·Review·

Advances in researches on the optic nerve protection

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Abstract

• The mechanisms of regeneration and protection of optic nerve, the represent of central nerves, are researched more and more profoundly and extensively in recent years. The retinal ganglion cells(RGCs) protection after injury is stopping or preventing it from apoptosis mainly. The methods include glutamic acid inhibitor, nitric oxide (NO) inhibitor, neurotrophic factor, gene therapy, acupuncture, traditional Chinese medicine and so on. However, there are no medicines or operations that play definite curative role in the RGCs protection after injury up to now. So the ganglion cells protection is at its exploratory research stage, which will shoulder heavy responsibilities.

• KEYWORDS: injury of optic nerve; retinal ganglion cells; protection

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INTRODUCTION

Optic nerve, the represent of central nerves, may be injured gravely by various diseases such as glaucoma, inflammatory affection, trauma, ischemia, oncothlipsis, etc., causing seriously visual lost consequently. There is a temporary budding phenomenon after optic neural injury, but the nascent germ recovers quickly, the axon antidromically dies rapidly and most of retinal ganglion cells (RGCs) undergo apoptosis. Meanwhile the glial scar is formed at the injured position after glial cells hyperplasia and hypertrophy. It was not until So and Aguayo [1] transplanted peripheral nerve to retina successfully in 1985 that the concept, the optic nerve could not be regenerated after injury, was changed thoroughly. At present, it is known that RGCs have the activity of inherent regeneration, which can avoid from dying, regenerate and associate with target tissue after injury in appropriate circumstances.

In recent years, the cognitions of the RGCs injury mechanisms are boosted partly, as the apoptosis is being researched more and more profoundly. Nowadays, it is considered that the mechanisms of RGCs injury consist of toxicity of excitatory amino acids, deprivation of neurotrophic factors, Ca^{2+} overloading, autoimmune response and the toxic actions of nitric oxide (NO), reactive oxygen species (ROS) and cysteine proteinase, but the exact mechanism has not been interpreted. The three procedures that the treatment of RGCs injury demands are the following: (1) preventing the damaged RGCs (or having been damaged potentially) from dying; (2) inducing the degenerated RGCs axon to elongate new axon and attach to the target site in central nervous system; (3) synapse must be formed and connections be rebuilt between new axon and target site. Usually preventing RGCs from dying is called as neural protection and optic neural function recovery after injury is called as neural reparation [2], which is not only functional reparation but also structural reparation.

The RGCs protection after injury is mainly stopping or preventing them from apoptosis by eliminating or inhibiting its priming procedure or the other methods to inhibit apoptosis.

GLUTAMIC ACID INHIBITOR

Glutamic acid as the main excitatory transmitter in brain and eye is connected with the formation of axon between photoceptor and bipolar cell, and bipolar cell and RGC as well. Its toxic action does not take place for its extracellular concentration is ingested by Müller cells intensely in normal condition. Injured RGCs and adjacent cells release glutamic acid after axon abscised and ischemic/reperfusion injury. Consequently the glutamic acid level in vitreous is

increaced. Meanwhile glutamic acid transporter eliminates in glaucoma patients [3], indicating that the increase of extracellular glutamic acid concentration may be caused by functional barrier of glutamic acid transporter. Profuse intracellular and extracellular glutamic acid makes RGCs die by combining with the receptor of N-methyl-diacetyl-aminosuccinic acid (NMDA), α- amino- 3-hydriding-5-methylisox-azolepropionate (AMPA) and kainite. It is known that excitable toxicity of glutamic acid is mediated by NMDA-receptor. Calcium channel depending on voltage will reopen while glutamic acid and NMDA-receptor combine together, resulting in the increase of intracellular calcium ion. The increased calcium ion as the second messenger activates caspases and a series of materials related with cytotoxicity, taking NO for example, and make RGCs die. Otherwise, the interaction of glutamic acid and non-NMDA-receptor could form positive feedback and stimulate much glutamic acid releasing, as a result, accelerating the procedure of cells death as an infernal circle. Accordingly the RGCs apoptosis can be prevented by eliminating the glumatic acid toxicity in the RGCs. The main methods are as the following: (1) inhibiting the glutamic acid releasing from the RGCs; (2) inhibiting the glutamic acid from being ingested by injured or non-invasive RGCs; (3) blockading the combining site of glutamic acid in the injured and non-invasive RGCs. Most of the materials can complete one or more methods. For example, memantin and dizocilpine (MK801) can blockade NMDA-receptor, while NBQX and DBQX can blockade AMPA-receptor and kainite-receptor. These materials play evident part in retinal ganglion cells protection in rats, mice [4, 5] and primates [6] after experimental optic neural injury. Nevertheless, inhibiting the function of the NMDA-receptor extensively will influence glutamic acid physiologic function in the central nervous system and lead to serious complications, such as the psychosis alike symptom, eleptiform bout and cognitive functional disturbance and so on. Consequently, their clinical applications are confined [7].

NO INHIBITOR

As we know, NO is inorganic micromolecular gas, containing electronic free radical that is not matched. It is active and instable extraordinarily, whose half period is short. NO synthase is activated by overload of intracellular calcium ion and catalyzes L-arginine to generate NO. Nowadays, it is known that there are two kinds of mechanisms of RGCs injury by NO. One is that NO and O_2^- combined into $ONOO^-$, a kind of hadro-oxidizer, which can oxidize proteinaceous hydrosulfuryl making varieties of enzyme inactivated and peroxidize lipid influencing biomembranous function seriously. SOD can eliminate O_2^-, so that NO cannot generate $ONOO^-$, more fierce toxicity, and NO neurotoxicity is weakened. The other is that NO can make caryon nitrosyl, destroy DNA helical structure and lead to cell damage finally. It can also devitalize important enzymatic iron-sulifide protein of respiratory chain, hinder mitochondrial electron transfer and lead to cell energy metabolic block and cell death. Morgan *et al*[8] investigated that N-nitro-L-arginine could increase the survival quantity of the RGCs as dose dependent through the experiment that they cocultured rat's RGCs and horizontal cell and exposed them to anoxic circumstance for 1-24 hours or excitatory amino acids for 6 hours after dealt with N-nitro-L-arginine of different density, a NO synthase catastaltica. These indicated that the RGCs resistance of hypoxia and excitatory amino acids could be improved obviously by the way of decreasing NO originated from colloid cell by NO synthase catastaltica. Although the research of NO synthase catastaltica has made a certain progress, its research in the field of ophthalmology remains in the stage of basic experiment and effective clinical medicine has not emerged.

NEUROTROPHIC FACTORS

Neurocyte under normal growth needs enough neurotrophic factors from its target tissue and/or colloid cell of distal end which will be abscised after nerve injury. RGCs will die because of injury and dystrophy. But the injured RGCs remain in better growth if ectogenic neurotrophic factors are given in time [9]. The biological actions of neurotrophic factors to nervous system can be summarized into two aspects: one is increasing the survival rate of neurocyte, called protective action; the other is facilitating the nervous process growth of neurocyte, called regenerative action. For instance, they can facilitate the regeneration of RGCs axon and the extension of nerve fiber, induce the directional growth of axon and decide the growth direction of nerve fiber. There are a series of neurotrophic factors, but their survival auxoactions to the RGCs are different from each other obviously, which are determined by whether there is the receptor to the certain neurotrophic factor. At present, the neurotrophic factors effective to RGCs are brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor

(CNTF), basic fibroblast growth factor (bFGF), neurotrophic facto -4/5 (NT-4/5), gliocyte-derived neurotrophic factor (GDNF), etc. Huang *et al* [10] indicated that the densities of RGC began to decrease 7 days after injury, the number of RGC was 70.2% and 40.5% of normal controls on days 14 and 28, respectively, and in the group with BDNF injection, RGC densities decreased on 7 days, but RGC densities were much higher than that of controls on 7, 14, 21and 28 days after injury. Those results showed that BDNF intraocular injection after injury could decrease the death of RGCs and protect them. However, there are limitations of BDNF. For example, using it for long time can activate NOS, decreasing its neuroprotective effect consequently. Yang *et al* [11] discovered that as a result of crushing injury, the expressing level of CNTF down-regulated soon after a transitory up-regulation, but CNTF α-mRNA was continuingly expressed in retina after injury. These facts suggest that exogenous CNTF might provide a supportive environment for axonal regeneration and might play a protective role in the retinal recovery. Negishi *et al* [12] discovered that intravitreal injection of BDNF, NT-4 and extracellular matrix could promote optic neural regeneration and application of BDNF, CNTF and forskolin, etc. could attain the same effectiveness, mechanisms of which might be related with adjusting process of cAMP [13]. How to utilize neurotrophic factors after neural injury will be a significant optic to be explored profoundly.

As we know, monosialoganglioside (GM1)is one of galactose-cerebroside, which imitates and strengthens the neuroprotective effect of neurotrophic factor, enhances the energy of nerve cell and protects nerve from varied injury without influencing other physiologic functions. GM1 enhances the dimerization or autophosphorylation of the neurenergen receptors, so that the receptor can be activated without neurotrophic factor and the activation can be potentialized if there is neurotrophic factor. GM1 inhibits the degeneration of pressurized or axotomized optic nerve axon [14], whereas it does not reduce the toxic action of glutamic acid in retina. At present, GM1 is utilized extensively in the treatment of central nervous system diseases, which plays a role in restoring injured nerve and promoting nerve regeneration. However, its clinical application is very few in the field of ophthalmology, remaining in the phase of study and research.

GENE THERAPY

Gene therapy plays its treatment effect by means of introducing exogenous gene into objective cell and making it express then. It was studied that many related genes took part in the control of apoptotic process, inducing or inhibiting apoptosis by their expressions. Inducing apoptotic genes are as the following: wild-type P53 ,ced-3 ,ced-4 and c-myc that can activate apoptosis; c-fos that can mediate apoptosis; bax , bcl-xc and bad that can promote apoptosis. Inhibiting apoptosis genes include bcl-2 ,bcl-xl and mutant P53. CrmAt and bcl-2 can promote the survival of apoptosis mediated by short of neurenergen (NT) [15]. Nowadays the research of the relationship between RGCs and apoptotic genetic expression is still in the elementary period. Nevertheless there have been experiments showing that promoting bcl-2 overexpression can heighten apoptotic threshold and increase the capability of resisting various kinds of apoptotic stimulation. The fact that bcl-2 overexpression caused by transgenic technology inhibits neuron apoptosis indicates the potential clinical therapeutic value of bcl-2. Since now, the most popular research is introducing bcl-2 into vivo cell mediated by adenovirus, which possesses nerve-cell-like high infected potentia and clinically relative security, nevertheless the most inadequate feature is that gene expression can not be permanent. Bcl-xl and bad belong to bcl-2 family too. The bcl-xl effect and deuto-cell distribution are similar to bcl-2. Bad , as the first apoptotic inducer, mediates apoptosis by regulating the ratios between homodimer and heterodimers of bax [16]. There is potentially useful value and prospects of gene therapy to eye diseases seriously threatening health. But some questions such as the lower efficacy of genetic transmission, security and target directionality are existing. Serious of expression vector whose specificity can be regulated must be constructed as gene medicine utilized in clinic.

OTHERS

It has been verified that the inhibitors correlated with neural sheath include Nogo, myelin-associated glycoprotein (MAG), CSPG and so on, among which Nogo protein and its acceptor are known as one of the most important factors to hinder survival and regeneration of injured CNS neuron [17,18]. It is investigated that the optic nerve can be protected by inoculating CNS-autoantigen-like synthetic polypeptide and playing T-cell neural protective immunity reaction, taking peptide vaccine of non-caused meningitis for example, at the basis of avoiding from autoimmune

disease in the way of using soluble antigen-induced tolerance so that the induction of destructive autoantigen response can be avoided [19]. Furthermore, the peripheral neural transplantation, the peripheral nerve or Schwann cell transplanted into cavum vitreum and the stem cell induction or transplantation can regenerate optic nerve.

In addition, the possible pathways to protect optic nerve may still by utilizing Ca^{2+} channel blocker, antioxidant/ free radical scavenger, β-acceptor blocker, α2-adrenoceptor agonist (e.g. brimonidine), heat shock protein (HSPs), cranial neurilemma cell transplantation, traditional Chinese medicine, etc. Although generous results of experimental researches are conspicuous, there will be many questions, which should be solved before they are utilized clinically.

It is known that the traditional Chinese medicine plays an effective role in optic nerve protection, the mechanisms of which may be related with improving microcirculation and adjusting visual cell function. Li *et al* [20] divided 30 rabbits with chronic experimental ocular hypertension into acupuncture group treated with acupuncture, treated group treated with eye drops of 5g/L timolol and experimental control group without treatment at random. The results showed that the effect of acupuncture was on the same level with Western medicine, but was superior to that of treated group with timolol in nodal cell structure and nerve cell transmission. All of those showed that acupuncture could promote optic neurotransmitter to increase, enhance visual information transfer and protect visual function. In the Province Natural Science Foundation topic of Sun *et al*, 60 rabbits with experimental ocular hypertension having been controlled in normal range were divided into model group without treatment, control group treated with masculine medicine, acupuncture group and electrical acupuncture group at random. At the end of the experiment, the glutamic acid and NO levels of rabbits in each group were deviated from each other significantly, and those in treated groups were significantly lower than in model group. Compared with the model group, the retinal and optic neural ultramicrostructure in treated group were damaged lighter, and what is more, the quantity of RGCs and optic neural axon and the area percentage between optic neural axon and optic nerve were higher. The expression level of Bcl-xl, BDNF in retina was enhanced in acupuncture and electrical acupuncture group. On the whole, the results showed that acupuncture could protect the optic nerve from ocular hypertension effectively, the mechanisms of which might be related with accelerating NO and glutamic acid removing in RGCs and up-regulating the level of Bcl-xl and BDNF.

Zhu *et al* [21] researched whether Erigeron Breviscapus (Vant.) Hand-Mazz (EBHM) could improve the optic nerve axoplasmic transport in rats with experimentally elevated intraocular pressure (IOP) via superior colliculus retrograde horse radish peroxidase (HRP) labeling. The result revealed that EBHM could improve and redintegrate the optic nerve axoplasmic transportation blocked by elevation of IOP. The research of Jiang *et al* [22] indicated that the mechanisms of how EBHM protected optic neural might be related with redintegrating the neuron closing to death and protecting the health neuron surrounding. It is reported that the complex Danshen root injection and traditional Chinese medicine Fuming could improve optic neural axoplasmic transportation evidently and protect RGCs [23]. Li *et al* [24, 25] reported that under the condition of ocular tension having been controlled in certain degrees, Angelicae Sinensis Decoction for Supplementing Blood could elevate retinal ganglion cells survival rate, lighten retinal nerve fibre layer injury, protect retinal ganglion cells and inhibit retinal ganglion cells from apoptosis caused by experimental ocular hypertension as well. In the research of Xu *et al* [26], the rabbits with chronic IOP elevation were treated with 5g/L timolol eye drops and injection puerarin in the treatment groups respectively and both, and 50g/L glucose in control group. The retinal ganglion cell density enhanced in the three treated groups when their periods of ocular hypertension were identical, in which both treated group effect was the most significant, and the density enhanced as the medication time extending. The results showed that puerarin improved both optic neural axoplasmic flow of rabbits with ocular hypertension and optic disc microcirculatory condition, and promoted the blocked axoplasm transmission to recover. Ma *et al* [27] showed through experiment that Ginaton, which contains ginkgetin and internal ester, could arrive at retina and protect RGCs of rats after optic nerve clipped. The Provincial Department of Education Project of Sun *et al* indicated that MINGMUⅣ decoction could improve retinal microcirculation, decrease the injury of retinal ultramicrostructure and promote the recovery of axoplasm transmission. Simultaneously, MINGMUⅣ could decrease the retinal level of glutamate and NO after ocular hypertension, up-regulate expression of Bcl-xl and BDNF, thereby the retrograde or anterograde neural degeneration could be blocked, apoptotic rate of RGCs could be decreased and the death of nerve cell could be avoided.

CONCLUSION

In a word, the clinical therapeutic effect to protect optic nerve of the traditional Chinese medicine has been accepted by extensive clinicians, nevertheless the related fundamental researches and prostective efficacy studies are still deficient. To sum up, the mechanisms of optic neural regeneration and

protection are researched more and more profoundly and extensively in recent years, having became the research focus. The retinal ganglion cells protection after injury is stopping or preventing it from apoptosis mainly. The methods include glutamic acid inhibitor, NO inhibitor, neurotrophic factor, gene therapy, eliminating inhibitive factor, immunity inhibitor, stem cell induction/ transplantation, peripheral nerve transplantation, calcium channel blocker, antioxidant/ free radical scavenger, heat shock protein, acupuncture, traditional Chinese medicine and so on. Prominent though extensive experimental study results are, there are still a lot of questions to be solved before used in clinic, such as medicine's serious complications, the lower efficacy of genetic transmission, security, target directionality and so on. The traditional Chinese medicine is effective and potential in this field. Furthermore, the combined therapy of combination of traditional Chinese medicine with Western medicine adopts their advantages and complements each other, whose retinal protective effect has been confirmed in clinical practices for several years. Experimental and clinical researches should be increased systemically and objectively, so that it can be used and expanded in clinic gradually, enhancing optic atrophic patients' quality of life and relieving the social burden.

REFERENCES

1 So KF, Aguayo AJ. Lengthy regrowth of cut axons from ganglion cells after peripheral nerve transplantation into the retina of adult rats. *Brain Res* 1985;328 (2):349-354

2 Vidal-Sanz M, Bray GM, Villegas-Perez MP, Thanos S, Aquayo AJ. Axonal regeneration and synapse formation in the superior colliculus by retinal ganglion cells in the adult rat. *Neurosci* 1987;7(9):2894-2909

3 Naskar R, Vorwerk CK, Dreyer EB. Concurrent downregulation of a glutamate transporter and receptor in glaucoma. *Invest Ophthalmol Vis Sci* 2000;41 (7): 1940-1944

4 Gu Z, Yamamoto T, Kawase C, Matsubara M, Kawase K, Sawada A, Kitazawa Y. Neuroprotective effect of Nmethyl-D-Aspartate receptor antagonists in an experimental glaucoma model in the rat. *Nippon Ganka Gakkai Zasshi* 2000;104 (1):11-16

5 Schuettauf F, Naskar R, Vorwerk CK, Zurakowski D, Dreyer EB. Ganglion cell loss after optic nerve crush mediated by AMPA-kainate and NMDA receptors. *Invest Ophthalmol Vis Sci* 2000;41(13):4313-4316

6 Hare W, WoldeMussie E, Lai R, Ton H, Ruiz G, Feldmenn B, Wijono M, Chun T, Wheeler L. Efficacy and safety of memantine, an NMDA-type open-channel blocker, for reduction of retinal injury associated with experimental glaucoma in rat and monkey. *Surv Ophthalmol* 2002;45(Supp l3):284-289

7 Huang ZR.Optic neural protection of glaucoma. *Chin J Practi Ophthalmol* 2003; 21(11):808-812

8 Morgan J, Caprioli J, Koseki Y. Nitric oxide mediates excito-toxic and anoxic damage in rat retinal ganglion cells cocultured with astroglia. *Arch Ophthalmol* 1999;117(11):1524-1529

9 Berry M, Gonzalez AM, Clarke W, Greenless L, Barrett L, Tsang W, Seymour L, Bonadio J, Logan A, Baird A. Sustained effects of geneactivated matrices after CNS injury. *Mol Cell Neurosci* 2001;17(4):706-716

10 Huang W, Wang L, Hui YN, Zhang Ml. Protective effects of Brain-derived neurotrophic factor on injured retinal ganglion cells of rats. *Eye Science* 2000;16 (4):232-234

11 Yang XL, Zhang X, Gu Q, Zhang XM, Sun XD. Expression of ciliary neurotrophic factor receptor in retina following the injury of optic nerve in rat. *Chin Ophthal Res* 2005;23(2):195-197

12 Negishi H, Dezawa M, Oshitari T, Adachi-Usami E. Optic nerve regeneration within artificial Schwann cell graft in the adult rat. *Brain Res Bull* 2001;55(3): 409-419

13 Watanabe M, Tokita Y, Kato M, Fukuda Y. Intravitreal injections of neurotrophic factors and forskolin enhance survival and axonal regeneration of zxotomized beta ganglion cells in cat retina. *Neurosci* 2003;116(3):733-742

14 Choi JS, Kim JA, Joo CK. Activation of MAPK and CREB by GM1 induces survival of RGCs in the retina with axotomized nerve. *Invest Ophthalmol Vis Sci* 2003;44(4):1747-1752

15 Gagliardini V, Fernandez PA, Lee RK, Drexler HC, Rotello RJ, Fishman MC, Yuan J. Prevention of vertebrate neuronal death by the crmA gene. *Science* 1994; 263(5148):826-828

16 Tuder G, Aguilera A, Halverson DO, Laing ND, Sausville EA. Susceptibility to drug-induced apoptosis correlates with differential modulation of Bad, Bcl-2 and Bcl-xl protein levels. *Cell Death Differ* 2000;7(6):574-586

17 Prinjha R, Moore SE, Vinson M, Blake S, Morrow R, Christie G, Michalovich D, Simmons DL, Walsh FS. Inhibitor of neurite outgrowth in humans. *Nature* 2000; 403(6768):383-384

18 Grandpre T, Nakamura F, Vartanian T, Strittmatter SM. Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein. *Nature* 2000;403 (6768):439-444

19 Hu MM, Duan XC. Immunologic pathway of optic neural protection against glaucoma. *Fore Med Sci (Ophthalmol)* 2005;29(2):89-93

20 Li ZY, Zhang GZ, Zhang GQ. Effect of acupuncture on experimental chronic ocular hypertension and retinal ultrastructure in rabbits. *J Traditio Chin Ophthalmol* 2002;12(3):135-139

21 Zhu YH, Jiang YQ, Liu ZH, Luo XG, Liu ZZ. The affect of Erigeron Breviscapus (Vant.) Hand-Mazz on axoplasmic transport of optic nerve in rats with experimentally elevated intraocular pressure. *Chin J Ophthalmol* 2000;36 (4): 289-291

22 Jiang B, Jiang YQ. The neuroprotective effect of erigeron breviscapus (vant) hand-mazz on retinal ganglion cells after optic nerve crush injury. *Chin J Ophthalmol* 2003;39(8):481-484

23 Xu L, Xia DZ. The affect of Fuming on optic neural axoplasmic flow after traction injury. *Chin J Practi Ophthalmol* 1999;17(11):654-656

24 Li DJ, Jiang BL, Zhu YL. The anti-apoptotic function of dangguibuxuetang on the retinal ganglion cells of rabbits with experimental intraocular hypertension. *J Traditio Chin Ophthalmol* 2005;1(1):30-32

25 Li DJ, Jiang BL, Zhu YL. The protection of dangguibuxuetang on the retinal ganglion cells of rabbits with experimental intraocular hypertension. *J Traditio Chin Ophthalmol* 2004;14(2):73-76

26 Xu XR, Cai FY. An experimental study on the effect of injection puerarin in the axoplasmic transport of optic nerve of rabbits with chronic introcular pressure elevation. *J Traditio Chin Ophthalmol* 1997;7(1):3-6

27 Ma K, Xu L, Liu YJ, Ma YN, Chen YJ, Chen CX. The research of Ginaton's retinal ganglion cells protective effect in rats. *Chin J Ophthalmol* 2003;39 (12): 757-758