Acta histochemica **107** (2005) 207—214

Available online at www.sciencedirect.com

SCIENCE DIRECT®

acta histochemica

www.elsevier.de/acthis

# Protective effects of a calcium channel blocker on apoptosis in thymus of neonatal STZ-diabetic rats

**Fatma Kaya Dağıstanlı[a], Belgin Süsleyici Duman[b], Melek Öztürk[a,*]**

[a]*Department of Medical Biology, Cerrahpasa Faculty of Medicine, Istanbul University, Cerrahpasa 34303, Istanbul, Turkey*
[b]*Department of Medical Biology and Genetics, Kadir Has University Faculty of Medicine, Istanbul, Turkey*

**KEYWORDS**
Apoptosis;
Neonatal STZ diabetic rats;
Thymus;
Isradipine

## Summary

Streptozotocin (STZ) is known to induce insulin-dependent diabetes in experimental animals. In STZ-induced diabetes, atrophy of the thymus is caused by elevated intracellular calcium levels leading to apoptosis. Hyperglycemia is known to result in a decrease in numbers of T cells in the thymus and circulation. Intracellular calcium levels increase in diabetic animals after induction by STZ. Hyperglycemia inhibits $Ca^{2+}$-ATPase and increases intracellular calcium levels. We have investigated apoptosis in thymus tissue of neonatal STZ (n-STZ)-diabetic rats and the effects of isradipine as a calcium channel blocker (CCB) on apoptosis. Five groups of newborn Wistar rats were used. On the second day after birth, 100 mg/kg STZ was given i.p. to the first two groups. The first group was n-STZ diabetic. To the second group, starting from the 12th week, 5 mg/kg/day isradipine (i.p) was given for 6 weeks. To the third group, the same dose of isradipine was given on the second day, followed by STZ treatment. The fourth group was non-diabetic and treated with 5 mg/kg/day isradipine for six weeks. The fifth group consisted of non-diabetic rats. To the sixth group, dexamethasone (5 mg/kg i.p.) was given to adult rats. For detection of apoptotic cells in paraffin-embedded thymus sections, the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labelling (TUNEL) assay was used. The DNA ladder method was performed for analysis of DNA fragmentation. In the isradipine-treated non-diabetic group, typical apoptotic banding patterns were found, whereas thick bands between 123 and 246 bp length were found in the n-STZ- and n-STZ+isradipine-treated groups. More apoptotic cells were observed in the thymus of isradipine-treated, n-STZ-treated and n-STZ+isradipine-treated groups when compared with the non-diabetic control and isradipine+n-STZ-treated groups. In conclusion, we observed that long-term STZ

*Corresponding author. Fax: +90 0212 414 30 42.
*E-mail address:* mozturk@istanbul.edu.tr (M. Öztürk).

0065-1281/$ - see front matter 
doi:10.1016/j.acthis.2005.03.005

diabetes results in apoptosis in the thymus. We also found that isradipine administered before STZ has protective effects against apoptosis, whereas isradipine alone induces apoptosis.


## Introduction

In animal models of diabetes-depressed T lymphocyte function as a result of hyperglycemia (Tabata et al., 1984) or toxic side effects of the diabetic agents (Nichols et al., 1981; Wellhausen, 1986) were shown to be associated with atrophy of the thymus and peripheral lymphoidal tissues.

Intracellular calcium concentrations were shown to increase in streptozotocin (STZ) diabetic animal models and this has been explained by inhibition of $Ca^{2+}$-ATPase by hyperglycemia resulting in an increase in calcium ion levels (Kaymaz et al., 1995). Tightly controlled calcium concentrations in cells are essential for their functioning. Various studies have shown that calcium induces apoptosis by activating effector proteases (Bortner et al., 1995; King et al., 1996; Cummings et al., 1997; Szabadkai and Rizzuto, 2004). It has been concluded that the apoptosis rate is increased in the thymus and that morphological changes occur and T lymphocytes require calcium ions for their vitality in vivo (Balakumaran et al., 1996).

Calcium channel blockers (CCBs) are a diverse group of antihypertensive medication with variable pharmocokinetics and clinical effects. CCBs are classified as either selective or non-selective and act on different types of calcium channels. The effects of CCBs in the treatment of hypertension are mediated by alterations in vascular smooth muscle calcium homeostasis (Flynn and Pasko, 2000). Furthermore, some CCBs improve insulin sensitivity in diabetics (Srinivasan et al., 1997). Several in vitro studies indicated that CCBs diminish intracellular calcium levels, thus leading to apoptosis. Verapamil, a CCB, stimulates apoptosis in cultures of proliferating vascular smooth muscle cells (Leszczynski et al., 1994). The mechanisms by which CCBs induce thymic apoptosis in vivo are not clear since in vitro studies have shown that excessive calcium influx into cells by inactivating different calcium channels prevents apoptosis (Berggren et al., 1993; Ray et al., 1993; Mason, 1999). On the other hand, CCBs induce an increase in apoptosis in rat thymus (Balakumaran et al., 1996).

The aim of the present study is to determine whether apoptosis occurs in chronic hyperglycemic thymocytes of neonatal STZ diabetic rats and whether isradipine is effective in preventing apoptosis caused by STZ treatment when administered before and after STZ treatement of neonatal STZ diabetic rats.

## Material and methods

### Animals and treatment

Five groups of newborn Wistar rats were used. On the second day after birth, 100 mg/kg STZ (STZ; Sigma, St Louis, MO, USA) was given i.p. to the first two groups. The first group ($n = 7$) was n-STZ diabetic. To the second group ($n = 9$), 5 mg/kg/day isradipine (i.p.) (Dynacirc; Sandoz, Basel, Switzerland; Chandra et al., 1999) was given starting in the 12th week for 6 weeks. To the third group ($n = 5$), the same dose of isradipine was given on the second day followed by STZ treatment. The fourth group ($n = 9$) consisted of non-diabetic rats treated with 5 mg/kg/day isradipine for 6 weeks. After week 12, 0.9% NaCl solution was given to the fifth group ($n = 5$) which consisted of non-diabetic control rats. To the sixth group ($n = 5$), dexamethasone (5 mg/kg, i.p.; Fehsel et al., 1994) was given to five adult rats and the animals were sacrificed after 3 h and their thymuses were taken out. This group has been evaluated only as the apoptotic control. All animals were fed with 21% protein-containing food and were given fresh water daily.

### Blood glucose levels

Blood glucose (BG) levels were measured weekly starting at week 6 in tail vein blood of overnight fasted animals blood using a glucostix (Glucostix, Bayer, İstanbul, Turkey) and a glucometer (Glucometer II Model 5550; Ames, Indianapolis, IN, USA).

### Tissues

Thymus tissue was obtained under ether anesthesia for histochemical examination and DNA isolation. Samples of fresh tissue for DNA isolation were stored at −70 °C until use, and other samples were prepared for the TUNEL method.

### In situ DNA end labelling method (TUNEL)

Thymus tissues were dissected, fixed in 10% neutral buffered formalin, embedded in paraffin wax then cut into 5 μm-thick sections. Sections were put on slides coated with poly-L-lysine (PLL; Sigma) for the in situ DNA end labelling method. Detection of DNA fragmentation in situ was visualized with the use of the ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit (Intergen, Purchase, NY, USA). Deparaffinized tissue sections were incubated with proteinase K (20 μg/ml). Tissue sections were subjected to 3% $H_2O_2$ for endogenous peroxidase inhibition and were incubated with 1 × equilibration buffer at room temperature for 30 min. The digoxigenin-labelled dNTP tail was incubated with Tdt (terminal deoxynucleotidyl transferase) for 1 h at 37 °C, and sections were washed in stop/wash buffer for 10 min at room temperature. Tissue sections were incubated with anti-digoxigenin-peroxidase antibody at room temperature for 30 min and were stained with diaminobenzidine (DAB) as a peroxidase substrate. Staining was evaluated using a light microscope after counterstaining with methyl green.

### Staining specificity controls

Thymus tissue sections of dexamethasone-treated rats were used as a positive control. For negative controls, distilled water was used instead of Tdt enzyme.

### Analysis of DNA fragmentation

Genomic DNA was isolated from thymus tissues with phenol–chloroform extraction (Blin and Stafford, 1976) and stored at −70 °C until electrophoresis. DNA samples were dissolved in 10 mM Tris–HCl, pH 8.0, containing 1 mM EDTA, mixed with 6 volumes of DNA loading buffer (40% sucrose in 50 mM EDTA/0.25% bromophenol blue) and then loaded onto 1.2% agarose gels containing 0.2 g/ml ethidium bromide. Electrophoresis was conducted in the running buffer (90 mM Tris, 90 mM boric acid, and 2 mM EDTA, pH 8.0) at 7 V/cm. As a control marker, a 123 bp DNA ladder (Sigma) was used.

### Statistical analysis

Values are expressed as means ± SD. BG levels of the different groups of rats were compared using one-way ANOVA tests. The labelled apoptotic cells were counted using a light microscope (Zeiss, Jena, Germany). Cell counts were performed using a × 10 ocular lens in five different fields. For comparison of the six groups including the dexamethasone-positive control, the one-way ANOVA test was used. Multiple comparisons of BG levels and apoptotic cell counts were performed with the Tukey HSD test.

## Results

### Blood glucose levels

The study groups were compared for their BG levels in the 6th and 18th week (Table 1). BG levels of untreated n-STZ diabetic rats were significantly higher than those of all other groups until the end of the experiment. BG levels of isradipine-treated n-STZ-diabetic groups were significantly lower ($p<0.05$) than those of the untreated n-STZ diabetic group at the end of the experiment. In the n-STZ+isradipine group, BG levels in the 18th week were significantly lower than in the 6th week ($p<0.001$). BG levels did not change during the experiments in n-STZ diabetic, isradipine-treated non-diabetic and non-diabetic control groups.

### TUNEL assay and morphological findings

Apoptotic cell numbers for each group are shown in Table 2. In dexamethasone-treated adult rats,

**Table 1.** Blood glucose levels (mg/dl) of non-diabetic, isradipine-treated non-diabetic (I) and untreated neonatal STZ (n-STZ) diabetic, neonatal STZ diabetic treated with isradipine before (I+n-STZ) and after (n-STZ+I) a single injection of STZ

| Week | Non-diabetic ($n=5$) | n-STZ diabetic ($n=7$) | I+n-STZ diabetic ($n=5$) | n-STZ+I diabetic ($n=9$) | I ($n=9$) |
|---|---|---|---|---|---|
| 6 | 90.2 ± 7.0 | 214.7 ± 32.7$^{\dagger}$ | 187.6 ± 50.9$^{\dagger}$ | 224.4 ± 48.2$^{\dagger}$ | 88.4 ± 11.8 |
| 18 | 75.8 ± 13.2 | 233.1 ± 34.1 | 137.4 ± 3.2**,*** | 145.7 ± 41.4*,*** | 82.9 ± 20.4*** |

Values are represented as mean ± SD; *$p<0.001$ and **$p<0.05$ versus week 6; ***$p<0.05$ versus n-STZ diabetic, week 18; $^{\dagger}p<0.05$ versus non-diabetics, week 6.

**Table 2.** Numbers of apoptotic cells in dexamethasone-treated, non-diabetic, isradipine-treated non-diabetic (I) and untreated neonatal STZ (n-STZ) diabetic, neonatal STZ diabetic treated with isradipine before (I+n-STZ) and after (n-STZ+I) a single injection of STZ

| | Dexamethasone diabetic | n-STZ diabetic | n-STZ+I diabetic | I+n-STZ | I | Non-diabetic |
|---|---|---|---|---|---|---|
| Numbers of apoptotic cells | 15.8±2.5* | 9.5±1.9* | 7.4±1.7 | 6.2±1.3** | 14.8±3.5* | 5.0±1.4** |

Values are represented as mean±SD; *$p<0.05$ versus non-diabetic control rats; **$p<0.05$ versus n-STZ diabetic rats.

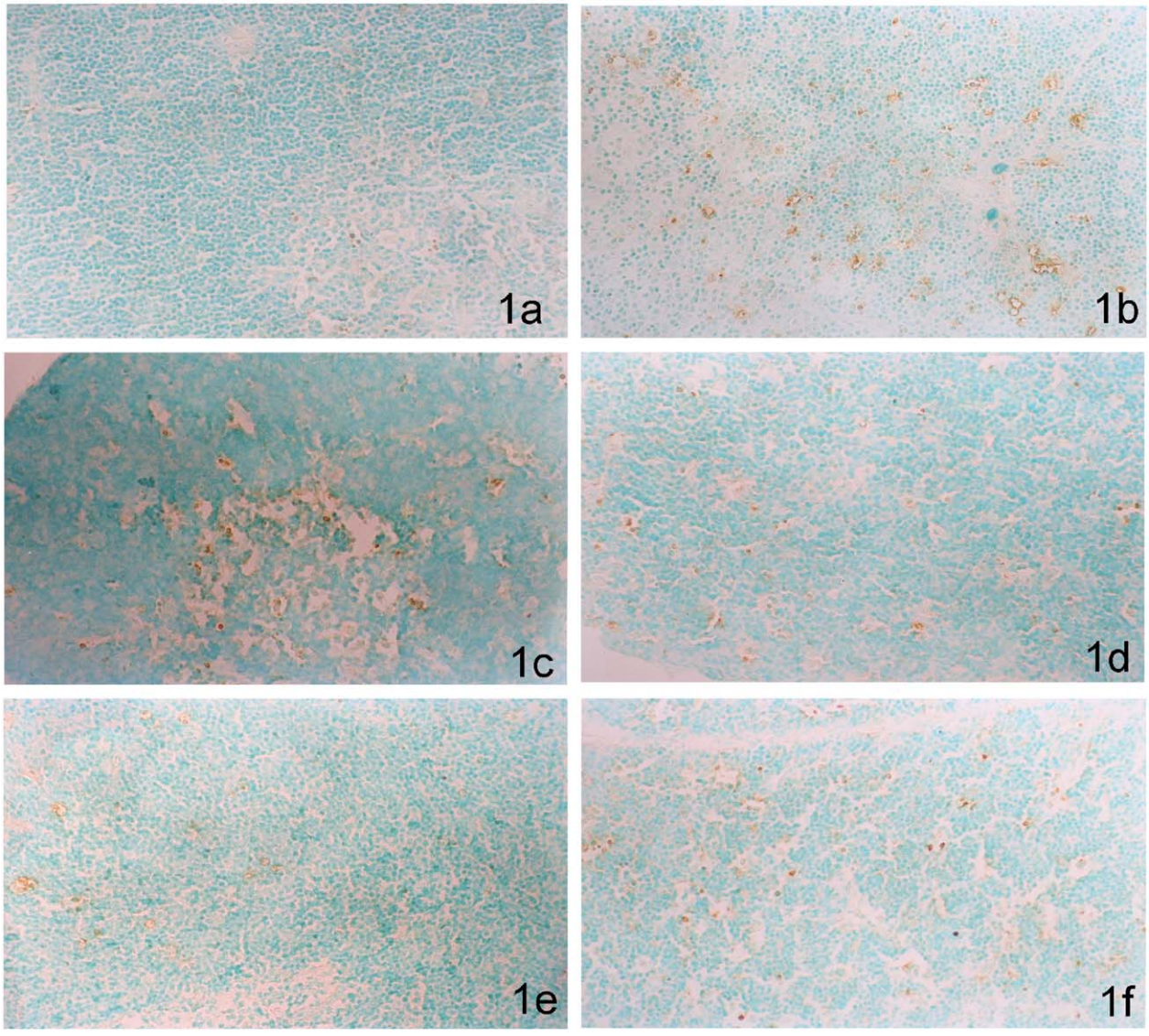


**Figure 1.** TUNEL staining of thymus tissue sections of non-diabetic control (a), dexamethasone-treated non-diabetic (b), untreated n-STZ diabetic (c), n-STZ diabetic treated with isradipine (d), isradipine+n-STZ diabetic (e) and isradipine-treated non-diabetic (f) rats. Labelled nuclei (brown) of apoptotic cells are present in one thymus of dexamethasone-treated non-diabetic (b) and isradipine-treated non-diabetic (f) rats. In isradipine+n-STZ diabetic rats (e), the numbers of apoptotic nuclei is similar to that in non-diabetic rats (a). Counterstaining: methyl green. Magnifications ×40.

the cortex-medulla border in the thymus was observed to disappear and was taken as control tissue for apoptosis. Numbers of apoptotic cell nuclei (15.8±2.5) were found to be higher when compared to non-diabetic control thymus (5.0±1.4) (Figs. 1a and b). In the thymus of n-STZ

diabetic group, apoptotic cells were found to be more frequent in the cortex as compared with medulla and some labelled nuclei were intensely stained (Fig. 1c). In the diabetic group, numbers of apoptotic cells (9.5±1.9) were higher than in the non-diabetic control group (5.0±1.4). Tissue integrity in the thymus of the STZ-induced diabetic group was destroyed. Medullary atrophy was clearly observed in the dexamethasone-treated group. In the isradipine+STZ-treated group, the apoptotic cell numbers (6.2±1.3) and tissue morphology was similar to non-diabetic control rats (5.0±1.4). Low numbers of apoptotic cells were observed in the thymus (Fig. 1e). Apoptotic cell numbers of the isradipine-treated n-STZ diabetic group were lower than in both the dexamethasone-treated and diabetic groups. Medullary atrophy was visible and apoptotic cells were found to be located frequently in the cortex and cortex/medulla borderline in the thymus of n-STZ+isradipine group (Fig. 1d). When the n-STZ+isradipine group was compared with the n-STZ diabetic group, apoptotic cell numbers were lower than in the n-STZ diabetic groups but the difference was not significant. In the n-STZ+isradipine group, tissue morphology was less conserved than that in the isradipine+STZ group (7.4±1.7) and the numbers of apoptotic cells was higher than in the isradipine+STZ group (6.2±1.3), but again the difference was not statistically significant. Apoptotic cells were more frequently present in the thymus of the isradipine-treated group (14.8±3.5) than in the thymus of controls (5.0±1.4). In this group, numbers of apoptotic cells were close to those in the dexamethasone group (Table 2). The thymus of STZ-induced diabetic rats was similar in appearance to those of dexamethasone-treated and isradipine-treated rats with respect to the distribution of apoptotic cells (Fig. 1f).

## DNA ladder formation

DNA of dexamethasone-treated rat thymus and DNA ladder markers were used as control markers for apoptosis, and were compared with those of the study groups. Typical apoptotic DNA fragmentation was observed in both the isradipine-treated non-diabetic and dexamethasone-treated groups. In n-STZ and n-STZ+isradipine-treated groups, thick DNA bands were found, and these were located between 123 and 246 bp. DNA ladder formation was not observed in the non-diabetic control group (Fig. 2).

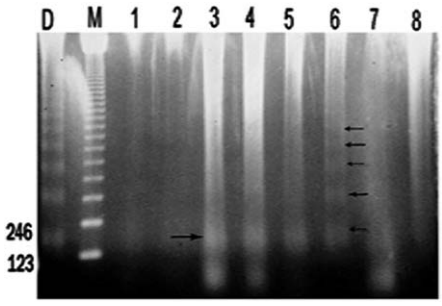


**Figure 2.** Apoptotic DNA fragments of thymus tissue after fractionation using 1.2% agarose gel electrophoresis and stained with ethidium bromide. Lane D, dexamethasone-treated rat thymus DNA (5 μg); lane M, DNA ladder as a control marker (5 μg); lanes 1–4, n-STZ diabetic rats DNA (50, 32, 24 and 16 μg, respectively); lane 5, n-STZ+isradipine-treated rat thymus DNA (16 μg). In n-STZ and n-STZ+isradipine-treated rats fewer DNA bands are present between 123 and 246 bp bands; lanes 6, 7, isradipine-treated non-diabetic rats thymus DNA (15 and 20 μg, respectively), showing apoptotic DNA fragmentation; lane 8, non-diabetic control group without any DNA fragmentation bands.

## Discussion

STZ is a nitrosoamide-containing diabetogenic agent extensively administered in various doses and via various routes to produce diabetes in experimental models (Wilson et al., 1988; Portha et al., 1989). Hyperglycemia caused by STZ-induced diabetes affects not only β cells but also has a selective effect on immune cells (Wellhausen, 1986). The toxic effects of STZ on T cells and lymphoid cells have been reported to diminish cellular levels in the thymus and lymphoidal tissues (Wellhausen, 1986). STZ reduces the numbers of circulating lymphocytes (Nichols et al., 1981) and inhibits DNA synthesis in the thymus and bone marrow (Wellhausen, 1986; Wood et al., 1999). The toxic effects of STZ have been observed as modest leukopenia at 30 days after a single dose. STZ-treated rats hypersecrete corticosterone as evidenced by their decreased thymus weights (Scribner et al., 1991). Briede et al. (1999) reported that the blood glucocorticoid level is increased in STZ diabetic rats. Elevated systemic levels of glucocorticoids activate a calcium-dependent endogenous endonuclease in thymocytes that lead to apoptosis (Wyllie, 1980). Glucocorticoids suppress the differentiation and proliferation of T and B lymphocytes and downregulate numbers of peripheral lymphocytes (Briede et al., 1999). Glucocorticoids stimulate apoptosis in rat thymocytes (Ichiyoshi et al., 2003). Dexamethasone is a glucocorticoid and

induces apoptosis in cells in the cortex (Fehsel et al., 1994). In our study, the apoptotic cell density in the cortex and medulla of dexamethasone-treated thymus was observed to be high. On the other hand, Tabata et al. (1984) showed a decrease in thymus weight and numbers of lymphocytes in diabetic rats 28 days after STZ injection. It was suggested that impairment of maturation of lymphoid cells in the thymus cortex is induced in STZ diabetes in rats and is associated with the chronic insulin-deficient diabetic state. Their results show that hyperglycemia is effective in lymphoid tissue rather than the toxic effects of STZ. In agreement with previous studies, we also observed the absence of the corticomedullary junction, medullar atrophy and high numbers of apoptotic thymocytes in neonatal STZ diabetics. Our results are in agreement with those of Tabata et al. (1984) and give additional information on impairment of thymic cell maturation at the apoptotic level.

An important feature of the experimental neonatal diabetes model is the mild basal hyperglycemia (Portha et al., 1989; Ozturk et al., 1998). Our findings show that in long term diabetes, chronic hyperglycemia causes a decrease in numbers of cells of the thymus due to apoptosis. Srinivasan et al. (1997) showed that serum glucose levels in STZ-diabetic rats are significantly higher than in controls, and found that treatment with amlodipine as CCB significantly decreased glucose levels in diabetic rats. In our study, hyperglycemia of the STZ-induced neonatal diabetic rats was found to decrease after isradipine treatment. We suggest that chronic hyperglycemia may be an important factor in thymic apoptosis on the basis of the observed decrease in BG levels as a response to isradipine treatment and its association with a decrease in numbers of apoptotic cells. As far as we know, this is the first study evaluating the effects of hyperglycemia on thymic apoptosis in neonatal STZ-induced diabetes in rats.

It has been reported that CCBs induce thymic apoptosis in rats (Balakumaran et al., 1996). The effects of CCBs and $Ca^{2+}$ on apoptosis are complex as both an increase and a decrease in intracellular $Ca^{2+}$ levels can be linked with apoptosis. Accumulating data from animal models of diabetes and patients with diabetes reveal that intracellular calcium levels are increased in most tissues. Activities of membrane-type, ATPase-associated cation pumps, which determine intracellular calcium levels, are also altered. The nature of the alterations is often tissue specific and may depend on the levels of BG and/or insulin (Levy et al., 1994). Intracellular calcium concentrations have been found to increase in STZ-induced diabetic models and this has been explained by inhibition of $Ca^{2+}$-ATPase by hyperglycemia resulting in an increase in calcium ion levels (Chan and Junger, 1984; Tsuji et al., 1993). Furthermore, tight control of calcium concentrations in cells is essential for their vitality. In fact, both an increase and decrease in cellular $Ca^{2+}$ levels have been shown to promote apoptotic cell death. In general, the prevailing view is that elevations in intracellular $Ca^{2+}$ levels may be one of the key signals leading to promotion of apoptosis (Mason, 1999). Similar to the findings of Balakumaran et al. (1996), we found that isradipine treatment alone induces apoptosis in the thymus. The decrease in intracellular calcium due to administration of CCBs is essential for the viability of thymus cells. In the present study, we demonstrated that isradipine, as CCB, has an effective role in preventing apoptosis caused by STZ whether it was given before or after STZ treatment. Isradipine is a blocker of L- and T-type channels and is effective in several cell types. Adrenal glomerulosa cells contain both L- and T-type calcium channels, which are both blocked by isradipine (Yingst et al., 2001). Isradipine is able to reduce both $Ca^{2+}$ release from internal stores and the $Ca^{2+}$ entry in stimulated human endothelial cells (Iouzalen et al., 1995). In the present study, an increase in apoptosis was observed in isradipine-treated rats, but numbers of apoptotic cells were lower in the STZ-group that used given after isradipine treatment. Increased numbers of apoptotic cells were present in the group treated with STZ before isradipine administration. The mechanisms of the protective effects of isradipine against apoptosis are not clear. Isradipine may block the release of $Ca^{2+}$ from intracellular stores and/or decrease BG levels. Isradipine administered before STZ may prevent the increase in intracellular $Ca^{2+}$ levels due to STZ treatment, whereas isradipine blocks apoptosis generated by glucose toxicity after STZ treatment.

Although we detected large numbers of apoptotic cells in the neonatal STZ diabetic group, typical DNA ladder patterns were not observed in the same group after gel electrophoresis. A typical banding pattern was not observed either in the other experimental groups in which higher numbers of apoptotic cells were present as compared with the controls. Large DNA fragments and even single-strand cleavage of DNA have also been suggested to occur during the apoptotic process (Peitsch et al., 1993; Bortner et al., 1995). In our study, a thick band between 123 and 246 bp after isradipine treatment of non-diabetic and STZ-induced diabetic rats corresponds to 180–200 bp

internucleosomal breaks. We also detected dense labelled apoptotic cells in the same tissue sections.

In conclusion, we observed that STZ induces diabetes in neonatal rats and causes marked apoptosis in the thymus. Our results demonstrate that isradipine treatment before STZ has a protective role against apoptosis, whereas this effect of isradipine was not sufficient in preventing apoptosis induced by STZ diabetes.

## Acknowledgment

We thank M.Sc. Ömer Uysal for interpretation of the statistics. This study was supported by the İstanbul University, Research Fund Projects T-323/301096 and B-240/130799.

## References

Balakumaran A, Champbell GA, Maslen MT. Calcium channel blockers induce thymic apoptosis in vivo in rats. Toxicol Appl Pharmacol 1996;139:122–7.

Berggren LJ, Larsson O, Rorsman P, Ammala C, Bokvist K, Wahlander K, Nicotera P, Dypbukt J, Orrenius, Hallberg A, Berggren PO. Increased activity of L-Type $Ca^{+2}$ channels exposed to serum from patients with Type I diabetes. Science 1993;261:86–90.

Blin N, Stafford DW. A general method for isolation of high molecular weight DNA from eukaryotes. Nucl Acids Res 1976;3:2303–8.

Briede J, Daija D, Stivrina M, Duburs G. Effect of cerebrocrast on the lymphocyte blast transformation activity in normal and streptozotocin-induced diabetic rats. Cell Biochem Funct 1999;17:89–96.

Bortner CD, Oldernburg NBE, Cidlowski JA. The role of the fragmentation in apoptosis. Trends Cell Biol 1995;5:21–8.

Chan KM, Junger KD. The effect of streptozotocin-induced diabetes on the plasma membrane calcium uptake activity of rat liver. Diabetes 1984;33:1072–7.

Chandra S, White RF, Everding D, Feuerstein GZ, Coatney RW, Sarkar SK, Barone FC. Use of diffusion-weighted MRI and neurological deficit scores to demonstrate beneficial effects of isradipine in a rat model of focal ischemia. Pharmacology 1999;58:292–9.

Cummings MC, Winterford CM, Walker NI. Apoptosis. Am J Surg Pathol 1997;21:88–101.

Fehsel K, Kroncke KD, Kolb H, Kolb-Bachofen V. In situ nick-translation detects focal apoptosis in thymuses of glucocorticoid and lipopolysaccharide-treated mice. J Histochem Cytochem 1994;42:613–9.

Flynn JT, Pasko DA. Calcium channel blockers: pharmacology and place in therapy of pediatric hypertension. Pediatr Nephrol 2000;15:302–16.

Ichiyoshi H, Kiyozuka Y, Kishimoto Y, Fukuhara S, Tsubura A. Massive telomere loss and telomerase RNA expression in dexamethasone-induced apoptosis in mouse thymocytes. Exp Mol Pathol 2003;75:178–86.

Iouzalen L, Devynck MA, David-Dufilho M. Isradipine affects histamine-induced cytosolic $Ca^{2+}$ movements in human endothelial cells. Eur J Pharmacol 1995;289:189–95.

Kaymaz AA, Tan H, Altug T, Buyukdevrim AS. The effects of calcium channel blockers, verapamil, nifedipine and diltiazem, on metabolic control in diabetic rats. Diabetes Res Clin Pract 1995;28:201–5.

King KL, Schwartzman RA, Cidlowski J. Apoptosis: In life, death and the cell cycle. Endocrinol Metab 1996;3(suppl A):93–7.

Leszczynski D, Zhao Y, Luokkamaki M, Foegh M. Apoptosis of vascular smooth muscle cells. Protein kinase C and oncoprotein Bcl-2 are involved in regulation of apoptosis in non-transformed rat vascular smooth muscle cells. Am J Pathol 1994;145:1265–70.

Levy J, Gavin 3rd JR, Sowers JR. Diabetes mellitus: a disease of abnormal cellular calcium metabolism? Am J Med 1994;96:260–73.

Mason RP. Calcium channel blockers, apoptosis and cancer: Is there a biologic relationship? J Am Coll Cardiol 1999;34:1857–66.

Nichols WK, Vann LL, Spellman JB. Streptozotocin effects on T lymphocytes and bone marrow cells. Clin Exp Immunol 1981;46:627–32.

Ozturk M, Tuncdemir M, Bolkent S, Kaya F, Yılmazer S, Akkan AG, Ozyazgan S, Senses V. Effect of AICA-riboside treatment on aortic alteration in neonatal STZ-induced diabetic rat. In: Maragoudakis ME, editor. Angiogenesis, models, modulators and clinical applications. New York, London: Plenum Press; 1998. p. 541–2.

Peitsch MC, Muller C, Tschopp J. DNA fragmentation during apoptosis is caused by frequent single-strand cuts. Nucl Acids Res 1993;21:4206–9.

Portha B, Blondel O, Serradas P, McEvoy R, Giroix MH, Kergoat M, Bailbe D. The rat models of non-insulin dependent diabetes induced by neonatal streptozotocin. Diabetes Metab 1989;15:61–75.

Ray SD, Kamendulis LM, Gurule MW, Yorkin RD, Corcoran GB. $Ca^{2+}$ antagonists inhibit DNA fragmentation and toxic cell death induced by acetaminophen. FASEB J 1993;7:443–63.

Scribner KA, Walker CD, Cascio CS, Dallman MF. Chronic strepotozotocin diabetes in rats facilitates the acute stress response without altering pituitary or adrenal responsiveness to secretagogues. Endocrinology 1991;129:99–108.

Srinivasan PS, Hakim ZS, Santani DD, Goyal RK. Effects of chronic treatment with amlodipine in streptozotocin-diabetic and spontaneously hypertensive rats. Pharmacol Res 1997;35:423–8.

Szabadkai G, Rizzuto R. Participation of endoplasmic reticulum and mitochondrial calcium handling in

apoptosis: more than just neighborhood? FEBS Lett 2004;567:111–5.

Tabata T, Okuna Y, Fujni S, Kimura S, Kinoshita Y. Maturational impairment of thymic lymphocytes in streptozotocin—induced diabetes in rats. Cell Immunol 1984;89:250–8.

Tsuji K, Taminato T, Ishido H, Okomoto Y, Tsuura Y, Kato S, Kurose T, Okada Y, Imura H, Scino Y. Selective impairment of the cytoplasmic $Ca^{+2}$ response to glucose in pancreatic $\beta$-cells of streptozotocin-induced non-insulin dependent diabetic rats. Metabolism 1993;42:1424–8.

Wellhausen SR. Defination of streptozotocin toxicity for primary lymphoidal tissues. Diabetes 1986;35: 1404–11.

Wilson GL, Hartig PC, Patton NJ, Le Doux SP. Mechanism of nitrosourea-induced $\beta$-cell damage. Diabetes 1988;37:213–6.

Wood SC, Rao TD, Frey AB. Multidose streptozotocin induction of diabetes in BALB/cBy mice induces a T cell proliferation defect in thymocytes which is reversible by interleukin-4. Cell Immunol 1999;192: 1–12.

Wyllie AH. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. Nature 1980;284:555–6.

Yingst DR, Davis J, Schiebinger R. Effects of extracellular calcium and potassium on the sodium pump of rat adrenal glomerulosa cells. Am J Physiol Cell Physiol 2001;280:119–25.