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The Effect of Cadmium on Apoptotic Genes mRNA Expression of Bax and Bcl-2 in Small Intestine of Rats

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ABSTRACT

Background & objective: Cadmium is a potent toxicant and carcinogenic substance for human and experimental animals. The evidences indicate that cadmium induces aberrant gene expression, inhibition of DNA damage repair, and apoptosis. In this study, we investigated the effects of IP (intraperitoneal) injection of cadmium on mRNA levels expression of Bcl-2 and Bax genes in rat small intestine.

Methods: 28 male Wistar rats weighing 200 to 250 grams were randomly assigned into 4 groups. Group 1 received saline while the animals in groups 2-4 were injected cadmium (1, 2 and 4 mg/kg) for 15 successive days. One day after the last injection, the small intestine was dissected and the mRNA levels expression of Bax and Bcl-2 genes was evaluated using Real Time PCR technique.

Results: Cadmium increased the mRNA levels of Bax gene compared to the control group at 2 and 4 mg/kg ($P < 0.01$) in small intestine of rats. The mRNA levels of Bcl-2 gene decreased significantly compared to the control group at 1, 2 and 4 mg/kg ($P < 0.001$) in small intestine of rats.

Conclusion: These results showed Cadmium exposure induced cell apoptosis by increasing Bax/Bcl-2 ratio expression.

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Introduction

Cadmium is a heavy and toxic metal, widely used in industrial applications. The major sources of cadmium entry into the gastrointestinal tract are dietary intake and smoking (1). Another source of cadmium is inhalation of cadmium-contaminated air (2). Cadmium can be teratogenic and carcinogenic within different organs and tissues in humans and animals (3, 4). Furthermore, cadmium is known as a category 1 carcinogenic substance by the International Agency for Research on Cancer (IARC) (3, 4). Cadmium induces lung cancer and recent experimental studies have demonstrated its correlation with cancers of the bladder, pancreas and stomach (5). The carcinogenicity mechanisms of cadmium could be related to the suppression of gene expression, inhibition of DNA repair enzymes, suppuration of apoptosis, induction of oxidative stress, formation of reactive oxygen species (ROS), interference with anti-oxidative enzymes and deregulation of cell proliferation and suppressed apoptosis in body organs (3, 5). Cadmium induces ROS generation and gastric mucosal and DNA lesions, alters gene regulation, signal transduction, gene abnormalities, and cell growth, which ultimately lead to carcinogenesis. In addition, cadmium affects both gene transcription and translation with the role in apoptosis (6).

Cadmium activates multiple death signals. Multifactors and genotype may determine the initiation and the rate of death signals. Cadmium-induced death

starts with an apoptosis-related mitochondrial membrane depolarization and a DNA damage response (7).

Cadmium induces apoptosis in renal tubular cells in vivo (8). One of the targets of cadmium is gastrointestinal tract (9). Researches showed clear genotoxic activities of cadmium on both the upper and distal parts of the gastrointestinal tract. Additionally, findings showed that accumulation of cadmium in intestines is poor but it induces change in gene expression that shows the oxidative stress and inflammatory status of the gut epithelium of the duodenum, ileum and colon (10). Therefore, long-term exposure to cadmium enhanced the mortality risk of several cancers including esophageal and gastric cancer (11).

It has been known that lead can induce apoptosis and change the levels (imbalance) of Bax, Bcl-2 and mitochondrial dysfunction (12). Cadmium increases mRNA level of Bax gene and decreases mRNA level of Bcl-2 gene in rat brain cells (13). Previous studies have shown that Bax/Bcl-2 ratio defines the chance of death or survival of the cells, following an apoptotic stimulus (13, 14).

However, little is known about the impact of molecular mechanism of cadmium on small intestine cells. Therefore, we investigated a number of parameters inducing apoptosis-related gene expression and Bax/Bcl-2 ratio in the small intestine cells of rats.

Materials and methods

Animals and experimental design

To conduct the present study, Male Wistar rats at 8 weeks of age (N= 28) weighing 200-250 g were purchased from Veterinary Medicine of University of Tehran (Iran). Animals were housed at an ambient temperature of 22 ± 3 °C and maintained under a 12-hours light/dark cycle in the animal house of Parand Islamic Azad University, and allowed access food and water ad libitum. After two weeks of adaptation to the new environment, rats were randomly assigned to four groups of 7 each; one control group and three treatment groups. All experiments and treatment were in accordance with guidelines of the Ethical Committee of Parand Islamic Azad University.

Cadmium nitrate administration

Cadmium nitrate was obtained from Kimia Pars, Inc. (Merck, Germany). The dose of Cadmium in this study was chosen according to the previous study (15-18). The control groups received saline (vehicle of cadmium) and animals of experiment groups were injected cadmium (1, 2, 4 mg/kg) (body weight) for 15 consecutive days. Injections were performed intraperitoneally within a final volume of 1 mL for each dose. One day after the last injection, the rats were deeply anesthetized with chloroform and rapidly decapitalized. The small intestines (duodenum) were dissected, freezing in liquid nitrogen and stored at -80 °C until real time PCR tests.

RNA extraction and cDNA synthesis

Total RNA of small intestine was isolated using the RNX-TM plus (CinnaGen Inc., Iran). The quantity and purity of the extracted RNAs was determined using a spectrophotometer (NanoDrop ND-2000, Wilmington, DE, USA), and only extracted RNAs with an A260/A280 ratio ranging from 1.8 to 2.0 were used for cDNA synthesis. Real-Time transcription was performed with 1 µg of RNA using first strand cDNA synthesis kit (Fermentas, Thermo scientific, USA) according to the manufacturer's instructions.

Real-Time quantitative PCR using SYBER green

Real-Time PCR to evaluate quantitative expression of mRNA for Bcl-2, Bax and GAPDH was carried out in an Illumina real-time PCR system Illumina, Inc., (San Diego, California, USA), by measuring increased fluorescence light. The amplification was performed in a final volume of 25 µl, which included 1 µl cDNA, 12.5 µl SYBR Green Master Mix (Master Mix Green-No Rox, Ampliqon Denmark), 5 µmol of each complimentary primer in a volume of 0.5 µl, and 10.5 µl deionized water. Specific primers were designed and underwent search using BLAST tool. The oligonucleotide Sequences of GAPDH, Bcl-2 and Bax used for real-time PCR were as follows: Forward: 5′- TGC CAC TCA GAA GAC TGT GG -3′, and Reverse: 5′- GGA TGC AGG GAT GTT CT -3′ for the rat GAPDH gene; Forward: 5'- GAG TAC CTG AAC CGG CAT CT -3' and Reverse: 5'- GAA

ATC AAA CAG AGG TCG CA -3' for the rat Bcl-2 gene; Forward: 5'- TTG CTA CAG GGT TTC ATC CA -3' and Reverse: 5'- GAG TAC CTG AAC CGG CAT CT -3' for the rat Bax gene. The amplification conditions were optimized as follows: pre denaturation at 94°C for 5 min followed by 35 cycles of denaturation: 94°C for 1 min, annealing: 53°C for 1 min and extension: 72°C for 5 min.

The results of Real-Time quantitative PCR were analyzed on the CT (ΔΔCT), where CT is the threshold cycle (Asara et al. 2013). GAPDH was used as an internal control. The relative fold increase (RFI) was calculated using the $2^{-\Delta\Delta CT}$ methods.

Statistical analysis

Data were expressed as mean ± SD. Statistical analysis was performed using One-way variance analysis and Tukey's test. $P< 0.05$ was considered to be statistically significant. SPSS 22 statistical software package (IBM SPSS Statistics 22, Chicago, IL, USA) was used for all statistical analyses.

Results

Melting curve analysis of Bax, Bcl-2 and GAPDH genes

Melting curve analysis of Bax, Bcl-2 and GAPDH genes for Real-time PCR products were obtained using the specific primer pairs in rat small intestine (Figure 1).

The level of Bax gene expression

Cadmium exposure did significantly change the mRNA gene expression of Bax at 1 mg/kg in rat small intestine. In small intestine, the mRNA expression level of Bax gene was increased at 2 and 4 mg/kg (body weight) by 93.14 and 95.72 times when compared to the control group (Figure 2).

The level of Bcl-2 gene expression

Cadmium exposure significantly decreased the mRNA expression levels of Bcl-2 gene at 1, 2 and 4 mg/kg in rat small intestine when compared to the control group ($P<0.001$). In small intestine cells, the mRNA expression levels of Bcl-2 gene was decreased at 1, 2 and 4 mg/kg (body weight) by 0.27, 0.21 and 0.05 times when compared to the control group (Figure 3).

Ratio of Bax/Bcl-2 in small intestine cells

In small intestine cells, Bax/Bcl-2 mRNA ratio was significantly increased at 2(*$P<0.05$) and 4 (***$P<0.001$) mg/kg (body weight) cadmium (Table 1).

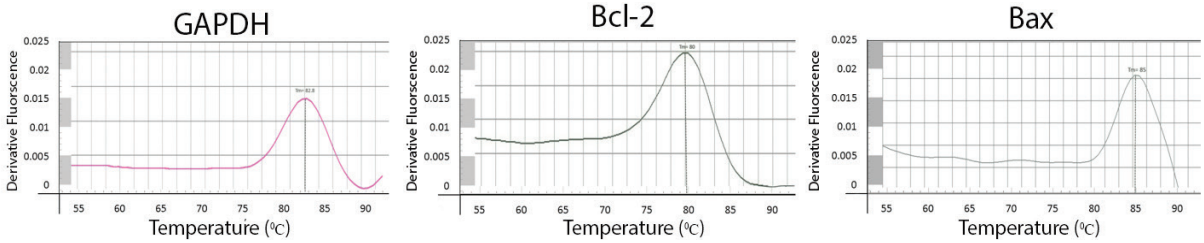

Figure 1. Melting curve analysis of real-time PCR for GAPDH, Bcl-2 and Bax genes in rat small intestine.

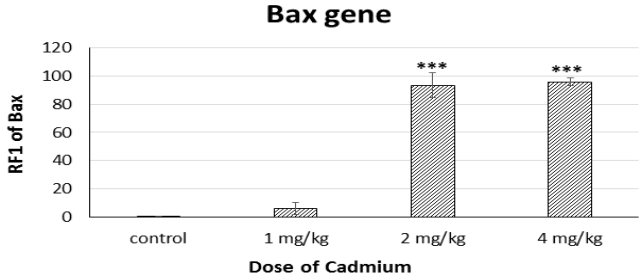

Figure 2. Effects of cadmium exposure on gene expression of Bax in the small intestine of the rats. The mRNA levels were measured and data were normalized to GAPDH. The expression of Bax in control group was designated as 1, and the others were expressed as folds compared to the control. Seven animals were tested per group. ***: $P<0.01$.

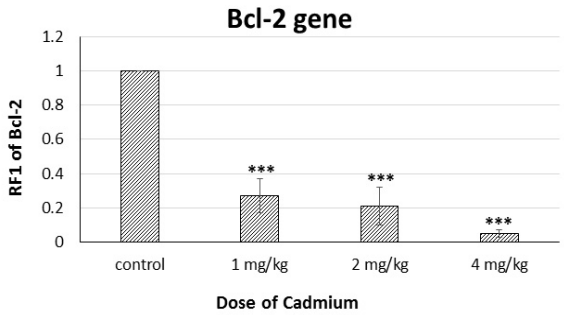

Figure 3. Effects of cadmium exposure on gene expression of Bcl-2 in the small intestine of the rats. The mRNA levels were measured and data were normalized to GAPDH. The expression of Bcl-2 in control group was designated as 1, and the others were expressed as folds compared to the control. Seven animals were tested per group. ***: $P<0.001$.

Table1. Ratio of Bax/Bcl-2 in small intestine in rats.

Concentration of cadmium nitrate (body weight)	imbalance of Bax/Bcl-2 ratio in small intestine
1 mg/kg	$P=0.999$
2 mg/kg	$P=0.013$
4 mg/kg	$P=0.000$

Discussion

Cadmium exposure increased the ratio of Bax/Bcl-2 and induced apoptosis in rat brain cells (13). In a similar way of changes od gene expression of Bax and Bcl-2, lead exposure increased Bax expression and the imbalance of Bax/Bcl-2 (19). In this study, the gene expression of Bax was significantly increased in rat small intestine,while the gene expression of Bcl-2 significantly decreased by cadmium exposure in rat small intestine. The ratio of the Bax/Bcl-2 increased in the small intestine of rats.

Cadmium is capable of inhibiting apoptosis and DNA repair, stimulating cell proliferation and promoting cancer in a number of tissues (20). At the cellular level, cadmium exposure induced damage to DNA and cell membranes by inhibiting different types of DNA repair, and inducing apoptosis in mammalian cells (21).

The results showed that cadmium exposure induces clear genotoxic activities, on both the upper and distal parts of the gastrointestinal tract (10).

One of the products of normal cellular metabolism is reactive oxygen species (ROS). Low and moderate concentrations of ROS are helpful for the cellular functions (22). In contrast, at high concentrations, ROS induces damage to cell structures. Cadmium can induce ROS generation (23) which causes gastric mucosal damage and various Gastrointestinal (GI) diseases including peptic ulcers, GI cancers, alterations in gene expression and signal transduction (22). Furthermore, a research has indicated that catalase is involved in antioxidant defense mechanisms and prevents excessive levels of ROS at the cellular level. Cadmium exposure can increase the catalase activity by generating high ROS levels in gastric cancer (24).

The Bcl-2 family regulates mitochondrial membrane permeability through a family of proto-oncogenes. The Bcl-2 family includes anti-apoptotic (Bcl-2) and pro-apoptotic (Bax) genes (25). Bax is in the cytosol, under physiological conditions. An apoptotic trigger leads to its translocation into the mitochondrial membrane. Bax can homodimerize or heterodimerize with pro-apoptotic members, thus forming mitochondrial pore and increasing membrane permeability, thereby releasing apoptogenic factors (26, 27). The anti-apoptotic protein, Bcl-2, inhibits the ability of Bax to increase membrane potential (28) and antagonizing the apoptotic cascade by a direct interaction (29) and cell fate may be determined by balance of these proteins. In this experiment, we showed that cadmium increases the expression levels of pro-apoptotic Bax gene in small intestine and decreases the expression levels of anti- apoptotic Bcl-2 genes in rat small intestine. Similar studies demonstrated modulation of the

Bax and Bcl-2 genes in apoptosis (13, 30, 31). In this study we found that alteration in Bax/Bcl-2 ratio is a key factor in the generation of apoptosis. Increasing in this ratio may stimulate apoptosis, and a decrease in this ratio may reverse the injurious effect of cytotoxic stimuli (31, 32). Further studies are needed to explore molecular mechanisms involved in apoptosis induction of cadmium in rat small intestine.

Conclusions

In conclusion, injection of cadmium increases the expression level of pro-apoptotic Bax gene in small intestine of rat. The expression level of anti-apoptotic Bcl-2 gene decreases in small intestine of rat. Due to an increment in the Bax/Bcl-2 ratio, it is likely that apoptosis increased by cadmium in small intestine of rat is caused by mitochondrial pathway.

Conflict of interest

The authors declare that there was no conflict of interest.

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