Effect of Sodium Fluoride on the Expression of Bcl-2 Family and Osteopontin in Rat Renal Tubular Cells

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ABSTRACT

Our earlier studies showed that the apoptosis of renal tubules can be induced by sodium fluoride (NaF). The present study was designed to estimated the effects of B-cell lymphoma/leukemia 2 (Bcl-2), Bcl-2-associated protein X (Bax), and osteopontin (OPN) on the apoptosis of renal tubular cells induced by NaF at different levels. The technique of reverse transcription–polymerase chain reaction and densitometer scanning volume density were used to evaluate the changes of Bcl-2, Bax, and OPN mRNA in tubular cells treated with different doses of NaF (0, 1, 5, 7.5, 12.5 mgF⁻/L) for 48 h. Compared to control, the level of Bax mRNA significantly increased at cells of the 7.5- and 12.5-mg F⁻/L groups and the expression of Bcl-2 mRNA obviously decreased at cells of the 5- and 7.5-mg F⁻/L groups. The NaF also enhanced the expression of OPN mRNA in a dose-dependent manner, but the strongest expression of OPN mRNA was observed at cells of the 7.5-mg F⁻/L group. The results suggested that NaF induces the apoptosis in renal tubules via activation of the Bax expression and Bcl-2 suppression; OPN probably acts as protective role against apoptosis in fluoride-treated renal cells.

Index Entries: Apoptosis; Bcl-2; Bax; osteopontin; sodium fluoride (NaF).

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INTRODUCTION

Fluoride is known to cause toxicity in animals or humans following acute or chronic exposure—for example, renal calcification, osteosclerosis, mottled teeth, and so forth. Experimental fluorosis rats were associated with nephrotoxicity. In our previous experiment, it was found that sodium fluoride (NaF) could accelerate renal epithelial cell apoptosis (1). Song et al. (2) demonstrated that NaF reduced cell viability; at the same time, it decreased DNA and protein biosynthesis capability in HL-60 cells, which was attributable to its induction of apoptosis. Our work was to prove the role of Bax and Bcl-2 in renal tubular apoptosis exposed to excessive fluoride.

Apoptosis allows the clearance of cells with damage in DNA, which is beneficial in preventing potential tumor formation. Conversely, the inappropriate activation of apoptosis might contribute to the deletion of functional cell, thus leading to organ atrophy. The role of Bax and Bcl-2 in the process of apoptosis has been demonstrated by widely investigating in numerous in vitro and in vivo systems. To date, Bcl-2 (B-cell lymphoma/leukemia 2)-related protein has been identified in the Bcl-2 family and is divided into two categories: antiapoptosis and proapoptosis. Bcl-2 homodimers have been shown to inhibit apoptosis and prolong cell survival in various settings. Bax (Bcl-2-associated protein X) homodimers, sharing a sequence homolog with Bcl-2, act as binding protein of Bcl-2 and favor cell death. Heterodimerization between Bax and Bcl-2 could abolish the function of either protein (3,4).

Osteopontin (OPN) is a secreted glycoprotein in both phosphorylated and nonphosphorylated forms. OPN is mainly present in the loop of Henle and the distal nephron in the normal kidney in animal and humans. After renal damage, OPN expression might be significantly upregulated in all tubule segments and glomeruli. It is reported that OPN has some renoprotective actions in renal injury, such as increasing tolerance to acute ischemia, inhibiting inducible nitric oxide synthase and suppressing nitric oxide synthesis, reducing cell peroxide level and promoting the survival of cell exposed to hypoxia, decreasing cell apoptosis, and participating in the regeneration of cells.

With this information, we investigated the expression level of Bax, Bcl-2, and OPN mRNA in a renal tubular epithelial cell exposed to NaF in vitro and provided some new insight into a possible relationship between apoptosis induced by NaF and the renoprotective value of OPN.

MATERIAL AND METHODS

Cells

The renal tubular epithelial cells were obtained from normal Wistar rat kidneys and isolated by the method of Detrisac et al. (5) with a slight mod-