Bcl-2 and Bax protein expression in human optic nerve axons in the eyeballs after severe trauma and in the eyes with absolute glaucoma

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Abstract

The aim of our study was to evaluate the immunohistochemical expression of Bcl-2 and Bax protein in optic nerve axons after severe eyeball injury and in the eyes with absolute glaucoma. A series of 19 eyeballs, enucleated because of absolute glaucoma and 41 eyeballs, enucleated, following extensive injury, at the Department of Ophthalmology of the Medical University of Bialystok, were taken into our study. The immunohistochemical reaction was performed with Bcl-2 and Bax protein antibodies and by the LSAB technique. DAB was used in order to visualise the reaction. The optic nerve axons in glaucomatous eyeballs showed statistically significant higher Bax protein expressions than those of Bcl-2 proteins. In the optic nerve axons after severe eyeball injury, a non-significantly higher Bcl-2 protein expression was observed.

Key words: optic nerve axons, trauma, absolute glaucoma, apoptosis, Bcl-2, Bax.

Introduction

Almost all body cells have a capacity to undergo a programmed cell death process. They differ in the ease with which they are able to initiate apoptosis. The tendency to initiate apoptosis and the speed with which this program is triggered depend on the type of cells and their stage of development. Cells, which are difficult to replace, including neurons of the developed nervous system, undergo apoptosis with some difficulty. Nevertheless, under normal physiological conditions, the average person loses 10000 retinal ganglion cells annually, due to apoptosis [1, 2, 3]. Proteins from the Bcl-2 family play a major role in apoptosis regulation at its decisive stage [4, 5, 6]. There are few studies, concerning apoptotic protein expression in human retinal ganglion cells in pathological states [7, 8]. It is not known how Bcl-2 and Bax protein expressions change in posttraumatic conditions. Glaucomatous optic neuropathy is multifactorial in etiology. Both mechanical and vasogenic theories remain viable explanations for the observed nerve damage and the destructive effect of trophic withdrawal could be espoused by either theory. Each theory feeds into the final common pathway of cell death by apoptosis [9, 10]. The aim of our study was an immunohistochemical evaluation of Bcl-2 and Bax protein expression in the optic nerve axons in eyeballs after severe trauma and in the eyes with absolute glaucoma.

Material and methods

We examined a series of 41 eyeballs, which were enucleated, following extensive injury over the period between 1991 and 2003 and a series of 19 eyeballs from patients with absolute glaucoma, suffering from severe ophthalmalgia treated of necessity by enucleation during the same period at the Department of Ophthalmology of the Medical University of Bialystok. After enucleation, the eyeballs were fixed in a 10% buffered formalin solution, embedded in paraffin at 56°C, then cut into 5µm slices and stained with hematoxylin and eosin (H+E). The evaluation of Bcl-2 and Bax protein expression was performed by the immunohistochemical reaction, using monoclonal antibodies from The Santa Cruz Biochemicals and DAKO (Bax No P-19, sc-526, DAKO/Bcl-2 No MO887) and LSAB technique. The IgG1 kappa antibody was used as a negative control, whereas samples of breast cancer tissue, which showed a strong positive

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Table 1. Correlations between examined marker expressions and the type of injury

Protein expression	Type of injury				p value
	Closed injury		Perforating injury		
	%	Number	%	Number	
Bcl-2 (-)	8,7	2	44,4	8	p= 0,012
Bcl-2 (+)	60,9	14	50	9	
Bcl-2 (++)	30,4	7	5,6	1	
Bax (-)	34,8	8	55,6	10	p= 0,36
Bax (+)	47,8	11	27,8	5	
Bax (++)	57,1	4	16,7	3	

Figure 1. Cytoplasmatic Bcl-2 protein immunostaining in the optic nerve axons after severe eyeball injury (mag.x200).

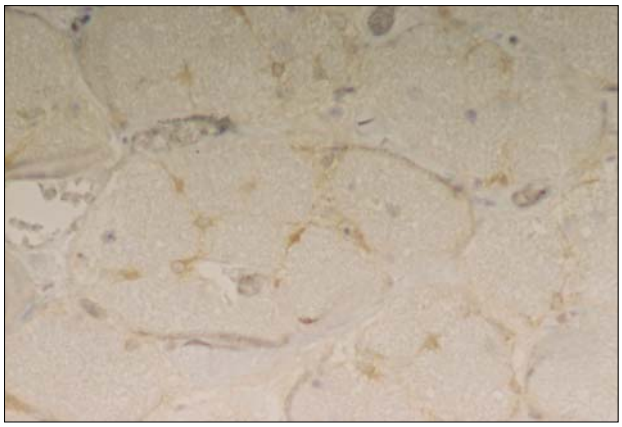
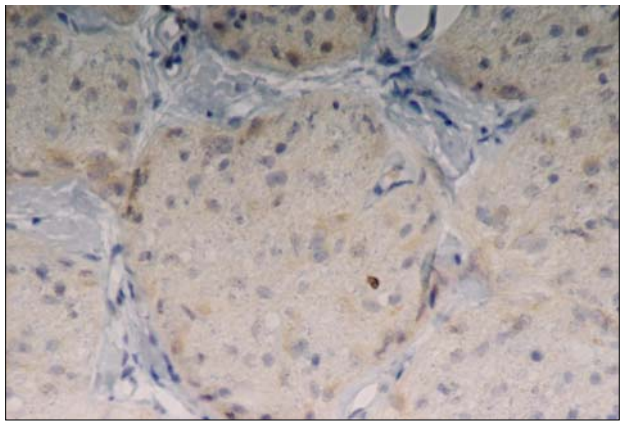


Figure 2. Cytoplasmatic Bax protein immunostaining in the optic nerve axons after severe eyeball injury (mag.x 400).



Bcl-2 and Bax immunoreaction, were used as a positive control. The estimation of immunostaining was done under a light microscope in representative fields, using a lens with a magnification of 20x in the optic nerve axons and sections were obtained from the retrolaminar region of the optic nerve head. The criteria for a positive reaction were as follows: (++) above 50% of axons of the field was showing positive immunostaining, (+) between 50%-10% and (-) below 10% of cells with positive immunostaining. We analyzed Bcl-2 and Bax protein expressions in relation to the patients age, sex and the relationships between the examined markers in both groups, as well as Bcl-2 and Bax protein expressions in relation to the time, elapsed from trauma to enucleation and to the type of injury. The obtained results were statistically analyzed, using Fisher and Chi-square tests. Differences at $p<0.05$ were considered statistically significant.

Results

The group of trauma patients: the series of 41 examined eyeballs came from male patients, aged between 18 and 77 years (the mean age: 45 years, SD±17,8). In 23 cases, we observed ruptured globes, secondary to severe blunt trauma. In 18 cases, the eye injury was caused by a sharp tool (severe corneal and scleral lacerations). All the injured and ruptured eyes were blind. In 13 patients, enucleation was performed on the day of trauma or 1 day post-injury (Group I), in 20 cases, 2-16 days post injury (Group II), and in 8 patients - 1 month till 4 years after trauma (Group III). Bcl-2 and Bax protein expressions were observed in cellular cytoplasm. The analysis of Bcl-2 and Bax protein expression in closed and perforating injury groups is shown in Table 1. In the entire trauma group, the mean age of patients without Bcl-2 protein expression in the optic nerve axons was 34.44±18.6 years and was lower than the mean age of patients with Bcl-2 overexpression: 49.04±19.78 years (p=0,065). Bcl-2 protein expression was stronger than Bax expression (statistically non-significant) in the group of trauma patients. In 92.3% patients with enucleation on the day of trauma, or 1 day post-trauma, Bcl-2 protein expression was observed, Bax in 61.5%. In the group with enucleation, 2-16 days post- injury, 60% cases showed Bcl-2 positive expression and Bax 50%. In the 1 month-4 years enucleation post-trauma group, Bcl-2 expression was observed in 87.5% cases, Bax in 62.5%. Bcl-2 and Bax expression did not correlate with the time period from severe trauma to eye enucleation. Bcl-2 protein expression did not correlate with Bax protein expression (Bcl-2/Bax p=0.445).

The group of glaucoma patients: the series of 19 examined eyeballs came from male patients (11) and female patients (8), aged between 21 and 86 years (the mean age: 59.7 years, SD±19.7). In the group of glaucoma patients without Bcl-2 expression, the mean age was 70.1±17 years and was statistically higher than the mean age of patients with Bcl-2 protein overexpression (53.7±18.8 years). Bax protein expression was statistically significantly stronger in the optic nerve axons than Bcl-2 one: Bax/Bcl-2 (p=0.0007) and correlated with Bcl-2 expression: p=0.0155. In the group of glaucoma patients, Bcl-2

and Bax expressions did not depend on sex. The trauma group consisted of male patients only. Bcl-2 and Bax expressions did not correlate with the age in either of the groups.

Discussion

Up until 1993, apoptosis had not been taken into consideration in the studies on the pathogenesis of nerve-related diseases. Disorders of apoptosis have widely been discussed in the literature with regards to neoplastic process [1, 11, 12]. A different situation is observed in cases of posttraumatic retinal detachment and glaucomatous optic neuropathy. A pathological intensification of apoptosis takes place, manifesting itself in retinal nerve cell damage and their axons, leading to sight loss [13, 14]. In our study, in the glaucomatous optic nerve axons, proapoptotic protein expression dominated, what might confirm the apoptotic nerve damage theory. The small percentage of statistically older glaucomatous patients with Bcl-2 protein positivity might be a proof for the proapoptotic tendency, even though, physiologically, a decreased apoptosis process is observed in the course of aging. The correlation Bcl-2 protein expression with Bax protein expression suggested a possibility of Bcl-2/Bax heterodimer creation and continuation of programmed cell death in the optic nerve axons, despite the absolute functional damage of the optic nerve.

Our study showed the expression of Bcl-2 protein to be more significant in the closed injury type group than in the perforating injury group. Bcl-2 protein expression was observed mainly in cases of older people. It is believed that older organisms lose their ability to induce apoptosis. In the whole group of trauma patients, a domination of Bcl-2 protein expression was observed. It was not statistically significant, however, it might be suggestive of some protection mechanism in the optic nerve axons after eye trauma. The results of our study might provoke thinking that, in the stage of absolute glaucoma in the optic nerve axons, proapoptotic Bax protein expression dominated, what is concordant with the apoptotic theory of glaucomatous optic neuropathy. Bcl-2 dominance in the optic nerve axons after severe eyeball trauma might suggest posttraumatic neuroprotection tendency in the optic nerve.

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