Amino Acids (2002) 23: 293–299
DOI 10.1007/s00726-001-0141-3

Amino
Acids
Printed in Austria

Molecular imaging of perfusion disturbances in glaucoma

O. Golubnitschaja[1], **K. Wunderlich**[2], **C. Decker**[1], **H. Mönkemann**[1], **H. H. Schild**[1], and **J. Flammer**[2]

[1] Department of Radiology, University of Bonn, Germany
[2] University Eye Clinic, Basel, Switzerland

Received June 29, 2001
Accepted August 8, 2001
Published online August 9, 2002;

Summary. Ocular ischemia resulting from perfusion disturbances may play a major role in initiation of glaucoma. Possibly secondary to ischemia autoimmunogenic events are activated in glaucoma patients with increased prevalence of systemic autoimmune diseases. The determination of potential molecular markers in blood leukocytes could be useful for early noninvasive diagnostics of glaucoma. Our study using subtractive hybridization showed altered gene expression in leukocytes of glaucoma patients in comparison to age and sex matched healthy subjects. Subtracted genes encoding lymphocyte IgE receptor (Fc epsilon RII/CD23), T cell-specific tyrosin kinase, thromboxan A2 receptor, alkaline phosphatase and Na^+/K^+-ATPase are differentially expressed in circulating leukocytes of glaucoma patients. These genes show expression profiles characteristic for adherent leukocytes which could be an important contributor to blood-brain barrier breakdown which has been found in glaucoma patients.

Keywords: Molecular imaging – Perfusion disturbances – Vasospasm – Glaucoma – Gene hunting – Leukocytes

Introduction

Glaucoma is the second leading cause of permanent vision loss in the world (Quigley, 1996). Although elevated intraocular pressure has been shown to be the major risk factor, there is a cohort of relatively young patients developing normal-tension glaucoma. Small assymptomatic ischemic lesions in brain have been demonstrated in glaucoma patients (Ong et al., 1995; Stroman, 1995). Perfusion of the retina and optic nerve head suffering from observed vasospastic dysfunction (Flammer, 1987; Guthauser et al., 1988) may be further reduced by changes in the intraocular pressure. Ocular ischemia resulting from these blood flow deficits may play a major role in initiation of glaucoma. Thus, hypoxia could represent a final pathway affecting retinal ganglion cells (Chung et al., 1999). Magnetic resonance imaging appears to be a powerful new approach for measuring retinal oxygenation quantitatively and noninvasively. It is applicable to study ischemic or ischemia-related retinopathies such as glaucoma (Berkowitz et al., 2000). Elevated glutamate level found in the glaucomatous eye contributes obviously to the apoptotic loss of ganglion cells via an "excitotoxic" pathway, mediated primarily through the NMDA subtype of glutamate receptor (Gross et al., 1999; Toriu et al., 2000; Vorwerk et al., 2000). Furthermore, possibly secondary to ischemia the autoimmunogenic events are activated in glaucoma patients having an increased prevalence of systemic autoimmune diseases (Wax, 2000). Recent evidence strongly suggests that activated immunity occurs during the neurodegenerative process of glaucomatous optic neuropathy (Yang et al., 2001a). They are accompanied by alterations in the immune system such as increased percentage of CD8 T lymphocytes and altered serum levels of soluble IL-2 cytokine receptors (Yang et al., 2001b). Taken together these facts argue in favour of the determination of potential molecular markers in blood leukocytes which could be useful for early diagnostics of glaucoma or for the treatment of patients with elevated intraocular pressure without glaucomatous eye changes. Our recent study using "gene hunting" techniques with concomitant mRNA and protein quantification showed indeed altered gene expression in blood leukocytes of vasospastic glaucoma patients in comparison to age and sex matched healthy subjects (Golubnitschaja-Labudova et al., 2000). Thereby the expression of p53 and 20 S proteasome subunit genes is upregulated which is usu-

ally found in ischemia-reperfusion injury leading to cell death. There is a possibility that activated adhesive leukocytes in the very early phase of ischemic insult contribute to secondary brain damage (Ishikawa et al., 1999). The role of circulating leukocytes in the pathogenesis of glaucoma has not been elucidated yet. In this work we present results supporting our hypothesis that adherent leukocytes could contribute to blood-brain barrier breakdown which has been found in glaucoma patients.

Materials and methods

Subjects

Blood samples (20 ml) were collected from six patients with glaucoma and six healthy control subjects (Table 1). All glaucoma patients had bilateral typical glaucomatous optic nerve head cupping and visual field defects (Octopus G1 program: MD = −7 dB), despite measurements of IOP that never exceeded 21 mm Hg. In addition, after local cooling of the fingers all these patients exhibited a stop in blood flow for more than 20 sec, which was detectable by nailfold capillaromicroscopy. In contrast, healthy control subjects had an unremarkable ophthalmological examination and did not show any vasospastic response. No patient had received either a systemic or a locally applied ocular therapy at least four weeks before the blood draw.

Subtractive hybridization

A) Leukocytes isolation

Blood samples (20 ml) anti-coagulated with heparin were collected from patients and controls. Leukocytes (including monocytes and stem cells) were separated using Ficoll-Hypaque gradients (Histopaque 1077, Sigma, Buchs, Switzerland) as described previously (Kalmar et al., 1998). Briefly, blood samples were diluted with equal volume of physiological buffer solution (PBS, GibcoBRL, Basel, Switzerland). Then, 2 ml of histopaque were placed into 10 ml sterile centrifuge tubes and 5 ml of diluted blood samples were carefully layered onto each histopaque gradient. Gradients were centrifuged at 475 g and 20°C for 15 minutes. The leukocytes bands were removed from interface between plasma and the histopaque layers of each tube and collected into one 50 ml tube. The total volume was brought to 50 ml with cold Dubecco's Modified Eagle Medium (DMEM, GibcoBRL, Basel, Switzerland). The cell suspension was washed three times with DMEM and the total number of cells was determined. Cells were finally suspended in PBS and aliquoted into eppendorf tubes at 10^7 cells/tube. After centrifugation cell pellets were dried and stored at −70°C until mRNA isolation.

Table 1. Information about the healthy subjects and NTG-patients

Subjects/NTG-patients			Normal controls		
No	Sex	Date of birth	No	Sex	Date of birth
1	F	12-07-47	7	F	17-01-45
2	F	16-07-43	8	F	29-10-44
3	F	09-02-57	9	F	18-08-60
4	F	18-08-55	10	F	22-09-54
5	M	12-06-62	11	M	12-05-66
6	F	14-06-34	12	F	07-06-32

B) mRNA isolation

Isolation of mRNA was performed using the Quick Prep Micro mRNA purification kit (Pharmacia Biotech, Uppsala, Sweden, Cat.No 27-9255-01). One microgram of mRNA from each of the 12 preparations was quality-checked by First-Strand cDNA Synthesis Kit (Pharmacia Biotech, Uppsala, Sweden, Cat.No 27-9261-01) using the incorporation of [α-^{32}P] dATP (Amersham, Buckinghamshire, UK, Cat.No AA0004) with subsequent electrophoresis on 1% agarose gel followed by autoradiography (Sambrook et al., 1989). The reflection film (NEN Life Science Products, NEF 496) was exposed to the gel for a period of 2 h at room temperature.

C) Construction of the substractive library

Construction of the subtractive library is principally based on the cloning of transcripts (in the form of cDNA) of those genes, which are activated/suppressed under pathological conditions. In our study we have considered both hypothesis: 1. glaucoma patients have additionally activated genes, and 2. glaucoma patients have suppressed genes in comparison to the control group. To prove both hypothesises the induced mRNA-pool was prepared in parallel experiments from glaucoma patients (1) and from the control group (2). The preparation of both induced and uninduced mRNA-pools for the molecular biological comparison with subsequent subtraction of the different transcripts is described below.

Construction of the subtractive library was done as follows: 10 micrograms of mRNA from leukocyte samples of glaucoma patients and controls were biotinylated by UV irradiation at 360 nm according to the instructions supplied in the subtractor kit (Invitrogen, Leek, Netherlands, Cat.No K4320-01). To avoid false positive results of the subtractive hybridization, the mRNA of the uninduced pool was added in excess. One microgram of each mRNA-pool was subjected to reverse transcriptase reaction with subsequent denaturation of mRNA-templates. Each newly-synthesized cDNA-pool (induced pool) was hybridized with the corresponding uninduced biotinylated mRNA-pool at 68°C for 48 h. The hybridization mixture was incubated with streptavidin and thus all the biotinylated molecules (uninduced mRNAs as well as RNA/DNA hybrids) were complexed with streptavidin. The streptavidin/nucleic acid-complexes were removed by repeated phenol-chloroform extraction and subtracted cDNAs were precipitated with ethanol. For each pair of glaucoma/control we have subtracted both induced "glaucoma" genes (induced pool of genes from glaucoma patient) and induced "normal" genes (induced genes of corresponding control). The 2nd strand cDNA synthesis was performed with cDNA Synthesis Kit (Boehringer Mannheim, Germany, Cat. No 1117831).

The aim of the constructed libraries was to compare gene expression individually and in the groups of glaucoma patients with healthy controls of corresponding age and sex. We have done the following grouping of blood samples for the libraries' construction (see Table 1): for the individual comparison: young female pair (subjects 4 vs. subjects 10); old female pair (subjects 6 vs. subject 7); male pair (subjects 5 vs. subjects 11).

For the comparison in groups: young female group (subjects 4 and 3 vs. subjects 10 and 9) and old female group (subjects 6, 1, 2 vs. subjects 7, 8, 12).

Only those genes which have been substracted from both the individual pairs and the corresponding groups have been considered as relevant.

D) Cloning of subtracted cDNA

In order to clone subtracted cDNAs, they were ligated with *Not*I-linkers followed by *Not*I-digestion. These *Not*I-linkered cDNAs were ligated to *Not*I-site of pSPORT 1 cloning vector (GIBCO, Life Technologies, Eggenstein, Germany). To enable visualization of subtracted cDNAs the cloned cDNAs were amplified using universal primers I – 5′ GTAAAACGACGGCCAGT 3′ and II – 5′ ACAGCTATGACCATG 3′ flanking the multiple cloning site of the cloning vector pSPORT 1. The amplificates were analysed in 1% agarose gel. The corresponding cDNAs were cut from the agarose gel, cleaned with DNA Clean Kit (AGS, Heidelberg, Germany) and recloned in Sma I-site of pUC 18 vector. The recombinant molecules were used for the transformation of highly competent INVαF *Escherichia coli* cells (Invitrogen, Cat.No C2020-03) and plated. Recombinant plasmid DNAs were analysed for the length of the inserted fragment using the method of restriction analysis. Plasmid DNAs were purified using QiaFilter Plasmid Midi System (Qiagen, Germany, Cat.No 12245). Plasmid DNAs were sequenced by MWG-Biotech (Ebersberg, Germany).

E) Gene identification

Homologies were determined by computer assisted comparison of data with DNA and protein gene banks (EMBL and SWISS-PROT, Heidelberg, Germany) (Altschul et al., 1990). Alignments were prepared using "DNASIS"-programs from MWG-Biotech (Ebersberg, Germany).

Results

The subtracted cDNAs have been cloned and sequenced. The comparison of their sequences with Genbank data revealed homologies with genes coding for the following known proteins:

Up-regulated transcripts correspond to the following known genes:

- lymphocyte IgE receptor (Fc epsilon RII/CD23)
- T cell-specific tyrosine kinase (ITK)
- thromboxan-A2-receptor
- alkaline phosphatase

Down-regulated transcripts are identical with a gene encoding a Na^+/K^+-ATPase. The alignments of the subtracted sequences are shown in Fig. 1–5 respectively.

Discussion

The role of leukocytes as inflammatory mediators of secondary injury after ischemic events has been recognized in the brain as well as other body organs (Gute et al., 1995). *In vivo* evidence of adherent leukocytes induced by global cerebral ischemia has been provided early after brain damage (Dirnagl, 1994). Activated leukocytes may impair a blood flow by disturb the microcirculation, exacerbate endothelial cell injury by releasing hydrolytic enzymes or by producing oxygen free radicals, may migrate into the ischemic parenchyma to interact with neurons and other supportive cells (del Zoppo et al., 1995). The blood-brain barrier breakdown has been detected also in glaucoma-patients (Yang et al., 2001a; Yang et al., 2001b). Nevertheless there is little information about the role of circulating leukocytes and a possible activation of their adhesion in the pathogenesis of the disease. Considerably more is already known about the mechanisms of the activation of adherent leukocytes

```
subtr.     1ggctctactttcagaagaaagtgtctctcttcctgcttaaacctctgtctctgacg56
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Fc-eps. 1292ggctctactttcagaagaaagtgtctctcttcctgcttaaacctctgtctctgacg1357
```

Fig. 1. Alignment of cDNA of the subtracted clone homologous to the lymphocyte IgE receptor (Fc epsilon RII/CD23) transcript. The sequence of the transcript is obtained from Gene Bank Heidelberg under the accession number M30447 (HSLYRE). Signs "|" show identical nucleotides

```
             *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
             E  D  R  P  A  F  S  R  L  L  R  Q  L  A  E  I  A  E  S
subtr.1CAGAAGATCGGCCAGCCTTCTCCAGACTGCTGCGTCAACTGGCTGAAATTGCAGAATCA59
       ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ITK 3819CAGAAGATCGGCCAGCCTTCTCCAGACTGCTGCGTCAACTGGCTGAAATTGCAGAATCA3877

            *  *
            G  L  *  *
subtr.60GGACTTTAGTAGAGACTGAGTACCAGGCCACGGGCTCAGATCCTGAATGGAGGAAGGA117
       ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ITK 3878GGACTTtagtagagactgagtaccaggccacgggctcagatcctgaatggaggaagga3935

subt. 118TATGTCCTC126
       |||||||||
ITK  3936tatgtcctc3944
```

Fig. 2. Alignment of cDNA and amino acid sequences of subtracted clone homologous to the T cell-specific tyrosine kinase (ITK) sequence obtained from Gene Bank Heidelberg under the accession number L10717 (HSTKTCS). Signs "|" show identical nucleotides. Asterisks show identical amino acids. Untranslated 3′UTR region of mRNA is written in small letters

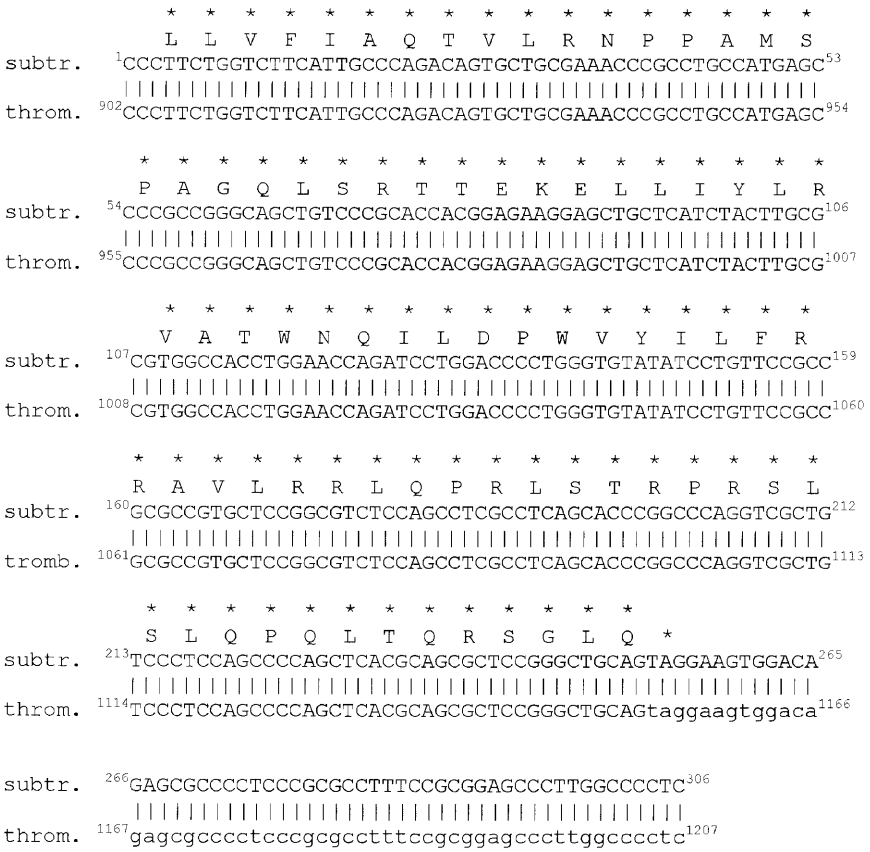

Fig. 3. Alignment of cDNA and amino acid sequences of subtracted clone homologous to the thromboxan A2 receptor sequence obtained from Gene Bank Heidelberg under the accession number U27325. Signs "|" show identical nucleotides. Asterisks show identical amino acids. Untranslated 3′UTR region of mRNA is written in small letters

by ischemia and postischemic reperfusion. Thereby perturbations of the T-cell repertoire accompanied by high frequencies of IL-2^+ and IL-4^+ $CD4^+$ and $CD8^+$ T cells have been shown (Liuzzo et al., 1999). An increased percentage of CD8 T lymphocytes and altered serum levels of soluble IL-2 cytokine receptors has been detected also in peripheral blood of glaucoma-patients (Yang et al., 1999). An induced synthesis of IL-4 increases an expression of lymphocyte receptors Fc-epsilon-RII-alpha via up-regulation of the Fc-epsilon-RII-alpha promoter activity (Suter et al., 1989). We found indeed this gene to be up-regulated in the circulating leukocytes of glaucoma patients. IL-2 induces an expression of T cell-specific tyrosin kinase (ITK) (Tanaka et al., 1993) the up-regulation of which has been detected in investigated leukocytes of glaucoma patients as well. The known alterations in the immune system of glaucoma patients include altered serum levels of soluble IL-2 cytokine receptors (Yang et al., 2001b). Investigations of the functional role of ITK in T cell activation showed that ITK plays an important role in IL-2 production, indicating a critical involvement of ITK in the initial stage of T cell activation by mediating signals from the TCR/CD3 complex, CD2, and CD28 (Tanaka et al., 1997). The up-regulation of ITK indicates the activation of leukocytes in glaucoma patients in comparison to the control group.

Elevated levels of endothelin-1 (ET-1) have been demonstrated in glaucoma-patients (Sugiyama et al., 1995). ET-1 may play a role in myocardial ischemia/reperfusion injury because both the release and vasoconstrictor effect of ET-1 are increased after ischemia. The increased vasoconstrictor effect of ET-1 can be mediated by ET-1-induced release of thromboxane A2 (Hornstein et al., 2001). Thromboxan A2 receptor antagonists reduce myocardial damage and polymorphonuclear leukocyte accumulation following coronary arterial occlusion and reperfusion in rats (Squadrito et al., 1993; Mazolewski et al., 1999; Fontana et al., 2001) indicating the function of this receptor in the ischemia induced leukocyte-endothelial cell interaction. The transcripts of the thromboxan A2 receptor gene have been isolated among the up-regulated genes from leukocytes of glaucoma patients by subtractive hybridization.

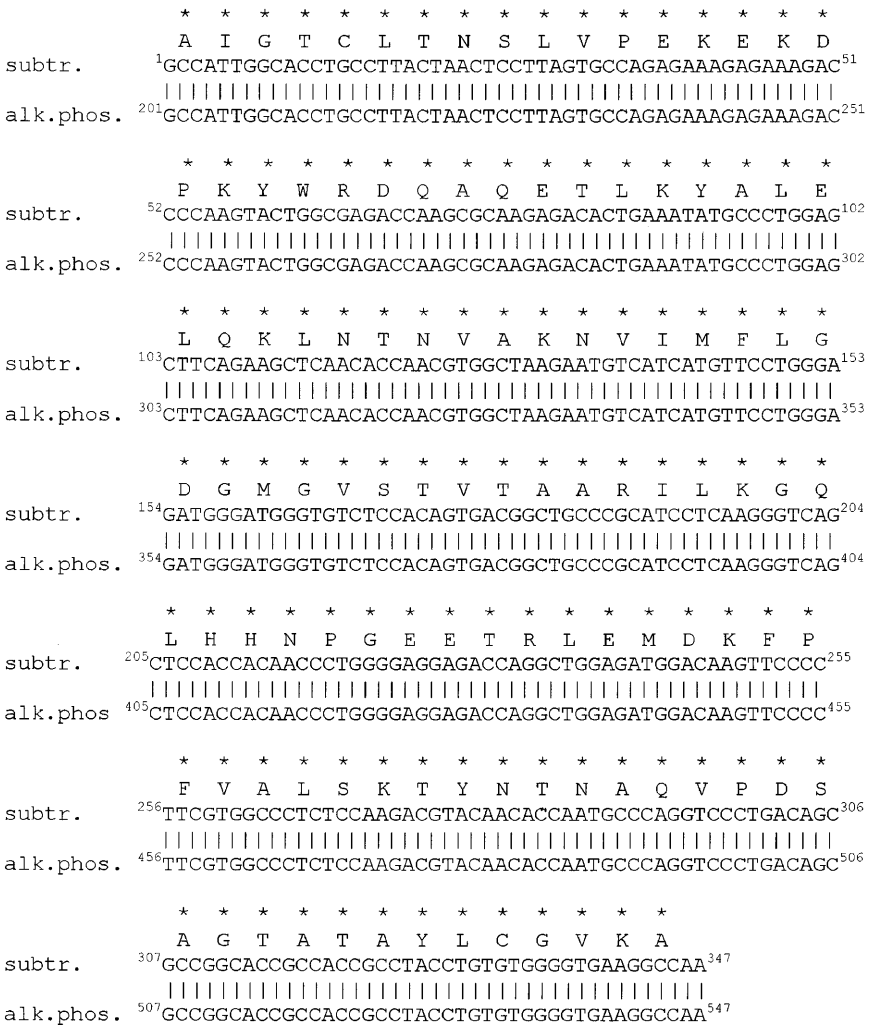

Fig. 4. Alignment of cDNA and amino acid sequences of subtracted clone homologous to the alkaline phosphatase sequence obtained from Gene Bank Heidelberg under the accession number AB011406. Signs "|" show identical nucleotides. Asterisks show identical amino acids

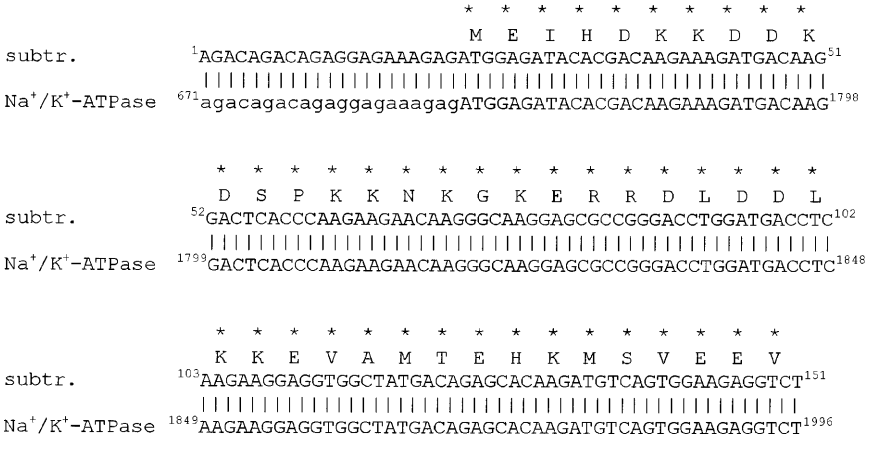

Fig. 5. Alignment of cDNA and amino acid sequences of subtracted clone homologous to the Na^+/K^+-ATPase sequence obtained from Gene Bank Heidelberg under the accession number X12910 (HSATPNK1). Signs "|" show identical nucleotides. Asterisks show identical amino acids. Untranslated 5′UTR region of mRNA is written in small letters

Among the up-regulated genes we have also identified the gene encoding an alkaline phosphatase. It has been shown earlier that patients with chronic ischemic heart disease and myocardial infarction showed high values of granulocyte alkaline phosphatase (Terletskaia, 1989). Although it is not clear yet which role this enzyme plays in the pathogenesis of myocardial infarction, its up-regulation in leukocytes of glau-

coma patients indicates the involvement of the same metabolic pathways – which should be further elucidated – also in pathomechanisms of glaucoma. This suggestion is supported by one additional finding: a gene encoding a Na^+/K^+-ATPase known to be down-regulated in lymphocytes in patients with acute myocardial infarction (Salomon et al., 1998), has also been found to be down-regulated in leukocytes of glaucoma patients. The lower Na^+/K^+-ATPase activity by patients with acute myocardial infarction may be one of the reasons of ventricular arrhythmias and coronary artery spasms. Decrease of intracellular potassium concentration and increase of intracellular calcium concentration may play a major role in the pathomechanism of coronary artery spasms; its role should be further elucidated in the pathogenesis of glaucoma.

Taken together the differential gene expression determined on the transcriptional level in circulating leukocytes of glaucoma patients indicates the involvement of metabolic pathways characteristic for the ischemic/reperfusion injury. Moreover, there is evidence that in these leukocytes adherence is induced. These findings allow to postulate the important role of adhesive leukocytes in the pathogenesis of glaucoma realized obviously by blood-brain barrier breakdown.

Molecular imaging of glaucoma provides insights in mechanisms of disease initiation and allows the early noninvasive diagnostics and development of a preventive treatment.

Supported by "Bio-Rad" and "Amersham Pharmacia Biotech"

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215: 403–410

Berkowitz BA, Zhang W (2000) Significant reduction of the panretinal oxygenation response after 28% supplemental oxygen recovery in experimental ROP. Invest Ophthalmol Vis Sci 41: 1925–1931

Chung HS, Harris A, Evans DW, Kagemann L, Garzozi HJ, Martin B (1999) Vascular aspects in the pathophysiology of glaucomatous optic neuropathy. Surv Ophthalmol 43 [Suppl 1]: S43–50

Del Zoppo GJ, Garcia JH (1995) Polymorphonuclear leukocyte adhesion in cerebrovascular ischemia: pathophysiologic implications of leukocyte adhesion. In: Granger DN, Schmid-Schonbein GW (eds) Physiology and pathophysiology of leukocyte adhesion. Oxford University Press, New York, pp 408–425

Flammer J (1987) Do ocular vasospasms help cause low tension glaucoma? Doc Ophthalmol Proc Ser 49: 397–399

Dirnagl U, Niwa K, Sixt G, Villringer A (1994) Cortical hypoperfusion after global forebrain ischemia in rats is not caused by microvascular leukocyte plugging. Stroke 25: 1028–1038

Fontana L, Giagulli C, Minuz P, Lechi A, Laudanna C (2001) 8-Iso-PGF2 alpha induces beta 2-integrin-mediated rapid adhesion of human polymorphonuclear neutrophils: a link between oxidative stress and ischemia/reperfusion injury. Arterioscler Thromb Vasc Biol 21: 55–60

Golubnitschaja-Labudova O, Liu R, Decker C, Zhu P, Haeflinger IO, Flammer J (2000) Altered gene expression in lymphocytes of patients with normal-tension glaucoma. Curr Eye Res 21: 867–876

Gross RL, Hensley SH, Gao F, Wu SM (1999) Retinal ganglion cell dysfunction induced by hypoxia and glutamate: potential neuroprotective effects of beta-blockers. Surv Ophthalmol 43 [Suppl 1]: S162–170

Gute D, Korthuis RJ (1995) Role of leukocytes adherence in reperfusion-induced microvascular dysfunction and tissue injury. In: Granger DN, Schmid-Schonbein GW (eds) Physiology and pathophysiology of leukocyte adhesion. Oxford University Press, New York, pp 359–380

Guthauser U, Flammer J, Mahler F (1988) The relationship between digital and ocular vasospasm. Graefes Arch Clin Exp Ophthalmol 226: 224–226

Hornstein PS, Zaugg CE, Zhu P, Allegrini PR, Buser PT (2001) Combined blockade of endothelin-1 and thromboxane A(2) receptors against postischaemic contractile dysfunction in rat hearts. Br J Pharmacol 132: 234–240

Ishikawa M, Sekizuka E, Sato S, Yamaguchi N, Inamasu J, Bertalanffy H, Kawase T (1999) Effects of moderate hypothermia on leukocyte-endothelium interaction in the rat pial microvasculature after transient middle cerebral artery occlusion. Stroke 30: 1679–1686

Kalmar JR, Arnold RR, Warbington ML, Gardner MK (1988) Superior leukocyte separation with a discontinuous one-step Ficoll-Hypaque gradient for the isolation of human neutrophils. J Immunol Methods 110: 275–281

Liuzzo G, Kopecky SL, Frye RL, O'Fallon WM, Maseri A, Goronzy JJ, Weyand CM (1999) Perturbation of the T-cell repertoire in patients with unstable angina. Circulation 100: 2135–2139

Mazolewski PJ, Roth AC, Suchy H, Stephenson LL, Zamboni WA (1999) Role of the thromboxane A2 receptor in the vasoactive response to ischemia-reperfusion injury. Plast Reconstr Surg 104: 1393–1396

Ong K, Farinelli A, Billson F, Houang M, Stern M (1995) Comparative study of brain magnetic resonance imaging findings in patients with low-tension glaucoma and control subjects. Ophthalmology 102 :1632–1638

Quigley HA (1996) Number of people with glaucoma worldwide. Br J Ophthalmol 80: 385–387

Salomon P, Mysiak A, Halawa B (1998) Sodium efflux through lymphocytic cell membranes in patients with acute myocardial infarction. Pol Arch Med Wewn 100: 543–550

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, pp 1.53–1.73

Squadrito F, Ioculano M, Altavilla D, Zingarelli B, Canale P, Campo GM, Saitta A, Oriti S, Spignoli G, Caputi AP (1993) G619, a dual thromboxane synthase inhibitor and thromboxane A2 receptor antagonist, reduces myocardial damage and polymorphonuclear leukocyte accumulation following coronary artery occlusion and reperfusion in rats. Pharmacology 47: 167–175

Stroman GA, Stewart WC, Golnik KC, Cure JK, Olinger RE (1995) Magnetic resonance imaging in patients with low-tension glaucoma. Arch Ophthalmol 113: 168–172

Sugiyama T, Moriya S, Oku H, Azuma I (1995) Association of endothelin-1 with normal tension glaucoma: clinical and fundamental studies. Surv Ophthalmol 39 [Suppl 1]: S49–56

Suter U, Texido G, Hofstetter H (1989) Expression of human lymphocyte IgE receptor (Fc epsilon RII/CD23). Identification of the Fc epsilon RIIa promoter and ist functional analysis in B lymphocytes. J Immunol 143: 3087–3092

Tanaka N, Asao H, Ohtani K, Nakamura M, Sugamura K (1993) A novel human tyrosine kinase gene inducible in T cells by interleukin 2. FEBS Lett 324: 1–5

Tanaka N, Abe H, Yagita H, Okumura K, Nakamura M, Sugamura K (1997) Itk, a T cell-specific tyrosine kinase, is required for CD2-mediated interleukin-2 promoter activation in the human T cell line Jurkat. Eur J Immunol 27: 834–841

Terletskaia LM (1989) Granulocyte alkaline phosphatase and myeloperoxidase in patients with ischemic heart disease. Vrach Delo 3: 13–14

Toriu N, Akaike A, Yasuyoshi H, Zhang S, Kashii S, Honda Y, Shimazawa M, Hara H (2000) Lomerizine, a Ca^{2+} channel blocker, reduces glutamate-induced neurotoxicity and ischemia/reperfusion damage in rat retina. Exp Eye Res 70: 475–484

Vorwerk CK, Naskar R, Schuettauf F, Quinto K, Zurakowski D, Gochenauer G, Robinson MB, Mackler SA, Dreyer EB (2000) Depression of retinal glutamate transporter function leads to elevated intravitreal glutamate levels and ganglion cell death. Invest Ophthalmol Vis Sci 41: 3615–3621

Wax MB (2000) Is there a role for the immune system in glaucomatous optic neuropathy? Curr Opin Ophthalmol 11: 145–150

Yang J, Han Z, Patil R, Wax MB (1999) Increased levels of soluble IL-2 receptors and IL-10 in sera from patients with glaucoma. Faseb J 13: A147

Yang J, Yang P, Tezel G, Patil RV, Hernandez MR, Wax MB (2001a) Induction of HLA-DR expression in human lamina cribrosa astrocytes by cytokines and simulated ischemia. Invest Ophthalmol Vis Sci 42: 365–371

Yang J, Patil RV, Yu H, Gordon M, Wax MB (2001b) T cell subsets and sIL-2R/IL-2 levels in patients with glaucoma. Am J Ophthalmol 131: 421–426

Authors' address: Assoc. Prof. Dr. Olga Golubnitschaja, Department of Radiology, Molecular/Experimental Radiology, University of Bonn, Sigmund-Freud-Strasse 25, D-53 105 Bonn, Germany,
E-mail: Olga.Golubnitschaja@ukb.uni-bonn.de