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Patomechanisms in proliferative vitreoretinopathy

Patomechanizmy witreoretinopatii proliferacyjnej

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Summary: Proliferative vitreoretinopathy (PVR) remains the most common cause of recurrent retinal detachment following retinal detachment surgery. The development of PVR is a complex process involving humoral and cellular factors. Surgical treatment of PVR, which consists of removal of the fibrous membranes and restoration of physiological anatomic ocular conditions is often unsuccessful. Therefore the surgery should by backed up by local medication to inhibit new formation of proliferative lesions. Unfortunately, there is no satisfactory antiproliferative treatment available so far. Proliferative vitreoretinopathy remains a therapeutic challenge.

Słowa kluczowe: witreoretinopatia proliferacyjna, czynniki ryzyka, fizjopatologia, odwarstwienie
Key words: proliferative vitreoretinopathy, risk factors, physiopathology retinal detachment.

Proliferative vitreoretinopathy (PVR) is the chief cause of surgical failures of retinal surgery specially in the cases of perforation-derived retinal detachment after serious mechanical traumas (1). The development of proliferative changes in the eyeball shows several similarities with tissular healing process, in which inflammatory reaction stimulates migration and proliferation of cells and leads to the generation of connective tissue which restores the continuity of injured tissue. Unfortunately, because of the subtlety of the connection between the neurosensoric retina and the layer of pigment cells, here localized development of fibrous structures and caused by them traction, is usually highly detrimental to the retina. The prevalence of vitreoretinal proliferative disorders after retinal detachment surgery is 12.9 to 21.6 per cent depending on the applied technique (intraocular surgery or vitrectomy through the flat section of the ciliary body), and the lenticular condition (2). The prevalence of PVR in post-traumatic eyes, especially with the presence of intraocular foreign bodies, is higher and positively correlated with the foreign body's size and the degree of damage to the ocular wall (3).

The factors which influence the occurrence of PVR are not fully known, but it is assumed that the duration of detachment and the number of retinal perforations, pre-operative choroidal detachment, bleedings into the vitreous chamber, chorioretinitis, pre-operative proliferative changes, aphakia, and cryo- or laser-therapy contribute to proliferative changes (4). The development of PVR is a complex process involving humoral and cellular factors. Its initiation is facilitated by inflammatory changes in the vitreous body and effector mesodermal cells: mostly glia cells and pigmented epithelium cells (retinal epithelial cells – RPE).

The inflammatory factors in the form of immune system cells, cytokines, growth factors, interleukins and others, appear in the vitreous chamber when the retina-blood barier malfunctions (5). These factors' role in PVR is generally similar to the one in other healing processes, but some of them manifest here certain specificity. Among the identified inflammatory factors which contribute to the pathomechanism of PVR the following seem to be of chief importance: platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), tumor necrosis factor alpha (TNF-α), and monocyte chemotactic protein-1 (MCP-1).

PDGF acts in a pro-mitotic manner on the glia cells and the RPE, stimulating the synthesis and excretion of collagen by fibroblasts. PDGF appears to play a significant role in the PVR cascade. The tests carried out in rabbits proved that fibroblasts extracted from mice embryos lacking PDGF receptors induce PVR to a small degree (6). In vivo tests demonstrated also that the concentration of PDGF in the vitreous body in persons suffering from retinal detachment in the course of PVR is significantly higher than in persons not affected (7).

TGF-β stimulates the synthesis and excretion of fibronectin and provokes contraction of collagen fibers (8). The level of this factor has been proved higher in the eyes of patients with PVR than in patients not suffering from retinal detachment (9).

A small concentration of TNF-α is demonstrable in the vitreous body; it is one of the most important inflammatory cytokines detectable in the extracellular matrix in proliferative changes. A positive correlation between the concentration of the soluble form of TNF-α receptor and proliferative changes has also been observed in the eyes with retinal detachment.

MCP-1 takes part in the regulation of angiogenesis in the central nervous system, vascular permeability, and neuronal apoptosis, and contributes to infiltration of the retina by macrophages and microglia cells. In the eyes with retinal detachment

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and PVR the level of MCP-1 has been proved elevated in comparison to normal eyes. MCP-1 stimulates RPE cellular migration *in vitro*.

The aforementioned and many other substances may initiate cellular migration of fibroblasts, macrophages, glia cells, and RPE cells into the subretinal space, onto the retinal surface, and into the vitreous chamber. Further proliferation and metaplasia follow the creation of fibroblastic complexes all of which form extracellular matrix made up of fibronectin, collagen type I-IV, laminin, and thrombospondin which does not differ from "ordinary" granulation tissue. Proliferating cells and extracellular matrix create fibrous membranes along the vitreous-retinal interface. Next, these membranes undergo reconstruction with participation of the matrix metalloproteinases (MMP), making space for the action of fibro- and myofibroblasts. These cells are responsible for further synthesis of constituents of fibrous membranes genesis and then their contraction. The generated tractive forces tear or elevate the retina – which depends on the localization of connective tissular elements in the neurosensoric retina.

On histochemical examination of the surgically removed proliferative membranes retinal pigment epithelial (RPE) and glia cells have been found, as well as fibroblasts, fibroblast-like cells, macrophage-like cells, and also less numerous neutrophils, lymphocytes T and B, thrombocytes, and unidentified pigment cells.

A crucial role in the genesis of PVR is ascribed to RPE cells. On histological examination they are present in almost all epiretinal membranes of patients with proliferative retinopathy (10). They may acquire various morphological and functional characteristics. Influenced by TGF-β1 they transform into contractile cells – myofibroblasts which cause shrinking of the proliferative membranes, influenced by TGF-β2 they transform into fibroblasts and participate in the synthesis of collagen. It has also been demonstrated that the effects of surgical treatment of PVR correlate with the PVR cells activity in the removed membranes: the more active RPE cells, the worse the prognosis.

Glia cells (astrocytes, Müller's cells, microglia) are the dominant part of the non-tractive epiretinal membranes. The retinal glia cells, as the glia cells of the central nervous system, when traumatized excrete a number of substances which take part in the formation of extracellular matrix (e.g. fibroectine and laminia).

Fibroblasts and fibroblast-like cells are the chief constituent of mature tractive membranes. They stimulate their growth and the production of collagen and other structural components of extracellular matrix. Due to their tractive properties they play the main role in the retraction of proliferative membranes. The origin of fibroblasts and fibroblast-like cells in proliferative membranes is unclear. They might derive from dividing and changing glia cells or RPE cells.

Macrophages, from all inflammatory cells, probably play the most important role in the development of PVR. They excrete several growth factors and cytokines, stimulating chemotaxis and cellular proliferation. Macrophages enter the interior if the eyeball from peripheral circulation where the blood-retina barrier is broken through.

The clinical picture of PVR is of wide spectrum: from foggy opacities and pigment aggregations in the vitreous body, to a complete retinal detachment caused by massive contractive epiretinal membranes and intraretinal fibrosis. The first clinical classification of PVR was proposed by Retina Society in 1983 (11). This classification distinguishes four stages of PVR (stages A, B, C, and D), where the most advanced stages C and D are divided into 3 subtypes according to the intensity and distribution of changes. In the later years, Marchemer (12) and the researchers from the Silicon Study group revised the original classification, introducing detailed localization of changes and types of vitreoretinal traction. Despite this newer classification being more precise in describing the ocular changes, the traditional classification is still widely used for it universal character.

Surgical treatment of PVR, that is removal of the fibrous membranes and restoration of physiological anatomic ocular conditions is often unsuccessful. Therefore, it is important to reduce the risk of proliferative changes through surgical procedures and pharmacological agents.

The principle of the surgical procedure in the treatment of PVR is to remove from the eyeball the environment which facilitates proliferative changes. They are induced by the RPE cells from the immune system in the peripheral blood, cytokines, growth factors and the vitreous body, whose structural fibers make up the framework for new proliferative membranes. Many surgeons share the view, that early vitrectomy, especially in the cases of heavy ocular traumas, may prevent PVR.

Even when the surgical procedure is performed fully successfully, not all pathological cells and collagen fragments, from which the proliferative process may originate causing PVR, can be removed. That is why surgery must by backed up by local medication to inhibit new formation of proliferative changes. Such a double therapeutic management may reduce the occurrence of postoperative complications. Unfortunately, the today available medicines are of narrow therapeutic spectrum which lies between retinal toxicity and anti-proliferative action. The substance showing strong efficacy against PVR and low toxicity for the retina is yet to be developed.

Another block which has to be overcome in the treatment of PVR is sustaining high therapeutic concentration of a pharmacological agent in the eyeball, stretching for periods of several months. One solution to this problem might by a slow drug release system of therapeutic substance. When PVR and retinal detachment occur, long lasting intraocular tamponade with the use of silicon oil is often applied. The use of silicon as the conveyor of therapeutic substance might combine the advantages of surgical treatment with those of pharmacological management of PVR.

Since the 1980s researchers have been investigating the possibilities of curbing RPE cells proliferation and formation of PVR by modulating the inflammatory and proliferative processes within the vitreous body. Main stress has been laid on pharmacological reduction of cellular proliferation by active agents administered intra- or post-operative. Best investigated thus far are 5-fluorouracyl (5-FU) (13) and daunomycine (14).

5-FU inhibits cellular proliferation breaking up the RNA synthesis. Even short exposure to 5-FU suffices to suppress proliferation for a long period of time. Like other cytostatics however, the substance may damage the retina and the optic nerve when administered intraocularly.

Daunomycine is an antibiotic which inhibits cellular migration and proliferation, without affecting their contraction. This substance acts upon the S phase of cellular cycle, decreases cellular metabolism and damages the cellular wall. Initial reports on its efficacy were promising, but later extensive, randomized tests and trials conducted in four European countries did not confirm its therapeutic influence on the acuity of vision when compared with control groups.

Besides 5-FU and daunomycine other substances were also tested to verify their usability in the treatment of PVR: adriamycine, taxol, methotrexate, colchicine, corticosteroids, retinoids and heparin were investigated.

Encouraging in vitro results of trials were often in sharp frustrating contrast with the *in vivo* effectiveness of tested medicines, a phenomenon which should be referred to the dosage and time of therapeutic activity of given agents when clinically employed; investigated substances tended to be quickly eliminated from the vitreous chamber. For example, salicylic acid with half-time duration of 2 hours in the vitreous body could not be expected to bring about required effects since the progress of PVR lasts 9 months. This highlights the difficulty of the issue, which is to devise a practicable method of slow drug-release system.

Such a system based on hydrogel substances would enable slow release of the medicine, but its drawbacks are imperfect biocompatibility and so called early substance escape, which means up to 30% of the stored substance is released within the first minutes after administration. This pharmacodynamics disqualifies hydrogels as pharmacological carriers of narrow therapeutic window.

Silicon oil is free of these disadvantages – it is a linear, synthetic, lipophilic polymer having good biocompatibility, used in vitreoretinal surgery since the year 1980. Due to its high superficial tension and insolubility in aqueous solutions, it makes an effective material for intraocular tamponades. In theory, the use of silicon oil in the therapy of PVR is based on limiting the circulation of growth factors, reduction of tractive forces, and achieving a physiological anatomy of the ocular fundus. Silicon oil should decrease the risk of eye loss and help many patients maintain practically useful acuity of vision. Unfortunately, despite these theoretical sound indications, clinical trials did not confirm the silicon oil's capability of reducing the incidence of PVR. It has been found out however, that it decreases the intensity of unfavorable consequences such as repeated detachment of the retina. There has been some promising research done into the oil's capacity for carrying pharmacological agents. The research however, focused only on selected substances with antiproliferative characteristics, such as heparin and acetylsalicylic acid, mycofenolate acid, and rapamycine.

Heparin inhibits the proliferation of fibroblasts and RPE cells by binding fibroectine and growth factors and reduces the cellular adhesion. It also decreases the contraction of collagen fibers. Johnson et al. have observed in clinical trial, that administration of heparin after a vitrectomy reduces post-operative fibrosis, increasing however the frequency of intraocular hemorrhages, which in turn may trigger PVR. As it is known that small molecule heparin does not increase the tendency to bleed, it has been put to the test. In animals tests showed efficacy of small molecule heparin in decreasing the development of retinal tractions in the course of PVR. An administration of 5 international units of small molecule heparin during vitrectomy reduced the frequency of tractive retinal detachment from 33--14% after two months.

Corticosteroids limit tissular inflammatory reactions by inhibiting migration and aggregation of macrophages and by influencing vascular permeability. They also decrease the inflammatory response of macrophages, reducing their capacity for phagocytosis and enzyme release (e.g. plazminogene activator), and growth factors (e.g. interleukin). Corticosteroids also inhibit phospholipase and the transformation of membrane phospholipids into arachidonic acid. Such blockade leads to the limitation of prostaglandin and leukotriens synthesis. The decreased vascular permeability evoked by corticosteroids results from the deactivation of kinins and reduction of histamine amount as it is released by basophilic leucocytes. These processes reduce local tissular damage, the amount and activity of mitogenes and growth factors, as well as the genesis of clot and fibrin. In effect, the fibroblastic activity is curbed and the healing process suppressed. Since macrophages, whose activity is strongly inhibited by steroids, probably initiate the process of PVR formation, one could expect the best efficacy of given drugs after their early administration. Trials with dexamethasone in the treatment of PVR were unsuccessful, but as in these trials only single doses of the drug were applied, its highly hydrophilic properties and short half-time duration in the eye may have been insufficient in the case of proliferative changes.

Mycophenolate mofetil (MMF) is an immunosuppressive agent used in the prophylaxis of graft rejection in trasplantology. MMF inhibits lymphocyte proliferation both in vitro and in vivo; its antiproliferative properties observed in its influence on mesangial and intraepithelial cells *in vitro* are known. Its mechanism relies on reversible inhibition of inosine monophosphate dehydrogenase (IMPDH) – a key enzyme in the *de novo* synthesis of guanosine monophosphorane (GMP). The deficiency in GMP leads to deficiency in guanosine triphosphorane (GTP), a necessary compound for desoxyguanosine production; absence of desoxyguanosine disrupts the syntheses of DNA and RNA. MMF blocks the proliferation of human pulmonary fibroblasts and the fibroblasts of the Tenon's capsule (15), but its effect on the RPE cells is unknown.

Rapamicine is a new inhibitor of proliferative signals possessing immunosuppressive properties. Preclinical trials in animals demonstrated its ability to decrease graft rejections of the kidneys, the heart, and the lungs. Rapamicine blocks the proliferative signals emitted by growth factors (e.g. interleukine-2). In acting so, it stops the cellular division in phase G1, making impossible the transition to phase S. It has been observed in the lab tests it inhibits the proliferation of the smooth muscles and human fibroblasts. The tests showed that rapamicine is non toxic for the retina at the dosage of 10-50 μg. The effects of rapamicine on the RPE cells and glia cells are unknown.

Thus far, it has not been verified whether intravenous administration of anti-proliferative drugs was successful in the treatment of PVR. It seems that penetration of these drugs into the vitreous body is too weak to provoke any satisfactory therapeutic reactions. Therefore, to use the antiproliferative property

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to its maximum, attempts were made in the years1990th to create slow drug-release systems for some of the agents. Such systems were tested for 5-FU and BCNU, but despite interesting preliminary reports, these trials have now been abandoned.

Proliferative vitreoretinopathy remains a therapeutic challenge. A clinically applicable and effective method of its treatment has yet not been invented. The multifactorial nature of the condition calls for new modifications of the future treatment models. Identification of the key processes which rule cellular migration and proliferation in the PVR cascade would offer a chance of suppressing the proliferative changes before a devastating damage to the retina occurs. Another opportunity for therapeutic progress lies in a better employment of drugs influencing various stages of PVR development plus devising slow drug release systems to enhance their action. Gene therapy might be an alternative medical approach as it has been proved effective during experiments in vivo which involved inhibition of the PDGF receptor. *Via* these measures we may acquire another tool for evolving new methods of treatment of proliferative vitreoretinopathy.

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