Nuclear Science and Techniques **21** (2010) 357–361

Re-canalized stenostic rabbit nasolacrimal duct by the ^{125}I radioactive probing: Affecting Bcl-2 and Bax protein expression

GUO Pengde[1] MA Qingjie[1] YANG Dongyan[1] GAO Shi[1] JIN Longyun[2]
ZHOU Xueyan[1] CUI Qu[1] HAN Zhenguo[1,*] ZHAO Guoqing[1,*]

[1]*China-Japan Union Hospital of Jilin University, Changchun 130033, China*

[2]*Hong Qi Hospital of Mudanjiang Medical College, Mudanjiang 157011, China*

Abstract To evaluate the ^{125}I radioactive probing re-canalizing stenostic nasolacrimal duct, the nasolacrimal duct stenosis models in epithelium and connective tissues are experimentally structured by inbred white rabbits (New Zealand), including the nasolacrimal duct stenosis, the mechanical probing with outer layer of thermoplastic tube, and the ^{125}I radioactive probing with the ^{125}I seeds sealing into the thermoplastic tube. After re-canalized for four weeks, tissue specimens from bilateral nasolacrimal ducts are obtained, and the Bcl-2 and Bax protein expression levels are evaluated by immunohistochemical staining analysis. Comparing with the blank control, the expression levels of the Bcl-2 and Bax in the nasolacrimal duct stenosis and the mechanical probing are significantly up-/down-regulated ($p<0.05$), but in the ^{125}I radioactive probing are down-/up-regulated ($p<0.05$) and can be used to re-canalize the stenostic lacrimal passage. The results show that the ^{125}I radioactive probing is a therapeutical mechanism for radioactive probing strategy for treating nasolacrimal duct stenosis to induce cell apoptosis.

Key words Lacrimal passage, Radiation, Bcl-2 and Bax, ^{125}I radioactive probing.

1 Introduction

Dacryagogatresia is a refractory disease in aged populations. The hyperplastic tissues in nasolacrimal duct can block the physiological pathway for lacrimal fluids, thus disturbing the patient life quality and likely causing other ophthalmologic disorders. In clinical practice curing the patients, several surgical strategies have been applied to treat nasolacrimal obstruction and nasolacrimal duct stenosis, such as laser and high-frequency electric cautery, nasolacrimal-stent implantation, nasolacrimal ostomy, and dacryocystectomy[1]. However, the laser and high- frequency electric cautery induce fibrous scars, and results in a local hemorrhage due to their surgical trauma. Other physical probing methods, which may accompany with such complications as epithelial cells and fibroblasts hyperplasia, can impair the functional recovery of nasolacrimal duct. On receiving common surgical re-canalization treatment, post-operational re-stenosis and/or re-dacryagogatresia emerge as a tough complication for patients[2].

Brachytherapy has been used widely in clinic to treat post-operational scars, subcutaneous angeioma, and coronary artery restenosis after PTCA, and this renders the radionuclide-based trials effective for the obstructive diseases in the nasolacrimal duct system. For example, the ^{125}I radioactive probing trial on nasolacrimal duct targets at stenosis and prevents re-stenosis from the epithelial and fibroblastic hyperplasia[3,4]. Besides the regular and physical re-canalization, the radioactive probing exerts the brachytherapy affect on adjacent tissues to induce the tissue shrinkage and re-canalize the stenostic nasolacrimal duct.

To the authors' knowledge, molecular mechanisms for the ^{125}I radioactive probing have not been clarified on its clinical application. In this

Supported by Natural Science Foundation of Jilin Province (No.200705327) and Project of Science and Technology of Changchun City (2007GH25).

* Corresponding author. *E-mail address*: zhaoguoqing@yahoo.com.cn; cpi2008@tom.com

Received date: 2010-08-01

study, we prepared the rabbit stenostic nasolacrimal duct models, evaluated expression profiles of apoptosis associated with the Bcl-2 and Bax protein in regional tissues, and revealed the correlated mechanism with the ^{125}I radioactive probing.

2 Material and method

2.1 Laboratory animal and instrument

The forth-five inbred white rabbits (New Zealand, 2.5±0.5 kg, 8-12 weeks old) were provided by the Animal Department of Jilin University. Optic microscope (Shimadzu BX41-72H02) was from Olympus Company, Japan. GE advantx LCV 1250 mA large digital subtraction angiography (DSA) applicator was from GE Healthcare, USA. Computer image analysis system (HPIAS-100) was from Qian Ping Video Works, Tongji Medical University, China. RM-905a activity counter was from China Calculation Technology Development Company. Mouse anti-rabbit Bcl-2 and Bax antibody kit was from the Wuhan Boshide Biotechnology Corp., Ltd.

2.2 Nasolacrimal duct stenosis models

To the inbred white rabbits, bilaterally nasolacrimal duct stenosis was prepared by hooked probe scratch method[5]. All the rabbits were fed regularly until the epiphora symptom occurred. The nasolacrimal duct stenosis located at 4-mm lacrimal punctum was obtained by the DSA analysis, and nasolacrimal duct tissues were collected after 30 days. The 30 rabbits with nasolacrimal duct stenosis were selected randomly to construct three models of 10 rabbits in each group, i.e. the control group, the group of mechanical probing model with outer layer of thermoplastic tube, and the group of ^{125}I probing model with the seeds sealing into the thermoplastic tube at 7–8 Gy (see Ref.[6] for detailed description). Five inbred white rabbits without nasolacrimal stenosis were used as the blank control.

2.3 Immunohistochemical and Statistical analysis

After the two probings as a marker were inserted into the nasolacrimal duct stenosis, the whole lacrimal models were peeled off by formalin before dehydration, and imbedded into paraffin, so as to make slices. According to stain reagent instruction, the positive slices were yellowish brown or brown, observed by optic microscope. Using the mouse anti-rabbit Bcl-2 and Bax antibody kit, the immuno-histochemical images were collected by computer image analysis system. The integral optical density (IOD) of epithelial cells and connective cells were further measured. All data of models were analyzed with the SPSS 10.0 software package and compared by t-test.

3 Results

3.1 Bcl-2 protein expression profile

The Bcl-2 protein in all models was constitutively expressed, and has a higher expression level in epithelium than in connective tissues. Fig.1 shows that the Bcl-2 protein expression of nasolacrimal duct stenosis. The mechanical probing are higher than those of the blank control in epithelium and connective tissues (p<0.05), and the levels of two models are basically the same (p>0.05). However, the Bcl-2 protein expression in the ^{125}I radioactive probing was remarkably lower than in the blank control (p<0.05). As can be seen in Table 1, the Bcl-2 expression in the ^{125}I radioactive probing is quantitatively down-regulated, reflecting an attenuated proliferating status in the target tissues.

3.2 Bax protein expression profile

Also, the Bax protein in all models was constitutively expressed, and had a higher expression in epithelium than in connective tissues. Fig.2 shows that the Bax protein expression of nasolacrimal duct stenosis and mechanical probing are lower than those of the blank control in epithelium and connective tissues (p<0.05), and both levels of models does not significantly vary (p>0.05). But the Bax protein expression of the ^{125}I radioactive probing is significantly higher than those of the blank control in epithelium and connective tissues (p<0.05). Table 2 shows that the Bax expression in the target tissues of radioactive probing is also up- regulated, indicating an enhanced apoptotic status.

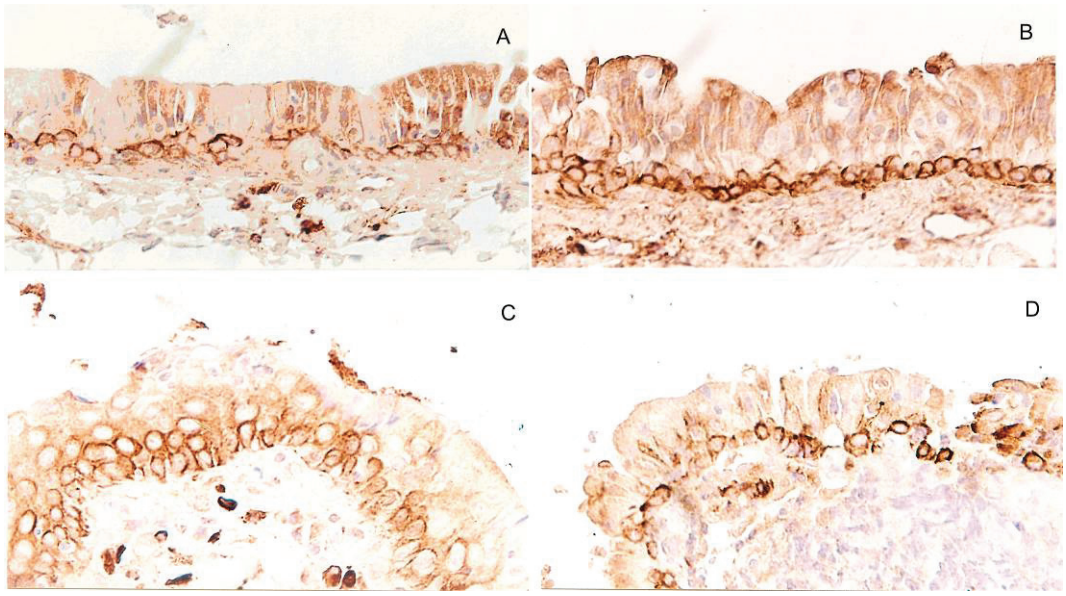

Fig.1 Bcl-2 protein expression in rabbit lacrimal passage (× 400) after 30 days. Blank control (A), nasolacrimal duct stenosis (B), and mechanical probing (C) and ^{125}I radioactive probing (D).

Table 1 The IOD value of Bcl-2 protein in epithelium and connective tissues ($\chi\pm s$).

Groups	*n*	Epithelium	Connective tissues
Blank control	10	70.9±8.1	34.2±3.3
Nasolacrimal duct stenosis	20	93.8±7.9[a]	48.5±3.6[b]
Mechanical probing	20	91.6±7.4[c,g]	46.3±3.1[d,h]
^{125}I radioactive probing	20	55.1±8.6[e]	25.2±2.8[f]

Note: Comparing with blank control, t values are 7.42 (a), 10.53 (b), 7.00 (c), 9.87(d), 4.83 (e), and 7.82(f) ($p<0.05$), and comparing with nasolacrimal duct stenosis, t values are 0.91(g) and 1.77 (h) ($p>0.05$), respectively.

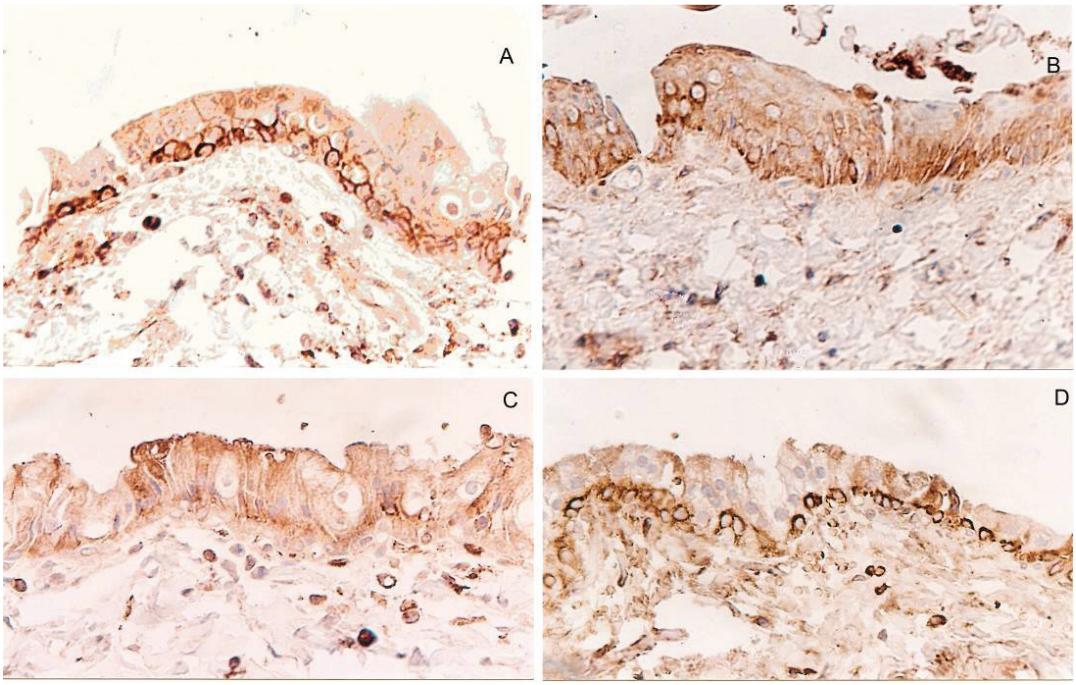

Fig.2 The Bax protein expression in rabbit lacrimal passage (× 400) after 30 days. Blank control (A), nasolacrimal duct stenosis (B), and mechanical probing (C) and ^{125}I radioactive probing (D).

3.3 Ratios of Bcl-2/Bax protein

The ratios of Bcl-2/Bax proteins in epithelium and connective tissues reflect the balanced profile between tissue proliferation and apoptosis. Table 3 shows that the two ratios in the nasolacrimal duct stenosis and mechanical probing are higher than that in the blank control (p<0.05), but they are a little higher in connective tissues than in epithelium (p>0.05), indicating an enhanced proliferation status in these tissues. However, the tow ratios in the ^{125}I radioactive probing is lower than that in the blank control (p< 0.05), this demonstrates that both epithelium and connective tissues in the lacrimal passage undergo a low-proliferation but high-apoptosis process.

Table 2 The IOD value of Bax protein in epithelium and connective tissues (χ±s).

Groups	*n*	Epithelium	Connective tissues
Blank control	10	63.3±4.5	30.9±2.4
Nasolacrimal duct stenosis	20	48.9±3.6[a]	20.9±2.7[b]
Mechanical probing	20	51.1±3.9[c,g]	21.3±2.6[d,h]
^{125}I radioactive probing	20	76.6±2.3[e]	36.2±1.8[f]

Note: Comparing with blank control, t values are 9.50 (a), 9.90 (b), 7.68 (c), 9.77(d), 10.81(e), and 6.80 (f) (p<0.05), and comparing with stenosis control, t values are 1.85 (g) and 0.48 (h) (p>0.05), respectively.

Table 3 Protein Bcl-2/Bax ratios in epithelium and connective tissues (χ±s).

Groups	*n*	Epithelium	Connective tissues
Blank control	10	1.1±0.2	1.1±0.2
nasolacrimal duct stenosis	20	1.9±0.2[a]	2.3±0.3[b]
Mechanical Probing	20	1.8±0.2[c,g]	2.2±0.3[d,h]
^{125}I radioactive probing	20	0.7±0.2[e]	0.7±0.2[f]

Note: Comparing with blank control, t values are 10.33 (a), 11.34 (b), 9.04 (c), 10.45 (d), 5.16 (e), and 5.16(f) (p<0.05), and comparing with stenosis control, t values are 1.58 (g) and 1.24 (h) (p>0.05), respectively.

4 Discussion

In biological process, cell proliferation and apoptosis are crucial to tissue maintenance regulated by programmed protein cascades[7]. The hyperplasia tissues in the stenostic nasolacrimal duct always have the hyper-function in cell proliferation and apoptosis reducing[6]. A group of proteins for the Bcl-2 family are associated with cell proliferation and apoptosis, and the Bcl-2 protein can inhibit apoptotic process, and prevent cell membrane rupture, cell body shrinkage, cell nuclear enrichment, and DNA endogenous hydrolysis, thus causing the cells to enter G_0 phase instead of death[8–10]. On the contrary, the Bax protein has a 21% homology to the Bcl-2 can oppositely induce the apoptosis owing to the Bcl-2 protein inhibiting[11,12]. Under physical circumstances, the Bcl-2 and Bax proteins in normal tissues maintain a certain proportion, but the disturbance of both ratio can break the balanced signal strength and affect the process in regulating apoptosis[13].

Our results in epithelium and connective tissues show that the Bcl-2 protein is higher, and the Bax protein is lower in both nasolacrimal duct stenosis and mechanical probing than those in blank control. Thus, nasolacrimal duct stenosis may result from the irregular balance between the Bcl-2 and Bax protein, and the Bcl-2/Bax ratio increasing makes the apoptosis inhibition up-regulate, and apoptosis induction down-regulate[11] to increase the cells of epithelial and connective tissue, thus leading to the nasolacrimal duct hyperplasia, stenosis and eventually obstruction[12].

Tables 2 and 3 show that the mechanical probing cannot affect Bcl-2 and Bax protein, this further confirms that it just reduces local tissue mass around stenosis area and relieves the stenosis, but not regulating the apoptosis[14]. We have reported that the ^{125}I radioactive lacrimal probing has the same

mechanical and in situ radiation effects as the mechanical probing[3]. Here, the Bcl-2 and Bax protein of the ^{125}I radioactive probing are lower and higher than those of blank control, indicating that its radiation can significantly change the Bcl-2/Bax ratio in lacrimal passage and induce apoptotic process, to make lacrimal lumen recover and fundamentally cure the nasolacrimal duct stenosis.

5 Conclusions

The ^{125}I radioactive probing can reduce the Bcl-2 protein expression and enhance the Bax protein expression in the hyperplastic nasolacrimal duct of inbred rabbit to down regulate Bcl-2/Bax ratio and promote apoptosis. The ^{125}I lacrimal probing may be one probable mechanism and potential strategy for lacrimal passage stenosis re-canalizing and a prophylactic trial of restenosis.

References

1 Fayet B, Racy E, Assouline M. Ophthalmology, 2004, **111:** 837–845.

2 Sener EC, Onerci M. Int J Pediatr Otorhinolaryngol, 2001, **58:** 65–68.

3 Gao S, Ma Q J, Zhang B J. Chin J Nucl Med, 2007, **27:** 297–299.

4 Jin L Y, Zhao J, Ma Q J. Chin J Gerontol, 2005, **25:** 565–566.

5 Gao S, Ma Q J, Cui Q. Chin J Nucl Med, 2006, **26:** 378–380.

6 Liang B B, Ma Q J, Wen Q. Jilin Med J, 2006, **27:** 113–114.

7 Khaled A R, Durum S K. Nat Rev Immunol, 2002, **2:** 817–830.

8 Xiao G, Fang H, Xing C, *et al.* Mini Rev Med Chem, 2009, **9:** 1596–1604.

9 Buggins A G, Pepper C J. Leuk Res, 2010, **34:** 837–842.

10 Reed J C, Pellecchia M. Blood, 2005, **106:** 408–418.

11 Lasi M, David C N, Böttger A. Apoptos, 2010, **15:** 269–278.

12 Antony R, Lukiw W J, Bazan N G. J Biol Chem. 2010, **285:** 18301–18308.

13 Kroemer G, Reed J C. Nature Med, 2000, **6:** 513–515.

14 Hetz C, Vitte P A, Bombrun A. J Biol Chem, 2005, **280:** 42960–42970.