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Expression of apoptosis-related genes in livers from rats exposed to sulfur dioxide

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Abstract

Sulfur dioxide (SO_2) is a ubiquitous air pollutant that is present in low concentrations in the urban air, and in higher concentrations in the working environment. In the present study, male Wistar rats were housed in exposure chambers and treated with 14.00 ± 1.01, 28.00 ± 1.77 and 56.00 ± 3.44 mg/m^3 SO_2 for 6 h/day for 7 days, while control rats were exposed to filtered air in the same condition. The mRNA and protein levels of three apoptosis-related genes (p53 and bax were promoters of apoptosis, whereas bcl-2 was apoptotic suppressor) were analyzed in livers using a real-time reverse transcription-polymerase chain reaction (real-time RT-PCR) assay and immunohistochemistry method. The results showed that mRNA levels of p53 and bax were increased in a dose-dependent manner and at the concentrations of 28.00 and 56.00 mg/m^3 SO_2 the increases were significant (for p53: 1.30-fold at 28 mg/m^3 and 3.43-fold at 56 mg/m^3, for bax: 1.63-fold at 28 mg/m^3 and 2.17-fold at 56 mg/m^3, respectively), while mRNA levels of bcl-2 were decreased significantly (0.63-fold at 28 mg/m^3 and 0.45-fold at 56 mg/m^3) in livers of rats exposed to SO_2. Dose-dependent increases of p53 and bax proteins in the livers were observed after SO_2 inhalation, while decrease of bcl-2 protein levels was obtained using immunohistochemistry method. These results lead to a conclusion that SO_2 exposure could change the expression of apoptosis-related genes, and it suggests that SO_2 can induce apoptosis in liver of rat and may have relations with some apoptosis-related diseases. It is critical for our understanding of the mechanisms of SO_2 toxicity and helpful for the therapeutic intervention to elucidate the expression pattern of those factors involved in apoptosis signaling pathway after SO_2 inhalation.

Keywords: Sulfur dioxide; Liver; p53; bax; bcl-2

1. Introduction

Apoptosis is a type of cell death that represents the culmination of naturally occurring, or highly programmed mechanisms. Subsequently, the intracellular substances that regulate the appearance of cell death were identified. Apoptosis is recognized to occur as a result of the execution of exquisitely regulated genetic programs. It plays a key role in the physiological control of development and growth and in the immune function. As the molecular mechanisms underlying cell death have come to be elucidated in the fields of developmental biology, immunology and pathology, interest in the study of this phenomenon has surged. Now, apoptosis is implicated in the pathogenesis of an increasing number of diseases and considered to be involved in pathological cell death as well (Thompson, 1995). Apoptosis requires de novo synthesis of mRNA and protein to take place

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(Martin et al., 1988), although there is an overall decrease in RNA transcription (Deckwerth and Johnson, 1993). Taken together, these observations suggest that there may be a distinct genetic program involving selective transcription of certain critical genes required for apoptosis to proceed, including p53, bax and bcl-2 (Estus et al., 1994).

P53 is a tumor suppressor gene which exerts control over cell cycling by controlling the progression through G1 phase, and it is reported to be involved into the induction of apoptosis (Van Gijssel et al., 1997). It encodes a nuclear phosphoprotein involved in cell cycle regulation. Mutated forms of wt-p53 protein are found in over 50% of human tumors demonstrating that p53 mutation is the most common genetic change in human cancers (Hollstein et al., 1991; Lane, 1992). P53 appears to play an essential role in programmed cell death (PCD) since overexpression of p53 induces apoptosis in a range of cells types.

Proteins in the bcl-2 family are important regulators of apoptosis in different cells. These proteins are mainly localized to the mitochondrial membrane and regulate apoptosis partly by controlling the release of cytochrome C into the cytosol (Strasser et al., 2000). bcl-2 family proteins consist of both pro- and antiapoptotic proteins. Many of these proteins bind to each other forming a complex network of homo- and heterodimers (Reed, 1997). Among the genes involved in the regulation of apoptosis in mammalian cells, *bcl-2* was first identified as a repressor gene of apoptosis (Reed, 1994). It was first described in B cell leukemia (Cleary and Sklar, 1985) and later was detected in other malignant tumors (Doglioni et al., 1994). It is known to prevent both an increase in mitochondrial permeability and a release of cytochrome C, as well as inhibit various caspases (Chinnaiyan et al., 1996; Armstrong et al., 1996) and can block programmed cell death without affecting cellular proliferation (Hockenbery et al., 1990).

Subsequently, bax, a 21 kDa protein coimmunoprecipitated with bcl-2, was identified as a promoter of cell death whose pro-apoptotic function was directly antagonized by bcl-2 through formation of bax/bcl-2 heterodimers (Oltvai et al., 1993; Sato et al., 1994; Yin et al., 1994). bax protein is a homologue of bcl-2. bax may bind to bcl-2 forming bax/bcl-2 heterodimers, or it may bind to itself forming bax/bax homodimers. The ratio of bax to bcl-2 determines the susceptibility of a cell to apoptosis. Thus, in cells with bax overexpression, bax homodimers predominate, and the susceptibility of such cells to apoptotic stimuli is enhanced. bax/bcl-2 heterodimers predominate in cells that overexpress bcl-2, and the susceptibility of these cells to apoptosis is reduced (Oltvai et al., 1993; Yang and Korsmeyer, 1996).

Recent advances in the use of fluorogenic probes in conjunction with PCR have enabled the measurement of an accumulating PCR product in real time (Lie, 1998). This allows the rapid generation of quantitative data showing changes in transcript numbers in tissue samples. An increase in throughput not only allows a greater number of transcripts to be studied, but also allows a greater number of animals and experimental conditions to be included in a study, enabling a more sensitive and reliable statistical analysis of the data.

Sulfur dioxide (SO_2) is a ubiquitous air pollutant that is present in low concentrations in the urban air, and in higher concentrations in the working environment. SO_2 pollution is produced by combustion and processing of sulfur-containing fossil fuels (Amdur, 1969); exposure to SO_2 is prevalent in many occupational settings, e.g. paper pulp factories, smelters, steel works, and chemical industries. As a ubiquitous air pollutant, gaseous SO_2 influences both human being health and the global ecological system of animals and plants (Noji et al., 2001). Inhaled SO_2 can easily be hydrated in the respiratory tract to produce sulfurous acid, which subsequently dissociates to form bisulfite and sulfite derivatives (1:3 M/M in neutral fluid) (Shapiro, 1977). These derivatives can be absorbed into the blood or other body fluids. Recently, Meng et al. have reported that SO_2 and its derivatives are the systemic toxic agent, can cause DNA damage and oxidative damage in multiple organs from mice, including liver (Meng and Zhang, 2003; Meng, 2003; Meng et al., 2004). However, so far expression changes of apoptosis-related genes in livers of rats caused by SO_2 at different concentrations have not been reported. For our investigations, we analyzed the expression of three genes, p53, bax and bcl-2.

2. Materials and methods

2.1. Preparation of animals

Male Wistar rats, weighing 180–200 g, were used for the present experiment. The rats were housed in metallic cages under standard conditions and divided randomly into four equal groups of six animals each: one control group and three groups exposed to SO_2 at concentrations of $14.00 \pm 1.01\ mg/m^3$ (about 5 ppm), $28.00 \pm 1.77\ mg/m^3$ (about 10 ppm) and $56.00 \pm 3.44\ mg/m^3$ (about 20 ppm) SO_2 in 1 m^3 exposure chambers for 7 days (6 h/day). Control group was exposed to filtered air in another identical chamber for the same period of time. The SO_2 gas was delivered to the animals through a tube positioned in the upper part of each chamber and was distributed homogeneously via a fan in each cham-