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Retinal degenerative diseases – mechanisms and perspectives of treatment

Choroby zwyrodnieniowe siatkówki – mechanizmy powstawania i perspektywy leczenia

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Streszczenie: Choroby zwyrodnieniowe siatkówki to duża grupa chorób oczu, które prowadzą do zaburzeń widzenia oraz w efekcie końcowym do nieodwracalnej utraty widzenia. Ze względu na to, że procesy zwyrodnieniowe siatkówki zachodzą w przebiegu zarówno powszechnie występujących chorób oczu – takich jak jaskra czy zwyrodnienie plamki związane z wiekiem, jak i wielu innych, które występują rzadziej, są istotnym problemem w okulistyce. W niniejszym artykule prezentujemy mechanizmy prowadzące do zmian zwyrodnieniowych siatkówki, tj.: egzotoksyczność, stres oksydacyjny, stan zapalny, oraz podsumowujemy najświeższe doniesienia nt. zastosowania neuroprotekcji w leczeniu chorób zwyrodnieniowych siatkówki.

Słowa kluczowe: choroby zwyrodnieniowe siatkówki, neuroprotekcja egzotoksyczność, stres oksydacyjny.

Summary: Retinal degenerative diseases are an extensive group of ocular diseases, leading to vision disorders and finally irreversible vision loss. They are an significant problem, because degenerative processes exist in common ocular disorders like glaucoma or age-related macular degeneration and many other less frequently occurring disorders. In this article, we present mechanisms leading to retinal degeneration like excitotoxicity, oxidative stress, inflammation and summarize the latest reports concerning neuroprotection in the treatment of retina degenerative diseases.

Key words: retinal degenerative diseases, neuroprotection, excitotoxicity, oxidative stress.

Introduction

Retina degenerative diseases are a large group of ocular diseases, in which the loss of cells results in visual impairment and potentially complete blindness. The retina is subject to inherited and acquired degenerative diseases. The first group includes: retinitis pigmentosa (RP), cone and rod dystrophy (CORD) and many other rarely occurring genetic disorders. The second group includes acute conditions for example ischemia, optic nerve crush and chronic conditions such as glaucoma, age-related macular degeneration (AMD) and diabetic retinopathy. In most degenerative diseases of the retina only a subset of cells are affected, although in more advanced stages, the loss and reorganization of the entire retina can occur. The etiology of these diseases is not completely clear. Although the clinical manifestations differ in each retinal degenerative disease, factors causing them may be common and may include: oxidative stress, glutamate-induced excitotoxicity, increased calcium concentration, inflammation, deprivation of neurotrophins and growth factors, abnormal accumulation of proteins, and apoptotic signals. In many diseases except for the primary noxious factor, dying cells induce secondary degeneration in surrounding tissue through secreting toxic substances, cell injury progresses even though primary factors have retreated. Many agents have been investigated for neuroprotective properties, including free radical scavengers, metal ion chelators, anti-excitotoxic agents, apoptosis inhibitors, anti-inflammatory agents, neurotrophic factors, etc. In this article we have concentrated on explaining the above-mentioned mechanisms and on presenting the latest reports concerning neuroprotective drug-based strategies in the potential retina degeneration diseases management.

Excitotoxicity and substances acting on NMDA receptors

Excitotoxicity is the pathological process by which nerve cells are damaged and killed by excessive stimulation by neurotransmitters such as glutamate or neurotoxins acting on the same receptors. Glutamate, an excitatory amino acid, activates different types of ion channel forming receptors (named iono-

tropic) and G-protein-coupled receptors (named metabotropic). N-methyl-D-aspartate (NMDA), Kainate and Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors belong to ionotropic receptors. Postsynaptic NMDA receptors are composed of several different subunits, two NR1 subunits and two NR2 subunits. Glutamate binds onto the target site on the NR1 subunit while glycine binds onto the NR2 subunit, which acts as a coagonist. In physiological conditions, the presence of glutamate in the synapse is regulated by active, ATP-dependent transporters in neurons and glia. In pathological condition the levels of ATP production decrease, leading to an impairment of glutamate uptake. Moreover, the membrane potential of presynaptic neurons is lost and an efflux of glutamate occurs, contributing to the excessive activation of postsynaptic glutamate receptors. Pathologically high concentration of glutamate overactivates NMDA receptors, which results in increased calcium influx into cells. Calcium ions activate a number of enzymes, including phospholipases C, endonucleases and proteases, which damage cell structures such as components of the cytoskeleton, membrane, and DNA. Activation of nitric oxide synthase by calcium ions, induces accumulation of NO and leads to oxidative stress. Moreover, overstimulation of NMDA receptors increases expression of p53 mRNA activating p53-dependent pathway of cell death and significantly elevates the levels of TNF-α, and interleukin-1β leading to inflammation. All of these mechanisms can induce cell death through apoptosis as well as necrosis, depending on the severity of exposure to an excitatory amino acid and which of the ionotropic receptors is activated (1-3). Excitotoxicity has been linked to various diseases of the central nervous system, such as Huntington, Alzheimer's and Parkinson's disease, amyotrophic lateral sclerosis and acute neurological disorders, such as epileptic convulsions, ischemic stroke and post-traumatic lesions (4,5). As for the retina, there is evidence that glutamate induced excitotoxicity plays a role in diseases such as retinal ischemia/reperfusion, optic nerve injury, diabetic retinopathy and glaucoma (2,6-8). Blocking the glutamatergic system, specifically NMDA receptors, offers a novel approach to the treatment of retinal degeneration. The most studied inhibitor of NMDA receptors, with advantageous neuroprotective properties and almost deprived of side effects, is memantine. It is a low-affinity, voltage-dependent, non-competitive antagonist at glutamatergic NMDA receptors. By binding to the NMDA receptor with a higher affinity than Mg^{2+} ions, memantine is able to inhibit the prolonged influx of Ca^{2+} ions (9-11). Clinically, memantine is approved for the treatment of neurological disorder – moderate to severe Alzheimer's disease. Memantine is, in general, well-tolerated. Common adverse drug reactions ($\geq$1% of patients) include confusion, dizziness, drowsiness, headache, insomnia, agitation, and/or hallucinations. Memantine had shown to be highly effective neuroprotective agent in animal models of retinal degenerative diseases (12-14). In response to the positive results of studies conducted on animal models, phase III clinical trial evaluating the efficacy of memantine as a neuroprotectant in patients with glaucoma was started in March 2000. It was a randomized, double-masked clinical trial enrolling 1179 patients with open-angle glaucoma. Although the study was completed in 2006, its results have not been presented so far (15). Another example of inhibitors of NMDA receptors are glycine site specific NMDA receptor antagonists. At the beginning, their neuroprotective efficacy was tested in ischemic brain tissue. Sun A. and Cheng J. (16) suggested that glycine site antagonists inhibit high-level glutamate, but do not alter the normal neurotransmitter activities in other cerebral regions, and thanks to this have fewer neural side-effects. Qui W. et al. (17) evaluated the NMDA glycine site antagonist protective effect on retinal ischemia reperfusion rat model. They administered 5-nitro-6, 7-dichloro-1, 4-dihydro-2, and 3-quinoxalinedione intravitreal at various points in time following ischemia/reperfusion. They showed that glycine site specific NMDA receptor antagonists protect RGCs against death through inhibition of apoptotic signaling. Their results suggest that an earlier start of the intervention, gives a better outcome.

Oxidative stress and antioxidants

Oxidative stress is an imbalance between the production of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell. One source of reactive oxygen is the leakage of activated oxygen from mitochondria during cellular respiration. Reactive oxygen species are formed in the photochemical reaction and under the influence of prooxidants like xanthine oxidase, NADPH oxidases or cytochromes P450. Moreover some of the less reactive of these species (such as superoxide) can be converted by oxidoreduction reactions with transition metals (Fe^{2+}) or other redox cycling compounds (including quinones) into more aggressive radical species (18). The defense against oxidative stress are cellular antioxidants which include enzymes: superoxide dismutase (SOD), catalase, and glutathione peroxidase and non-enzymatic factors: vitamins A, C and E. Lutein and zeaxanthin – retinal pigments belonging to the carotenoids – are natural barriers, which, through absorption of visible blue light protect the retina against its negative effects. Antioxidants also include trace elements such as zinc (Zn) and selenium (Se) (19). The retina is particularly susceptible to oxidative stress because of intense oxidative metabolism, continuous exposure to light, the presence of photosensitizer – lipofuscin – and a high content of easily oxidizable polyunsaturated fatty acids. Oxidative stress plays an important role in the development of different degenerative retinal diseases, for example glaucoma, AMD, diabetic retinopathy, ischemia, retinitis pigmentosa (20-24).

We can protect the retina against oxidative stress primarily by strengthening the antioxidative biological mechanisms. The Age-Related Eye Disease Study (AREDS) provided evidence that antioxidants – Vit. C (500 mg), Vit. E (400 IU), beta-karotenoids (15 mg) especially used with zinc (80 mg) and cooper (2 mg) have a significant neuroprotective effect on retina patients with moderate dry AMD and patients with severe AMD in one eye. This multi-center, randomized, placebo controlled clinical trial gathered 3640 participant with AMD, over an analysis period of 5–10 years. AREDS has the greatest scientific value of research on this topic conducted so far (25). The next study AREDS 2 is currently being conducted. AREDS 2 is a multi-center, rando-

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mized trial designed to assess the effects of oral supplementation of the lutein and zeaxanthin and/or the omega-3 Long-Chain Polyunsaturated Fatty Acids [docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)] on the progression to advanced AMD. Studies demonstrating the efficacy of lutein in therapy of atrophic AMD have been carried out, however, as the authors say further studies are needed with more patients and longer follow up (26).

One candidate for a therapeutic antioxidant approach in treating neuronal degeneration is the stable nitroxide tempol (4 hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl) – a low weight anti-oxidant. Tempol acts in different ways. It exhibits SOD and catalase mimic action and oxidases Fe^{2+} preventing the Fenton reaction. Moreover more recent studies have shown its potent anti-inflammatory activity. All this may contribute to Tempol's neuroprotective effects. El-Remessy A.B. et al. (27) confirmed this hypothesis and demonstrated tempol to be a retinal neuroprotectant in a rat model of NMDA-induce retinal neurotoxicity. They showed that Tempol at a dose 0,4 mg/eye, due to the ability to reduce the level of superoxide anions almost completely eliminated retina (inner nuclear layer and gangilion cell layer) damage. Thaler S. et al. (28) found a significant protection of retinal ganglion cells (RGCs) in partial optic nerve crush (PONC) in rats, animal model of glaucoma, after treatment with tempol (20 mg/kg of body weight). In their in vivo study there were no observed systemic side effects of tempol. The very small therapeutic time window possibly limits tempol's effectiveness of antioxidant therapy. This suggests that tempol may be beneficial in cases of acute neuronal damage. It is still debatable whether tempol can be equally useful in long-term therapy in chronic neurodegeneration, such as in glaucoma. Tanito M. et al. evaluated the neuroprotective efficacy of new catalytic antioxidants: tempol's diamagnetic hydroxylamine form tempol-h (TP-H or OT-674; 1,4-dihydroxy-2,2,6,6- tetramethylpiperidine) and OT-551 (1 hydroxy-4-cyclopropanecarbonyloxy-2,2,6,6-tetramethylpiperidine hydrochloride), which is converted to TP-H in the body. They used in vivo animal models (rats) of light-induced retina degeneration. In their study, systemic administration of OT-551 and TP-H provided protection of photoreceptor cells and retinal pigment epithelim (RPE) cells (29,30). The protection by OT-551 was greater than TP-H. OT-551's safety and efficacy was also assessed in the II phase of clinical trials: OT-551 Antioxidant Eye Drops to Treat Geographic Atrophy in Age-Related Macular Degeneration. It was a single-center, open-label study, enrolling 10 participants with bilateral geographic atrophy. This pilot study was designed to characterize the effect of 0.45% concentration of OT-551 eye drops given 3 times a day over a two-year period. The study showed that OT-551 was well tolerated and was not associated with any serious adverse effect. The results suggest a possible effect in maintaining visual acuity (the mean change in BCVA at 2 years was $+0.2 \pm 13.3$ letters in the study eyes and -11.3 ± 7.6 in control eyes). However, the absence of significant effects on the other outcomes, such as area of geographic atrophy, contrast sensitivity, and total drusen area, suggest that OT-551 in this concentration and mode of delivery may have limited or no benefit as a treatment for geographic atrophy (31).

A strong oxidant – peroxynitrite (ONOO-) has been demonstrated as an important factor in the pathogenesis of different retina degenerative diseases (22,27,32,33). It can damage macromolecules by reacting with them directly or it can generate other strong oxidants such as hydroxyl or carbonate radicals. Peroxynitrite is generated from nitric oxide (NO) and superoxide radicals during oxidative stress. Also overstimulation of NMDA and excessive levels of intracellular calcium leads to activation of nitric oxide synthase and superoxides and NO production. In addition to generating free radicals, NO induces the pro-apoptotic cascade by enhancing phosphorylation of Bcl-2, which in turn results in the loss of anti-apoptotic potential (34,35). The best way to deal with peroxynitrite is to prevent its formation by keeping levels of NO and superoxide at low levels. A nonspecific inhibitor of nitric oxide synthase (NOS), N-ω-nitro-L-arginine methyl ester (L-NAME) has been demonstrated as a retinal neuroprotectant in a rat model of NMDA-induce retinal neurotoxicity (36). El-Remessy A.B. et al. (27) confirmed this hypothesis. They co-injected L-NAME with NMDA what almost completely eliminated NMDA noxious impact on the retina. Komeima K. et al. (32) evaluated the role of oxidative stress in cone cells degeneration and showed that treatment with nitric oxide synthase (NOS) inhibitors, mostly neuronal NOS relatively specific inhibitors (7-nitroindazole) significantly increases cone survival in the rdl mouse model of RP. All these data suggests that antioxidant therapy should be combined with reduction of NO levels in the retina.

Another element which may be modified in order to limit oxidative stress is iron. This metal in Fenton reaction, reacts with hydrogen peroxide (H_2O_2) to produce hydroxyl radical ($HO^{\bullet}$), the most reactive and toxic of the free radicals. Abnormal retinal iron metabolism may promote a variety of retinal disorders. Retinal degeneration has been observed in hereditary disorders resulting in iron overload (aceruloplasminemia, hereditary hemochromatosis, Friedreich's Ataxia) and acquired ocular siderosis (intraocular metallic foreign bodies, subretinal hemorrhage). Recent studies suggest that iron overload may also play a role in the pathogenesis of AMD (37-39). Therefore iron chelation may be useful in retinal degenerative diseases. Until recently the only iron chelator in widespread clinical use was deferoxamine B (DFO). DFO is used in patients with transfusion-related iron overload. Due to the necessity of subcutaneous or intravenous administration, DFO has limited usefulness in chronic therapy. More recently, several other iron chelators characterized by a greater penetration of the blood-brain barrier, blood-retina and the possibility of oral treatment, have been put in to clinical use. Hadziahmetovic M. et al. (40) evaluated the possibility of using one of them, deferiprone, in ophthalmology. They assessed the retinal protection effect of deferiprones on Ceruloplasmin/Hephaestin double-knockout (DKO) mice with age-dependent retinal iron accumulation and some features of AMD and showed that deferiprone, by lowering the level of iron, and thus oxidative stress, leads to a reduction of degenerative changes in the retina. They also found that the drug was not toxic to the mouse retina.

Another group of substances influencing oxidative stress are cannabinoids. Cannabinoid components of marijuana, such as (-)Δ9-tetrahydrocannabinol (THC), or the synthetic can-

nabinoid WIN55,212-2 are known to prevent glutamate-induced neurotoxicity in isolated neurons or in the brain through activation of the cannabinoid receptor subtype CB1 (41,42). The major drawback of the use of cannabinoids is their psychotropic effects. However, research has shown that as well as THC, cannabidiol (CBD) – the component of marijuana, and the synthetic cannabinoid HU-211, both of which are not psychotropic, are potent antioxidants and NMDA receptor antagonists (43,44). El-Remessy A.B. et al. (27) analyzed cannabinoids impact on rats' retina. They confirmed that CBD as well as THC protect retinal ganglion cells from NMDA-induced neurotoxicity and their neuroprotective effect results partly from the reduction of superoxide antion, NO and ONOO-. In addition to possessing retinal neuroprotective activity cannabinoids induce a reduction in intraocular pressure. This suggests that they may offer a multifaceted therapy for glaucoma. El-Remessy A.B. et al. also showed that CBD treatment reduces diabetes induced neurotoxicity, inflammation, and blood-retinal barrier breakdown through activities that may involve, reduction of oxidative stress, levels of tumour necrosis factor-α, vascular endothelial growth factor, intercellular adhesion molecule-1 and inhibition of p38 MAP kinase (45).

Inflammation

Microglial and macroglial cells (Müller cells and astrocytes) play important immunoregulatory role and control the extracellular environment of the optic nerve head and retina. Under normal conditions, glial cells support neuronal function through different mechanisms including structural and nutritional roles as well as the removal of ions and neurotransmitters from the extracellular space (46). Under prolonged stress glial cells may become neurodestructive, releasing increased amounts of neurotoxic substances including TNF-α and NO. TNF-α is a potent proinflammatory cytokine. TNF-α binds onto the death receptor, TNF receptor-1 (TNF-R1) and can induce both the caspase-dependent and the caspase-independent components of cell death pathway. It has been implicated as a mediator of RGC death in glaucomatous retina (47). Lowering the level of TNF-α reduces inflammation and can thus provide neuroprotection to retinal cells. Laengle U.W. et al. (48) showed that dopamine agonist GLC756 inhibit TNF-α release from activated rat mast cells and suggested a potential of the compound on neuroprotection in glaucoma management.

The immune system – the body's own defence mechanism – also plays a key role in neuroprotection. This defence involves recruitment of both innate and adaptive immune cells. The immune system is mainly associated with the recognition of pathogens. Any immune response to the body's own antigen was traditionally considered harmful and was seen as an autoimmune disease. However, T cells activated by self-reactive antigens, can create a neuroprotective environment, what prevents or reduces the secondary spread of damage. T cells engage blood monocytes sending them to the damaged area, produce immunoregulatory cytokines, and induce production of growth factors, chemokines, and cytokines that properly activate resident microglia. Such activated microglia/macrophages can take up glutamate, remove debris, and produce growth factors (49).

The spontaneous immune response might not be sufficient to promote significant neuronal protection. Therefore, there is reason to believe that using passive transfer of self-reactive T cells or active immunization using self-antigens can boost protective autoimmune response. The choice of the antigen is critical, only the antigens that are present at the damage site will promote neuroprotection. Fisher J. et al. (50) used nonencephalitogenic myelin-associated peptides derived from proteolipid protein (PLP) or myelin oligodendrocyte glycoprotein (MOG) as an antigen in their study and demonstrated that active immunization leads to neuroprotection of RGCs in injured mouse optic nerve model. The use of nonencephalitogenic myelin peptides for immunization apparently allows neuroprotection without incurring the risk of an autoimmune disease. Another substance used as an active immunization is the synthetic four-amino acid polymer copolymer 1 (Cop-1, trade name Copaxone), which is known not to be encephalitogenic despite its cross-reactivity with myelin basic protein. Kipnis J. et al. showed that active immunization with Cop-1 administered in adjuvant, as well as adoptive transfer of T cells reactive to Cop-1, can inhibit the progression of secondary degeneration after crush injury of the rat optic nerve (51). It has been also demonstrated that Cop-1 significantly reduces RGC loss in glutamate-induced toxicity in mice and in rat ocular hypertension models (52). A similar phenomenon is evident in passive immunization (51,53). Schwartz M. and London A. (54) suggested that immune cells, not only have neuroprotective properties, but also regulate neurogenesis in retina. They found that Toll-like receptors (TLR4) are expressed on neural progenitor cells (NCP) and contribute to neurogenesis. Their study carried out on TLR4- deficient mice, showed increased proliferation and neuronal differentiation. This deficiency alone does not extend the postnatal neurogenesis period, but it works only in combination with growth factors (55). Therefore, the discovery of TLR4 as a regulator of neurogenesis may be hopeful in treatment of neurodegenerative diseases. Currently Cop-1 is US Food and Drug Administration-approved drug for the treatment of multiple sclerosis patients. A clinical trial evaluating the efficacy and safety of Copaxone in dry AMD was started in December 2007. Preliminary results are promising and show that Copaxone reduces drusen area in dry AMD (56).

Neurotrophic factors

Neurotrophic factors promote the development, survival, and differentiation of neurons. Many of them demonstrate neuroprotective properties, for example: brain-derived neurotrophic factor (BDNF), nerve growth factor NGF), fibroblast growth factor 2 (FGF-2), ciliary neurotrophic factor (CNTF). Recently, attention is focused on ciliary neurotrophic factor (CNTF). CNTF is a protein encoded by the CNTF gene. It promotes neurotransmitter synthesis and neurite outgrowth in nervous system, it also enhances survival of neurons and oligodendrocytes, and may reduce the inflammatory process. For this reasons, it has been used in human clinical trials. Phase I clinical trials showed the safety of an administration method of CNTF and suggested its efficacy in the treatment of retinitis pigmentosa. CNTF is released from intraocular implant called NT-501, what allows a direct effect on the retina (57). Following the positive results, phase II and III clinical trials evaluating safety and effectiveness of CNTF implants on vision in persons with retinitis pigmentosa and atrophic macular degeneration was conducted (58-60). Recently clinical trials evaluating safety and efficiency of CNTF

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implants in ischemic optic neuropathy and primary open angle glaucoma have been started (61,62). Results should be forthcoming in the near future.

Selective α2-adrenergic agonists used to reduce intraocular pressure also have a neuroprotective attribute. Brimonidine tartrate is a highly selective α2-adrenergic agonist. During the past decade, brimonidine has gained attention for its role in reducing intraocular pressure in the initial and long-term treatment of ocular hypertension and glaucoma (63). Recent studies suggest a neuroprotective effect of brimonidine. The presence of α2 receptors in the retina (ganglion cell layer and possibly within the inner nuclear layer and amacrine cells) or optic nerve head, lays the foundation for the potential neuroprotective role of brimonidine (64). The exact mechanism of the role of brimonidine in neuroprotection is yet to be studied. It is likely multifactorial. Activation of the α2-adrenergic receptors by brimonidine reduce levels of intravitreal glutamate, elevate levels of neurotrophic factors (brain-derived neurotrophic factor and fibroblast growth factor), and up-regulate intrinsic cell survival signaling pathways and anti-apoptotic genes such as Bcl-2 and BCL-XL.4 (65). Results of studies conducted on experimental models of optic nerve crush injury (66), ischemic optic neuropathy (67), ocular hypertension and glaucoma (68) suggest that bromonidine has neuroprotective properties. However, the results of clinical investigations suggest that brimonidine treatment has failed in human clinical trials assessing its efficacy for acute non-arteritic anterior ischemic optic neuropathy (NAION) treatment (69). The failure of clinical results, despite the successes of diverse animal models in demonstrating the neuroprotective effects of brimonidine treatment, may explain the late start of treatment and differences between species. The most notable successes in experimental models involved groups in which treatment preceded the optic nerve injury (67). Therefore, earlier brimonidine administration after ischemic event might increase the effectiveness of the treatment. Another clinical trial evaluated the usefulness of the brimonidine administered as an intravitreal implant in the treatment of geographic atrophy due to age-related macular degeneration (AMD). The trial has been carried out since May 2008. Results have not been published yet (70).

Conclusions

Neuroprotection offers new opportunities for the treatment of degenerative retinal diseases. Neuroprotective drugs have been approved for clinical use, mainly in neurology. Unfortunately in ophthalmology, despite the positive outcome in animal models, many potentially neuroprotective agents did not demonstrate effectiveness in clinical trials. This situation can be explained by several differences, which exist between the experimental and clinical models, as well as unique challenges when working with human subjects. Mechanisms leading to retina degeneration must still be analyzed. Therefore, we believe that their particular understanding will lead to more effective treatment.

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