Light-induced Retinal Damage in Rats" 2012 ARVO annual meeting, Fort Lauderdale, Florida (May 6-10, 2012)

3. **Hideyuki Onami**, Nobuhiro Nagai, Ryosuke Wakusawa , Hirokazu Kaji, Takuya Yamada, Yumi Ishikawa, Matsuhiko Nishizawa, Yasufumi Sato, Toru Nakazawa,and Toshiaki Abe "Suppression of Rat Choroidal Neovascularization by Transscleral Vasohibin-1 Delivery Device" 2012 ARVO annual meeting, Fort Lauderdale, Florida (May 6-10, 2012)
4. Nobuhiro Nagai, Hirokazu Kaji, **Hideyuki Onami**, Takuya Yamada, Yuki Katsukura, Yumi Ishikawa, Matsuhiko Nishizawa, Yukihiko Mashima, Toshiaki Abe "Protective Effects of Transscleral Drug Delivery Device Against Photoreceptor Cell Death in S334ter Rhodopsin Mutant Rats" *2013 ARVO annual meeting, Seattle, Washington* (May 5-9, 2013)
5. Nobuhiro Nagai, **Hideyuki Onami**, Hirokazu Kaji, Takuya Yamada, Yuki Katsukura, Machiko Sato, Yumi Ishikawa, Toru Nakazawa, Matsuhiko Nishizawa, and Toshiaki Abe "Protective Effects of Transscleral Drug Delivery Device Against Light-induced Retinal Damage in Rats" *2012 ARVO annual meeting, Fort Lauderdale, Florida* (May 6-10, 2012)
6. **Hideyuki Onami**, Nobuhiro Nagai, Ryosuke Wakusawa , Hirokazu Kaji, Takuya Yamada, Yumi Ishikawa, Matsuhiko Nishizawa, Yasufumi Sato, Toru Nakazawa,and Toshiaki Abe "Suppression of Rat Choroidal Neovascularization by Transscleral Vasohibin-1 Delivery Device" *2012 ARVO annual meeting, Fort Lauderdale, Florida* (May 6-10, 2012)
7. Nobuhiro Nagai, **Hideyuki Onami**, Hirokazu Kaji, Takuya Yamada, Yuki Katsukura, Machiko Sato, Yumi Ishikawa, Toru Nakazawa, Matsuhiko Nishizawa, and Toshiaki Abe "Protective Effects of Transscleral Drug Delivery Device Against Light-induced Retinal Damage in Rats" 2012 ARVO annual meeting, Fort Lauderdale, Florida (May 6-10, 2012)
8. **Hideyuki Onami**, Nobuhiro Nagai, Ryosuke Wakusawa , Hirokazu Kaji, Takuya Yamada, Yumi Ishikawa, Matsuhiko Nishizawa, Yasufumi Sato, Toru Nakazawa,and Toshiaki Abe "Suppression of Rat Choroidal Neovascularization by Transscleral Vasohibin-1 Delivery Device" 2012 ARVO annual meeting, Fort Lauderdale, Florida (May 6-10, 2012)
9. Nagai N, Kaji H, **Onami H**, Yamada T, Katsukura Y, Ishikawa Y, Nishizawa M, Mashima Y, Abe T "Protective Effects of Transscleral Drug Delivery Device Against Photoreceptor Cell Death in S334ter Rhodopsin Mutant Rats" 2013 ARVO annual meeting, Seattle, Washington (May 5-9, 2013)

（国内学会発表）

1. 永井展裕、大浪英之、梶弘和、山田琢也、勝倉由樹、小柳恵理、西澤松彦、阿部俊明:「経強膜マルチドラッグ徐放デバイスの作製と網膜保護効果の検討」日本バイオマテリアル学会シンポジウム 2012、仙台国際センター（2012 年 11 月 26-27 日）
2. 永井展裕、大浪英之、梶弘和、山田琢也、勝倉由樹、小柳恵理、西澤松彦、阿部俊明:「薬物徐放デバイスの作製と網膜光障害モデルに対する網膜保護効果の検討」第 32 回日本眼薬理学会学術集会、ピアザ淡海（2012 年 9 月 15 日～16 日）
3. 永井展裕、大浪英之、梶弘和、山田琢也、勝倉由樹、小柳恵理、西澤松彦、阿部俊明：「網膜光障害モデルに対する経強膜 DDS の網膜保護効果」第 28 回日本 DDS 学会学術集会、札幌コンベンションセンター（2012 年 7 月 4 日～5 日）
4. 大浪英之、永井展裕、梶弘和、山田琢也、勝倉由樹、西澤松彦、中澤徹、阿部俊明：「プロテインドラッグ眼内徐放デバイスによる加齢黄斑変性治療の試み」第 28 回日本 DDS 学会学術集会、札幌コンベン

ションセンター（2012 年 7 月 4 日～5 日）

5. 大浪英之、永井展裕、梶弘和、西澤松彦、涌沢亮介、佐藤靖史、中澤徹、阿部俊明：「分子徐放デバイス作製と網膜保護」第 63 回東北臨床超微形態懇話会、東北大学医学部（2012 年 6 月 28 日）
6. 永井展裕、大浪英之、梶弘和、山田琢也、勝倉由樹、佐藤真智子、中澤徹、西澤松彦、阿部俊明：「網膜光障害モデルに対する経強膜ドラッグデリバリーデバイスの網膜保護効果」第 116 回日本眼科学会総会、東京国際フォーラム（2012 年 4 月 5 日～8 日）
7. 大浪英之、永井展裕、梶弘和、西澤松彦、涌沢亮介、佐藤靖史、中澤徹、阿部俊明：「経強膜 vasohibin 徐放デバイスによるラット脈絡膜新生血管抑制」第 116 回日本眼科学会総会、東京国際フォーラム（2012 年 4 月 5 日～8 日）
8. 永井展裕、梶弘和、大浪英之、山田琢也、勝倉由樹、小柳恵理、西澤松彦、眞島行彦、中澤徹、阿部俊明：「ウノプロストン徐放デバイスの作製と網膜保護効果」第 117 回日本眼科学会総会、東京国際フォーラム（2013 年 4 月 4 日-7 日）

H．知的財産権の出願・登録状況
（予定を含む。）

1. 特許取得

なし

2. 実用新案登録

なし

3.その他

なし

20141502B（2/2）

別紙１

研究報告書表紙

厚生労働科学研究費補助金

難治性疾患等克服研究事業

網膜色素変性治療をめざした経強膜ウノプロストン徐放法の開発に関する研究

平成２４－２６年度　　**総合研究報告書**

研究代表者　阿部　俊明

平成２７（２０１５）年　５月

（２／２　冊）

別紙４

研究成果の刊行に関する一覧表（阿部　俊明）

雑誌【平成 24 年度】

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Onami H, Nagai N, Kaji H, Nishizawa M, Sato Y, Osumi N, Nakazawa T, Abe T	Transscleral sustained vasohibin-1 delivery by a novel device suppressed experimentally-induced choroidal neovascularization	PLoS One	8(3)	e58580	2013
Ahmed Y Shanab, Toru Nakazawa, Morin Ryu, Yuji Tanaka, Noriko Himori, Keiko Taguchi, Masayuki Yasuda, Ryo Watanabe, Jiro Takano; Saido Takaomi, Naoko Minegishi; Toshio Miyata, Toshiaki Abe, Masayuki Yamamoto	Metabolic stress response implicated in diabetic retinopathy: the role of calpain, and the therapeutic impact of calpain inhibitor	Neurobiol Dis	48(3)	556-67	2012 Dec
Hiroshi Kunikata, Masayuki Yasuda, Naoko Aizawa, Yuji Tanaka, Toshiaki Abe, and Toru Nakazawa	Intraocular Concentrations of Cytokines and Chemokines in Rhegmatogenous Retinal Detachment and the Effect of Intravitreal Triamcinolone Acetonide	Am J Ophthalmol	155(6)	1028-1037	2013 Jun;
Aizawa N, Kunikata H, Abe T, Nakazawa T	Efficacy of combined 25-gauge microincision vitrectomy, intraocular lens implantation, and posterior capsulotomy	J Cataract Refract Surg	38(9)	1602-7	2012 Sep
Kobayashi W, Abe T, Tamai H, Nakazawa T.	Choroidal excavation with polypoidal choroidal vasculopathy: a case report.	Clin Ophthalmol	6	1373-6	2012
Hideyuki Onami, Nobuhiro Nagai, Shigeki Machida, Norihiro Kumasaka, Ryosuke Wakusawa, Yumi Ishikawa, Hikaru Sonoda, Yasufumi Sato, Toshiaki Abe	Reduction of laser-induced choroidal neovascularization by intravitreal vasohibin-1 in monkey eyes	Retina	32(6)	1204-13	2012 Jun
Yumi Tokita-Ishikawa, Nobuhiro Nagai, Hideyuki Onami, Norihiro Kumasaka, Hikaru Sonoda, Tomoaki Takakura, Yasufumi Sato, Toshiaki Abe	Vasohibin-1 and retinal pigment epithelium	Adv Exp Med Biol	723	305-310	2012

別紙４

研究成果の刊行に関する一覧表（阿部　俊明）

雑誌【平成 24 年度】

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
相澤奈帆子、國方彦志、岡村知世子、阿部俊明、中澤徹	25G 硝子体手術中の脈絡膜剥離	眼科臨床紀要	5(8)	792-796	2012. 8
金澤紘子、國方彦志、安田正幸、新田文彦、鬼怒川次郎、阿部俊明、中澤徹	特発性黄斑円孔に対する硝子体手術成績とトリアムシノロンアセトニドの効果	臨床眼科	66(8)	1219-1224	2012. 8
伊藤梓、國方彦志、阿部俊明、安田正幸、中澤徹	黄斑円孔術後に発症した脈絡膜新生血管の一例	眼科臨床紀要	5(9)	855-859	2012

雑誌【平成 25 年度】

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
AbeT, Tokita-Ishikawa Y, Onami H, Katsukura Y, Kaji H, Nishizawa M, Nagai N	Intrascleral Transplantation of a Collagen Sheet with Cultured Brain-Derived Neurotrophic Factor Expressing Cells Partially Rescues the Retina from Damage due to Acute High Intraocular Pressure. Retinal Degenerative Diseases	Advances in Experimental Medicine and Biology	Volume 801	837-843	2014
Kunikata H, Aizawa N, Meguro Y, Abe T, Nakazawa T.	Combined 25-gauge microincision vitrectomy and toric intraocular lens implantation with posterior capsulotomy.	Ophthalmic Surg Lasers Imaging Retina	44	145-54	2013
Kunikata H, Yasuda M, Aizawa N, Tanaka Y, Abe T, Nakazawa T	Intraocular concentrations of cytokines and chemokines in rhegmatogenous retinal detachment and the effect of intravitreal triamcinolone acetonide	Am J Ophthalmol	155	1028-37	2013

別紙４

研究成果の刊行に関する一覧表（阿部　俊明）

書籍【平成 26 年度】

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
Toshiaki Abe * Nobuhiro Nagai	Chapter 2 Neuroprotection for age-related macular degeneration (AMD) and retinal pigmentary degeneration 2.1 Neuroprotection for photoreceptors	Nakazawa, Toru, Kitaoka, Yasushi, Harada, Takayuki (Eds.)	Neuroprotection and Neuroregeneration for Retinal Diseases	Sptringer Japan	Japan	2014	191-204

雑誌　【平成 26 年度】

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Suzuki N, Kunikata H, Aizawa N, Abe T, Nakazawa T.	Predicting Visual outcomes for macula-off rhegmatogenous retinal detachment with optical coherence tomography.	J Ophthalmol.	2014	269837	2014
Kunikata H, Aizawa N, Fuse N, Abe T, Nakazawa T.	25-gauge microincision vitrectomy to treat vitrectoretinal disease in glaucomatous eyes after trabeculectomy.	J Ophthalmol.	2014	306814	2014
Nagai N, Kaji H, Onami H, Katsukura Y, Ishikawa Y, Nezhad ZK, Sampei K, Iwata S, Ito S, Nishizawa M, Nakazawa T, Osumi N, Mashima Y, Abe T.	Platform for Controlled Dual-Drug Delivery to the Retina: Protective Effects against Light-Induced Retinal Damage in Rats.	*Adv Healthc Mater.*	3(10)	1555-1560	2014 Apr 19.
Nagai N, Kaji K, Onami H, Ishikawa Y, Nishizawa M, Osumi N, Nakazawa T, and Abe T,	A polymeric device for controlled transscleral multi-drug delivery to the posterior segment of the eye.	*Acta Biomaterialia*	10	680-687	2014
Fujie T, Mori Y, Ito S, Nishizawa M, Bae H, Nagai N, Onami H, Abe T, Khademhosseini A, Kaji H	Micropatterned Polymeric Nanosheets for Local Delivery of an Engineered Epithelial Monolayer.	*Adv Mater*	Volume 26, Issue 11,	1699–1705	2014

別紙４

研究成果の刊行に関する一覧表（中澤　徹）

【平成 26 年度】

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Yukita M, Machida S, Nishiguchi KM, Tsuda S, Yokoyama Y, Yasuda M, Maruyama K, **Nakazawa T.**	Molecular, anatomical and functional changes in the retinal ganglion cells after optic nerve crush in mice	*Doc Ophthalmol*	**130**	149-56	2015
Kunikata H, Aizawa N, Kudo M, Mugikura S, Nitta F, Morimoto R, Iwakura Y, Ono Y, Satoh F, Takahashi H, Ito S, Takahashi S, **Nakazawa T.**	Relationship of ocular microcirculation, measured by laser speckle flowgraphy, and silent brain infarction in primary aldosteronism.	*PLoS One*	10	e0117452	2015
Takada N, Omodaka K, **Nakazawa T.**	Regional susceptibility of the optic disc to retinal nerve fiber layer thinning in different optic disc types of eyes with normal tension glaucoma	*Clin Experiment Ophthalmol*	43	291-3	2015
Omodaka K, Yabana T, Takada N, **Nakazawa T.**	Regional correlation of macular areas and visual acuity in patients with open angle glaucoma.	*Clin Experiment Ophthalmol*	43	279-82	2015
Yokoyama Y, Tanito M, Nitta K, Katai M, Kitaoka Y, Omodaka K, Tsuda S, Nakagawa T, **Nakazawa T.**	Stereoscopic analysis of optic nerve head parameters in primary open angle glaucoma: the glaucoma stereo analysis study.	*PLoS One*	9	e99138	2014
Yokoyama Y, Maruyama K, Yamamoto K, Omodaka K, Yasuda M, Himori N, Ryu M, Nishiguchi KM, **Nakazawa T.**	The role of calpain in an in vivo model of oxidative stress-induced retinal ganglion cell damage.	*Biochem Biophys Res Commun*	**451**	510-5	2014

別紙４

研究成果の刊行に関する一覧表（中澤　徹）

【平成 26 年度】

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Yasuda M, Tanaka Y, Ryu M, Tsuda S, **Nakazawa T**.	RNA sequence reveals mouse retinal transcriptome changes early after axonal injury.	*PLoS One*	**9**	e93258	2014
Aizawa N, Kunikata H, Yokoyama Y, Nakazawa T.	Correlation between optic disc microcirculation in glaucoma measured with laser speckle flowgraphy and fluorescein angiography, and the correlation with mean deviation. Clin Experiment	Ophthalmol	42	293-4	2014
Doi H, Kunikata H, Kato K, Nakazawa T.	Ophthalmologic Examinations in Areas of Miyagi Prefecture Affected by the Great East Japan Earthquake.	JAMA Ophthalmol	132	874-6	2014
10. Aizawa N, Nitta F, Kunikata H, Sugiyama T, Ikeda T, Araie M, Nakazawa T.	Laser speckle and hydrogen gas clearance measurements of optic nerve circulation in albino and pigmented rabbits with or without optic disc atrophy	Invest Ophthalmol Vis Sci	55	7991-6	2014
Aizawa N, Kunikata H, Shiga Y, Yokoyama Y, Omodaka K, Nakazawa T.	Correlation between structure/function and optic disc microcirculation in myopic glaucoma, measured with laser speckle flowgraphy.	BMC Ophthalmol	14	113	2014
Aizawa N, Kunikata H, Omodaka K, Nakazawa T.	Optic disc microcirculation in superior segmental optic hypoplasia assessed with laser speckle flowgraphy.	Clin Experiment Ophthalmol	42	702-4	2014
Yamamoto K, Maruyama K, Himori N, Omodaka K, Yokoyama Y, Shiga Y, Morin R, **Nakazawa T**.	The novel Rho kinase (ROCK) inhibitor K-115: a new candidate drug for neuroprotective treatment in glaucoma.	*Invest Ophthalmol Vis Sci*	**55**	7126-36	2014

別紙４

研究成果の刊行に関する一覧表（中澤　徹）

【平成 26 年度】

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Tsuda S, Kunikata H, Shimura M, Aizawa N, Omodaka K, Shiga Y, Yasuda M, Yokoyama Y, **Nakazawa T**.	Pulse-Waveform Analysis of Normal Population using Laser Speckle Flowgraphy.	*Curr Eye Res*	**39**	1207-15	2014
Tanaka Y, Tsuda S, Kunikata H, Sato J, Kokubun T, Yasuda M, Nishiguchi K M, Inada T, **Nakazawa T**	Profiles of Extracellular miRNAs in the Aqueous Humor of Glaucoma Patients Assessed with a Microarray System.	*Sci Rep*	4	5089.	2014
Takayama S, Shiga Y, Kokubun T, Konno H, Himori N, Ryu M, Numata T, Kaneko S, Kuroda H, Tanaka J, Kanemura S, Ishii T, Yaegashi N, **Nakazawa T**.	The traditional kampo medicine tokishakuyakusan increases ocular blood flow in healthy subjects.	*Evid Based Complement Alternat Med*	2014	586857	2014
Suzuki N, Kunikata H, Aizawa N, Abe T, **Nakazawa T**.	Predicting visual outcomes for macula-off rhegmatogenous retinal detachment with optical coherence tomography.	*J Ophthalmol*	2014	269837.	2014
Shiga Y, Sato M, Maruyama K, Takayama S, Omodaka K, Himori N, Kunikata H, **Nakazawa T**.	Assessment of Short-Term Changes in Optic Nerve Head Hemodynamics in Hyperoxic Conditions with Laser Speckle Flowgraphy.	*Curr Eye Res*	Nov.7	1-8	2014
Omodaka K, Yokoyama Y, Shiga Y, Inoue M, Takahashi S, Tsuda S, Maruyama K, **Nakazawa T**	Topographical Correlation Between Macular Layer Thickness and Clockwise Circumpapillary Retinal Nerve Fiber Layer Sectors in Patients with Normal Tension Glaucoma.	*Curr Eye Res*	Sept 11	1-8	2014
Omodaka K, Takada N, Takahashi H, **Nakazawa T**	Regional structural vulnerability of the macula in patients with normal tension glaucoma.	*Clin Experiment Ophthalmol*	3	89-90	2014

別紙４

研究成果の刊行に関する一覧表（中澤　徹）

【平成 26 年度】

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Omodaka K, Nishiguchi KM, Yasuda M, Tanaka Y, Sato K, Nakamura O, Maruyama K, **Nakazawa T**	Neuroprotective effect against axonal damage-induced retinal ganglion cell death in apolipoprotein E-deficient mice through the suppression of kainate receptor signaling.	*Brain Res*	**1586**	203-12	2014
Omodaka K, Kurimoto T, Nakamura O, Sato K, Yasuda M, Tanaka Y, Himori N, Yokoyama Y, **Nakazawa T**.	Artemin augments survival and axon regeneration in axotomized retinal ganglion cells.	*J Neurosci Res*	**92**	1637-46	2014
Nitta F, Kunikata H, Aizawa N, Omodaka K, Shiga Y, Yasuda M, **Nakazawa T**.	The effect of intravitreal bevacizumab on ocular blood flow in diabetic retinopathy and branch retinal vein occlusion as measured by laser speckle flowgraphy.	*Clin Ophthalmol*	8	1119-27	2014
Maekawa S, Shiga Y, Kawasaki R, **Nakazawa T**.	Usefulness of novel laser speckle flowgraphy-derived variables of the large vessel area in the optic nerve head in normal tension glaucoma.	*Clin Experiment Ophthalmol*	42	887-9	2014
Kobayashi W, Kunikata H, Omodaka K, Togashi K, Ryu M, Akiba M, Takeuchi G, Yuasa T, **Nakazawa T**.	Correlation of optic nerve microcirculation with papillomacular bundle structure in treatment naive normal tension glaucoma.	*J Ophthalmol*	2014	468908	2014
Himori N, Maruyama K, Yamamoto K, Yasuda M, Ryu M, Omodaka K, Shiga Y, Tanaka Y, **Nakazawa T**.	Critical neuroprotective roles of heme oxygenase-1 induction against axonal injury-induced retinal ganglion cell death.	*J Neurosci Res*	**92**	1134-42	2014

別紙４

研究成果の刊行に関する一覧表（西澤　松彦）

雑誌　【平成 24 年度】

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kuniaki Nagamine, Shuntaro Ito, Mai Takeda, Shingo Otani and Matsuhiko Nishizawa	An Oxygen Responsive Microparticles-Patterned Hydrogel Sheet for Enzyme	Activity Imaging Electrochemistry	80	318-320	2012
Keigo Haneda, Syuhei Yoshino, Takuya Ofuji, Takeo Miyake and Matsuhiko Nishizawa	Sheet-Shaped Biofuel Cell Constructed from Enzyme-Modified Nanoengineered Carbon Fabric	Electrochimica Acta	82	175-178	2012
Yuichiro Ido, Daisuke Takahashi, Masato Sasaki, Kuniaki Nagamine, Takeo Miyake, Piotr Jasinski and Matsuhiko Nishizawa	Conducting Polymer Microelectrodes Anchored to Hydrogel Films	ACS Macro Letters	1	400-403	2012
Takeo Miyake, Keigo Haneda, Syuhei Yoshino and Matsuhiko Nishizawa	Flexible, Layered Biofuel Cells	Biosensors and Bioelectronics	40	45-49	2013
Syuhei Yoshino, Takeo Miyake, Takeo Yamada, Kenji Hata and Matsuhiko Nishizawa	Molecularly Ordered Bioelectrocatalytic Composite inside a Film of Aligned Carbon Nanotubes	Advanced Energy Materials	3	60-64	2013

雑誌　【平成 25 年度】

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Abe T, Tokita-Ishikawa Y, Onami H, Katsukura Y, Kaji H, Nishizawa M, Nagai N.	Intrascleral Transplantation of a Collagen Sheet with Cultured Brain-Derived Neurotrophic Factor Expressing Cells Partially Rescues the Retina from Damage due to Acute High Intraocular Pressure	*Advances in Experimental Medicine and Biology*	801	837-843	2014

別紙４

研究成果の刊行に関する一覧表（西澤　松彦）

雑誌　【平成 26 年度】

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Nagai N, Kaji H, Onami H, Katsukura Y, Ishikawa Y, Nezhad ZK, Sampei K, Iwata S, Ito S, Nishizawa M, Nakazawa T, Osumi N, Mashima Y,	Platform for Controlled Dual-Drug Delivery to the Retina: Protective Effects against Light-Induced Retinal Damage in Rats.	*Adv Healthc Mater.*	3(10)	1555-1560	2014 Apr 19.
Nagai N, Kaji K, Onami H, Ishikawa Y, Nishizawa M, Osumi N, Nakazawa T, and Abe T,	A polymeric device for controlled transscleral multi-drug delivery to the posterior segment of the eye.	*Acta Biomaterialia*	10	680-687	2014
Fujie T, Mori Y, Ito S, Nishizawa M, Bae H, Nagai N, Onami H, Abe T, Khademhosseini A, Kaji H	Micropatterned Polymeric Nanosheets for Local Delivery of an Engineered Epithelial Monolayer.	*Adv Mater*	Volume 26, Issue 11,	1699–1705	2014

別紙４

研究成果の刊行に関する一覧表（梶　弘和）

雑誌　【平成 26 年度】

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Nagai N, Kaji H, Onami H, Katsukura Y, Ishikawa Y, Nezhad ZK, Sampei K, Iwata S, Ito S, Nishizawa M, Nakazawa T, Osumi N, Mashima Y, Abe T.	Platform for Controlled Dual-Drug Delivery to the Retina: Protective Effects against Light-Induced Retinal Damage in Rats.	Adv Healthc Mater.	3(10)	1555-1560	2014 Apr 19.
Nagai N, Kaji K, Onami H, Ishikawa Y, Nishizawa M, Osumi N, Nakazawa T, and Abe T,	A polymeric device for controlled transscleral multi-drug delivery to the posterior segment of the eye.	*Acta Biomaterialia*	10	680-687	2014
Fujie T, Mori Y, Ito S, Nishizawa M, Bae H, Nagai N, Onami H, Abe T, Khademhosseini A, Kaji H	Micropatterned Polymeric Nanosheets for Local Delivery of an Engineered Epithelial Monolayer.	*Adv Mater*	Volume 26, Issue 11,	1699–1705	2014

別紙４

研究成果の刊行に関する一覧表（永井　展裕）

雑誌【平成 24 年度】

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Hideyuki Onami, **Nobuhiro Nagai**, Shigeki Machida, Norihiro Kumasaka, Ryosuke Wakusawa, Yumi Ishikawa, Hikaru Sonoda, Yasufumi Sato, Toshiaki Abe	Reduction of laser-induced choroidal neovascularization by intravitreal vasohibin-1 in monkey eyes	RETINA The Journal of Retinal and Vitreous Diseases	32(6)	1204-1213	2012
Yumi Ishikawa, **Nobuhiro Nagai**, Hideyuki Onami, Norihiro Kumasaka, Ryosuke Wakusawa, Hikaru Sonoda, Yasufumi Sato, Toshiaki Abe	Vasohibin-1 and retinal pigment epithelium	Adv Exp Med Biol	723	305-310	2012

雑誌　【平成 25 年度】

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Abe T, Tokita-Ishikawa Y, Onami H, Katsukura Y, Kaji H, Nishizawa M, Nagai N.	Intrascleral Transplantation of a Collagen Sheet with Cultured Brain-Derived Neurotrophic Factor Expressing Cells Partially Rescues the Retina from Damage due to Acute High Intraocular Pressure	*Advances in Experimental Medicine and Biology*	801	837-843	2014

書籍【平成 26 年度】

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
Toshiaki Abe Nobuhiro Nagai	Chapter 2 Neuroprotection for age-related macular degeneration (AMD) and retinal pigmentary degeneration 2.1 Neuroprotection for photoreceptors	Nakazawa, Toru, Kitaoka, Yasushi, Harada, Takayuki (Eds.)	Neuroprotection and Neuroregeneration for Retinal Diseases	Sptringer Japan	Japan	2014	191-204

別紙４

研究成果の刊行に関する一覧表（永井　展裕）

雑誌　【平成 26 年度】

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Nagai N, Kaji H, Onami H, Katsukura Y, Ishikawa Y, Nezhad ZK, Sampei K, Iwata S, Ito S, Nishizawa M, Nakazawa T, Osumi N, Mashima Y, Abe T.	Platform for Controlled Dual-Drug Delivery to the Retina: Protective Effects against Light-Induced Retinal Damage in Rats.	Adv Healthc Mater.	3(10)	1555-1560	2014 Apr 19.
Nagai N, Kaji H, Onami H, Ishikawa Y, Nishizawa M, Osumi N, Nakazawa T, and Abe T,	A polymeric device for controlled transscleral multi-drug delivery to the posterior segment of the eye.	*Acta Biomaterialia*	10	680-687	2014
Fujie T, Mori Y, Ito S, Nishizawa M, Bae H, Nagai N, Onami H, Abe T, Khademhosseini A, Kaji H	Micropatterned Polymeric Nanosheets for Local Delivery of an Engineered Epithelial Monolayer.	*Adv Mater*	Volume 26, Issue 11,	1699–1705	2014

別紙４

研究成果の刊行に関する一覧表（大浪　英之）

雑誌　【平成 24 年度】

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Onami H, Nagai N, Kaji H, Nishizawa M, Sato Y, Osumi N, Nakazawa T, Abe T	Transscleral sustained vasohibin-1 delivery by a novel device suppressed experimentally-induced choroidal neovascularization	PLoS One	8(3)	e58580	2013
Hideyuki Onami, Nobuhiro Nagai, Shigeki Machida, Norihiro Kumasaka, Ryosuke Wakusawa, Yumi Ishikawa, Hikaru Sonoda, Yasufumi Sato, Toshiaki Abe	Reduction of laser-induced choroidal neovascularization by intravitreal vasohibin-1 in monkey eyes	RETINA The Journal of Retinal and Vitreous Diseases	32(6)	1204-1213	2012
Yumi Ishikawa, Nobuhiro Nagai, Hideyuki Onami, Norihiro Kumasaka, Ryosuke Wakusawa, Hikaru Sonoda, Yasufumi Sato, Toshiaki Abe	Vasohibin-1 and retinal pigment epithelium	Adv Exp Med Biol	723	305-310	2012

雑誌【平成 25 年度】

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Abe T, Tokita-Ishikawa Y, Onami H, Katsukura Y, Kaji H, Nishizawa M, Nagai N.	Intrascleral Transplantation of a Collagen Sheet with Cultured Brain-Derived Neurotrophic Factor Expressing Cells Partially Rescues the Retina from Damage due to Acute High Intraocular Pressure	*Advances in Experimental Medicine and Biology*	801	837-843	2014

平成24年度

OPEN ACCESS Freely available online

PLOS ONE

Transscleral Sustained Vasohibin-1 Delivery by a Novel Device Suppressed Experimentally-Induced Choroidal Neovascularization

Hideyuki Onami[1,2☯], Nobuhiro Nagai[1☯], Hirokazu Kaji[3], Matsuhiko Nishizawa[3], Yasufumi Sato[4], Noriko Osumi[5], Toru Nakazawa[2], Toshiaki Abe[1]*

1 Division of Clinical Cell Therapy, United Centers for Advanced Research and Translational Medicine (ART), Tohoku University Graduate School of Medicine, Sendai, Japan, **2** Department of Ophthalmology, Tohoku University Graduate School of Medicine, Sendai, Japan, **3** Department of Bioengineering and Robotics, Graduate School of Engineering, Tohoku University, Sendai, Japan, **4** Department of Vascular Biology, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan, **5** Division of Developmental Neuroscience, United Centers for Advanced Research and Translational Medicine (ART), Tohoku University Graduate School of Medicine, Sendai, Japan

Abstract

We established a sustained vasohibin-1 (a 42-kDa protein), delivery device by a novel method using photopolymerization of a mixture of polyethylene glycol dimethacrylate, triethylene glycol dimethacrylate, and collagen microparticles. We evaluated its effects in a model of rat laser-induced choroidal neovascularization (CNV) using a transscleral approach. We used variable concentrations of vasohibin-1 in the devices, and used an enzyme-linked immunosorbent assay and Western blotting to measure the released vasohibin-1 (0.31 nM/day when using the 10 μM vasohibin-1 delivery device [10VDD]). The released vasohibin-1 showed suppression activity comparable to native effects when evaluated using endothelial tube formation. We also used pelletized vasohibin-1 and fluorescein isothiocyanate-labeled 40 kDa dextran as controls. Strong fluorescein staining was observed on the sclera when the device was used for drug delivery, whereas pellet use produced strong staining in the conjunctiva and surrounding tissue, but not on the sclera. Vasohibin-1 was found in the sclera, choroid, retinal pigment epithelium (RPE), and neural retina after device implantation. Stronger immunoreactivity at the RPE and ganglion cell layers was observed than in other retinal regions. Significantly lower fluorescein angiography (FA) scores and smaller CNV areas in the flat mounts of RPE-choroid-sclera were observed for the 10VDD, VDD (1 μM vasohibin-1 delivery device), and vasohibin-1 intravitreal direct injection (0.24 μM) groups when compared to the pellet, non-vasohibin-1 delivery device, and intravitreal vehicle injection groups. Choroidal neovascularization can be treated with transscleral sustained protein delivery using our novel device. We offer a safer sustained protein release for treatment of retinal disease using the transscleral approach.

Citation: Onami H, Nagai N, Kaji H, Nishizawa M, Sato Y, et al. (2013) Transscleral Sustained Vasohibin-1 Delivery by a Novel Device Suppressed Experimentally-Induced Choroidal Neovascularization. PLoS ONE 8(3): e58580. doi:10.1371/journal.pone.0058580

Editor: Olaf Strauß, Eye Hospital, Charité, Germany

Received August 11, 2012; **Accepted** February 6, 2013; **Published** March 5, 2013

Funding: This work was supported in part by Grant-in-Aid for Scientific Research (No. 21592214) and Young Scientists (A) (No. 23680054) from the Ministry of Education, Culture, Sports, Science, and Technology, Health Labour Sciences Research Grant from the Ministry of Health Labour and Welfare (No. H23-iryokiki-wakate-003, H23-kankaku-ippan-004, H24-nanchitoh-ippan-067), the Suzuken Memorial Foundation, and the Ichiro Kanehara Foundation. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: toshi@oph.med.tohoku.ac.jp

☯ These authors contributed equally to this work.

Introduction

Age-related macular degeneration (AMD) is a well-known sight-threatening disease in developed countries [1]. Although many treatment regimens have been used to treat AMD [2–6], intravitreal injection of anti-vascular endothelial growth factor (VEGF) produced lesion improvement and better visual acuity in some patients [7,8]. However, intra-vitreal injection of anti-VEGF also produced irritation, infection, and other adverse side effects [9]. Further, that treatment required repeated injections, usually occurring once a month [7,8]. Thus, other types of drugs or drug delivery systems (DDSs) need to be developed to treat AMD.

Eye drops and systemic drug administration are unsuitable for retinal diseases if the physician is looking for effective drug penetration into the eye, especially for macular diseases such as AMD [10,11]. Although drug delivery device implantation into the vitreous showed effective delivery of drug to the retina, these treatments may cause severe side effects, such as infection, vitreous hemorrhage, or retinal detachment [12–14]. Drug delivery using viral vectors has been attempted for treatment of devastating retinal diseases [15]; however, this method may induce immune cell or humoral responses [16,17].

Subconjunctival drug delivery is less invasive than intravitreal drug injection and can deliver more drug than seen with eye drops or systemic administration [10,11]. There are published data investigating clinical use of subconjunctival drug administration [18,19]. Thus, the subconjunctival route may be an attractive method for drug delivery to the retina. The major difficulties with subconjunctival DDS are uncontrollable release of the target drug [20], as well as an unknown drug delivery route and mechanism to

reach the retina [20,21]. Sustained release, with no drug bolus effect, would be required to reduce side effects [22,23].

We previously reported our results of the use of a novel drug delivery device placed on the sclera that we thought would be an effective tool in treating retinal diseases [24]. The device consisted of a drug-releasing semi-permeable membrane and impermeable membranes acting as the drug reservoir. Because of the non-biodegradable and one-way release nature of the device, we could achieve sustained release of the drug to the retina. We examined the effects of this device using a laser-induced choroidal neovascularization (CNV) model in rats.

Anti-VEGF antibody is a well-known treatment agent in CNV therapy, but suppression of VEGF function may induce many harmful effects in physiological function [25]. We selected vasohibin-1 for the loading drug in the device in this study because of its well-known anti-angiogenic activity [26,27]. Vasohibin-1 is a 42-kDa polypeptide, a VEGF-inducible molecule expressed by cultured human endothelial cells (ECs) [26]. Vasohibin-1 inhibits the formation of EC networks *in vitro*, corneal neovascularization *in vitro* [26], retinal neovascularization in a mouse model of oxygen-induced ischemic retinopathy [27], and laser-induced mouse [25] and monkey CNV [28]. Each of the *in vivo* studies treated the tissue by direct intravitreal injection of vasohibin-1.

Here we shall show that continuous trans-scleral vasohibin-1 delivery by the device can suppress laser-induced CNV in rat eyes (Fig. 1A) as well as that by intravitreal injection. This technique and device may hold promise for safer and more effective treatment of patients with AMD.

Methods

Vasohibin-1 and Device Preparation

Vasohibin-1 was purified as reported previously [25]. For the preparation of the vasohibin-1 formulation, an 80-μL volume of vasohibin-1 (either 1.25 or 12.5 μM) in vehicle (phosphate buffered saline [PBS] control) was mixed with 20 μL of polyethylene glycol dimethacrylate (PEGDM), then underwent UV curing at an intensity of 11.5 mJ/cm^2 (Lightningcure LCS; Hamamatsu Photonics, Hamamatsu City, Japan) for 3 minutes.

The devices consisted of a semi-permeable drug-releasing membrane and an impermeable reservoir (Fig. 1A, 1B), as we reported previously [24]. The loaded vasohibin-1 doses included vehicle only (identified as NVDD), 1 μM vasohibin-1 (VDD), and 10 μM vasohibin-1 (10VDD), with a total volume of 1.5 μL in each device. The size of the device was 2 mm×2 mm wide ×1 mm high (drug-releasing surface area; 1.5 mm×1.5 mm = 2.25 mm^2) for the rat experiments (Fig. 1B, device) and 4 mm×4 mm×1.5 mm (drug-releasing surface area; 3.5 mm×3.5 mm = 12.25 mm^2) for the vasohibin-1 releasing *in vitro* assay. The release amount from the transplanted device was small and it was very difficult to detect released vasohibin-1 by the standard ELISA technique, so we decided to use a larger device for the ELISA procedure. As a control, we used pelletized vasohibin-1 without the reservoir and permeable membrane (Fig. 1B, pellet). The concentration of pelletized vasohibin-1 was adjusted to be the same concentration as that of the 10VDD (10 μM vasohibin-1). The total amount of vasohibin-1 released from the 10VDD device during the 2-week *in vivo* experiment was aimed to be equivalent to that of the intravitreal vasohibin-1 injection. A FITC-labeled 40 kDa dextran-loaded device (FD40DD) was also used for monitoring the position of the implanted device.

In Vitro Experiments

***1 In Vitro* Release Assay, Enzyme-linked Immunosorbent Assay, and Western Blotting.** The devices loaded with vasohibin-1 were placed in the wells of a 24-well culture plate filled with 200 μL PBS at 37°C. Aliquots (200 μL) of the buffer in each well were collected at Days 1, 7, 14, and 28 during change-out of old buffer for new buffer solution. The collected samples were considered to include only protein for vasohibin-1. We then determined the amount of vasohibin-1 in the buffer using an enzyme-linked immunosorbent assay (ELISA) [29] and western blotting [30]. The intensity of the color of the ELISA reaction products was measured with a microplate reader (MAXline; Molecular Devices Corporation, Sunnyvale, CA, USA). The measurements were made in duplicate, and the mean value was used for comparisons. The 50-μL collected samples and 100 fmol of recombinant vasohibin-1 (positive control) were loaded, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% separating gel, and transferred to nitrocellulose membranes for western blotting. The membranes were blocked for 1 hour at room temperature with 5% ECL blocking agent (GE Healthcare Biosciences, Pittsburgh, PA, USA), and then incubated overnight at 4°C in PBS containing 0.05% Tween 20 (T-PBS), 2.5% skim milk, and 1 μg/mL horseradish peroxidase-conjugated anti–vasohibin-1 monoclonal antibody. The membrane filters were washed 3 times with T-TBS and the blots were detected using an enhanced chemiluminescence method (ECL Western Blotting Detection Kit; Amersham Biosciences, Piscataway, NJ, USA). The results were visualized using an imaging system (ImageQuant LAS-1000; GE Healthcare Biosciences).

2 Endothelial Tube Formation. Endothelial tube formation was assessed with normal human umbilical vein endothelial cells (HUVECs) (Takara Bio; Otsu, Japan) co-cultured on neonatal normal human dermal fibroblasts (NHDF, Takara Bio) layer using anti-human CD31 immunostaining, as reported previously [28]. Two nM vascular endothelial growth factor (VEGF, Wako; Tokyo, Japan) was then added to the endothelial cell growth medium (EGM, Takara Bio) containing no vasohibin-1 (control), and 0.2, 2, or 10 nM vasohibin-1, respectively. VEGF (2 nM) and samples of vasohibin-1 released from the vasohibin-1-loaded device over 3 hours at 37°C were used to examine released vasohibin-1 activity. We collected the released vasohibin-1 from the pellet and used it at a concentration of 0.56 nM (as measured by ELISA). On Day 3, the cells were fixed and stained using an anti-human CD31 immunostaining kit (Kurabo; Tokyo, Japan) according to the manufacturer's instructions. The number of stained HUVECs was determined using a computerized system (Kurabo Angiogenesis Image Analyzer program; Kurabo).

In Vivo CNV Experiments

1 Animals. The procedures used in the animal experiments followed the guidelines of the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research, and they were approved by the Animal Care Committee of Tohoku University Graduate School of Medicine (Permit Number: 2011–136). Twenty Sprague-Dawley (SD) rats (Experiments 1 and 2) and 36 Brown Norway (BN) rats (Experiment 3) weighing between 250 and 300 g were used (Table 1). All animals were followed up to 2 weeks after device transplantation and/or laser burn. We examined the effects of devices either at 1 week or 2 weeks for FA evaluation and 2 weeks for flat-mount evaluation. Macro examination was performed at 1 and 2 weeks after the device transplantation. For all procedures, the rats were anesthetized with an intramuscular