# FLUORIDE-INDUCED OXIDATIVE STRESS IN RAT MYOCARDIUM THROUGH THE Bax/BcI-2 SIGNALLING PATHWAY

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SUMMARY: The purpose of this study was to investigate whether fluoride (F) induces cardiotoxicity in rats and to discuss its underlying mechanisms by detecting morphological change, enzyme activity of oxidative stress, and the expression of Bcl-2 family protein. With increasing dosages of F, obvious pathological changes occurred in the myocardial tissue of rats with a trend to increased expression in the cardiomyocytes of Bax and a trend to decreased expression of Bcl-2. Excessive fluoride caused peroxidation damage with inhibition of the activity of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) in myocardial tissue leading to a rise of malonaldehyde (MDA) content. These results indicate that a molecular basis for the cardiac damage by fluoride involves the Bax/ Bcl-2 signalling pathway.

Keywords: Bcl-2/Bax; Myocardial damage from F in rats; Oxidative stress.

# INTRODUCTION

During experimental fluorosis in animals, cardiovascular system dysfunctions have been observed in myocardium such as the decreasing of cardiac output, the occurrence of arrhythmias, and heart block.<sup>1-3</sup> ECG changes and myocardial damage have also been reported in patients with endemic fluorosis.<sup>4,5</sup> However, the mechanism by which F can induce damage in the cardiovascular system has not been elucidated at present. Excessive F may induce a high level of oxidative stress, which might important in the pathogenesis of chronic fluorosis.<sup>6-9</sup> B-cell CLL/lymphoma-2 (Bcl-2) is an oxidative stress-responsive protein and a key regulator of apoptosis.<sup>10</sup> Our investigations have previously demonstrated that F is a cytotoxic agent inducing damage in brain and testis tissues by oxidative stress through the Bcl-2/Bax signalling pathway.<sup>11,12</sup> In order to investigate what the relationship is between myocardial damage induced by F and the expression of Bcl-2 and Bcl-2 associated X protein (Bax), we observed the change in rat myocardial tissue morphology, and measured the activity of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), and the variation in the content of malonaldehyde (MDA). We also determined the expression of Bax and protein induced by F on myocardium with the Bcl-2 method of immunohistochemistry.

# MATERIALS AND METHODS

Eighty healthy 30-day-old Wistar albino rats $(56\pm7.1g)$  were obtained from the Experimental Animal Center of Shanxi Medical University, and were kept in a standard animal house at 22-25°C with ventilation and hygienic conditions. Based

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on the LD50 of sodium fluoride (NaF) sub-chronic experiments, the rats were divided into four equal groups and treated with NaF in their drinking water as follows: 50 mg NaF/L (low F group, LF), 100 mg NaF/L (medium F group, MF), 150 mg NaF/L (high F group, HF), and the normal control group (NC, tap water with <0.5 mg F/L). All procedures were performed in accordance with the Animal Care Guidelines of the Institutional Animal Care and Use Committee of China.

On the 90th and 120th days of treatment, the rats were anaesthetized by ethyl carbamate and sacrificed. Haematoxylin-eosin staining (HE staining) was used for morphological examination. The myocardial tissues were fixed in 4% paraformaldehyde embedded in paraffin, cut into 5  $\mu$ m sections, and stained with haematoxylin and eosin.<sup>13</sup> Morphological examination was conducted under a light microscope. Immumohistochemical staining was performed on the paraffin section. The sections derived from 3–4 slides of three different rats in each group were analysed by IPP5.1. Part of left ventricle was assessed by 25 consecutive high-power fields (×400 magnification) for each slide (Media Cybernetics Image-Pro plus v5.1, US).

The myocardium was removed and placed in a cold tissue homogenizer, which contained phosphate buffer (pH 7.3, 0.2 M, 4°C). After the myocardium was ground, the homogenate was centrifuged at 3,000–4,000 r/min at 4°C for 15 min. SOD, GSH-Px, CAT activities and MDA content were determined with kits bought from the Nanjing Institute of Biological Engineering.

Data are expressed as mean±standard error (SEM) and analyzed by one-way ANOVA using the SPSS 17.0 statistical software (SPSS, Chicago, IL). Statistical differences between the experimental and control groups were estimated with the least significant difference (LSD) test. Statistical significance was set at p<0.05 or p<0.01.

# RESULTS

*Morphological changes of myocardium:* HE staining clearly showed increased interstitial collagen and fibrin accumulated in the F groups compared with NC group (Figure 1). Denaturation occurred in cardiac myocytes in the experimental group, as well as extensive hemorrhage and rupture between cardiomyocytes in the MF and HF groups.

SOD, GSH-PX, and CAT activity induced by F: After 90 days of exposure, GSH-Px activity in the HF group decreased significantly compared with the NC group(p<0.01, Figure 2).

Similar changes occurred after 120 days of exposure. Total SOD, CAT, and GSH-Px activity decreased significantly (p<0.05 or p<0.01, Figure 2) in the MF and HF groups while there was no significant change observed in the LF groups (p>0.05).

However, the MDA content in all the treatment groups increased significantly, particularly at 120 days for the MF and HF groups, compared with that of the NC group (p<0.05 for the LF and MF groups; p<0.01 for the HF group, Figure 2).



Figure 1. Morphological changes of myocardium in rats after treatment with fluoride for 120 days.



**Figure 2.** SOD, CAT, GSH-PX activity and MDA contents in the myocardium of rats after treatment with fluoride for 90 and 120 days. Each bar represents the mean  $\pm$  SEM, n = 3. \* or # indicates p< 0.05; \* \* or # # indicates p<0.01.

*Expression of Bcl-2 and Bax protein levels induced by F:* The results indicated that, compared with the NC group, the expression levels of the Bax in the treated group increased significantly in a dose- and time-dependent manner. In particular, the Bax expression of HF group up-regulated 20.5% compared to the NC group after 120 days of treatment (p<0.05). In contrast, the expression levels of Bcl-2 in the treated group decreased significantly in a dose- and time-dependent manner compared with NC group. The Bcl-2 expression of HF down-regulated 21.7% compared to the NC group after 120 days of treatment (p<0.05, Figure 3).



**Figure 3.** Optical density of immunoreactive products of Bax, and Bcl-2 after 120 days of treatment. (Values are mean±SEM). Compared with the control, \*p<0.05, n=3.

#### DISCUSSION

F can interact with a wide range of cellular processes such as gene expression, proliferation and migration, respiration, metabolism, apoptosis/necrosis, and oxidative stress, and these mechanisms are involved in a wide variety of signalling pathways.<sup>14</sup> In addition, F is known to induce oxidative stress and to impair the functioning of antioxidants in soft tissues.<sup>15-18</sup> In the present study, the administration of 50, 100, or 150 mg/L F for 90 days and 120 days to rats resulted, in the heart tissues in the MF and HF groups, in a decrease in the activity of SOD, CAT, and GSH-Px while the MDA content increased. At the same time, while the F concentration increased there was a gradual increase in the pathological injury to the rat myocardium. Increased breaks and extensively bleeding were observed in the myocardium of the F groups. These phenomena indicate that high F has the ability to elevate lipid peroxidation and impair the antioxidant enzyme system<sup>19-21</sup> leading to increased oxidative stress and these mechanisms are mediating factors in the pathogenesis of fluoride toxicity in cardiovascular system.

It is increasingly recognized that the Bcl-2 family and oxidative stress are key components of the relation to cardiac myocyte apoptosis.<sup>21-24</sup> Although F causes injury to the cardiovascular system by several mechanisms,<sup>25</sup> of particular interest is its ability to cause oxidative damage.<sup>5,8,25</sup> There is a dearth of information on myocardial damage induced by F being mediated by the Bcl-2/Bax signalling

pathway. Our results show that the expression of Bax and Bcl-2 in the experimental groups changed significantly with the changes induced by F exposure to the myocardium of rats. The signal pathway of Bax and Bcl-2 may therefore be one of the molecular mechanisms by which fluorosis affects myocardial apoptosis or the cardiovascular system.

In conclusion, we consider that the exposure of animals to MF and HF (100 mg NaF/L and 150 mg NaF/L) can increase pathological injury of rat myocardium with enhanced myocardial oxidative stress through the Bcl-2/Bax signaling pathway, so that the normal physiological functions of the myocardium are affected. However, the mechanisms of fluorosis in the cardiovascular system are very complex and further investigations are required.

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