Bcl-xl and Bak protein expression in human optic nerve axons in eyeballs post- trauma and in the eyes with absolute glaucoma

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Abstract

The aim of our study was an evaluation of Bcl-xl and Bak protein expressions in optic nerve axons in eyeballs after severe injury and absolute glaucoma. We examined a series of 41 eyeballs, which were enucleated, following extensive injury and a series of 19 eyeballs from patients with absolute glaucoma. The immunohistochemical reaction was performed, using antibodies against human Bcl-xl and Bak protein with LSAB and DAB. In the injured eyeballs in Group I, Bcl-xl protein expression was observed in 53.8%, Bak in 38.5%, in Group II, Bcl-xl in 40%, Bak 55%; in Group III, Bcl-xl in 62.5%, Bak in 62.5%. Nine (9), out of 19 (47.4%) cases showed Bcl-xl protein positivity, Bak 15, out of 19 (78.9%). The percentage of cases with Bak protein positivity was statistically higher than that for Bcl-xl (Bak/Bcl-xl p=0.0356). The results showed that there may be a dominance expression of proapoptotic proteins in optic nerve axons in glaucoma.

Introduction

Perforative or obtuse severe trauma of the eyeball frequently leads to complete and permanent loss of vision, which is usually caused by a direct mechanical damage to photoreceptors and nerve cells, as well as in result of necrosis and apoptosis. Retinal detachment can also be caused by trauma. In animal experimental models, apoptosis - the programmed death of cells - has been implicated as the major factor in the mechanism, leading to death of photoreceptors in this condition [1, 2]. It is known, that the death of retinal ganglion cells, the axons of which form the optic nerve, can be caused by experimental axotomy. It has been demonstrated that retinal ganglion cell degeneration, due to trauma of this kind, is caused by apoptosis [1, 2, 3, 4]. However, to date, there have been no reports concerning the mechanism of axon damage of ganglion cells, due to severe trauma of the eyeball. Glaucoma is an optic neuropathy, characterized by retinal ganglion cell death, axon loss, and an excavated appearance of the optic nerve head. Retinal ganglion cell mechanisms in experimental animal models of glaucoma and in human glaucoma have been shown to involve apoptosis [5, 6, 7, 8]. The apoptotic cascade invokes a series of cellular events which lead to cell death. Proteins from the Bcl-2 family play a major role in apoptosis regulation at its decisive stage [9, 10]. Bcl-2 protein family may inhibit apoptosis - anti-apoptotic: Bcl-2, Bcl-xl, Bcl-w and protein enhancing apoptosis -proapoptotic: Bcl-xs, Bak and Bax [9, 11, 12]. There are no reports on apoptotic protein expression in human retinal ganglion cells and their axons under physiological conditions. It is not known how protein expression in the Bcl-2 subgroup changes in posttraumatic conditions, the opportunity to assess the expression of these proteins in the unique material provided in the form of eyeballs enucleated as a result of severe trauma is interesting and useful in attempting to explain the process of apoptosis in retinal ganglion cells. The aim of our study was an evaluation of Bcl-xl and Bak expression in optic nerve axons in eyeballs after severe trauma and absolute glaucoma.

Material and Methods

We examined a series of 41 eyeballs, which were enucleated after an extensive injury at the Department of Ophthalmology of the Medical University of Bialystok over the period from 1991 to 2003, and a series of 19 eyeballs from patients with absolute glaucoma, suffering from severe ophthalmalgia and treated, of neces-

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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		
	%	N number	%	N number	
Bcl-xl -	47.8%	11	55.6%	10	p=0.74
Bcl-xl +	13%	3	16.7%	3	
Bcl-xl ++	39.1%	9	27.8%	5	
Bak -	43.5%	10	55.6%	10	p=0.22
Bak +	30.4%	7	38.9%	7	
Bak ++	26.1%	6	5.6%	1	

sity, by enucleation. After enucleation, the eyeballs were fixed in a 10% buffered formalin solution, embedded in paraffin at 56°C, then cut into 5 mm slices and stained with haematoxylin and eosin (H+E). The evaluation of Bcl-xl and Bak protein expressions was performed with the immunohistochemical reaction, using monoclonal antibodies from the Santa Cruz Biochemicals (Bak No N-20, sc-1035, Bcl-xl No A-20, sc- 7122). In order to visualize the antigen-antibody reaction, the LSAB technique was applied, using DAB (diaminobensidine). The IgG1 kappa (DAKO) antibody was used as a negative control, whereas samples of breast cancer tissue, which showed a strong positive Bcl-xl and Bak immunoreactions, were used as a positive control. Two independent pathologists performed the immunohistochemical evaluations of Bcl-xl and Bak proteins. The estimation of immunostaining was done under a light microscope in representative fields, using a lens with 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. Bcl-xl and Bak positive reaction in the optic nerve axons was observed as a granular, cytoplasmatic staining. We statistically analyzed Bcl-xl and Bak protein expression in relation to the time, elapsing from trauma to enucleation (all the cases were divided into 3 time-groups: on the day and one day post-trauma; 2-16 days post-trauma and 1 month till 4 years post-trauma enucleation was performed). Statistically analyzed were the relationships between the expression of examined proteins and the patients' sex and age, as well as the correlation between Bcl-xl and Bak protein expressions. Bcl-xl and Bak protein expressions were compared in both groups: in the absolute glaucoma and the post-trauma group. The obtained results were statistically analyzed, using the Fisher or Chi-square tests, as well as the Student's t-test for paired samples (the correlation between Bcl-xl and Bak protein expression), employing the SPSS software package v.8.0 for Windows (SPSS Inc., Chicago, IL). The values at $p< 0.05$ were considered statistically significant.

Results

The series of 41 examined eyeballs came from male patients, aged between 18 and 77 years (the average 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). In 13 patients, enucleation was performed on the day of trauma and 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-xl and Bak protein expressions were observed in cellular cytoplasm. The analysis of expression of the examined proteins in both closed and perforating injury groups is shown in Table 1. Comparing the injury time groups and the expressions of proteins, the results are as followed: in Group I, Bcl-xl protein expression was observed in 53,8%, Bak in 38,5%, in Group II, Bcl-xl in 40%, Bak 55%; in Group III, Bcl-xl in 62,5%, Bak in 62,5%. There was no statistically significant correlation between the type of injury and either Bcl-xl or Bak protein expression. Closed injury was observed mainly in older patients. There was no correlation between Bcl-xl and Bak protein expressions. 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 +/-19,7). Nine (9), out of 19 (47,4%) cases showed Bcl-xl protein positivity, Bak - 15, out of 19 (78,9%). In the group with absolute glaucoma, there was no correlation between the patients' age, sex or Bcl-xl, Bak protein expression. The percentage of cases with Bak protein positivity was statistically higher than that for Bcl-xl (Bak/Bcl-xl p=0.0356). The results showed that there might be dominance expression of proapoptotic proteins in optic nerve axons in glaucoma, what may suggest axon cell death by apoptosis.

Discussion

Apoptosis, which is the reverse process to mitosis, is connected, amongst others, with tissue mass control, organ formation during morphogenesis and with removal of infected or genetically damaged cells, the survival of which would be harmful to the organism. In case of glaucomatous optic nerve atrophy, increased apoptosis is observed, causing an irreversible damage to optic nerve axons and, in consequence, resulting in vision loss. In our study, a significant Bak protein expression was noticed. Bcl-xl protein, which inhibits apoptosis, showed weak expression in optic nerve axons in glaucoma. In cases of post-traumatic retinal detachment and axotomy, a pathological intensification of apoptosis takes place, manifesting itself in photoreceptor and retinal nerve cell damage. Our study showed expres-

sions of Bcl-xl and of proapoptotic proteins similar both in closed and perforating injuries. Bak protein expression was high after 1 month from injury. The positive correlation between Bcl-xl and Bak protein expression may point out at an intensification of apoptosis process in optic nerve axons in injured eyeballs. On the day, 1 day and 1 month after trauma, programmed cell death seemed to achieve the highest activity, moreover, proapoptotic processes may have dominated the antiapoptotic ones in the matter of the time period from eyeball injury. Napankangas et al. observed increased Bax and Bak protein expressions on the 4th day after optic nerve transection [11]. The results of our study may suggest that there are significant differences between the expression of apoptotic proteins, such as Bcl-2, Bcl-xl, Bak and Bax in human optic nerve axons in the eyeballs after severe trauma. There may be a dominating expression of proapoptotic proteins in optic nerve axons in glaucoma, what may suggest axon cell death by apoptosis.

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