〈Original Article〉

Apoptosis and expression of bcl-2 in cyclosporine induced renal damage and its reversal by beneficial effects of 4', 5', 7'- trihydroxyflavone

Srikumar Chakravarthi[1)], Chong Fu Wen[1)] and Nagaraja Haleagrahara[2)]

Summary Cyclosporine A, a calcineurin inhibitor produced by the fungi *Trichoderma polysporum* and *Cylindrocarpon lucidum*, is an immunosuppressant prescribed in organ transplants to prevent rejection. Its adverse effect of renal dysfunction has limited its use in a clinical setting. Apigenin (4',5',7'-trihydroxyflavone), a herbal extract, with anti-inflammatory and anti-tumour properties, has shown to reverse this adverse effect. This research was conducted to study the effects of apigenin on reversal of cyclosporine A induced damage, and this was assessed by immunohistochemical estimation of expression of bcl-2, and estimation of apoptosis in histopathological sections. Rats were divided into groups and administered with cyclosporine A with apigenin in different doses. The kidneys from the rats were harvested, weighed, and observed for gross pathology changes. The renal tissue was processed, and stained for haemotoxylin and eosin staining, to assess the apoptotic index, and stained by immunohistochemistry, for the analysis of the apoptosis regulatory gene bcl-2. The apoptotic index was then compared with the bcl-2 intensity to observe for any correlation. It was found that there was a high apoptotic index and bcl-2 intensity in the cyclosporine A group. Apigenin managed to reduce the values of both parameters. The apoptotic index correlated with the bcl-2 intensity, especially in the glomeruli. The study proved that cyclosporine A enhanced the expression of bcl-2 in the rat kidney, which signifies accelerated apoptosis. Therefore, bcl-2 and apoptotic index may be used to assess apigenin and its effect on cyclosporine A induced renal damage.

Key words: Cyclosporine, Nephrotoxicity, Apigenin, 4',5',7'-Trihydroxyflavone, Apoptosis, bcl-2

1. Introduction

Cyclosporine A or ciclosporin is widely used as an immunosuppressing agent in organ transplantation. It was formally found in the fungi *Trichoderma polysporum* and *Cylindrocarpon lucidum*[1)]. Besides being prescribed for organ transplant patients, it is also used in patients with rheumatoid arthritis and

1)Department of Pathology, Faculty of Medicine, International Medical University
2)Department of Human Biology, Faculty of Medicine, International Medical University
Received for Publication July 14, 2009
Accepted for Publication July 29, 2009

Address for correspondence & reprint request, contact:
Dr. Srikumar Chakravarthi
Department of Pathology, Faculty of Medicine, International Medical University,
57000 Kuala Lumpur, Malaysia

psoriasis[2]. Cyclosporine A acts by inhibiting interleukin-2 and cytokine production. It is specific for T-lymphocytes and does not affect haematopoietic tissue[3]. Cyclosporine A successfully inhibited rejection in patients who received kidney transplants from mismatched donors, but nephrotoxicity and hepatotoxicity were clearly visible side effects in patients. Other minor side effects of cyclosporine A have been identified as hirsutism, hyperglycemia, hypertension, hyperuricemia, hyperkalemia, hypertrichosis, tremors and gingival hyperplasia. The major advantage of cyclosporine A compared to other immunosuppressant drugs is its lack of bone marrow toxicity. Cyclosporine A acts on proliferating T-cells but not on mature T-cells. In addition, functions of mature B-cell and macrophages remain unaffected by cyclosporine A[4].

Cyclosporine A nephrotoxicity can be characterized by the presence of interstitial fibrosis, isometric tubular vacoalisation and the thickening of arteriolar walls[5, 6]. It may be difficult to distinguish acute cyclosporine A toxicity from acute organ transplant rejection. However, it was suggested that the former can be discriminated from each other by radiological methods[7].

Apigenin (4', 5, 7-trihydroxyflavone), a herbal extract, was reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumour and antioxidant activities[8]. Apigenin has shown to arrest the proliferation of several cancel cell lines through several mechanisms such as by decreasing the expression of bcl-2 and inducing the expression of p53 gene[9]. In addition, studies found that apigenin inhibits the proteosome activity that is required by cancer cells for survival[10]. Apigenin was also found to inhibit the motility and invasiveness of carcinoma cells *in vitro*. This was observed in HeLa wild-type cells and HeLa Cx43 transfectants, which were found to be highly invasive in the control group but were significantly reduced in by apigenin[11]. Apigenin is a useful therapeutic management of inflammatory diseases. Its proposed mechanism is by inhibiting NO-mediated COX-2 expression and monocyte adherence[12]. Apigenin also has an antiallergic property. Hirano et al. discovered that the anti allergic property of apigenin was due to the inhibition of IL-4 and IL-13 production by apigenin. IL-4 and IL-13 are cytokines produced by basophils that lead to an allergic reaction[13]. Apigenin has shown to reverse the adverse effect of cyclosporine A on the kidney in preliminary studies. This research was conducted as a pioneer one to study the effects of apigenin on reversal of cyclosporine A induced damage, and this was assessed by immunohistochemical estimation of expression of bcl-2, and estimation of apoptosis in histopathological sections.

Members of the bcl-2 family of proteins are important regulators of programmed cell death pathways with individual members that can suppress (e.g., bcl-2, bcl-XL) or promote (e.g., Bax, Bad) apoptosis. While the mechanism(s) of bcl-2's anti-apoptotic function is not yet clear, introduction of bcl-2 into most eukaryotic cell types will protect the recipient cell from a wide variety of stress applications that lead to cell death[14]. Augmented bcl-2 expression may be one of the important regulators of this apoptosis in the glomeruli of the kidneys in rats[15]. This may help assess the extent of renal damage that has occurred.

Apoptosis is the physiological death of a cell and is tightly regulated intracellularly. Apoptosis does not induce an immune response compared to necrosis, as the cells are quickly cleared up by phagocytes. The cell activates intracellular enzymes with function to digest or degrade the cell's own nuclear DNA and proteins of nuclear as well as cytoplasmic origin. An apoptotic cell is identified as a cell with membrane blebbing, condensed chromatin and cell shrinkage[17].

2. Materials and methods

1. Animals

Male Sprague-Dawley albino rats were used for the research, after due ethical committee approval. They were acquired from the Institute of Medical Research (IMR), Malaysia. They were 6-8 weeks old, weighed between 200-250 grams and were fed with standard rat chow and free access to water. The weight of the rats was recorded once every week. Cyclosporine A was acquired from Novartis, Switzerland and was used at dosage of 25 mg/mL. Apigenin was acquired in powdered form from Sigma

Table 1 Groups used in this research and their respective administered drugs

Groups	Dosage
Group 1 - Control	Corn oil
Group 2 - CsA	Cyclosporine A at 25 mg/kg body weight
Group 3 - Apg 20	Apigenin at 20 mg/kg body weight
Group 4 - CsA + Apg (10)	Cyclosporine A at 25 mg/kg body weight + Apigenin at 10 mg/kg body weight
Group 5 - CsA + Apg (15)	Cyclosporine A at 25 mg/kg body weight + Apigenin at 15 mg/kg body weight
Group 6 - CsA + Apg (20)	Cyclosporine A at 25 mg/kg body weight + Apigenin at 20 mg/kg body weight

CsA: Cyclosporine A.

Table 2 Grading of percentage of bcl-2 expression[18)]

Grade	Percentage of cells in tissue expressing bcl-2
0	0% of the cells expressed bcl-2
+	Less than 33% of the cells expressed bcl-2
++	34%-66% of the cells expressed bcl-2
+++	More than 67% of the cells expressed bcl-2

Aldrich, with a purity of 98%. Apigenin was prepared at 3 different doses, 10 mg/mL, 15 mg/mL and 20 mg/mL.

2. Groups and sample collection

There were a total of 6 experimental groups for this study (Table 1). Rats were dosed every 24 hours for 21 days. After the 21st day, they were sacrificed and their kidneys harvested. The gross morphology was observed, and the kidneys were then stored in 10% formalin. They were weighed (Mettler Toledo College B204-5) and measured using a caliper before they were sliced, processed and mounted on paraffin blocks.

3. Staining procedure

The paraffin blocks were sectioned and slides stained with haematoxylin and eosin. Mouse monoclonal (Dako) antibody to bcl-2 diluted at a ratio of 1:50 (pH 7.6, 90 minutes, room temperature) was used for immunohistochemical staining of the paraffin blocks, after target retrieval at 95℃ for 45 minutes. The slides were observed for the expression of bcl-2 in the glomeruli and tubules using a Nikon brightfield light microscope. Images were captured with a 5.1 megapixel evolution MP digital camera. Image Proexpress software was used to process the images. The images were then analysed.

4. Analysis of sections

Apoptotic index in H&E sections : The apoptotic index was evaluated in the haematoxylin and eosin (H&E) stained slides. The number of apoptotic cells were counted in the glomeruli (500 cells) and tubules (500 cells) of the tissues. The apoptotic cells were identified as cells shrinkage, deeply eosinophilic cytoplasmic staining and dense nuclear staining compared to normal cells due to the chromatin condensation. The cells were counted by selecting the first suitable field from the left side of the tissue and moving the stage towards the right side.

bcl-2 expression by immunohistochemistry: Cells that expressed bcl-2 positivity were characterized by a brownish bronze coloured pigmentation within the cytoplasm. Slides that exhibited positivity were categorised as (+), (++) or (+++) depending on the percentage of the staining. Slides that were negative for bcl-2 were labelled as 0 (Table 2).

5. Statistical analysis

In this study, 30 samples were studied and analysed. Statistical tests that were used in the experiment were:

a) Kruskal-Wallis test for global comparison of groups

b) Non parametric Mann-Whitney U test for comparison of apoptotic index in different groups

c) Spearman's rho test for correlation between

Table 3 The average weight and average volume of kidneys in each group with the average Apoptotic Index and the p-values of the groups when compared with group 1 and group 2. Statistical method used was the Mann-Whitney U test

Group	Average Weight	Average Volume	Average Apoptotic Index	*p-value* of Apoptotic Index (vs. Group 1)	*p-value* of Apoptotic Index (vs. Group 2)
Group 1	2.516	1.168	21.0	-	0.009**
Group 2	1.998	1.072	59.4	0.009**	-
Group 3	2.578	1.218	19.8	0.530	0.009**
Group 4	2.126	1.238	34.0	0.009**	0.021*
Group 5	1.606	1.041	29.4	0.028*	0.009**
Group 6	1.873	1.276	22.0	0.623	0.014*

** Significant difference at $p < 0.01$
* Significant difference at $p < 0.05$

Table 4 The median bcl-2 expression in the glomeruli and tubules as well as the results of the Mann-Whitney U test conducted for comparison between 2 groups for bcl-2 expression

Group	Glomeruli bcl-2 expression	*p-value* in glomeruli (vs Group 1)	*p-value* glomeruli (vs Group 2)	Tubules bcl-2 expression	*p-value* in the tubules (vs Group 1)	*p-value* in the tubules (vs Group 2)
Group 1	1+	-	0.007*	2+	-	0.014*
Group 2	3+	0.007*	-	3+	0.014*	-
Group 3	2+	0.189	0.054	3+	0.058	0.221
Group 4	2+	0.015*	0.072	3+	0.058	0.221
Group 5	2+	0.065	0.065	3+	0.058	0.221
Group 6	1.5+	0.371	0.018*	1.5+	0.777	0.018*

* Significant difference at $p < 0.05$

apoptotic index and expression of bcl-2

The statistical tests were employed on the data using SPSS software. For all individual tests, a *p-value* of less than 0.05 ($p<0.05$) was taken and considered as significant.

3. Results

1. Weight and volume of kidneys and apoptotic index

From the Table 3, it was observed that Group 1 has significant difference compared to Group 2 ($p<0.01$), Group 4 ($p<0.01$) and Group 5 ($p<0.05$). These 3 groups were administered with cyclosporine A. This shows that cyclosporine A has caused significant apoptosis in the kidney. Group 3 showed no significant change compared to Group 1 as Group 3 was administered with apigenin only. This shows that apigenin has no significant morphological effects on the kidney. Although Group 6 was administered with cyclosporine A, there was no significant difference when compared to Group 1. This is probably due to the high apigenin dosage administered in this group. This shows that apigenin at a dose of 20 mg/kg body weight successfully protected the kidneys from the apoptotic damage caused by cyclosporine A.

Group 2 was also compared to Groups 3, 4, 5 and 6 and showed significant difference to the other groups. Group 2 was administered with cyclosporine A and no additional drugs. This proved that cyclosporine A has tremendous detrimental effects on the kidneys leading to severe apoptosis of cells in the kidneys. Apigenin, which was given in groups 4, 5 and 6 along with cyclosporine A managed to protect the kidneys from the toxic effects of cyclosporine A, thus showing a significant statistical value when compared with Group 2.

2. Correlation of kidney weights, kidney volumes and apoptotic index

Non-parametric Spearman's rho test was utilized to determine if there was any correlation between the average kidney weight, average kidney volume and average apoptotic index in all groups and showed the kidney weight and volume were positively correlated to one another.

3. Immunohistochemistry for bcl-2 expression

Immunohistochemistry staining was performed to evaluate the expression of bcl-2 in the slides (Fig. 1). The grading was assessed on the percentage of cells that expressed bcl-2[18] (Table 4). Group 2 has the highest median value while Group 1 and Group 6 have the lowest median value. In Group 3, the apigenin 20 group, showed a moderately high bcl-2 expression. From the Mann-Whitney U test conducted, it was found that there was a significant difference in both the glomerular and tubular areas of the kidney in when group 1 was compared to group 2 and when group 2 was compared to group 6. However, there was significant difference only in the glomerular area when group 1 was compared to group 4. In the rest of the group comparisons, it was found that there was no significant difference between the groups.

4. Correlation of apoptotic index and expression of bcl-2

The median of both apoptotic index and bcl-2 expression was used to compare and to examine if any correlations existed between the two parameters. Non-parametric Spearman¬s rho test was also used to find out if there was any correlation between the apoptotic index and bcl-2 expression. For each group, the median value was used. Glomerulus apoptotic index vs. glomerulus bcl-2 expression had a correlation coefficient of 0.820. Tubules apoptotic index vs. tubules bcl-2 expression had a correlation coefficient of 0.278. The values show that there was a positive correlation in both the comparisons. In the glomeruli, the apoptotic index (from H&E staining) has a strong correlation with the expression of of bcl-2 (from immunohistochemistry staining).

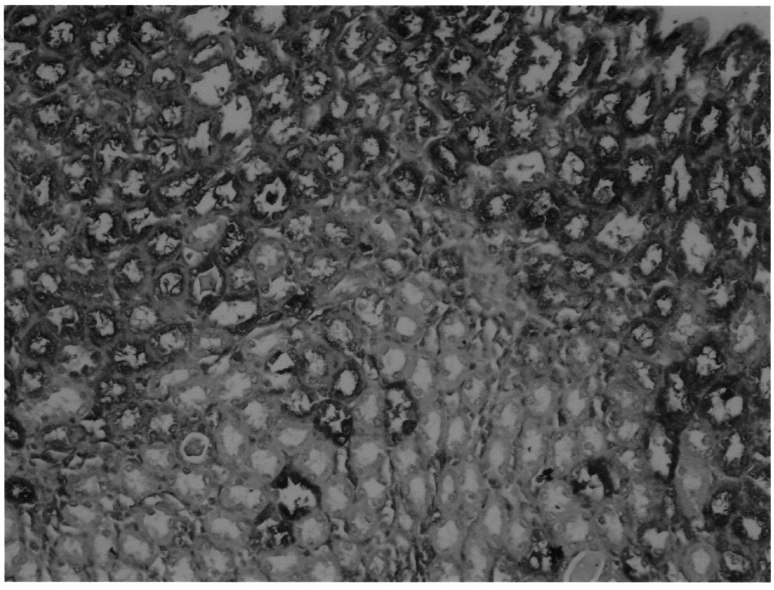

Fig. 1 Photomicrograph at 200x magnification showing tubules with diffuse grade +++ staining for bcl-2 (stained by immunohistochemistry).

4. Discussion

Apoptosis & Cyclosporine A induced nephrotoxicity

Numerous studies have been conducted to examine the clinical possibilities of apigenin. However, its association with apoptosis and bcl-2 has not been studied yet. Apoptosis is a natural process of the body to eliminate unwanted or potentially harmful cell and cells that has outlasted its usefulness or importance. It occurs naturally in many situations[17]. From our research conducted, the results showed that administration of cyclosporine A increases the apoptotic index in rat kidneys (Table 3). Consequently, the administration of apigenin successfully reduced the apoptotic index significantly. This shows that apigenin may have a protective effect at a very high dose. As described in the methodology, in our study, the apoptotic index was calculated by an accurate method, which was counting the apoptotic cells in 400x and reconfirming by 1000x using oil immersion. This was done for every 1000 cells in the glomeruli and tubules in each case. The total apoptotic index was then calculated for the glomeruli and tubules, and statistically evaluated. Although the mechanisms of cyclosporine A nephrotoxicity are not fully known, few studies have shown that the toxicity produces glomerular and tubular damage[20]. One of these manifestations is by inducing apoptosis in cells, which resulted ultimately in the atrophy of the glomeruli and tubules, in addition to focal interstitial changes[19]. This feature was also confirmed by our study, where the apoptotic index was increased in rats treated with cyclosporine A, during the analysis of the histopathological sections. The cells included in the category of apoptotic cells in our study were cells with various stages of the process: cells displaying chromatin condensation; cells with densely eosinophilic cytoplasm and shrunk nuclei; and cells with cytoplasmic blebs and nuclear fragments, forming apoptotic bodies. Our study has shown that Apigenin, when combined with cyclosporine A therapy, was able to prevent the glomerular and tubular changes to a significant extent, which was reflected by the variation in the apoptotic indices in the various groups.

bcl-2 and its role in apoptosis

bcl-2 protein was stained in normal with greater intensity and focally in diseased renal tissue, showing an analogous expression. The antigen was expressed in a few parietal epithelial cells, in scattered proximal tubular epithelial cells, and in the majority of distal and collecting tubular epithelial cells, but not in the glomerular capillary tuft. The pattern of bcl-2 expression in normal and diseased glomeruli suggests and supports the reported notion that the mechanism of apoptosis may be increased in the injured glomerulus[16].

Based on the non-parametric Spearman's test conducted, there was a correlation between the apoptotic index and bcl-2 expression, especially in the glomeruli. This showed that the damage to the glomeruli and tubules in cyclosporine A mediated toxicity was facilitated through the expression of bcl-2. This expression of bcl-2 in turn reflects the increased apoptotic death of the cells. These findings also correlated well with studies conducted previously[14, 15, 16].

There are a number of theories concerning how the bcl-2 gene family exert their pro- or anti-apoptotic effect. An important one states that this is achieved by activation or inactivation of an inner mitochondrial permeability transition pore, which is involved in the regulation of matrix Ca^{2+}, pH, and voltage. It is also thought that some bcl-2 family proteins can induce (pro-apoptotic members) or inhibit (anti-apoptotic members) the release of cytochrome c into the cytosol which, once there, activates caspase-9 and caspase-3, leading to apoptosis[21].

Another theory suggests that Rho proteins play a role in bcl-2, Mcl-1 and Bid activation. Rho inhibition reduces the expression of anti-apoptotic bcl-2 and Mcl-1 proteins and increases protein levels of pro-apoptotic Bid but had no effect on Bax or FLIP levels. Rho inhibition induces caspase-9 and caspase-3-dependent apoptosis[22].

The members of the bcl-2 family share one or more of the four characteristic domains of homology entitled the bcl-2 homology (BH) domains (named BH1, BH2, BH3 and BH4). The BH domains are known to be crucial for function. The anti-apoptotic

bcl-2 proteins, conserve all four BH domains. The BH domains also serve to subdivide the pro-apoptotic bcl-2 proteins into those with several BH domains (e.g. Bax and Bak) or those proteins that have only the BH3 domain (e.g., Bid, Bim and Bad). Depending on their function, once activated, bcl-2 proteins either promote the release of these factors, or keep them sequestered in the mitochondria. Either the activated pro-apoptotic Bak and/or Bax would mediate the release of cytochrome c, or the anti-apoptotic bcl-2 would block it, possibly through inhibition of Bax and/or Bak. So in our study, the relationship of bcl-2 and apoptosis is due to the pro-apoptotic action, which correlates well with few other studies[22, 23].

Evaluation of the bcl-2 expression was done immunohistochemically to give additional evidence on the renal damage of cyclosporine A and also to observe the less extent of damage in the cyclosporine A with apigenin group. Hence, it was used as a confirmatory parameter. Although statistical comparison was not necessary, however, when it was done, it showed an acceptable correlation.

Moreover, from the observation of the median, there may be a change in the percentage of expression. Therefore, findings can be continued with a larger sample size. Although all doses of apigenin were beneficial to the morphology of the kidney, it was found that the ideal dosage of apigenin in reversal of cyclosporine A toxicity was 20 mg/kg body weight. Apigenin by itself at the dose of 20 mg/kg did show a high expression of bcl-2. This may suggest that apigenin can ideally be used in association with cyclosporine A rather than being given alone. This also gives scope for future studies into the effects of apigenin when given alone.

The weight and volume of the kidneys are directly proportional to each other. The apoptotic index and over expression of bcl-2 showed a significant correlation to both kidney weight and volume. Both these parameters adhered well with our hypothesis.

Limitations to this study

This study focused more on the expression of bcl-2, which was used as a parameter to assess the renal damage due to cyclosporine A. Hence, the sub-members of the bcl-2 family were not separately analyzed. A deeper analysis would reveal the anti- and pro-apoptotic nature of the bcl-2 family, such as Bad, Bax, Bid, Bim and Bak. Further studies on the mitotic index may also help to analyse the comparison between mitosis and apoptosis, and strengthen the evidence for our hypothesis.

Acknowledgments

This research was funded by research grants from the International Medical University, Kuala Lumpur, Malaysia.

References

1) Borel JF, Feurer C, Magnée C and H Stähelin: Effect of the new-anti-lymphocytic peptide cyclosporin A in animals. Immunology, 32: 1017-1025, 1977
2) Webster AC, Woodroffe RC, Taylor RS, Chapman JR and Craig JC: Tacrolimus versus ciclosporin as primary immune suppression for kidney transplant recipients: meta-analysis and meta-regression of randomized trial data. BMJ, doi:10.1136/bmj.38569. 471007. AE, 2005
3) Edited by Delves PJ, Martin SJ, Burton DR: Ivan Roitt: Activation induced cell death. Roitt's Essential Immunology, 11th ed. 364-366, Blackwell Publishing, USA (2007)
4) Calne RY, White DJ, Thiru S, Evans DB, McMaster P, Dunn DC, Craddock GN, Pentlow BD and Rolles K: Cyclosporin A in patients receiving renal allografts from cadaver donors. Lancet, 2: 1323-1327, 1978
5) Edited by Fauci AS, Braunwald E, Isselbacher KJ, Wilson JD, Martin JB, Kasper DL, Hauser SL, Longo DL and Harrison TR: Carpenter CB, Milford EL and Sayegh MH: Transplantation in the Treatment of Renal Failure. Harrison's Internal Medicine, 17th ed. Chap 276, 1779- 1782, McGraw-Hill, USA (2008)
6) Shihab FS, Andoh TF, Tanner AM, Yi H and Bennett WM: Expression of apoptosis regulatory genes is chronic cyclosporine nephrotoxicity favors apoptosis. Kidney Int., 56: 2147-2159, 1999
7) Edited by Haeney M, Misbah S and Snowden N: Helen Chapel: Tissue damage caused by the immune system. Essentials of Clinical Immunology. 5th ed. 146-148, Blackwell Publishing, USA (2006)
8) Buhler DR and Miranda C: Antioxidant Activities of Flavonoids. The Linus Pauling Institute, Oregon State University website. November 2000. http://lpi.oregon-state.edu/f-w00/flavonoid.html.
9) Zheng PW, Chiang LC and Lin CC: Apigenin induced

apoptosis through p53-dependent pathway in human cervical cancer cells. Life Sci., 76: 1367-79, 2005

10) Chen D, Daniel KG, Chen MS, Kuhn DJ, Landis-Piwowar KR and Dou QP: Dietary flavonoids as proteosome inhibitors and apoptosis inducers in human leukemia cells. Biochem. Pharmacol., 69: 1421-1432, 2005
11) Czyz J, Madeja Z, Irmer U, Korohoda W and Hülser DF: Flavonoid apigenin inhibits motility and invasiveness of carcinoma cells in vitro. Int. J. Cancer, 114: 12-18, 2005
12) Lee JH, Zhou HY, Cho SY, Kim YS, Lee YS and Jeong CS: Anti-inflammatory mechanisms of apigenin: inhibition of cyclooxygenase-2 expression, adhesion of monocytes to human umbilical vein endothelial cells, and expression of cellular adhesion molecules. Arch. Pharm. Res., 30: 1318-1327, 2007
13) Hirano T, Higa S, Arimitsu J, Naka T, Shima Y, Ohshima S, Fujimoto M, Yamadori T, Kawase I and Tanaka T: Flavonoids such as luteolin, fisetin and apigenin are inhibitors of interleukin-4 and interleukin-13 production by activated human basophils. Int. Arch. Allergy Immunol., 134: 135-140, 2004
14) Ruvolo PP, Deng X, and May WS: Phosphorylation of Bcl2 and regulation of apoptosis of Bcl2 and regulation of apoptosis. Leukemia, 15: 515-522, 2001
15) Murata I, Takemura G, Asano K, Sano H, Fujisawa K, Kagawa T, Baba K, Maruyama R, Minatoguchi S, Fujiwara T and Fujiwara H: Apoptotic cell loss following cell proliferation in renal glomeruli of Otsuka Long-Evans Tokushima Fatty rats, a model of human type 2 diabetes. Am. J. Nephrol., 22: 587-595, 2002
16) Nakopoulou L, Stefananki K, Papadakis J, Boletis J, Zeis PM, Kostakis A and Vosnides G: Expression of bcl-2 oncoprotein in various types of glomerulonephritis and renal allografts. Nephrol. Dial. Transplant., 11: 997-1002, 1996
17) Shihab FS, Andoh TF, Tanner AM, Noble NA, Border WA, Franceschini N and Bennett WM: Role of transforming growth factor β1 in experimental chronic cyclosporine nephropathy. Kidney Int., 49: 1141-1151, 1996
18) Edited by Kumar V, Abbas AK and Fausto N: Stanley RL: Cellular adaptations, cell injury and cell death. Robbins & Cotran Pathologic Basis of Disease. 7th ed. 26-32, Elsevier Saunders, USA (2005)
19) Janku F, Novotny J, Julis I, Julisova I, Pecen L, Tomancova V, Kocmanova G, Krasna L, Krajsova I, Stork J and Petruzelka L: KIT receptor is expressed in more than 50% of early stage malignant melanoma: a retrospective study of 261 patients. Melanoma Res., 15: 251-256, 2005
20) Thomas SE, Andoh TF, Pichler RH, Shankland SJ, Couser WG, Bennett WM and Johnson RJ: Accelerated apoptosis characterizes cyclosporine-associated interstitial fibrosis. Kidney Int., 53: 897-908, 1998
21) Kinnally KW and Antonsson B: A tale of two mitochondrial channels, MAC and PTP, in apoptosis. Apoptosis, 12: 857-868, 2007
22) Hippenstiel S, Schmeck B, N'Guessan PD, Seybold J, Krüll M, Preissner K, Eichel-Streiber CV and Suttorp N: Rho protein inactivation induced apoptosis of cultured human endothelial cells. Am. J. Physiol. Lung Cell Mol. Physiol., 283: L830-838, 2002
23) Dejean LM, Martinez-Caballero S, Manon S and Kinnally KW: Regulation of the mitochondrial apoptosis-induced channel, MAC, by BCL-2 family proteins. Biochim. Biophys. Acta, 1762: 191-201, 2006