26. 少なくとも1眼の視力が良好な黄斑疾患症例の視覚関連 quality of life

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研究要旨　The 25-Item National Eye Institute Visual Function Questionnaire (VFQ-25) は視覚関連 QOL の定量的かつ包括的評価法であり、過去の黄斑疾患症例に対する検討報告では、視力が良好である方の眼の視力が多くの下位尺度スコアに強く相関している。今回少なくとも1眼の視力は0.7以上と比較的良好である黄斑疾患症例に限って、QOL スコアおよび年齢、性別、または視力との関連について検討した。東京大学附属病院眼科を受診した黄斑疾患症例に対して VFQ-25 日本語版による調査を行い、少なくとも1眼の小数視力が0.7以上の症例55例(男性38例，女性17例、年齢27-91歳)に対して、回答数が稀少であった「運転」を除く 11 項目の VFQ-25 下位尺度スコアを算出し、各スコアと性別、年齢、良好眼 logMAR 視力、および不良眼 logMAR 視力との関連の有無について相関分析および重回帰分析を用いて検討した。全症例では年齢と、「心の健康」または「役割制限」のスコアに有意な関連を認めたが、性別、良好眼視力、または不良眼視力とは関連を認めなかった。さらに、60 歳以下の症例群では、不良眼視力と「心の健康」または「役割制限」のスコアに有意な関連を認めた。少なくとも 1 眼の視力は 0.7 以上と比較的良好である黄斑疾患症例においては、年齢と「心の健康」および「役割制限」との関連を認め、また 60 歳以下の症例においては不良眼視力と「心の健康」 および「役割制限」が関連した。

A．研究目的

The 25-Item National Eye Institute Visual Function Questionnaire (VFQ-25) は、質問票を用いたプロファイル型の視覚関連 QOL 測定尺度であり、２５およびオプション１４項目の質問により、日常活動の制限、それに伴う精神面及び社会生活への影響といった下位尺度から、視機能に関連した QOL を総合評価することが可能である。日本語翻訳版が作成されており、既に信頼性・妥当性が検証済みであるのが特長である。[1 2]

黄斑疾患症例に対する VFQ-25 測定については、加齢黄斑変性症例に対して、行われた検討報告において、良い方の眼の視力と多くの下位尺度(全体的見え方、近見行動、遠見行動、社会生活、心の健康、役割制限、自立) が強く相関していること[3 4]、また、良好眼視力の変化が、VFQ-25 の前身であり51の質問項目からなる、VFQ-51 のスコア変化と相関すること[5]が報告されている。すなわち、視力良好眼の状態が QOL スコアに関

連しすることはよく知られている。しかし黄斑疾患の臨床経過においては、両眼が同時に視力低下することは稀であり、まず片眼(いわゆる first eye)が罹患し、その後僚眼（second eye）に及ぶことがほとんどである。過去の報告によれば、second eye の視力が QOL に強く相関することが示唆されるが、それでは second eye 視力が良好である状況下において、first eye のみ罹患した状態が QOL に関与するのか否か、するならばどのように関与するかについてはよく分かっていない。このような、少なくとも 1 眼の視力が良好な症例の QOL を検討した報告はない。今回我々は、少なくとも 1 眼の視力が 0.7 以上と比較的良好な黄斑疾患症例に対して、VFQ-25 日本語版を用いて視覚関連 QOL を調査し、その分布と、性別、年齢、および視力との関連について検討した。

B．研究方法

2003 年 7 月～2005 年 7 月に東京大学医学部附属病院黄斑外来を受診した黄斑疾患（後掲）を有する症例のうち、少なくとも 1 眼の矯正視力が 0.7 以上である計 55 例すなわち男 38 例、女 17 例、平均年齢 67 歳(27-91)の症例に対して、VFQ-25 日本語版による調査を行った。調査は 25 項目および 14 のオプション項目につき自己記入用紙を用いて回答を得た。自己記入が困難な症例は家族または眼科スタッフが介助した。本調査票の使用に関しては、NPO 健康医療評価研究機構に対し、VFQ-25 日本語版の使用登録を事前に申請した。

VFQ-25 のスコアリングについては、各設問(5 択式)毎に、解答に既定のスコア(0-100)を割り付け、回答数の稀少であった「運転」を除く１１の各下位尺度毎に割り当てられた設問のスコアを加算平均した。下位尺度および VFQ-25 またはオプション１４項目の質問を加えた VFQ-39 の相当項目数は別表の通りである。

表：VFQの下位尺度と相当質問項目数

下位尺度	設問数	
	VFQ-25	-39*
全体的健康観	1	2
全体的見え方	1	2
眼痛	2	2
近見視力による行動	3	6
遠見視力による行動	3	5
社会生活機能	2	3
心の健康	4	5
役割制限	2	4
自立	3	4
色覚	1	1
周辺視覚	1	1

解析方法は、①全症例、②６１歳以上の症例群、および③６０歳以下の症例群に対して、説明変数（性別、年齢、良好眼 logMAR 視力、および不良眼 logMAR 視力）および従属変数 VFQ 下位尺度１１項目の各スコア）に関し、（１）VFQ-25 スコアでの Pearson 相関分析、（２）VFQ-39 スコアでの Pearson 相関分析、および（３）ステップワイズ重回帰分析（変数増加方）を行い、少なくとも（１）かつ（３）において $p<0.05$ の有意な相関を示したものを「関連有り」とした

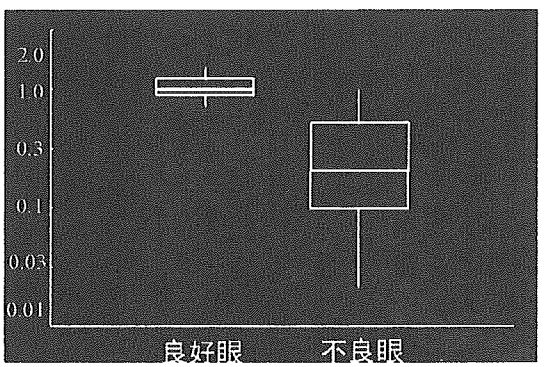
（倫理面への配慮）

本研究は、東京大学医学部倫理委員会の承認を受けた。対象症例からは文書による同意を得、診療記録の調査におけるプライバシーの保護に特に配慮した。

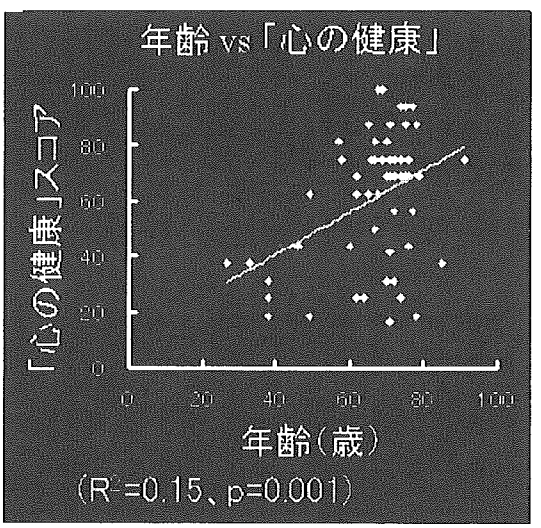
C．研究結果

背景疾患は加齢黄斑変性(AMD)およびポリープ状脈絡膜血管症41例、近視性新生血管黄斑症9例、特発性脈絡膜新生血管4例、慢性中心性漿液性網脈絡膜症1例である。視力分布は別図の通りである。

図：視力分布グラフ

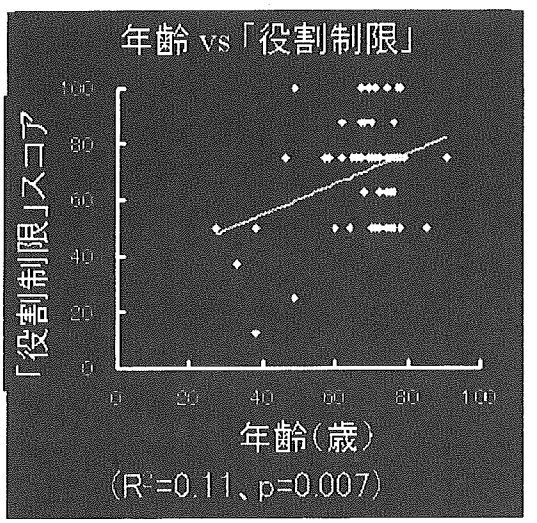


図：全症例における年齢と「心の健康」スコアの分布および回帰直線

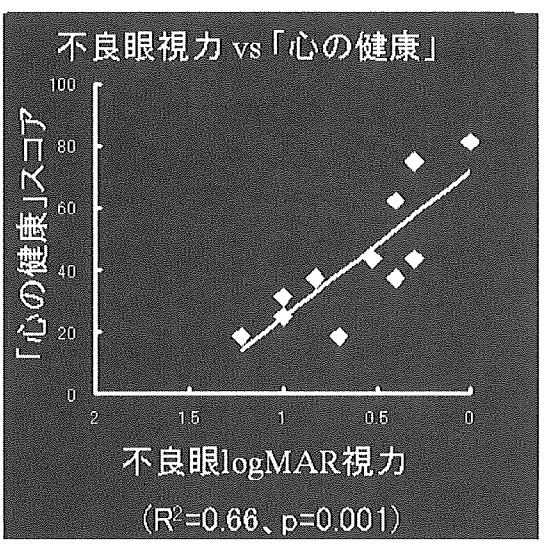


①全症例においては、年齢と「心の健康」および「役割制限」スコアに関連を認めた。
②61 歳以上症例群においては、いずれの変数間とも関連を認めなかった。
③60 歳以下症例群においては、不良眼 logMAR 視力と「心の健康」および「役割制限」スコアに関連を認めた。

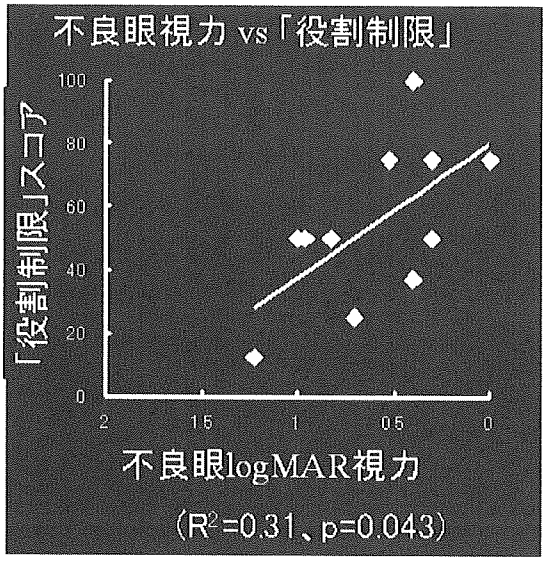
図：全症例における年齢と「役割制限」スコアの分布および回帰直線



図：６０歳以下の症例における不良眼 logMAR視力と「役割制限」スコアの分布および回帰直線



図：６０歳以下の症例における不良眼logMAR視力と「心の健康」スコアの分布および回帰直線



D．考察

過去に、白人の両眼加齢黄斑変性症例における VFQ-25 測定において、年齢と「全体的見え方」、「遠見行動」、「心の健康」、および「役割制限」とが正相関したと報告されている。[5]今回の検討では、全症例に関して、同様に年齢と「心の健康」「役割制限」との関連を認めた。少なくとも 1 眼の視力が良好な黄斑疾患症例において、年齢が QOL に関与する可能性が示唆される。

一方、60 歳以下の症例に関して、不良眼視力と「心の健康」「役割制限」スコアに関連を認めたが、61 歳以上の症例には認めなかった。症例数が少なくさらなる検討を要するが、少なくとも 1 眼の視力が良好な黄斑疾患症例のうち、年齢が比較的若い症例は不良眼視力が QOL に関与する可能性が示唆される。

E．結論

少なくとも 1 眼の視力が 0.7 以上の黄斑疾患症例に対し、VFQ-25 日本語版を用いて視覚関連 QOL を調査したが、年齢と、「心の健康」および「役割制限」との関連を認め、また 60 歳以下の症例においては不良眼視力と「心の健康」 および「役割制限」との関連を認めた。

F．健康危険情報

なし

G．研究発表

1．論文発表

なし

2．学会発表

小畑　亮　他．片眼視力が良好な黄斑疾患症例の視覚関連 quality of life．第 59 回日本臨床眼科学会、札幌、2005

H．知的財産権の出願・登録状況

1．特許取得

なし

2．実用新案登録

なし

3．その他

なし

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27. 家兎眼での新しい加齢黄斑変性モデル

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研究要旨　加齢黄斑変性の確実な危険因子である「加齢」により眼内にまず起こる変化は、網膜色素上皮（RPE: retinal pigment epithelium）細胞内へのリポフスチンの蓄積である。我々は、リポフスチン内に多く存在すると考えられる最終糖化産物（AGE: advanced glycation end products）からなる微粒子を作製し家兎の網膜下に注入することにより、新しい加齢黄斑変性モデルを開発した。AGE 微粒子を網膜下に投与 1 週目には RPE 細胞の貪食作用により RPE 内に移行しており、リポフスチン蓄積と類似した状態となった。コントロール微粒子では、移植 8 週後、移植部位に軽度の蛍光漏出を認めたが、12 週目には漏出は軽減し、組織的に脈絡膜新生血管組織は認められなかった。一方、AGE 微粒子投与群では、4 週目にはドルーゼン様沈着物をコントロール微粒子群に比較して有意に認めるようになり、組織的にはドルーゼン様沈着物の他に、時に網膜下および RPE 下の脈絡膜新生血管、遊走してきたマクロファージを認めた。このリポフスチン蓄積模倣による家兎眼加齢黄斑変性モデルは、リポフスチンの病態への関わりを解明する上で有用であると考えられた。

A. 研究目的

加齢黄斑変性(AMD)は欧米諸国では成人失明の主要原因であり、近年、我が国でも増加傾向にある。病態については不明な点が多い。現在、光線力学療法を初めとして、様々な治療が行われているが、一度低下した視力を改善させることは難しい場合が多く、今後、より早期に行える新しい治療法の開発が望まれるが、そのためにも病態を解明することが重要である。AMD の確実な危険因子は「加齢」であるが、「加齢」によりまず眼内に起こる変化は、網膜色素上皮（RPE）内へのリポフスチンの沈着である。我々は、リポフスチンの主要成分と考えられる最終糖化産物(AGE)の反応を利用してリポフスチンを模倣した微粒子を作製し、これを家兎の網膜下に注入することにより、AMD に類似した変化が誘導できるか検討した。

B. 研究方法

トルエン内でアルブミン水溶液のエマルジョンを形成させた後、AGE 反応の中間産物であるグリコールアルデヒドに反応させて、AGE 微粒子を作製した。グルタールアルデヒドを利用してコントロール微粒子も作製した。これらを家兎の網膜下に経強膜・経網膜的に注入した。これらの眼を術後 1 週、4 週、8 週、12 週に蛍光眼底撮影(FA)、眼底検査を施行、また摘出した眼球を組織的に検討した。対照群としては、コントロール微粒子の他に、アルブミン水溶液、水溶性 AGE を注入して、同様の観察を行った。

（倫理面への配慮）

動物の取り扱いについては、Association for Research and Vision in Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research に従って実行された。

C．研究結果

アルブミン水溶液を家兎の網膜下に注入した眼においては、注入部の色素のむらを認めるのみであった。水溶性 AGE を注入した眼においては、術後４週目にはFA にて過蛍光点を認めたが、徐々に改善した。組織的には視細胞の萎縮を認めたが RPE に構造上の異常を認めなかった。コントロール微粒子、AGE 微粒子共に、術後一周目には RPE の貪食作用により RPE の細胞内に移行したが、コントロール微粒子は時間とともに消退する傾向を認めた。微粒子は RPE の基底部側に移動。ブルッフ膜に向かって排泄される傾向を示したが、AGE 微粒子は術後 12 週目においても RPE 内に多く留まり、リポフスチンの蓄積と類似した状態を得た。コントロール微粒子注入眼では、術後４週、８週において、FA にて過蛍光点を認めたが、１２週目には消退した。組織的には、網膜の構造の乱れ、RPE の脱色素を認めたが、RPE の一層の構造は保たれていた。それに比べ、AGE 微粒子注入群では、４週目から蛍光漏出を認める場合や、１２週目になって蛍光漏出を認める場合があり、組織的に術後４週目での蛍光漏出はclassic 脈絡膜新生血管(CNV)、術後１２週目になって認める蛍光漏出部はoccult CNV に相当すると考えられた。術後４週において、AGE 微粒子注入群では、コントロール微粒子注入群に比較して有意にドルーゼン様の沈着物を認めた。水溶液注入眼ではドルーゼンは形成されなかった。AGE 微粒子注入群において、組織的に CD68 陽性のマクロファージと考えられる細胞が AGE 微粒子を含んだ RPE 細胞の基底部側に遊走している像を認めた。

D．考察

AGE 微粒子は RPE 内に長く留まり、その結果として、ドルーゼン、時にマクロファージの遊走、CNV の発生を認めると考えられた。このモデルにおいて認めたclassic CNV は、術後早期より発生したため、手術侵襲、大量の微粒子が網膜下に存在することにより物理化学的な影響により、炎症性に誘導された可能性が高いが、occult CNV に関しては、術後１２週目などに発生を認め、RPE に滞留する微粒子の影響により発生したと考えられた。

E．結論

以上の結果より、AMD におけるリポフスチンの蓄積の影響が示唆された。この新しい家兎加齢黄斑変性モデルは、AMD の病態におけるリポフスチンの役割を検討する上で有用であり、ドルーゼンなどの病態の理解にも役立つと考えられた。

F．健康危険情報

なし

G．研究発表

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H. 知的財産権の出願・登録状況

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

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28. ラット脈絡膜新生血管モデルを用いた光線力学的治療における細胞死のメカニズム

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研究要旨　ラット脈絡膜新生血管モデルにPDTを施行し、アポトーシスの機構とPI3-K/Akt経路の関与について検討した。実験動物はBrown-Norwayラットを使用し、CNVはレーザー光凝固により作成した。verteporfinを3, 6, 12 mg/m^{2}の濃度で静注し、15分後に照射出力600mW/cm^2、光照射エネルギー量25 J/cm^{2}の条件でPDTを行った。CNV中のアポトーシス細胞はTUNEL法で、カスパーゼ3, 9の活性は免疫染色法により検出した。リン酸化Aktは免疫染色法とWestern blot法で検出し、Aktの活性化にはIGF-1(5 , 10μg)を、不活性化にはwortomannin(12.5 μM)を硝子体内注入した。CNV中のTUNEL陽性細胞はPDT後3時間で増加し始め、6時間後に最大となった。Aktの脱リン酸化はPDT1時間以内に始まり、その後カスパーゼが活性化された。CNV中のカスパーゼ9陽性細胞はTUNEL陽性を示した。IGF-1は有意にAktをリン酸化し、TUNEL陽性細胞数を減少させた。また、その効果はwortomanninにより減じられた。ラットCNVモデルに対するPDTはカスパーゼ介在性のアポトーシスをきたし、その制御にAktの脱リン酸化が関与していることが示唆された。

A. 研究目的

脈絡膜新生血管（choroidal neovascularization, CNV）に対して光感受性物質verteporfinをもちいた光線力学的治療（photodynamic therapy, PDT）が広く行われているが、そのメカニズムに関しては不明な点が多い。近年、PDTによりCNV中の細胞がアポトーシスをきたすことが報告されている[1,2)]。今回我々はラット脈絡膜新生血管モデルにPDTを施行し、アポトーシスの機構とPI3-K/Akt経路の関与について検討した。

B. 研究方法

実験動物はBrown-Norwayラットを使用し、CNVはレーザー光凝固により作成した。verteporfinを3, 6, 12 mg/m^{2}の濃度で静注し、15分後に照射出力600mW/cm^2、光照射エネルギー量25 J/cm^{2}の条件でPDTを行った。CNV中のアポトーシス細胞はTUNEL法で、カスパーゼ3, 9の活性は免疫染色法により検出した。リン酸化Aktは免疫染色法とWestern blot法で検出し、Aktの活性化にはIGF-1(5 μg)を、不活性化にはwortomannin(12.5 μM)を硝子体内注入した。

（倫理面への配慮）

動物実験の取り扱いは ARVO に準じた。

C．研究結果

CNV 中の TUNEL 陽性細胞は PDT 後 3 時間で増加し始め、6 時間後に最大となった。Akt の脱リン酸化は PDT1 時間以内に始まり、その後カスパーゼが活性化された。CNV 中のカスパーゼ 9 陽性細胞は TUNEL 陽性を示した。IGF-1 は有意に Akt をリン酸化し、TUNEL 陽性細胞数を減少させた。また、その効果は wortomannin により減じられた。

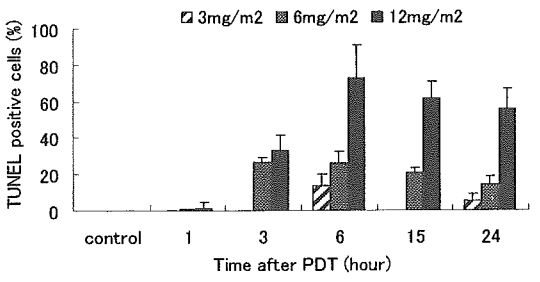

図 1　CNV 中の TUNEL 陽性細胞の割合（%）

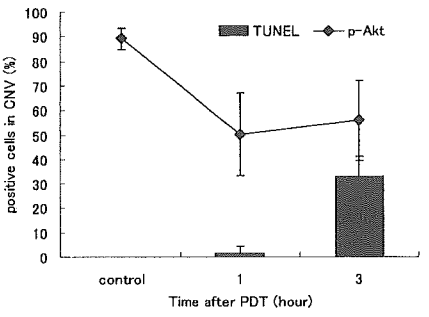

図 2　CNV 中の p-Akt, TUNEL 陽性細胞の割合（%）

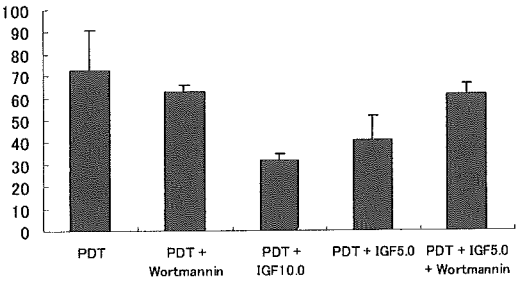

図 3　IGF-1 (5, 10 μg)、wortomannin (12.5 μM) を硝子体内注入した時の CNV 中の TUNEL 陽性細胞の割合（%）

D．考察

Akt の脱リン酸化は PDT1 時間以内に始まり、3 時間以内にカスパーゼが活性化された。CNV 中のカスパーゼ 9 陽性細胞は TUNEL 陽性を示したことから、PDT はカスパーゼ介在性のアポトーシスをきたしうると考えられた。IGF-1 は Akt をリン酸化して TUNEL 陽性細胞数を減少させ、その効果は wortomannin により減じられたことから、PDT 後の Akt の脱リン酸化がアポトーシスの一因と考えられた。

E．結論

ラット CNV モデルに対する PDT はカスパーゼ介在性のアポトーシスをきたし、その制御に Akt の脱リン酸化が関与していることが示唆された。

F．健康危険情報

なし

G．研究発表

1．論文発表

なし

２．学会発表

なし

Ｈ．知的財産権の出願・登録状況

１．特許取得

なし

２．実用新案登録

なし

３．その他

なし

Ｉ．参考文献

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Protective effect of polyethylene glycol-superoxide dismutase on leukocyte dynamics in rat retinal microcirculation under lipid hydroperoxide-induced oxidative stress

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Abstract

The levels of lipid hydroperoxide (LHPs) in vitreous are elevated in a variety of retinal disorders. Recently, we have shown that increased levels of LHPs in the vitreous enhanced leukocyte-endothelium interaction in the retina, which should contribute to the initial disturbance of the retinal microcirculation. Based upon the previous work, the purpose of the present study was to investigate the effect of polyethylene glycol-superoxide dismutase (PEG-SOD), one of the important enzyme antioxidants, on leukocyte-endothelial interaction in the retinal microcirculation under LHP-induced oxidative stress. Male Brown-Norway rats weighing approximately 250 g were used. LHP(18:2) was made from linoleic acid (LA) with lipoxygenase and 10 μg of LHP dissolved in 5 μl of sodium borate buffer (SBB, 0.02 M) was slowly injected into the vitreous using a 33-gauge needle. PEG-SOD (5000 units/kg) was given intravenously 5 min before LHP injection. At 2, 4, 6, 12, 24 and 48 hr after the vitreous injections, we evaluated the number of rolling leukocytes along the major retinal veins and the number of leukocytes that accumulated in the retinal microvasculature with acridine orange digital fluorography. In LHP-treated rats, leukocyte rolling along the major retinal veins was maximal at 6 hr after LHP injection. The number of rolling leukocytes in the PEG-SOD-treated rats was decreased to 5.5% of those in the LHP-treated rats at 6 hr after LHP injection ($P<0.01$). No rolling leukocytes were observed in either control or vehicle-treated eyes. The number of accumulated leukocytes in LHP-treated eyes started to increase at 12 hr, and peaked at 24 hr which was significantly higher than in both control and vehicle-treated eyes ($P<0.01$). The number of accumulated leukocytes in the PEG-SOD-treated rats was reduced by 88.0% at 24 hr ($P<0.01$). Intravenous injection of PEG-SOD significantly inhibited the leukocyte rolling and its accumulation under LHP-induced oxidative stress. These results suggest that PEG-SOD might attenuate various retinal microcirculatory disorders associated with LHP.

Keywords: lipid polyethylene-hydroperoxide; leukocyte; glycol-superoxide dismutase; retinal microcirculation

1. Introduction

Free radical and lipid peroxide formation, which can cause oxidative stress-induced damage to cell membranes, are initiated by various factors. As the eye is always exposed to initiators such as oxygen, light, ultraviolet ray and x-irradiation, the relationship between free radicals and ocular diseases has attracted much attention. Many studies have suggested important roles of free radicals and lipid peroxides in various ocular diseases including keratitis, cataract, uveitis, retinal degeneration, diabetic retinopathy, retinopathy of prematurity and retinal ischemic diseases (Lakatos et al., 1982; Sery and Petrillo, 1984; Bhuyan et al., 1986; Armstrong et al., 1992; Alio et al., 1995; Spaide et al., 1999).

The retina contains a high proportion of polyunsaturated fatty acids, which are susceptible to lipid peroxidation.

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Lipid hydroperoxide (LHPs) derived from oxidized unsaturated fatty acids are prominent intermediates of propagative reactions induced by activated species such as hydroxyl radical, lipid oxyl or peroxyl radicals, singlet oxygen, and peroxynitrite (Girotti, 1998). It is also known that lipid peroxidation injury to the endothelial cell membrane provides a signal or serves as a marker that can be recognized by circulating polymorphonuclear leukocytes (Del Maestro et al., 1981). Patel et al. (1991) reported that reactive oxygen species like H_2O_2 and LHP increased the expression of P-selectin and the adherence of neutrophils to endothelial cells in vitro. Recently, we have shown that oxidative stress induced by 18:2 LHP injection in the vitreous enhanced leukocyte-endothelium interaction in the retinal microcirculation in vivo using acridine orange digital fluorography (Tamai et al., 2002).

LHP is associated with a variety of retinal disorders such as diabetic retinopathy (Armstrong et al., 1992; Augustin et al., 1993), Eales' disease (Bhooma et al., 1997), proliferative vitreoretinopathy (Boker et al., 1994), retinopathy of prematurity (Lakatos et al., 1982), and age-related macular degeneration (Spaide et al., 1999). In fact, in the vitreous samples from patients with proliferative diabetic retinopathy (Augustin et al., 1993; Verdejo et al., 1999) or proliferative vitreoretinopathy (Boker et al., 1994; Verdejo et al., 1999), LHP levels were shown to be significantly elevated. In those patients, antioxidant activity was reduced compared with normal counterparts (Verdejo et al., 1999). It was demonstrated that levels of superoxide dismutase (SOD), one of the important enzyme antioxidants were notably reduced in diabetic patients (Kernell et al., 1992) and patients with Eales' disease which induces retinal vascular occlusion, inflammation and neovascularization (Sulochana et al., 1999). Therefore, we hypothesize that increasing the levels of SOD may reduce retinal microcirculatory disorders associated with LHP. In this study, we have investigated the effect of intravenous injection of SOD on rat retinal microcirculatory disorders under LHP-induced oxidative stress in terms of leukocyte dynamics in vivo. We used here polyethylene glycol-conjugated superoxide dismutase (PEG-SOD) that has a longer half-life in plasma (>30 hr) than native SOD and has been proven to be effective against ischemic conditions (Pyatak et al., 1980; Tamura et al., 1988; Chi et al., 1989).

2. Materials and methods

2.1. Animal model

Male Brown–Norway rats weighing approximately 250 g were used. Only one eye of each rat was used. Rats were anesthetized with a mixture (1:1) of xylazine hydrochroride (4 mg/kg) and ketamine hydrochroride (10 mg/kg). The pupils were dilated with 0.5% tropicamide and 2.5% phenylephrine hydrochroride. LHP (18:2) was made from linoleic acid (LA) with lipoxygenase and 10 μg of LHP dissolved in 5 μl of sodium borate buffer (SBB, 0.02 M) was slowly injected into the vitreous using a 33-gauge needle (Browne and Armstrong, 2002). Vehicle-treated rats were given the same amount of LA dissolved in 5 μl of SBB. PEG-SOD-treated rats were injected intravenously with 5000 units/kg of PEG-SOD (Sigma-Aldrich) 5 min before LHP injection. All experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

2.2. Acridine orange digital fluorography

Acridine orange digital fluorography was performed as previously described (Kimura et al., 1995; Nishiwaki et al., 1995). In this technique, a scanning laser ophthalmoscope (Rodenstock Instrument), coupled with a computer-assisted image analysis system, makes continuous high-resolution images of the fundus stained with the metachromatic fluorochrome, acridine orange (AO; Wako Pure Chemical), which emits a green fluorescence when it interacts with DNA. The spectral properties of AO-DNA complexes are very similar to those of sodium fluorescein, with an excitation maximum at 502 nm and an emission maximum at 522 nm (Darzynkiewicz and Kapuscinsky, 1990). An argon blue laser was used as the illumination source, with a regular emission filter for fluorescein angiography.

Immediately before acridine orange digital fluorography, rats were anesthetized, and the pupils were dilated. A contact lens was used to retain corneal clarity throughout the experiment. Each rat had a catheter inserted into the tail vein, and was placed on a stereotaxic platform. Body temperature was maintained at 38 ± 0.5 °C. Immediately after AO (0.1% solution in saline) solution was infused intravenously, leukocytes stained selectively among circulating blood cells were observed with the scanning laser ophthalmoscope. Nuclei of vascular endothelial cells were also stained. AO was injected continuously through the catheter for 1 min at a rate of 1 ml/min. The fundus was observed to evaluate the leukocyte dynamics in the retinal microcirculation for 5 min after AO injection in a 40° field. AO easily infiltrates through vessel walls and diffuses into the retina due to its membrane permeability. Accordingly, a few minutes after AO injection was completed, fluorescence of circulating leukocytes was faint, due to washout. In contrast, leukocytes that had been trapped in the retinal microcirculation remained fluorescent for approximately 2 hr, being recognized as distinct fluorescent dots 30 min after AO injection. At 30 min after the injection of AO, the fundus was observed again to determine leukocyte accumulation in the retinal microcirculation. The obtained images were recorded on digital videotape at a rate of 30 frames/sec for further image analysis. After the experiment, rats were killed with an anesthetic overdose, and the eye was enucleated to determine a calibration factor with which to

convert values measured on a computer monitor (in pixels) into real values (in μm).

2.3. Experimental design

Acridine orange digital fluorography was performed at 2, 4, 6, 12, 24 and 48 hr after vitreous injections in LA and LHP-treated rats. In PEG-SOD treated-rats, it was held at 6, 12, 24 and 48 hr after LHP injections. Non-operated rats were used as controls. Five different rats were randomly selected and used at each time point in each group. We evaluated the number of rolling leukocytes along the major retinal vessels, the number of leukocytes accumulated in the retinal microcirculation, and the diameters of major retinal vessels in a masked manner.

2.4. Image analysis

The digital video recordings were analysed with an image analysis system, described in detail elsewhere (Kimura et al., 1995; Nishiwaki et al., 1995) with a slight modification. In brief, we used a computer equipped with software (DVgate, SONY) which enters the digital images in real time (30 frames/sec) to 640 horizontal and 480 vertical pixels with an intensity resolution of 256 steps into a personal computer.

Rolling leukocytes were defined as leukocytes that moved at a velocity much slower than that of free-flowing leukocytes. The process of differentiating rolling leukocytes from free-flowing leukocytes has been described in a previous article (Tsujikawa et al., 1998). In brief, leukocytes rolling along the major retinal veins were easily recognized on the video monitor, because even the fastest rolling leukocyte moved almost 300 times more slowly than the average for free-flowing leukocytes. Since no leukocytes with intermediate velocity were observed, it was not difficult to distinguish rolling leukocyte from free-flowing leukocytes. Their numbers were calculated from the number of rolling cells passing a fixed line in all major veins (4–7 veins) at a distance of 1 disk diameter from the center of the optic disc per minute. The average number of rolling leukocytes in individual major veins was used as the value for each rat.

The number of leukocytes accumulated in the retinal microcirculation was evaluated at 30 min after AO injection. The number of fluorescent dots in the retina within 8 areas of 100 pixels square at a distance of 1 disk diameter from the edge of the optic disk was counted. The average density of leukocytes in individual areas was used as the value for each rat.

The diameters of major retinal vessels were measured at 1 disk diameter from the center of the optic disk in monochromatic images recorded before AO injection. Each vessel diameter was calculated in pixels as the distance between the half-high points determined separately on each side of the density profile of the vessel image and converted into real values using the calibration factor. The averages of the individual arterial and venous diameters were used as the arterial and venous diameters for each rat.

2.5. Statistical analysis

All values are presented as mean ± S.D.. Data were compared by ANOVA, with post hoc comparisons tested using the Bonferroni procedure. Probabilities of $P<0.05$ were considered to be statistically significant.

3. Results

3.1. Leukocyte rolling

Immediately after AO was infused, many free-flowing leukocytes were visualized. No rolling leukocytes were observed along the major retinal veins in control and LA-treated rats. In all eyes injected with LHP, a few leukocytes rolling along major retinal veins were observed initially at 2 hr after the injection. The number peaked at 110.6 ± 11.4 cells/min at 6 hr after the injection and decrease thereafter. The number of rolling leukocytes was significantly less in PEG-SOD-treated rats than in LHP-treated rats ($P=0.0104$). In PEG-SOD-treated rats, the number of rolling leukocytes was 6 ± 0.3 cells/min at 6 hr after LHP injection, which was significantly reduced by 94.5% compared with that in LHP-treated rats ($P<0.01$) (Fig. 1). There weren't any rolling leukocytes along retinal arteries in all groups throughout the experiments.

3.2. Leukocyte Accumulation in the retinal microcirculation

Leukocytes accumulated in the retinal microcirculation were recognized as distinct fluorescent dots 30 min after AO injection, although no circulating leukocytes fluoresced (Fig. 2). In LA-treated rats, a few leukocytes could be recognized at any time point. The number of accumulated

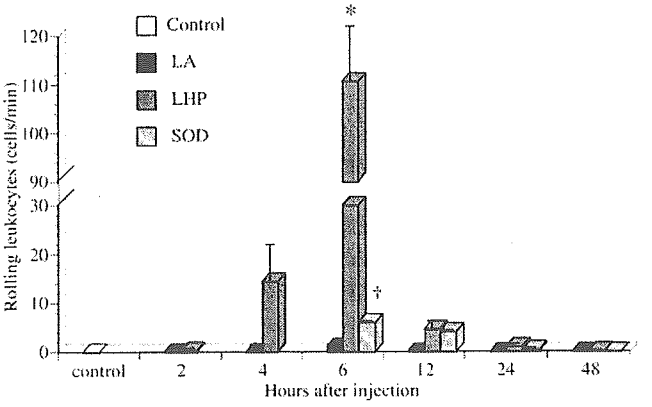

Fig. 1. Time course of the number of rolling leukocytes in major veins. Values are mean ± S.D. *$P<0.01$, compared with LA-treated rats. †$P<0.01$, compared with LHP-treated rats. Five different rats were used at each time point in each group.

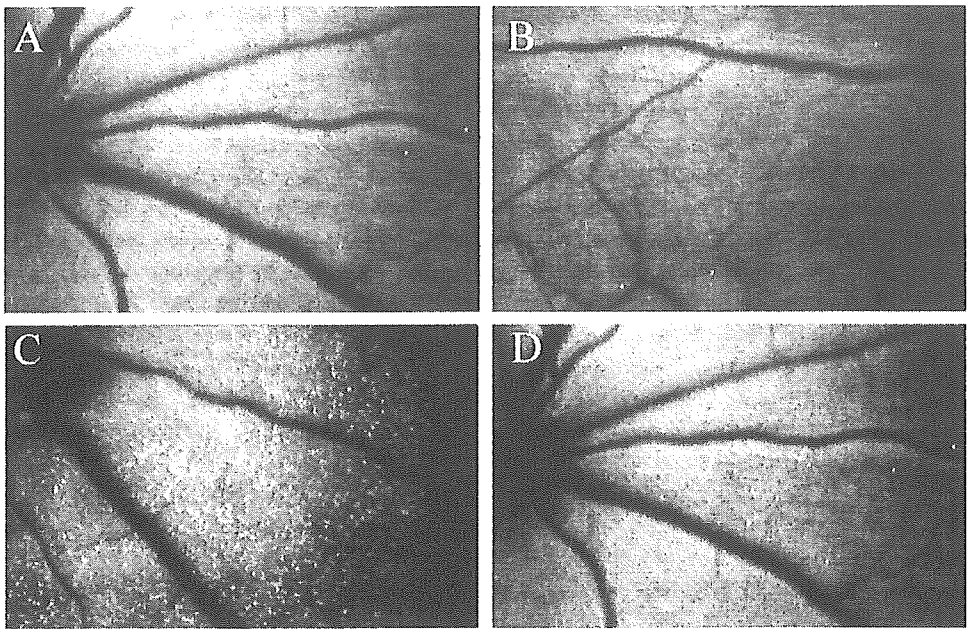

Fig. 2. Representative fundus images of leukocyte accumulation at 24 hr after vitreous injection. Leukocyte accumulated in the retina were observed as fluorescent dots 30 min after acridine orange injection. A small number of leukocytes could be found in control (A) and LA-treated rats (B). In LHP-treated rats, increasing number of leukocytes accumulated at 24 hr after LHP injection (C). Significant reduction of leukocyte accumulation was seen in PEG-SOD-treated rats (D).

leukocytes in LHP-treated eyes started to increase at 12 hr (152.2 ± 37.3 cells/mm^2), and peaked at 24 hr (919.0 ± 223.1 cells/mm^2) which was significantly higher than in both control and LA-treated eyes (21.3 ± 5.0 and 45.3 ± 9.8 cell/ mm^2, respectively, $P<0.01$). The number of accumulated leukocytes was significantly less in PEG-SOD-treated rats than in LHP-treated rats ($P=0.0105$). With the treatment of PEG-SOD, the number of accumulated leukocytes was significantly reduced by 73.7% at 12 hr ($P<0.01$) and 88.0% at 24 hr ($P<0.01$) after LHP injections (Fig. 3).

3.3. Diameters of major retinal vessels

In arteries, a slight vasodilation appeared to occur at 4–24 hr after LHP injection; however, no significant differences were observed among any experimental groups (Fig. 4(A)). In veins, significant vasodilation was observed at 6–24 hr after LHP injection ($P<0.05$, versus LA-treated rats). The vasodilation peaked at 12 hr after LHP injection (122% in LHP-treated rats, $P<0.05$, versus LA-treated rats) and subsided at 48 hr after injection. In PEG-SOD-treated rats, venous vasodilation was significantly suppressed compared with that in LHP-treated rats at 12 hr after LHP injection ($P<0.05$) (Fig. 4(B)).

4. Discussion

The present study demonstrated in vivo that intravenous injection of PEG-SOD significantly suppressed LHP-induced increase in both leukocyte rolling along the retinal vessels and leukocyte accumulation in the retina. Leukocyte-endothelial interactions are regulated by multi-step processes (Osborn, 1990), with each step mediated by distinct adhesion like molecules (Lawrence and Springer, 1991). Leukocyte rolling is the first step in a cascade of events that lead to firm adhesion and transmigration through the endothelium. Leukocyte rolling that represents mild adhesion between leukocytes and endothelial cells is induced mostly by the selectin family at the early stage, and then the strong adhesion is induced because of up-regulation of CDll/CD18b and activation of ICAM-1. P-selectin is thought to play an essential role in initial rolling during an inflammatory

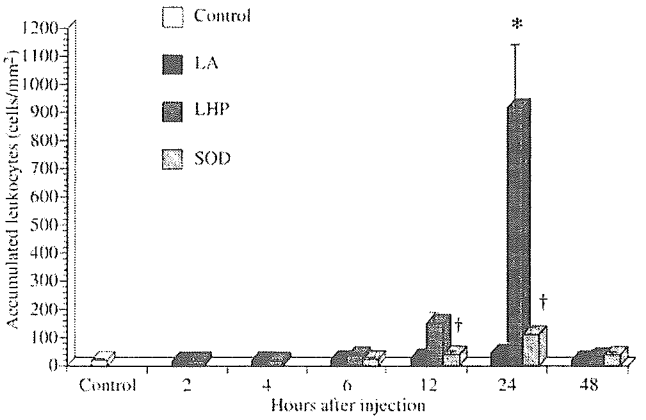

Fig. 3. Time course of the number of leukocytes accumulated in the retina after vitreous injection. Values are mean ± S.D. *$P<0.01$, compared with LA-treated rats. †$P<0.01$, compared with LHP-treated rats. Five different rats were used at each time point in each group.

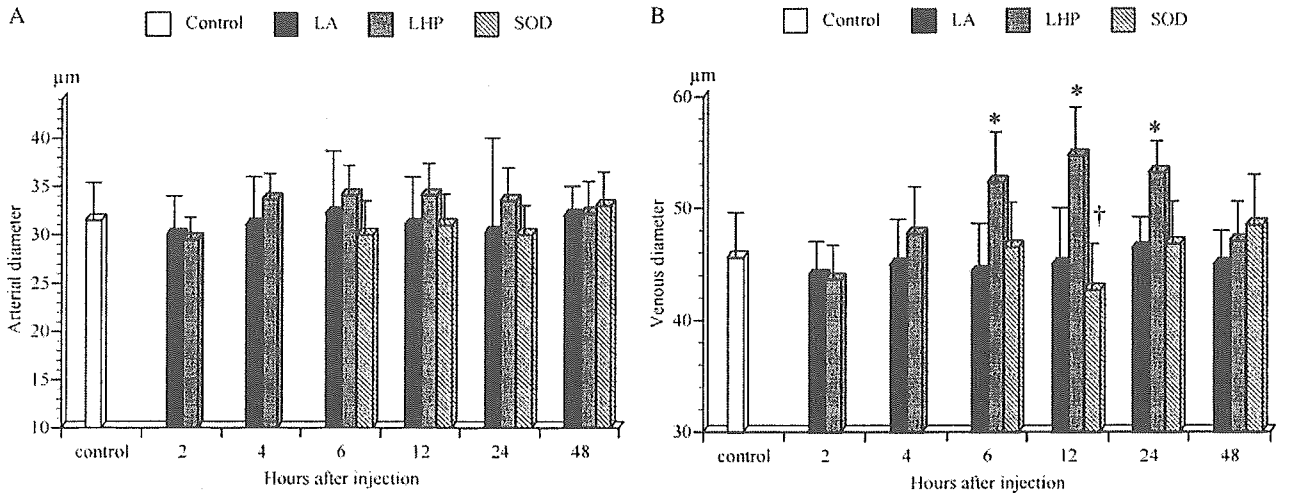

Fig. 4. Time course of major retinal arterial (A) and venous, (B) diameters after vitreous injection. Values are mean ± S.D. $^{*}P<0.05$, compared with LA-treated rats. $^{\dagger}P<0.05$, compared with LHP-treated rats. Five different rats were used at each time point in each group.

reaction (Lawrence and Springer, 1991). Many studies have demonstrated that inhibition of P-selectin significantly reduced leukocyte rolling (Tsujikawa et al., 1999; Matsubara et al., 2000).

Oxygen-derived free radicals are known to enhance leukocyte-endothelial adherence. Patel et al. (1991) reported that reactive oxygen species like H_2O_2 and LHP increased the expression of P-selectin and the adherence of neutrophils to endothelial cells in vitro. Reactive oxygen species also induce expression of ICAM-1 in endothelial cells (Chiu et al., 1997). LHPs have been proposed as mediators or sec messengers, whereby they induce gene expression and up-regulate various cytokines (Suzuki et al., 1997). Armstrong et al. (1998) reported that 18:2 LHP injected in the vitreous of albino rabbits started to increase the concentration of various cytokines (TNF-α, IL-1α, PDGF and VEGF) in the retina at early periods (3–12 hr). In the presence of TNF-α and IL-1, expression of adhesion molecules including ICAM-1 is increased and leukocyte-endothelial adhesion is induced in vitro (Morzycki et al., 1990; Hubbard and Rothlein, 2000), and thereby leukocyte-endothelium interaction is enhanced in addition to the direct action of LHP. Previously, we have reported that increased LHP levels in the vitreous enhanced leukocyte-endothelial interaction in the retinal microcirculation in vivo (Tamai et al., 2002).

Many studies have shown beneficial effects of SOD for ischemic disorders such as myocardial and cerebral ischemia-reperfusion (Naslund et al., 1986; Schettini et al., 1989). However, maintaining appropriate plasma SOD levels is difficult because of the short life of native SOD (half-life time: 6–10 min) (Galinanes et al., 1992). In the present study, we used long acting SOD covalently linked to polyethylene glycol (PEG-SOD), that has been reported to extend the plasma half-life of SOD activity to more than 30 hr (Pyatak et al., 1980; Tamura et al., 1988; Chi et al., 1989).

We found that PEG-SOD treatment significantly inhibited both rolling and accumulation of leukocytes in the retinal microcirculation using acridine orange digital fluorography. Akeo et al. (1996) reported that the damage of the RPE cells exposed by LHP was minimized in a dose-dependent manner after 24 and 48 hr addition of SOD in vitro. As SOD is a well-known enzyme which specifically reduces superoxide (O_2^-), our results suggest that superoxide constitutes an important step in the transduction of the LHP-mediated increase in leukocyte-endothelial cell interaction observed in vivo. Inflammatory conditions and proinflammatory cytokines are also known to stimulate production of reactive oxygen species in endothelial cells and blood vessels (Okada et al., 1998; Rahman et al., 1998; Gunnett et al., 2002). Xanthine oxidase, which produces superoxide in response to proinflammatory cytokines in cultured cells (Page et al., 1998), appears to be an important source of superoxide in vessels under pathological conditions like hypercholesterolemia, atherosclerosis (Graier et al., 1998), and hypertension (Suzuki et al., 1998). Injected LHP in the vitreous generates various cytokines (TNF-α, IL-1α, PDGF and VEGF) in the retina (Armstrong et al., 1998). TNF-α causes xanthine dehydrogenase-to-xanthine oxidase conversion in rat endothelial cells, which would result in the production of superoxide (Friedl et al., 1989; Kapp et al., 1989). TNF-α could also directly stimulate human neutrophils to produce superoxide. We speculate that LHP injected in the vitreous may sensitize retinal tissue initially and cause preinflammatory conditions, and thereby, superoxide is generated secondarily.

Several reports have showed that exogenous SOD could inhibit leukocyte-endothelial cell interaction (Lehr et al., 1992; Morita et al., 1995). Akgür et al. (2000) quantified P-selectin expression in a murine model of hemorrhage-resuscitation by use of the dual-radiolabeled monoclonal antibody technique and found that either administration of exogenous SOD to wild-type mice or genetic

overexpression of SOD resulted in a significant attenuation of hemorrhage-resuscitation-induced P-selectin expression. Yang et al. (2003) reported that endothelial cells obtained from the aorta of transgenic mice overexpressing Cu, ZnSOD showed significantly less expression of intercellular adhesion molecule-1 (ICAM-1) by using enzyme-linked immunosorbent assay. Therefore, attenuation of adhesion molecule expression by SOD is considered to be linked to decreased leukocyte adhesion.

In the present study, venous vasodilation in PEG-SOD-treated rats was significantly suppressed at 12 hr after vitreous injection, compared with that in LHP-treated rats. Increased production of nitric oxide (NO) may be responsible for the vasodilation after LHP injection. As SOD has been reported to protect against the induction of NO in activated microglial cells (Chang et al., 2001), inhibition of NO production by SOD might prevent venous vasodilation.

Many experimental studies have suggested that preventing leukocyte participation attenuates retinal damage in ischemia-reperfusion injury (Strachan et al., 1992; Tsujikawa et al., 1999; Matsubara et al., 2000). However, further studies are needed to assess whether attenuating leukocyte infiltration contributes to maintenance of retinal function under LHP-induced oxidative stress.

In conclusion, the present study has demonstrated an inhibitory effect of PEG-SOD on enhanced leukocyte-endothelial interaction under LHP-induced oxidative stress in the rat retina. The results suggest that PEG-SOD might attenuate various retinal microcirculatory disorders related to oxidative stress.

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29. ポリープ状脈絡膜血管症（PCV）に対する光線力学療法（PDT）の効果

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研究要旨 目的：ポリープ状脈絡膜血管症（PCV）に対する光線力学療法（ＰＤＴ）の効果を明らかにする。対象および方法：対象はフルオレセイン蛍光造影（FA）で中心窩にCNVの所見があり、インドシアニングリーン蛍光造影（IA）でPCVと診断し、ＰＤＴを施行した37例37眼。それらに対し、PDT施行回数、12か月後の視力の推移、IAでのポリープ状病巣と異常血管網の変化をしらべた。結果：PDT施行回数は、1回が14眼、2回が11眼、3回が9眼、4回が3眼であった。視力は、改善12眼（33%）、不変16眼（43%）、悪化9眼（24%）であった。12か月後のＩＡで、ポリープ状病巣は全部消失31眼（84%）、異常血管網は、不変28眼（76%）であった。3眼に残存した異常血管網の辺縁にポリープ状病巣の再発をみとめた。結論：ＩＡでＰＣＶと診断できる場合、ＰＤＴは１年後の視力の改善、維持に有効であった。ＰＤＴによってポリープ状病巣は、容易に消失したが、異常血管網は残存した。残存した異常血管網からポリープ状病巣の再発をみとめる場合があったことから、PDTの有効性の評価には長期の観察が必要である。

A．研究目的

ポリープ状脈絡膜血管症（ＰＣＶ）に対する光線力学療法（ＰＤＴ）の効果を明らかにする。

B．対象および方法

対象は平成16年6月から9月までにフルオレセイン蛍光造影（ＦＡ）で中心窩にCNVの所見があり、インドシアニングリーン蛍光造影（ＩＡ）でPCVと診断し、ＰＤＴを施行し、12か月間経過観察ができた37例37眼であった。性別は男性21例、女性16例であった。年齢は52歳～89歳、平均年齢は67.5歳であった。

検討項目は、PDT施行回数、視力の推移、IAでのポリープ状病巣と異常血管網の変化であった。視力の評価はlog MAR視力で0.2以上の変化で改善、悪化とした。ポリープ状病巣は全部消失、減少、不変、に分類し、異常血管網はすべて消失、長径の減少、不変に分類し評価した。

C．研究結果

PDT施行回数は、1回が14眼、2回が11眼、3回が9眼、4回が3眼で、平均施行回数は2.0回であった。GLDは、2250μm－7000μm（平均4134μm）であった。12か月後の視力は、改善12眼（33%）、不変16眼（43%）、悪化9眼（24%）であった。12か月後のＩＡで、ポリープ状病巣は全部消失31眼

（84%）半数以下に減少3眼（8%）、出血のため判定不能3眼（8%）、一方異常血管網は消失なく、長径が半分以下に減少6眼（16%）、不変28眼（76%）、出血のため判定不能3眼（8%）であった。9か月後に2眼、12か月後に1眼で、残存した異常血管網の辺縁にポリープ状病巣の再発をみとめた（図1－3）。

D．考察

今回我々の結果では、ＰＤＴ後の視力改善は３７眼中２８眼（33%）であった。過去の報告では、ＰＤＴ後の視力改善・不変は、Chan WM（2003 年）は２２眼中２１眼（95%）、Lee SC（2004 年）は 9 眼中８眼（89%）、Hessian N（2005 年）は、９眼中９眼（100%）であった。これらを合わせて考えると、PDT はPCV を有する症例の 12 か月間の視力の改善・維持に有効であると考えた。

今回の結果では、ポリープ状病巣の消失あるいは減少が９７%に見られた。これは、ポリープ状病巣を形成する血管は異常血管網の末端にあり、比較的細く PDT で閉塞しやすいためと考えた。一方、異常血管網は不変が 85%であった。これは、異常血管網はポリープ状病巣より中枢側にあり、また比較的太い血管も多いため、PDT で閉塞しにくく、残った異常血管網がポリープの再発につながるのではないかと考えた。

E．結論

PCV に対するPDT は12か月後の視力の改善、不変に有効であることがわかった。ＰＤＴ後、異常血管網は残存するので、ポリープ状病巣が再発する可能性があるので長期に経過観察をする必要があると考えた。

F．健康危険情報

なし

G．研究発表

1．論文発表

なし

2．学会発表

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2. 赤座英里子　他:　ポリープ状脈絡膜血管症に対する光線力学療法の効果. 第４３回網膜硝子体学会，2005

H．知的財産権の出願・登録状況

1．特許取得

なし

2．実用新案登録

なし

3．その他

なし

I．参考文献

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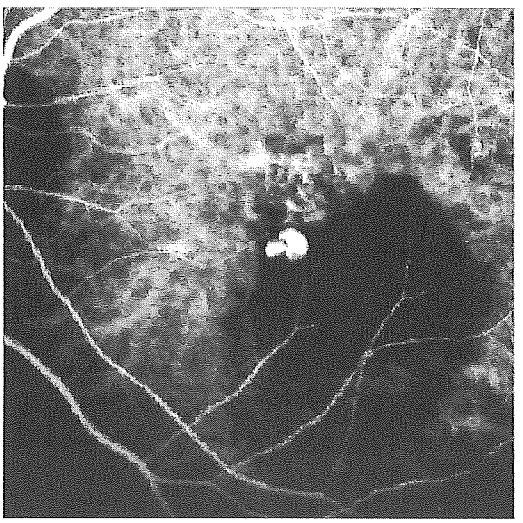

図１　再発例　ＰＤＴ施行前

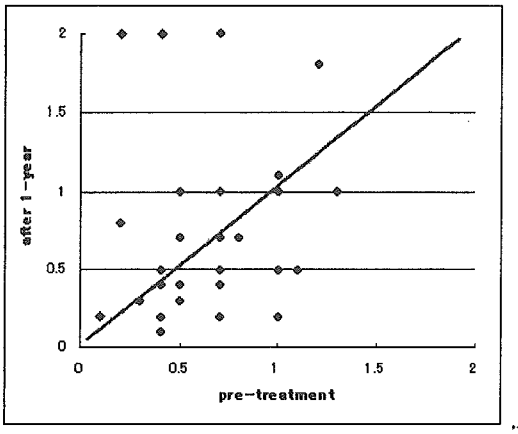

表１　視力の推移

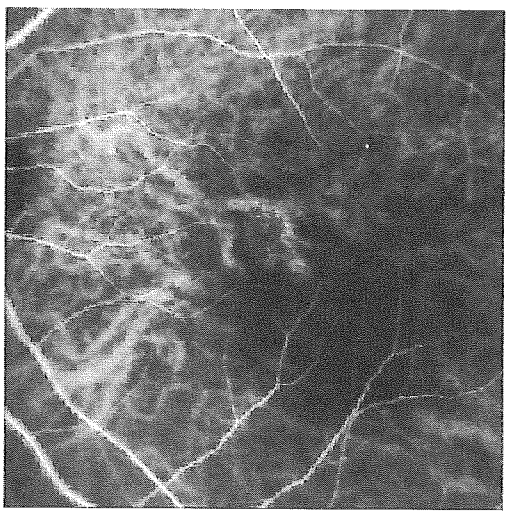

図２　再発例　ＰＤＴ施行後３か月

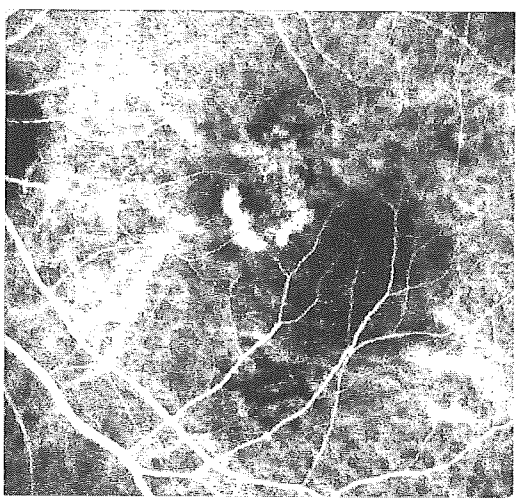

図３　再発例　ＰＤＴ施行後９か月