Induction of apoptosis and necrosis by zinc in human thyroid cancer cell lines

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Abstract

Zinc at concentrations of 150 μM or higher induced necrosis as well as apoptosis in thyroid cancer cell lines. Necrosis was induced by zinc in a dose-dependent manner, whereas apoptosis did not increase at higher concentrations of zinc. The expression of the antiapoptotic protein phosphorylated Bad was markedly increased, whereas the expression of the proapoptotic proteins Bax and Bad decreased following Zn^{2+} exposure. Zn^{2+} induced rapid degradation of IκB, and an increase in the binding of nuclear transcription factor-κB (NF-κB). These observations indicate that antiapoptotic pathways were activated in thyroid cancer cells following exposure to Zn^{2+}. This may be a self-defence mechanism against apoptosis and may underlie the general resistance of thyroid cancer cells to apoptotic stimuli. Zinc may be a potential cytotoxic agent for the treatment of thyroid cancer.

Journal of Endocrinology (2001) **169,** 417–424

Introduction

Zinc is an essential component of a wide variety of metalloenzymes, transcription factors and other proteins. A deficiency in zinc affects various organs including the nervous system, gonadal tissue, skin and the intestine. Ingestion or administration of excessive amounts of zinc compounds, on the other hand, does not seem to result in any adverse long-term effects (Vallee & Falchuk 1993).

Most physiologically relevant cell death in the body occurs by an active process of self-destruction known as programmed cell death or apoptosis. Apoptosis can be initiated by a number of external signals including glucocorticoids, irradiation, cytokines, or withdrawal of growth factors (Raff 1992, Williams & Smith 1993). Several studies have indicated that zinc can inhibit apoptotic cell death. Apoptosis was observed in the small intestine of animals on a zinc-deficient diet (Elmes 1977), and a zinc-chelator was found to induce apoptosis in human thymocytes and leukemia cells (McCabe *et al.* 1993). The induction of apoptosis by irradiation, anti-cancer drugs or tumor necrosis factor (TNF) was prevented by zinc (Shimizu *et al.* 1990, Telford *et al.* 1991, Zalewski *et al.* 1991, Leccia *et al.* 1993, Shiokawa *et al.* 1994, Tanuma & Shiokawa 1994, Fady *et al.*1995). In contrast, however, a few reports have suggested that zinc actually induces apoptotic cell death (Telford & Fraker 1995, Manev *et al.* 1997, Paramanantham *et al.* 1997). Furthermore, zinc induced necrosis in prostate carcinoma cells (Iguchi *et al.* 1998).

In the present study, we show that zinc induced both necrosis and apoptosis in thyroid cancer cells. After exposure to zinc, the expression of antiapoptotic proteins was increased, and that of proapoptotic proteins was decreased. Furthermore, nuclear factor-κB (NF-κB) was activated. These changes may be a protective mechanism of thyroid cancer cells against apoptotic cell death, and may underlie the general resistance of thyroid cancer cells to apoptotic stimuli (Arscott *et al.* 1997).

Materials and Methods

Materials

Antibodies to Bcl-2, Bax, Bcl-xL, NFKB1, RelA, c-Rel, IκB and actin were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Antibodies to Bad and phosphorylated Bad (serine 112/136) were from New England BioLabs Inc. (Beverly, MA, USA). A caspase inhibitor, Ac-DEVD-CHO (Ac-Asp-Glu-Val-Asp-CHO), was purchased from Peptide Institute Inc. (Osaka, Japan). RNase A and proteinase K were from Nippon Gene (Osaka, Japan). T4 polynucleotide kinase and poly(dI-dC)·poly(dI-dC) were from Promega (Madison, WI, USA). Hoechst 33342 and propidium iodide (PI) were purchased from Wako Chemical Co.

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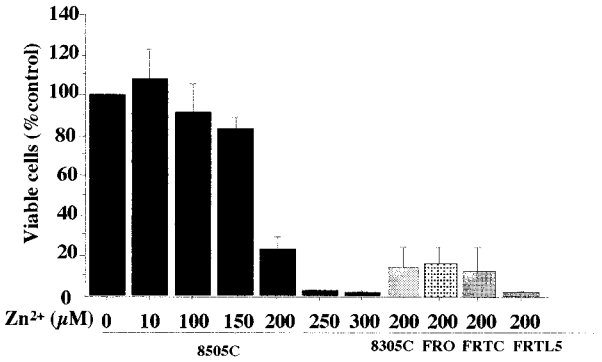

Figure 1 $ZnCl_2$-induced cell death in 8505C and other thyroid cancer cell lines. Means ± S.D. from three different experiments are shown.

(Osaka, Japan). A TACS annexin V(ANV)-FITC apoptosis detection kit was from Trevigen Inc. (Gaithersburg, MD, USA).

Cell culture and treatment

The rat thyroid cancer cell line, FRTC, derived from FRTL-5, has been described previously (Iitaka *et al.* 1997). Cell lines 8505C (poorly differentiated human papillary thyroid carcinoma), 8305C (undifferentiated human thyroid anaplastic carcinoma) and 3T3-L1 (mouse pre-adipocytes) were obtained from the Japanese Cancer Research Resources Bank (Tokyo, Japan). FRO (human anaplastic thyroid carcinoma) (Namba *et al.* 1995) and MO7-E (human megakaryoblastic leukemia cells) (Avanzi *et al.* 1990) were obtained from Drs Namba (Nagasaki University, Japan) and Murohashi (Saitama Medical School, Japan) respectively. Human hepatocellular carcinoma cell line HepG2 (American Type Culture Collection, ATCC HB 8065) was purchased from Dainippon Pharmatheuticals (Osaka, Japan). Thyroid cells were cultured in Ham F12 medium supplemented with 10% fetal calf serum (FCS). MC3T3-E1 (mouse osteoblastic cell line, Iitaka *et al.* 1994), 3T3-L1, and HepG2 cells were cultured in α-MEM supplemented with 10% FCS. MO7-E cells were cultured in RPMI 1640 supplemented with 10% FCS and GM-CSF. Cells were incubated in 24-well microtiter plates (Nunc, Roskilde, Denmark) at a concentration of 1×10^4 cells per well, and exposed to 10–500 μM $ZnCl_2$, $FeSO_4$, $MnCl_2$, or $CuSO_4$ for 24 h at 37 °C. In some experiments, cells were pretreated with 100 μM Ac-DEVD-CHO for 1 h before adding $ZnCl_2$. Live epithelial cells were counted by the crystal violet method (Iitaka *et al.* 1994), and MO7-E cells were quantified by the trypan blue exclusion method.

Detection of apoptosis and necrosis

Cells were stained with FITC-ANV (TACS annexin V-FITC apoptosis detection kit) to measure externalization of phosphatidyl serine, an early marker of apoptosis. Cells were also stained with Hoechst 33342 or PI after exposure to Zn^{2+}. Hoechst 33342 stains the nuclei in both apoptotic and necrotic cells, while PI stains the nuclei in cells with damaged cell membrane (necrosis). Cells were examined under a Zeiss axioplan fluorescence microscope (Oberkochen, Germany) or analyzed by a FACScan flow cytometer (Beckton Dickinson, San Jose, CA, USA) as previously reported (van Engeland *et al.* 1996). DNA ladder formation was observed as described previously (Gong *et al.* 1994). Briefly, 1×10^6 Zn^{2+}-treated or untreated cells were collected and washed twice with cold phosphate-buffered saline (PBS). Pellets were fixed with 70% ethanol for 20 h at 4 °C. After being washed twice

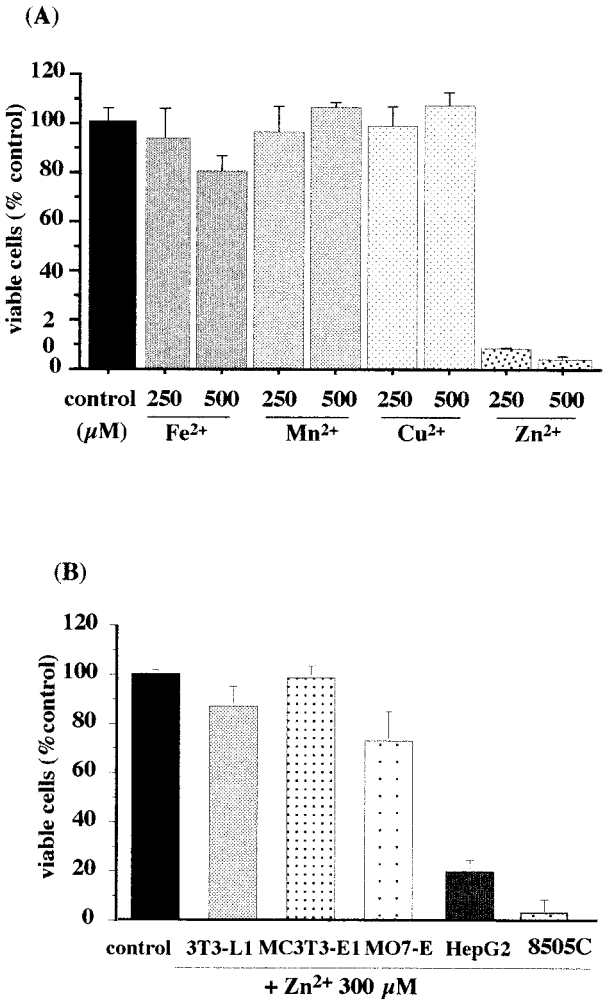

Figure 2 (A) Effect of divalent cations on 8505C thyroid cancer cells. A similar effect was observed in other thyroid cancer cell lines. (B) Effect of 300 μM zinc on various cell lines. Zinc had a significant cytotoxic effect on 8505C and HepG2 cells. Means ± S.D. from three different experiments are shown.

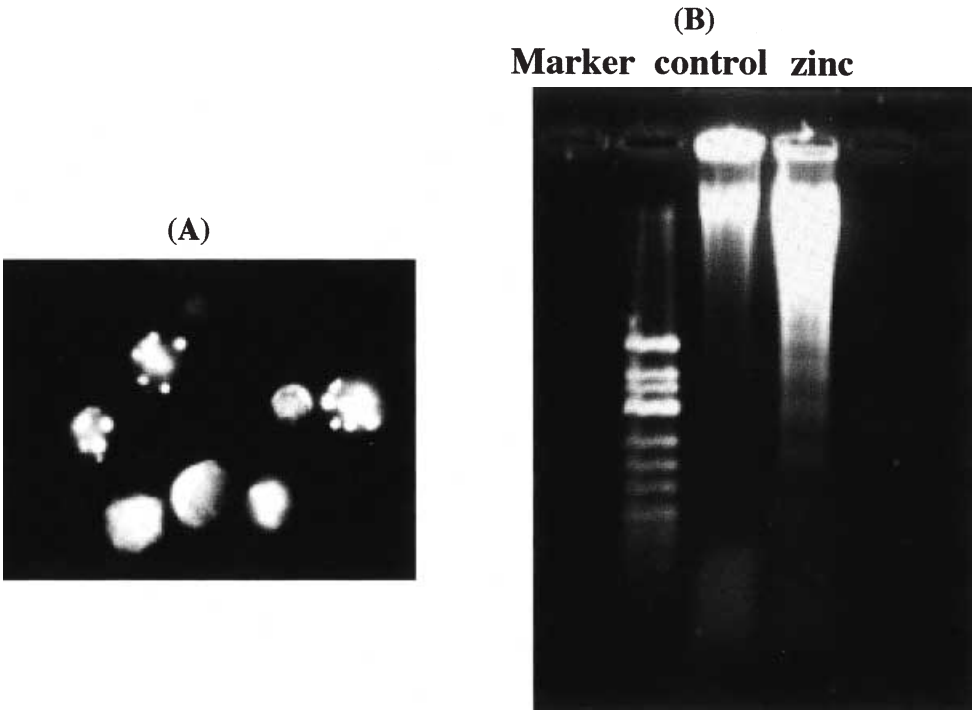

Figure 3 (A) Apoptotic bodies in Zn^{2+}-treated 8505C cells stained with Hoechst 33342. The same morphological change was observed in FRTC and FRO cells following Zn^{2+} exposure. (B) DNA laddering was observed in Zn^{2+}-treated 8505C cells.

with cold PBS, the cells were treated with 0·2 M phosphate-citrate buffer, pH 7·8, for 30 min at room temperature. The supernatants were recovered by centrifugation and incubated with 1 mg/ml RNase A (Nippon Gene) for 1 h at 37 °C, followed by proteinase K (1 mg/ml; Nippon Gene) for 1 h at 37 °C. DNA fragments were precipitated with 2·5 vol ethanol in the presence of 0·5 vol 10 M ammonium acetate at − 20 °C for 20 h. After centrifugation, samples were washed with 70% ethanol and resuspended in loading buffer. Electrophoresis was performed in 1 × TBE (50 mM Tris–HCl, pH 8·3, 50 mM boric acid, 1 mM EDTA) buffer on 1·5% agarose gels containing ethidium bromide.

Electrophoretic mobility shift assay (EMSA)

To examine the activation of NF-κB, an EMSA was performed as described previously (Watanabe *et al.* 1997). Whole cell and nuclear extracts were prepared after lysis of the cells with NP-40 lysis buffer. An oligonucleotide containing the NF-κB consensus binding site (5′-AGT TGAGGGGACTTTCCCAGGC-3′) was 5′ end-labeled using polynucleotide kinase and [γ-^{32}P]ATP. Nuclear protein extracts (4 μg) or whole cell extracts (10 μg) were incubated with labeled oligonucleotide for 20 min at room temperature in 10 μl binding mixture containing 10 mM Tris–HCl, pH 7·5, 1 mM $MgCl_2$, 50 mM NaCl, 0·5 mM EDTA, 0·5 mM dithiothreitol, 4% glycerol, and 0·05 mg/ml poly(dI-dC)·poly(dI-dC). The DNA–protein complexes were resolved by electrophoresis on 4% acrylamide gels in 1 × TBE at 10 V/cm for 60 min at room temperature.

Western blotting

The expression of Bcl-2, Bcl-xL, Bax, Bad, phosphorylated Bad (ser 112/136) and IκB was examined by Western blot analysis. Cell lysates were separated by SDS-PAGE on 7·5 − 12% gradient polyacrylamide gels and transferred onto Hybond-ECL nitrocellulose membranes (Amersham International plc, Amersham, Bucks, UK) in 25 mM Tris, 192 mM glycine. Membranes were blocked in Tris–buffered saline (20 mM Tris–HCl, pH 7·5, 137 mM NaCl) containing 0·1% Tween 20 and 3% non-fat dry milk for 30 min at room temperature. The blots were incubated with antibodies to Bcl-2, Bcl-xL, Bax, Bad, phospho-Bad, and IκB overnight at 4 °C. The membranes were washed in Tris–buffered saline and incubated with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (Amersham) for 1 h at room temperature. The blots were visualized by the Amersham ECL system (Amersham). The expression of these proteins was quantified using NIH image software (NIH, Bethesda, MD, USA).

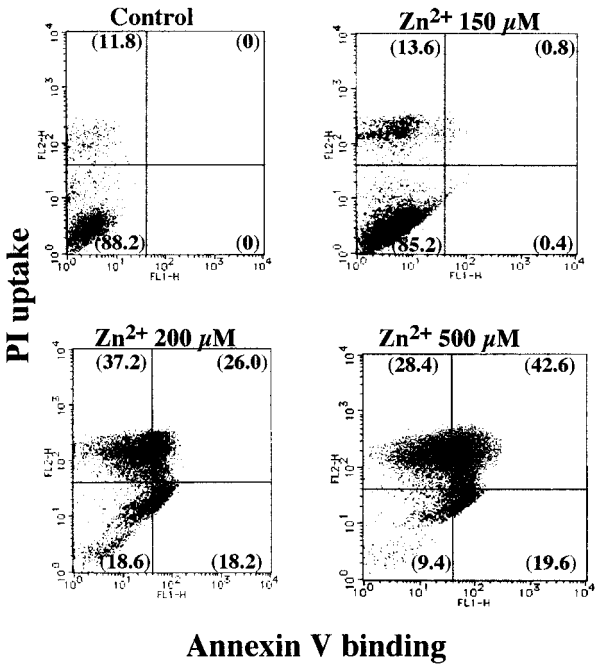

Figure 4 Flow cytometric analysis of apoptosis and necrosis in 8505C thyroid cancer cells following Zn^{2+} exposure. Data are representative of four separate experiments. Figures in parentheses indicate the percentage of cell numbers in each area. Annexin(+)/PI(−) cells (lower right area) indicate apoptotic cells. PI(+) cells (upper area) indicate necrotic cells.

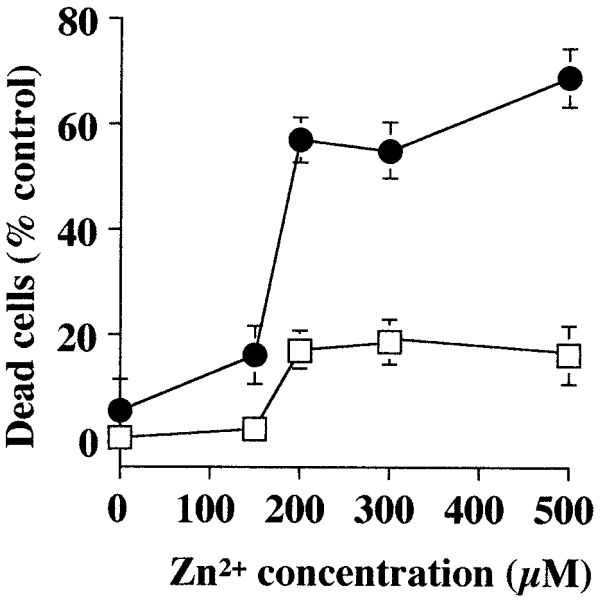

Figure 5 Effect of Zn^{2+} on the induction of ANV(+)/PI(−) apoptosis (□) or PI(+) necrosis (●) in 8505C thyroid cancer cells. Results are means ± S.E. from four different experiments.

Statistical analysis

The Scheffé's multiple comparison test (Figs 1 and 2) and the Student's *t*-test (Fig. 6B) were used to analyze data. A level of $P<0{\cdot}05$ was accepted as statistically significant.

Results

Zn^{2+}-induced cell death in thyroid cancer cells

As shown in Fig. 1, $ZnCl_2$ at concentrations of 150 μM or higher induced prominent cell death in a number of thyroid cancer cell lines as well as in FRTL-5 cells. Other divalent cations, including Cu^{2+}, Fe^{2+} and Mn^{2+} had no significant cytotoxic effect on these cells, even at concentrations as high as 500 μM (Fig. 2A). Non-thyroid cell lines, including MC3T3-E1, 3T3-L1 and MO7-E, were resistant to exposure to Zn^{2+} concentrations up to 300 μM (Fig. 2B). However, 300 μM Zn^{2+} had a significant cytotoxic effect on HepG2 cells. $ZnCl_2$ at 500 μM also had a significant cytotoxic effect on MO7-E cells (number of viable cells was 54 ± 10% of the control), but not on MC3T3-E1 and 3T3-L1 cells.

Cells with apoptotic bodies and DNA laddering were observed in Zn^{2+}-treated thyroid cancer cells (Fig. 3). Flow cytometric analysis showed that the number of PI(+) necrotic cells as well as ANV(+)/PI(−) apoptotic cells increased after exposure to 150 μM or higher concentrations of zinc (Fig. 4). Necrosis was induced by zinc in a dose-dependent manner (Fig. 5). However, apoptotic cells were not further increased by 200 μM or higher concentrations of zinc. As the concentration of zinc increased, the number of ANV(+)/PI(+) cells (including secondary necrotic cells following apoptosis) also increased. As shown in Fig. 6A, cells were rapidly killed through necrosis following 300 μM zinc administration. On the other hand, apoptosis as well as necrosis were gradually induced by 180 μM zinc. The cytotoxic effect of 180 μM zinc was significantly inhibited by the caspase inhibitor Ac-DEVD-CHO, whereas cell death induced by 300 μM zinc was not inhibited (Fig. 6B).

Zn^{2+}-induced DNA binding activity of NF-κB

The inhibitory protein IκB was degraded rapidly and increased binding of NF-κB in thyroid cancer cells was observed following Zn^{2+} exposure (Fig. 7). Supershift analysis revealed that activated NF-κB was mainly a heterodimer of NFKB1 and RelA (Fig. 7).

Expression of proapoptotic and antiapoptotic proteins after Zn^{2+} exposure

The expression of the proapoptotic proteins Bax and Bad was significantly decreased after exposure to 200 μM Zn^{2+} (Fig. 8). The expression of phosphorylated Bad at serine 112 and 136, which exerts antiapoptotic effects, was

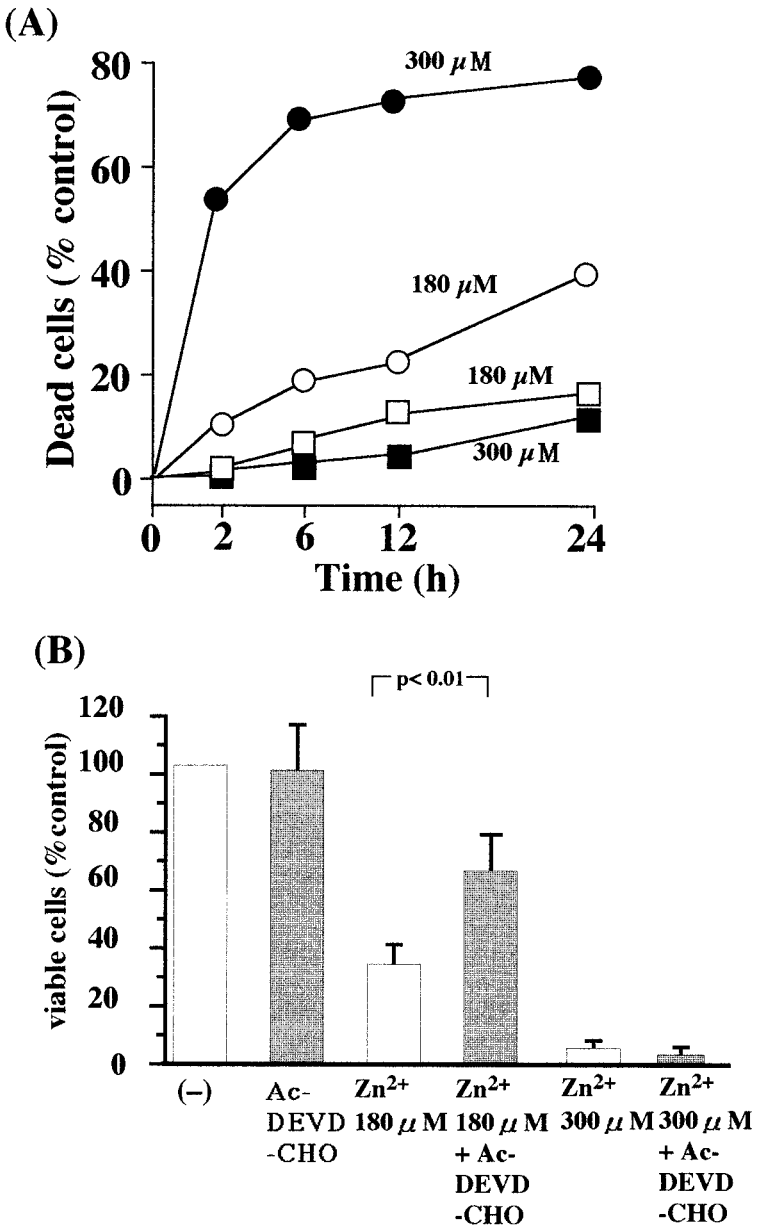

Figure 6 (A) Induction of apoptosis (ANV(+)/PI(−), boxes) and necrosis (PI(+), circles) by zinc. Necrosis was rapidly induced by 300 μM zinc (●), whereas both apoptosis (□) and necrosis (○) were induced by 180 μM zinc. (B) Effect of a caspase inhibitor, Ac-DEVD-CHO, on Zn^{2+}-induced cell death. The cytotoxic effect of 180 μM zinc was significantly inhibited (P<0·01), but that of 300 μM zinc was not. Means ± S.D. from three different experiments are shown.

gradually but markedly increased. A slight increase in expression of the antiapoptotic protein Bcl-2 was observed. There was no significant change in the expression of Bcl-xL. These changes were not observed in zinc-resistant MC3T3E-1 cells even after 500 μM zinc treatment (data not shown).

Discussion

This study demonstrated that Zn^{2+} had a potent cytotoxic effect on thyroid cancer cell lines and FRTL-5. High concentrations of $ZnCl_2$ induced both necrosis and apoptosis. Previous reports have shown that Zn^{2+} prevented

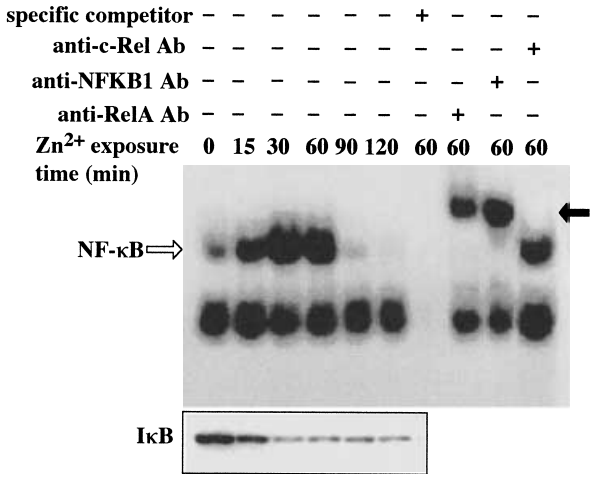

Figure 7 IκB was degraded and DNA binding of NF-κB (open arrow) increased in 8505C cells after exposure to Zn^{2+}. Activated NF-κB was a heterodimer of NFKB1 and RelA as demonstrated by a supershift assay (solid arrow). Similar results were obtained using FRO and FRTC cells.

apoptotic cell death in various cell types when apoptosis was induced with various cytotoxic agents including TNFα, cycloheximide, and UV and γ-irradiation (Shimizu *et al.* 1990, Telford *et al.* 1991, Zalewski *et al.* 1991, Leccia *et al.* 1993, Shiokawa *et al.* 1994, Tanuma & Shiokawa 1994, Fady *et al.* 1995). The mechanism of the antiapoptotic effect of Zn^{2+} was thought to be via its inhibitory effect on DNases (Shiokawa *et al.* 1994, Tanuma & Shiokawa 1994), or on poly(ADP-ribose) synthetase (Shimizu *et al.* 1990). Conversely, Zn^{2+} appeared to induce apoptotic cell death in a particular subset of mouse thymocytes, rat cerebellar granule cells and human liver cells (Telford & Fraker 1995, Manev *et al.* 1997, Paramanantham *et al.* 1997). We also confirmed the cytotoxic effect of Zn^{2+} on HepG2 cells. Telford *et al.* (1991) and Telford and Fraker (1995) reported that high concentrations (>200 μM) of Zn^{2+} inhibited glucocorticoid-induced apoptosis in mouse thymocytes, whereas lower concentrations (80–200 μM) of Zn^{2+} were shown to induce apoptotic cell death in these cells. Provinciali *et al.* (1995) also reported a similar dose-dependent effect of Zn^{2+} on apoptosis in mouse thymocytes. In the present study, concentrations of Zn^{2+} at 150 μM or higher were observed to induce cell death in various thyroid cancer cell lines in a dose-dependent manner.

Manev *et al.* (1995, 1997) have shown that the neurotoxicity of Zn^{2+} on rat cerebellar granule neurons involved both necrotic and apoptotic cell death. They suggested that calcium channel blocker-sensitive mitochondrial injury and DNA damage were involved in the protein synthesis-dependent neurotoxicity of Zn^{2+}. These authors reported that actinomycin D and calcium channel blockers reduced mitochondrial injury and cell death. In our study, we did not observe any inhibitory effect of actinomycin D or calcium channel blockers on Zn^{2+}-induced cell death in thyroid cancer cells (data not shown). Zinc has also been reported to induce necrosis in prostate carcinoma cells (Iguchi *et al.* 1998).

The present study shows that necrosis was induced in a dose-dependent manner by zinc. Higher concentrations of zinc rapidly induced necrotic cell death, whereas relatively lower concentrations of zinc induced apoptosis as well as necrosis in a time-dependent fashion. This suggests that zinc can induce two cytotoxic pathways in thyroid cancer cells. It is of interest that about 65% of cell death induced by 180 μM zinc could be rescued by the caspase inhibitor in spite of the fact that only 20% of cells are supposed to be killed by apoptosis. This suggests that secondary necrosis may be induced following apoptosis by relatively lower concentrations of zinc. Blom *et al.* (1999) reported that permeabilization of the plasma membrane (i.e. necrosis) was induced in some hepatocytes after the induction of apoptosis. Another possibility is that the caspase inhibitor actually inhibited necrosis. Warny and Kelly (1999)

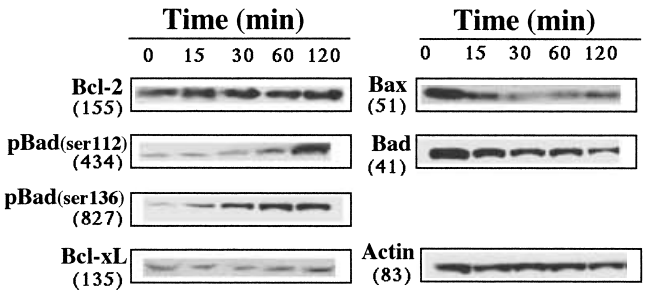

Figure 8 Expression of apoptosis-related proteins in 8505C thyroid cancer cells following exposure to 200 μM Zn^{2+}. Figures in parentheses indicate the percentage ratio of amounts of each protein expressed before Zn^{2+} exposure and 120 min after. Data are representative of two different experiments.

showed that caspase-like proteases were activated when necrosis was induced, and that the caspase inhibitor inhibited necrosis. In our experiments, however, the caspase inhibitor could not inhibit the cytotoxic effect of 300 μM zinc. We do not know why higher concentrations of zinc still induce apoptosis in a minor population of thyroid cancer cells. Palomba *et al.* (1996) found that the same initial damage can be a triggering event for both apoptosis and necrosis. They concluded that necrosis does not appear to be a passive response to overwhelming damage. Although the mechanism of induction of apoptosis or necrosis by zinc still remains to be elucidated, it was reported that intracellular ATP (Eguchi *et al.* 1997, Leist *et al.* 1997) or reduced glutathione (Fernandes & Cotter 1994) levels may serve as a switch between apoptosis and necrosis.

Exposure of thyroid cancer cells to Zn^{2+} led to a marked increase in expression of the antiapoptotic protein phosphorylated Bad, and a concurrent decrease in expression of the proapoptotic proteins Bax and Bad. This, in addition to a slight increase in Bcl-2 expression, results in an increase in the ratio of antiapoptotic proteins and proapoptotic proteins. These changes were not observed in zinc-resistant cells even after exposure to high concentrations of zinc. Furthermore, Zn^{2+} increased the binding of NF-κB to DNA in thyroid cancer cells. The inhibitory protein, IκB, was rapidly degraded following Zn^{2+} exposure, resulting in translocation of NF-κB into the nucleus. Activated NF-κB was mainly a heterodimer of NFKB1 (p50) and RelA (p65). Recent studies have provided convincing evidence that NF-κB is involved in the inhibition of apoptosis (Beg *et al.* 1995, Antwerp *et al.* 1996, Beg & Baltimore 1996, Wang *et al.* 1996). These observations suggest that antiapoptotic pathways were activated in thyroid cancer cells following Zn^{2+} exposure. This may be a self-defence mechanism against external apoptotic stimuli, although necrotic cell death could not be prevented. This self-defence mechanism may be one reason why thyroid cells are resistant to apoptotic stimuli (Arscott *et al.* 1997).

The prognosis of anaplastic or poorly differentiated thyroid carcinomas is extremely poor because of the resistance of these cancers to chemotherapeutic agents and/or radiation. Since administration of excessive amounts of zinc compounds does not seem to result in any adverse effects (Vallee & Falchuk 1993), the cytotoxic effect of zinc may be useful in the treatment of anaplastic and/or other thyroid cancers, especially when zinc is injected into the tumor percutaneously.

Acknowledgements

The authors would like to thank Ms T Suzuki for her excellent technical assistance. We are also grateful to Dr Namba and Dr Murohashi for providing us with cells.

References

Antwerp DJV, Martin SJ, Kafri T, Green DR & Verma IM 1996 Suppression of TNF-α-induced apoptosis by NF-κB. *Science* **274** 787–789.

Arscott PL, Knapp J, Rymaszewski M, Barton JL, Bretz JD, Thompson NW & Baker JR Jr 1997 Fas (APO-1, CD95)-mediated apoptosis in thyroid cells is regulated by a labile protein inhibitor. *Endocrinology* **138** 5019–5027.

Avanzi GC, Brizzi MF, Giannotti J, Ciarletta A, Yang YC, Pegoraro L & Clark SC 1990 MO7e human leukemic factor-dependent cell line provides a rapid and sensitive bioassay for human cytokines GM-CSF and IL-3. *Journal of Cellular Physiology* **145** 458–464.

Beg AA & Baltimore D 1996 An essential role for NF-κB in preventing TNF-α-induced cell death. *Science* **274** 782–784.

Beg AA, Sha WC, Bronson RT, Ghosh S & Baltimore D 1995 Embryonic lethality and liver degeneration in mice lacking the RelA component of NF-κB. *Nature* **376** 167–170.

Blom WM, De Bont HJGM, Meijerman I, Kuppen PJK, Mulder GJ & Nagelkerke JF 1999 Interleukin-2-activated natural killer cells can induce both apoptosis and necrosis in rat hepatocytes. *Hepatology* **29** 785–792.

Eguchi Y, Shimizu S & Tsujimoto Y 1997 Intracellular ATP levels determine cell death fate by apoptosis or necrosis. *Cancer Research* **57** 1835–1840.

Elmes ME 1977 Apoptosis in the small intestine of zinc-deficient and fasted rats. *Journal of Pathology* **123** 219–223.

van Engeland M, Ramaekers FC, Schutte B & Reutelingsperger CP 1996 A novel assay to measure loss of plasma membrane asymmetry during apoptosis of adherent cells in culture. *Cytometry* **24** 131–139.

Fady C, Gardner A, Jacoby F, Brinskin K, Tu Y, Schmid I & Lichtenstein A 1995 Atypical apoptotic cell death induced in L929 targets by exposure to tumor necrosis factor. *Journal of Interferon Cytokine Research* **15** 71–80.

Fernandes RS & Cotter TG 1994 Apoptosis or necrosis: intracellular levels of glutathione influence the mode of cell death. *Biochemical Pharmacology* **48** 675–681.

Gong J, Traganos F & Darzynkiewicz Z 1994 A selective procedure for DNA extraction from apoptotic cells applicable for gel electrophoresis and flow cytometry. *Annals of Biochemistry* **218** 314–319.

Iguchi K, Hamatake M, Ishida R, Usami Y, Adachi T, Yamamoto H, Kohsida K, Uchibayashi T & Hirano K 1998 Induction of necrosis by zinc in prostate carcinoma cells and identification of proteins increased in association with this induction. *European Journal of Biochemistry* **253** 766–770.

Iitaka M, Kitahama S & Ishii J 1994 Involvement of protein kinase A and C in the production of interleukin-1a-induced prostaglandin E_2 from mouse osteoblast-like cell line, MC3T3-E1. *Biochimica et Biophysica Acta* **1221** 78–82.

Iitaka M, Fukasawa N, Kitahama S, Miura S, Kawakami Y, Sato H, Sugano S, Ishii J & Katayama S 1997 Transplantable rat thyroid cancer cell line FRTC transformed with muramyl dipeptide. *British Journal of Cancer* **75** 40–46.

Leccia MT, Richard MJ, Beani JC, Faure H, Monjo AM, Cadet J, Amblard P & Favier A 1993 Protective effect of selenium and zinc on UV-A damage in human skin fibroblasts. *Photochemistry and Photobiology* **58** 548–553.

Leist M, Single B, Castoldi AF, Kühnle S & Nicotera P 1997 Intracellular adenosine triphosphate (ATP) concentration: a switch in the decision between apoptosis and necrosis. *Journal of Experimental Medicine* **185** 1481–1486.

McCabe MJ Jr, Jiang SA & Orrenius S 1993 Chelation of intracellular zinc triggers apoptosis in mature thymocytes. *Laboratory Investigation* **69** 101–110.

Manev H, Cagnoli CM, Atabay C, Kharlamov E, Ikonomovic MD & Grayson DR 1995 Neuronal apoptosis in an *in vitro* model of photochemically induced oxidative stress. *Experimental Neurology* **133** 198–206.

Manev H, Kharlamov E, Uz T, Mason RP & Cagnoli CM 1997 Characterization of zinc-induced neuronal death in primary cultures of rat cerebellar granule cells. *Experimental Neurology* **146** 171–178.

Namba H, Hara T, Tsukazaki T, Migita K, Ishikawa N, Ito K & Nagataki S 1995 Radiation-induced G_1 arrest is selectively mediated by the p53-WAF1/Cip1 pathway in human thyroid cells. *Cancer Research* **55** 2075–2080.

Palomba L, Sestili P, Cattabeni F, Azzi A & Cantoni O 1996 Prevention of necrosis and activation of apoptosis in oxidatively injured human myeloid leukemia U937 cells. *FEBS Letters* **390** 91–94.

Paramanantham R, Sit K-H & Bay B-H 1997 Adding Zn^{2+}-induced DNA fragmentation and cell condensation in cultured human Chang liver cells. *Biological Trace Element Research* **58** 135–147.

Provinciali M, Di Stefano G & Fabris N 1995 Dose-dependent opposite effect of zinc on apoptosis in mouse thymocytes. *International Journal of Immunopharmacology* **17** 735–744.

Raff MC 1992 Social controls on cell survival and cell death. *Nature* **356** 397–400.

Shimizu T, Kubota M, Tanizawa A, Sano H, Kasai Y, Hashimoto H, Akiyama Y & Mikawa H 1990 Inhibition of both etoposide-induced DNA fragmentation and activation of poly(ADP-ribose) synthesis by zinc ion. *Biochemical and Biophysical Research Communications* **169** 1172–1177.

Shiokawa D, Ohyama H, Yamada T, Takahashi K & Tanuma S 1994 Identification of an endonuclease responsible for apoptosis in rat thymocytes. *European Journal of Biochemistry* **226** 23–30.

Tanuma S & Shiokawa D 1994 Multiple forms of nuclear deoxyribonuclease in rat thymocytes. *Biochemical and Biophysical Research Communications* **203** 789–797.

Telford WG & Fraker PJ 1995 Preferential induction of apoptosis in mouse $CD^{4+}CD^{8+}$abTCR^{lo}CD3e^{lo} thymocytes by zinc. *Journal of Cellular Physiology* **164** 259–270.

Telford WG, King LE & Fraker PJ 1991 Evaluation of glucocorticoid-induced DNA fragmentation in mouse thymocytes by flow cytometry. *Cell Proliferation* **24** 447–459.

Vallee BL & Falchuk KH 1993 The biochemical basis of zinc physiology. *Physiological Reviews* **73** 79–118.

Wang C-Y, Mayo MW & Baldwin AS 1996 TNF- and cancer therapy-induced apoptosis: potentiation by inhibition of NF-κB. *Science* **274** 784–787.

Warny M & Kelly C 1999 Monocytic cell necrosis is mediated by potassium depletion and caspase-like proteases. *American Journal of Physiology* **276** C717–C724.

Watanabe N, Iwamura T, Shinoda T & Fujita T 1997 Regulation of NFKB1 proteins by the candidate oncoprotein BCL-3: generation of NF-κB homodimers from the cytoplasmic pool of p50-p105 and nuclear translocation. *EMBO Journal* **16** 3609–3620.

Williams GT & Smith CA 1993 Molecular regulation of apoptosis: genetic controls on cell death. *Cell* **74** 777–779.

Zalewski PD, Forbes IJ & Giannakis C 1991 Physiological role for zinc in prevention of apoptosis (gene-directed death). *Biochemical and Biophysical Research Communications* **24** 1093–1101.

Received in final form 2 January 2001
Accepted 9 January 2001