Response of BAX and Bcl-2 protein expression to treadmill running in fluorosis-susceptible mice exposed to high Fluoride

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Introduction

Better understanding of fluoride (F) metabolism is important in helping to minimise the side effects of excessive F exposure; e.g. fluorosis. In a previous study to investigate the effect of chronic exercise on F metabolism in fluorosis-susceptible mice exposed to high-F, we identified a significant reduction in body weight and increased exercise capacity of mice exposed to high F when compared with untreated control mice. Apoptosis or programmed cell death is an active multistep process characterized by morphological, biochemical and molecular events which requires coordinated regulation of specific genes. The initiation of apoptosis is controlled by many genes, whose expression is carefully regulated by different intra-and extra-cellular signals. Some gene products are activators of apoptosis (e.g. Bax), whereas others are inhibitors (e.g. Bcl-2,).

Methods

Thirty male mice were randomised into three groups, according to F concentration of water received and exercise regime: Group I: No-Fluoride (0ppmF) and No-Exercise; Group II: F-exposure (50ppmF) and No-Exercise, and; Group III: F-exposure (50ppmF) and Exercise. Body weight and physical performance of all mice were measured at baseline (week 0) and end of experiment (week 11). Gastrocnemius muscles were dissected after the last training session. The protein expression of BAX and Bcl-2 was assessed by Western immunoblot. All results were expressed relative to alpha tubulin expression in the same sample.

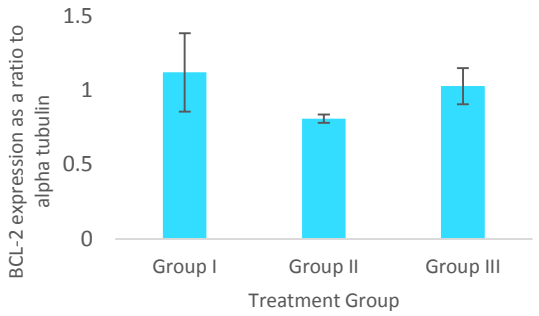

Figure 1: BCL-2 protein expression as a ratio to alpha tubulin in the same sample. Results are shown as mean (+/-) S.E.M. Group 1 and 2 n = 7, Group 3 n = 6. No significant difference in level of Bcl-2 protein expression between groups.

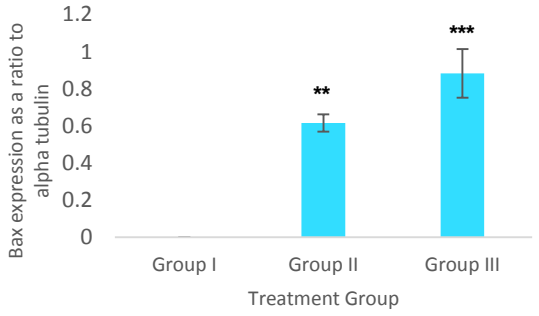

Figure 2: Bax protein expression as a ratio to alpha tubulin in the same sample. Results are shown as mean (+/-) S.E.M. Group 1 and 2 n = 7, Group 3 n = 6. ** p < 0.01 compared with group 1. *** p < 0.001 compared with group 2, by one way ANOVA followed by Tukey's multiple comparison test.

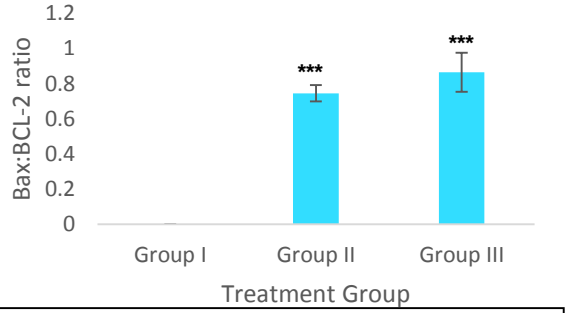

Figure 3: Bax: Bcl-2 ratio. Results are shown as mean (+/-) S.E.M. Group 1 and 2 n = 7, Group 3 n = 6. *** p < 0.001 compared with group 2, by one way ANOVA followed by Tukey's multiple comparison test.

Results

Mean Bcl-2 protein content was comparable across the three treatment groups (Group I; 1.12 (0.26), Group II; 0.81 (0.03), Group III; 1.02 (0.12)), however Bax protein levels were significantly higher in mice exposed to high F (Group II; 0.62 (0.05) p< 0.01) and high F plus exercise (Group III; 0.88 (0.13), p< 0.001) compared with the control mice (Group I Bax levels not detectable) and subsequent Bax:Bcl-2 ratio was significantly higher in Group II and III compared with group I (Group I; ND, Group II; 0.75 (0.05), Group III; 0.87 (0.11), p <0.001 by one way ANOVA followed by Tukey's post hoc test).

Discussion

These results show an increase in muscle apoptosis in both groups of mice exposed to high F, regardless of whether the mice are pre-trained, indicative of muscle damage and possibly higher rate of tissue-breakdown, which could account for the reduction in body weight previously observed in these mice.