

CHAPTER IV

RESULTS

Primary bone cell cytotoxicity

Primary bone cells were irradiated with dental x-radiation and cell cytotoxicity was determined by the MTT assay after 24h irradiation. Figure 4.1 showed that dental radiation was not toxic to primary bone cells as presented by the slight increase of the number of surviving cells following dental irradiation.

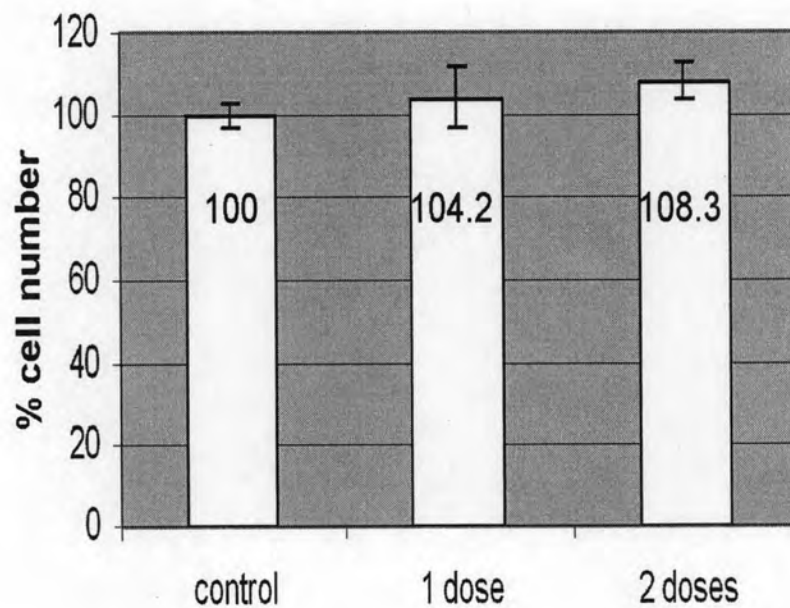


Figure 4.1 The effect of dental radiation on primary bone cell proliferation. Cells were irradiated with 0, 1 and 2 doses of a periapical radiograph and the number of surviving cells were evaluated by the MTT assay.

The changes of apoptotic-related gene expression after 4h dental irradiation

The six independent experiments from various cell lines established from 6 patients demonstrated both increase and decrease of apoptotic-related gene expression after 4h dental irradiation compared to the control (Table 4). After irradiation with 1 dose of a periapical radiograph, the number of decreased expression was bigger than that of the increased for all the apoptotic-related genes. Interestingly, no increase was found for Bax. Following 2 doses of a periapical radiograph, the number of decreased expression was more than that of the increased for all the genes.

Table 4 The changes of apoptotic-related gene expression after 4h dental irradiation

Apoptotic-related gene expression	1dose		2 doses	
	Increase	Decrease	Increase	Decrease
Anti-apoptotic				
Bcl-2	1	5	1	5
Bcl-xl	2	4	3	3
Pro-apoptotic				
Bax	0	6	1	5
Bad	1	5	2	4
Caspase-3	2	4	1	5

Apoptotic-related gene expression after 4h dental irradiation

Effect on BCL-2

RT-PCR analysis demonstrated that the decrease of BCL-2 expression after treated with various doses of dental x-radiation was not significant (Fig. 4.2).

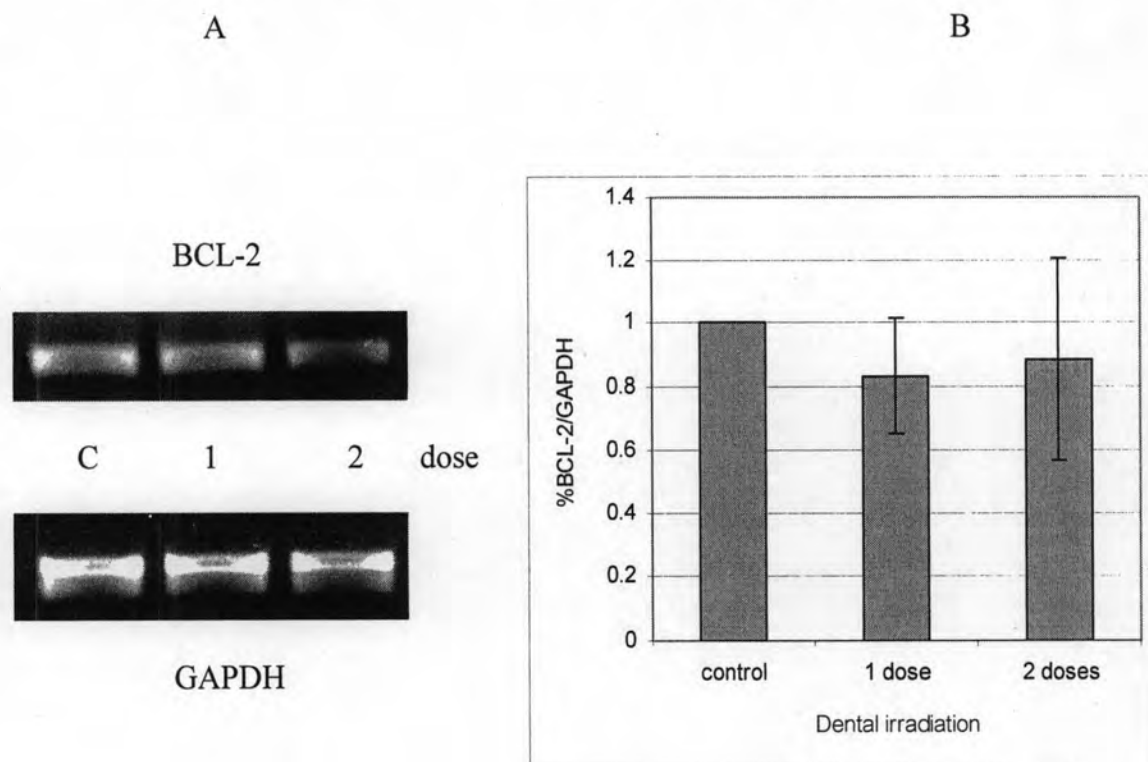


Figure 4.2 Expression of Bcl-2 in the primary bone cell cultures was evaluated using RT-PCR assay at 4 hours after irradiated by 0, 1 and 2 dose of a periapical radiograph.

A: Representative electrophoresis images of Bcl-2 and GAPDH expression regarding to dental dosage. B: The quantification of BCL-2 expression which normalized to the GAPDH. All values are presented as mean \pm SD (n=6).

Effect on BAX

BAX expression decreased significantly after irradiation with 1 and 2 doses of a periapical radiograph compared to the control (Fig. 4.3).

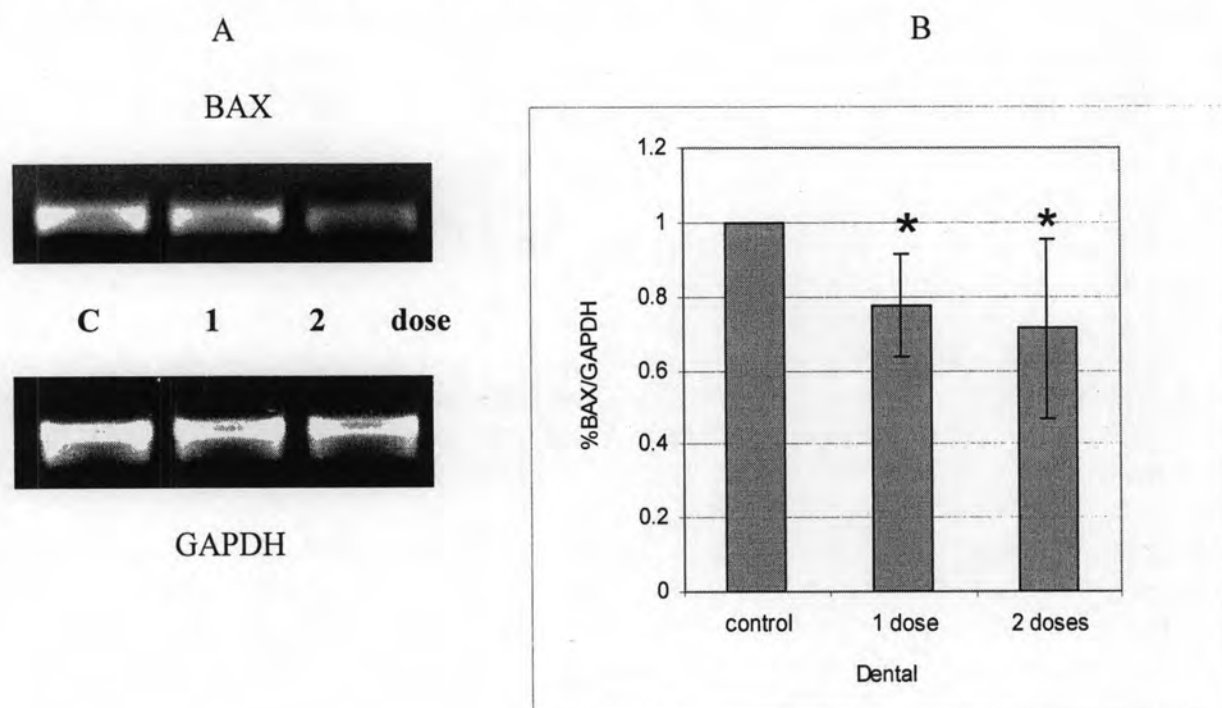


Figure 4.3 Expression of Bax in the primary bone cell cultures was evaluated using RT-PCR assay at 4 hours after irradiated by 0, 1 and 2 dose of a periapical radiograph. A: Representative electrophoresis images of Bax and GAPDH expression regarding to dental dosage. B: The quantification of Bax expression which normalized to the GAPDH. All values are presented as mean \pm SD (n=6). *Statistically significant, $p < 0.05$

Effect on BAD

Although the mean of BAD expression show dramatically decreased after dental irradiation, the big difference in each experiment caused no significant difference from non-irradiated control group (Fig. 4.4).

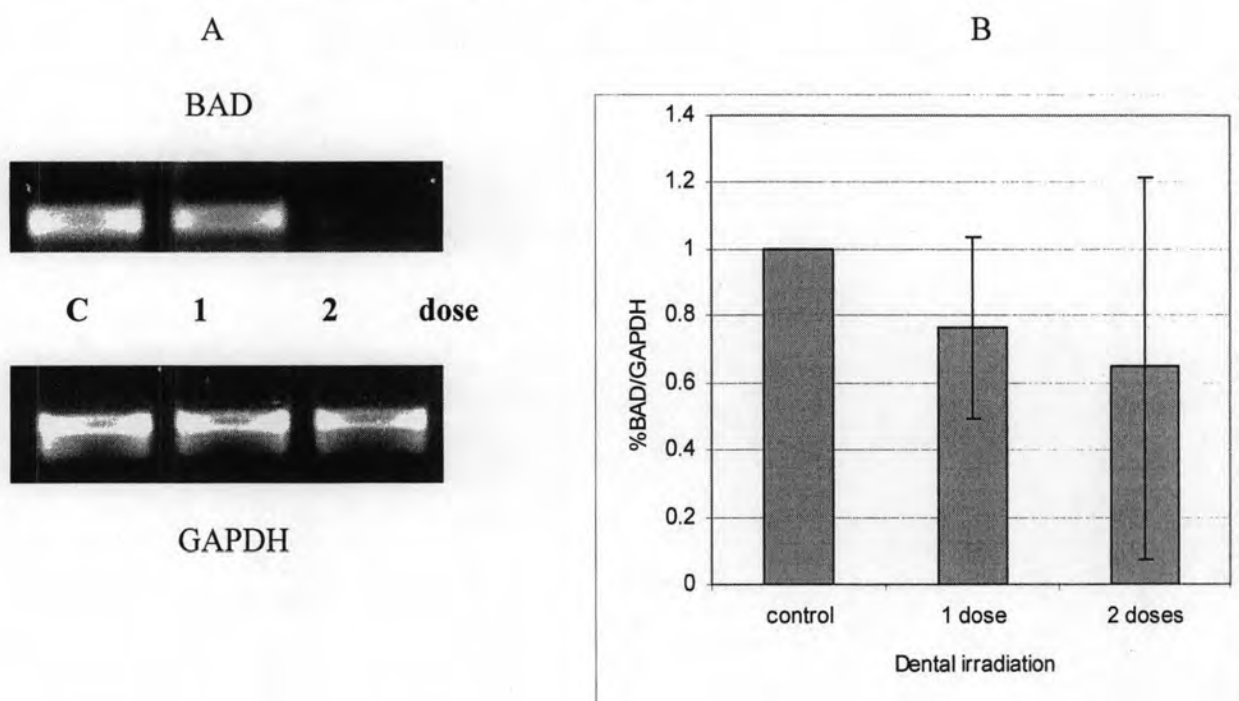


Figure 4.4 Expression of Bad in the primary bone cell cultures was evaluated using RT-PCR assay at 4 hours after irradiated by 0, 1 and 2 dose of a periapical radiograph. A: Representative electrophoresis images of Bad and GAPDH expression regarding to dental dosage. B: The quantification of Bad expression which normalized to the GAPDH. All values are presented as mean \pm SD (n=6).

Effect on BCL-XL

Dental x-radiation resulted in a non statistically significant difference in BCL-XL mRNA expression at 4 hours after irradiation compared to the control group (Fig. 4.5).

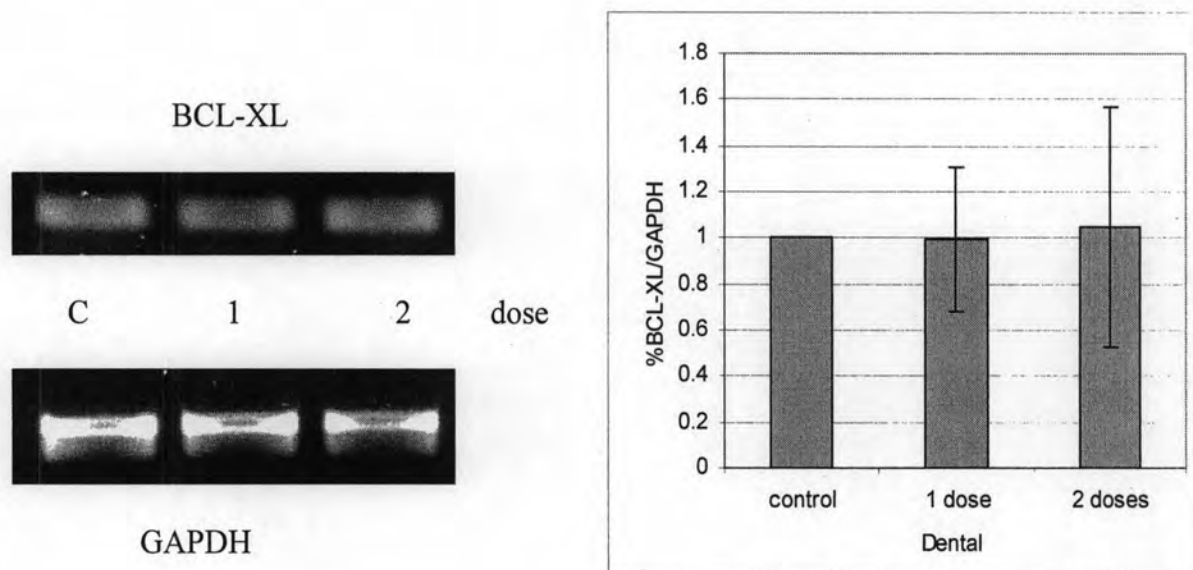


Figure 4.5 Expression of Bcl-xl in the primary bone cell cultures was evaluated using RT-PCR assay at 4 hours after irradiated by 0, 1 and 2 dose of a periapical radiograph. A: Representative electrophoresis images of Bcl-xl and GAPDH expression regarding to dental dosage. B: The quantification of Bcl-xl expression which normalized to the GAPDH. All values are presented as mean \pm SD (n=6).

Effect on CASPASE-3

After irradiation with 2 doses of a periapical radiograph, the CASPASE-3 expression decreased significantly compared to the control group (Fig. 4.6).

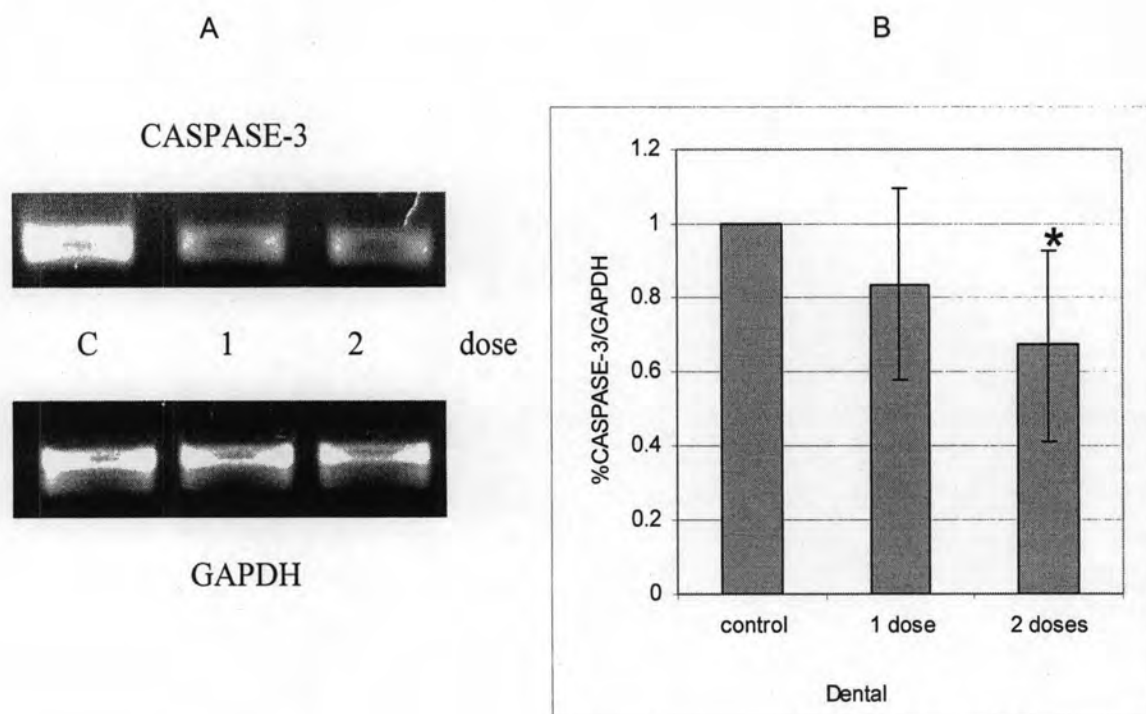


Figure 4.6 Expression of Caspase-3 in the primary bone cell cultures was evaluated using RT-PCR assay at 4 hours after irradiated by 0, 1 and 2 dose of a periapical radiograph. A: Representative electrophoresis images of Caspase-3 and GAPDH expression regarding to dental dosage. B: The quantification of Caspase-3 expression which normalized to the GAPDH. All values are presented as mean \pm SD (n=6).

*Statistically significant, $p < 0.05$

Bax/Bcl-2 ratio 4h after dental irradiation

The average Bax/Bcl-2 ratio of 0, 1 and 2 doses were 1 ± 0.0 , 0.98 ± 0.31 and 0.82 ± 0.14 . After irradiation of 2 doses, the Bax/Bcl-2 ratio decreased significantly compared to the control ($p < 0.05$)