing mature sperm from spermatogonia, which has three main stages of mitosis, meiosis, and spermiogenesis (6). Abnormality at any stage of spermatogenesis can result in abnormal sperm production or lack of sperm production leading to male infertility. One of the drugs used in the cancer treatment process is busulfan. Busulfan is a cytotoxic alkylating agent and belongs to the alkyl sulfonate group. This agent causes DNA damage by cross-linking with DNA. It also breaks the cross-links between guanine-guanine bases inside the DNA. Busulfan is used to treat chronic leukemia, ovarian cancer, and pre-bone marrow transplantation in cancer patients. This drug has many side effects, one of which is the effect on the spermatogenic process (7). This drug destroys spermatogonial stem cells and disrupts the connections between Sertoli cells and spermatogonial cells to the basal layers. Alkylating agents cause azoospermia in 100-90% of men, and 25-5% of women cause ovarian dysfunction. It has been found that the use of potent antioxidants can reduce the side effects of alkylating agents (8). One of the most popular antioxidants in recent years is curcumin. Curcumin (trifluoromethyl methane), a polyphenol of the diarylheptanoids, is the essential active ingredient of the turmeric root, scientifically known as Curcuma longa, of the ginger family. Many studies in the last century have shown the potential effects of curcumin as an antioxidant, anti-inflammatory, anti-cell proliferative compound that has made it a valuable adjunct to the prevention and

treatment of a wide range of diseases (9). In this study, we measured the protective effect of curcumin on testicular tissue of mice treated with busulfan by measuring BAX expression as an apoptotic inducer and Bcl2 as an apoptotic inhibitor.

MATERIAL AND METHODS

The study population consisted of 20 male NMRI mice. Mice were randomly divided into four groups of 5 each kept in separate cages under the same conditions: 12-hour light cycle 12-hour darkness, 22°C ambient temperature, and free access to water and food. The four study groups were: control group, busulfan group, curcumin group, and busulfan + curcumin group. The treatment period consisted of 40 days, during which the busulfan was injected intraperitoneally at a dose of 3.2mg/kg. In both groups, curcumin and curcumin + busulfan were injected into mice at a dose of 15mg/kg by intraperitoneal injection. At the end of treatment, mice were killed with chloroform, and the testes were removed under sterile conditions and kept at -40°C until extraction. For extraction of 50mg DNA from testis tissue was mixed with RNX-plus sinaclon solution and extracted according to the kit protocol. cDNA synthesized by The first-strand cDNA kit was according to the kit protocol. In this study, the relative real-time PCR method was used to measure the expression of Bax and bcl2 genes. The PCR reaction was performed using specific primers in Table 1. Real plus master mix green amplicon was used in this study. Statistical analysis was performed by SPSS V19 software, and $P \leq 0.05$ was considered significant.

Table 1. Primer Sequence

Gene symbol	Primer sequence
BAX	F:TGAAGACAGGGGCCTTTTTG
	R:AATTCGCCGGAGACACTCG
Bcl-2	F:ATGCCTTTGTGGAACTATATGGC
	R:GGTATGCACCCAGAGTGATGC
GAPDH	F:AGGTCGGTGTGAACGGATTTG
	R:TGTAGACCATGTAGTTGAGGTCA

RESULTS

Initial results of real-time PCR reactions were analyzed using 2 ^ ΔΔct methods after evaluation of the melting curve (Figure 1). Results showed a significant decrease in Bcl-2 gene expression in the busulfan group compared to the control group ($P = 0.002$). Whereas the busulfan + curcumin group showed a decrease in expression compared to the control group and an increase in the expression of Bcl-2 compared to the busulfan group. In the case of Bax gene, busulfan treatment significantly increased its expression ($P = 0.0001$). Also, the busulfan + curcumin group showed an increase in expression compared to the control group and a slight decrease in expression compared to the busulfan group (Figure 2).

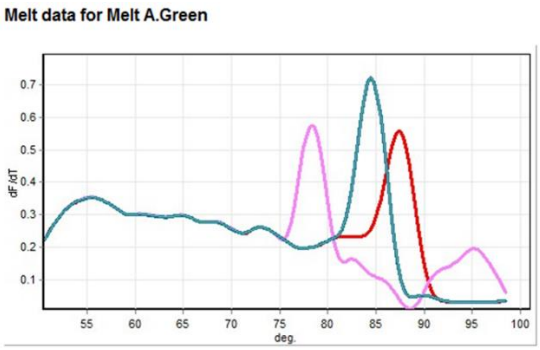

Figure 1. The Melt Curve of bax-bcl2 and GAPDH Gene

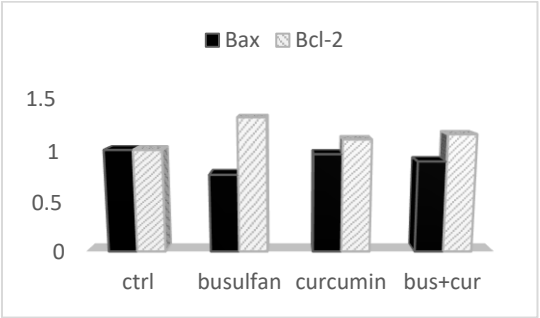

Figure 2. Fold Change of Interest Gene in Four Group

DISCUSSION

The study found that busulfan is an alkylating agent used in the treatment of many cancers, which could induce infertility in male mice by inducing apoptosis and necrosis in testicular cells (10). It was also found that curcumin could have a protective effect against the side effects of busulfan. One of the side effects of busulfan is increased production of free radicals and oxidative stress in spermatogonial stem cells. One of the cellular responses to increased oxidative stress activity is the induction of apoptotic programmed cell death. BAX protein is a critical element in the introduction of apoptosis in cells. In contrast, Bcl-2 protein inhibits apoptosis in cells by its antiapoptotic activity (11). The balance of these two proteins in the cell controls the process of apoptosis in the cell. The study found that mice treated with busulfan had an increased expression of the Bax gene and a decreased expression of the Bcl-2 gene so that cells were expected to apoptosis, thus leading to different rates of infertility. But many people, especially young people, tend to have fertility and children after cancer recovery, so strategies that can be used to protect a person from the side effects of chemotherapy drugs such as busulfan are essential. Potent antioxidants such as curcumin can help reduce the side effects of chemotherapy drugs. In this study, curcumin could reverse the effects of busulfan, thereby reducing Bax expression and increasing Bcl-2 expression. Another survey by Bahmanpour et al. 2017 (12) showed that different doses of busulfan could interfere with sperm parameters, reducing the number of mature sperm. Moloody Tappe et al. 2018 also

showed that the use of coenzyme Q10 as an antioxidant could reverse the effects of busulfan on testicular cells, thereby increasing the number of adult sperm (13). These studies show that the use of potent antioxidants such as curcumin can be beneficial as a dietary supplement in cancer patients treated with drugs such as busulfan.

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