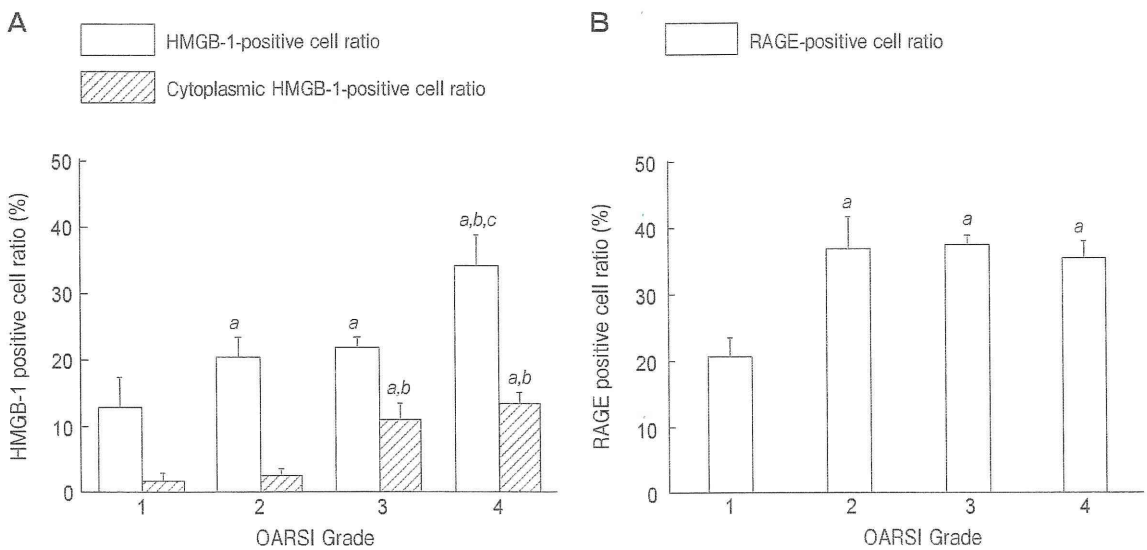

Fig. 3 Population of HMGB-1-and RAGE positive chondrocytes. (A) Cytoplasmic HMGB-1-positive cell ratios tended to be higher in the specimens having the higher OARSI grade. (a, $p < 0.05$ vs. Grade 1; b, $p < 0.05$ vs. Grade 2; c, $p < 0.05$ vs. Grade 3.), (B) The ratios and localization of RAGE expression by chondrocytes in Grades 2, 3 and 4 were significantly higher than those in Grade 1. There was no significant difference between Grades 2/3 and 4, nor was there any significant difference between Grades 3 and 4.

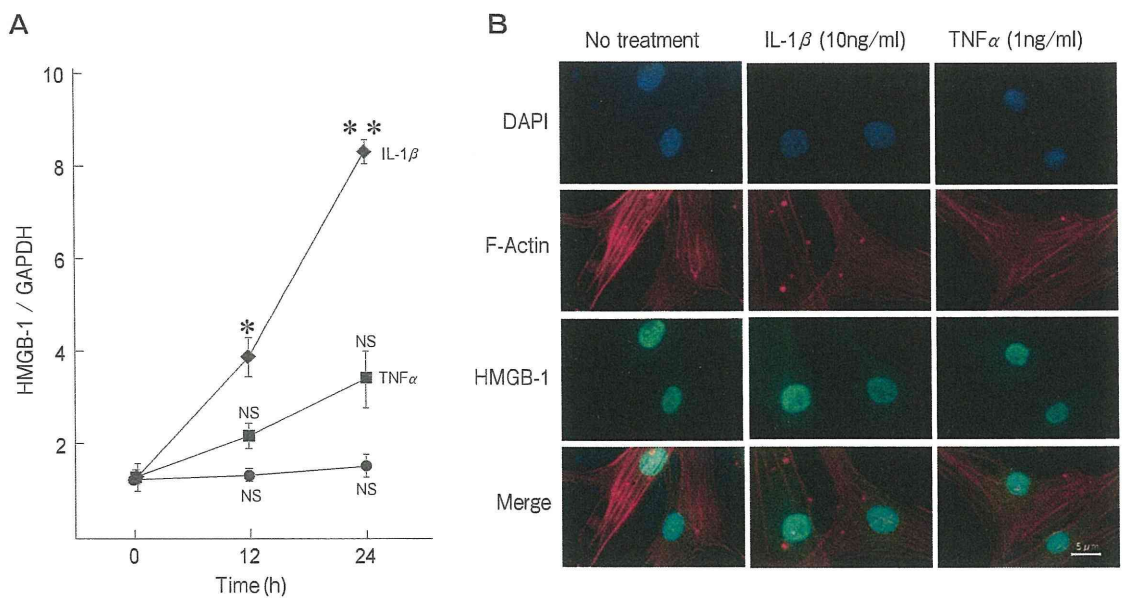

Fig. 4 Effect of IL-1β and TNFα on HMGB-1 and localization in OA chondrocyte. Expression of HMGB-1 mRNA by real-time PCR. Cultured chondrocytes were stimulated with IL-1β (10ng/μL), and total RNA was isolated. Up-regulation of HMGB-1 was still increasing after 12h, but not TNFα (A). ($^{*}p < 0.05$ vs. 0h ($p = 0.033$), $^{**}p < 0.05$ vs. 12h ($p = 0.002$), NS = not significant.) Translocation of HMGB-1 in chondrocytes after IL-1β and TNFα stimulation. The immunofluorescence assay indicated that HMGB-1was translocated from the nucleus to the cytosol following stimulation with both IL-1β and TNFα (B). Chondrocytes were stimulated with IL-1β (10ng/mL) and TNFα (1ng/mL) for 48h and then compared with unstimulated chondrocytes. Chondrocytes stimulated with IL-1β or TNFα exhibited HMGB-1 staining in the cytosol. Note that unstimulated chondrocytes exhibited HMGB-1 staining only in the nucleus.

the nucleus to the cytosol after stimulation by IL-1β (10ng/mL) and TNFα (1ng/mL) at 48h.

Effect of HMGB-1 on IL-1β and TNFα release by chondrocytes. The effects of HMGB-1 on IL-1β and TNFα production by chondrocytes were analyzed by ELISA. HMGB-1 stimulated the production of IL-1β up to 24h (Fig. 5A), and 1,000ng/mL of HMGB-1 significantly up-regulated the production of IL-1β by OA chondrocytes at 12h (Fig. 5B). Peak TNFα levels after stimulation with HMGB-1 (100ng/mL) occurred at 12h and tended to decrease for 24h thereafter. (Fig. 5C). At 12h, the amount of TNFα released into the cultured medium increased in a dose-dependent manner after stimulation by HMGB-1 (Fig. 5D).

Discussion

A previous report demonstrated the significant increase of HMBG-1 mRNA expression in human OA cartilage as compared with normal cartilage [19]. In the present study, we examined the expression and localization of HMGB-1 in OA cartilage. Our results confirmed that HMGB-1 expression in OA cartilage was significantly higher than that in the normal cartilage for both mRNA and protein levels. In the immunohistochemical analysis, it was revealed that the HMGB-1-positive cell ratio and cytoplasmic HMGB-1 positive cell ratio tended to increase along with the OA histological grade, but the nuclear HMGB-1 positive cell ratio was unchanged. These results coincide with a report of Heinola *et al.* in which they studied bovine

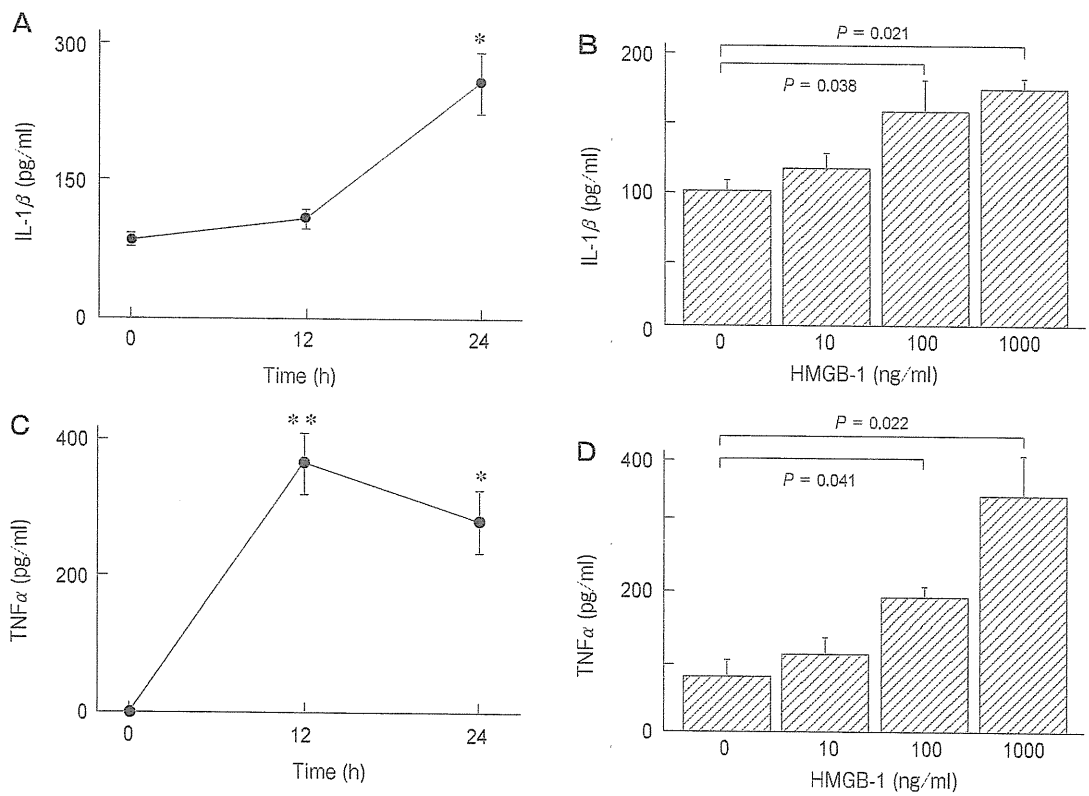

Fig. 5 Effect of HMGB-1 on IL-1β and TNFα expression by human chondrocytes. Chondrocytes were stimulated with HMGB-1, and ELISA was used to measure levels of IL-1β and TNFα. After stimulation with HMGB-1 (100ng/mL), IL-1β levels increased in a time-dependent manner (**A**). IL-1β was released in a dose-dependent manner for 24h after stimulation with HMGB-1, and the amount of IL-1β and TNFα production increased significantly at doses of > 100ng/mL (**B**). The peak TNFα levels after stimulation with HMGB-1 (100ng/mL) occurred at 12h and tended to decrease at 24h (**C**). TNFα was released in a dose-dependent manner after stimulation for 12h (**D**). (A) $^{*}p < 0.05$ vs. 0h ($p = 0.025$). (C) $^{*}p < 0.05$ vs. 0h ($p = 0.039$), $^{**}p < 0.05$ vs. 0h ($p = 0.018$).

cartilage [23].

It is not fully understood what stimulates HMGB-1 production or cytoplasmic translocation from the nucleus in chondrocytes. In normal joint cartilage, HMGB-1 has been reported to be located in the nuclei of chondrocytes and synovial cells [12]. However, in inflammatory synovial cells, HMGB-1 shows both nuclear and cytoplasmic distribution [12]. It was also shown that TNFα stimulation caused translocation of HMGB-1 from the nucleus to the cytosol in CD68-positive cells [14]. In the present study, we demonstrated that IL-1β but not TNFα up-regulated the expression of HMGB-1 at mRNA levels. Treatment with both IL-1β and TNFα caused translocation of HMGB-1 from the nucleus to the cytosol 48h after stimulation.

On the other hand, previous studies have demonstrated that extracellular HMGB-1 induced the production of IL-1β, TNFα, and IL-6 in macrophages [10, 14]. Intra-articular injection of HMGB-1 into murine knee joints resulted in synovitis, but mice rendered deficient in the IL-1 receptor did not develop synovitis after injection. These results suggest that HMGB-1 is not a mere expression of the inflammatory response, but a trigger for joint inflammation by activating macrophages and inducing production of IL-1 via NF-κB activation [13]. In the current study, IL-1β and TNFα production by OA chondrocytes was stimulated by HMGB-1 in a dose-dependent manner. It is speculated that proinflammatory cytokines stimulate the expression and release of HMGB-1 by chondrocytes and that the HMGB-1 released by chondrocytes further promotes the expression of proinflammatory cytokines [14]. This speculation is supported by our current result that the HMGB-1-positive cell ratio increases along with the OA cartilage grade. It is reported that the combination of HMGB-1 and heparin induced marked angiogenesis in a heparin-dependent manner [24]. The HMGB-1 increase in the deep layers of OA cartilage might be related to angiogenesis at the osteochondral junction.

RAGE and toll-like receptors 2 and 4 are known ligands of HMGB-1 [25]. RAGE has been found to be expressed in cartilage [16] and RA synovium [14]; however, RAGE and HMGB-1 do not have a one-to-one correspondence. A study reported by Loeser *et al.* [16] showed that RAGE expression was higher in OA cartilage than in normal cartilage. Our immunohistochemical results also demonstrated that expression of RAGE was significantly higher in cartilage with Grades 2, 3, and 4 OA than that in Grade 1 cartilage. In a separate experiment we used 5 other human OA cartilage specimens to perform double immunohistochemistry for HMGB-1 and RAGE to clarify the interrelationship of the 2 proteins. The result showed that some populations of chondrocytes showed a positive reaction only for HMGB-1 or for RAGE, whereas some were positive for both HMGB-1 and RAGE (data not shown). These findings suggest that HMGB-1 might act on chondrocytes in both an autocrine and paracrine manner. Stimulation of RAGE signaling from HMGB-1 might lead to upregulation of catabolic enzymes such as matrix metalloproteinase 13 production and contribute to cartilage matrix degradation in arthritis [16].

In conclusion, we demonstrated the increased expression of HMGB-1 in OA cartilage relevant to the histological OA grade. *In vitro* experiments using human OA chondrocytes suggested the close interrelationship between HMGB-1 and IL-1β. These results indicate the possible involvement of the proinflammatory cytokine-HMGB-1-RAGE cascade in the development of human osteoarthritis. Whether the blockade of this cascade could contribute to the moderation of cartilage degeneration requires further investigation.

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References

1. Gardner D: Osteoarthrosis and allied disease; in: Pathorogical Basis of the Connective Tissue Disease, Edward Arnold, London (1992) pp 842–923.
2. Paonessa G, Frank R and Cortese R: Nucleotide sequence of rat liver HMG1 cDNA. Nucleic Acids Res (1987) 15: 9077.
3. Ferrari S, Ronfani L, Calogero S and Bianchi ME: The mouse gene coding for high mobility group 1 protein (HMG1). J Biol Chem (1994) 269: 28803–28808.
4. Wen L, Huang JK, Johnson BH and Reeck GR: A human placental cDNA clone that encodes nonhistone chromosomal protein HMG-1. Nucleic Acids Res (1989) 17: 1197–1214.
5. Bustin M: Regulation of DNA-dependent activities by the functional motifs of the high-mobility-group chromosomal proteins. Mol Cell Biol (1999) 19: 5237–5246.
6. Melvin VS and Edwards DP: Coregulatory proteins in steroid hormone receptor action: the role of chromatin high mobility group proteins HMG-1 and -2. Steroids (1999) 64: 576–586.

7. Boonyaratanakornkit V, Melvin V, Prendergast P, Altmann M, Ronfani L, Bianchi ME, Taraseviciene L, Nordeen SK, Allegretto EA and Edwards DP: High-mobility group chromatin proteins 1 and 2 functionally interact with steroid hormone receptors to enhance their DNA binding in vitro and transcriptional activity in mammalian cells. Mol Cell Biol (1998) 18: 4471-4487.
8. Taguchi A, Blood DC, del Toro G, Canet A, Qu W, Tanji N, Lu Y, Lalla E, Fu C, Hofmann MA, Kislinger T, Ingram M, Lu A, Tanaka H, Hori O, Ogawa S, Stern DM and Schmidt AM: Blockade of RAGE-amphoterin signalling suppresses tumour growth and metastases. Nature (2000) 405: 354-360.
9. Fages C, Nolo R, Huttunen HJ, Eskelinen E and Rauvala H: Regulation of cell migration by amphoterin. J Cell Sci (2000) 113: 611-620.
10. Wang H, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J, Fraizier A, Yang H, Ivanova S, Borovikova L, Manogue KR, Faist E, Abraham E, Andersson J, Andersson U, Molina PE, Abumrad NN, Sama A and Tracey KJ: HMG-1 as a late mediator of endotoxin lethality in mice. Science (1999) 285: 248-251.
11. Abraham E, Arcaroli J, Carmody A, Wang H and Tracey KJ: HMG-1 as a mediator of acute lung inflammation. J Immunol (2000) 165: 2950-2954.
12. Kokkola R, Sundberg E, Ulfgen AK, Palmblad K, Li J, Wang H, Ulloa L, Yang H, Yan XJ, Furie R, Chiorazzi N, Tracey KJ, Andersson U and Harris HE: High mobility group box chromosomal protein 1: a novel proinflammatory mediator in synovitis. Arthritis Rheum (2002) 46: 2598-2603.
13. Pullerits R, Jonsson IM, Verdrengh M, Bokarewa M, Andersson U, Harris HE and Tarkowski A: High mobility group box chromosomal protein 1, a DNA binding cytokine, induces arthritis. Arthritis Rheum (2003) 48: 1693-1700.
14. Taniguchi N, Kawahara K, Yone K, Hashiguchi T, Yamakuchi M, Goto M, Inoue K, Yamada S, Ijiri K, Matsunaga S, Nakajima T, Komiya S and Maruyama I: High mobility group box chromosomal protein 1 plays a role in the pathogenesis of rheumatoid arthritis as a novel cytokine. Arthritis Rheum (2003) 48: 971-981.
15. Sunahori K, Yamamura M, Yamana J, Takasugi K, Kawashima M and Makino H: Increased expression of receptor for advanced glycation end products by synovial tissue macrophages in rheumatoid arthritis. Arthritis Rheum (2006) 54: 97-104.
16. Loeser RF, Yammani RR, Carlson CS, Chen H, Cole A, Im HJ, Bursch LS and Yan D: Articular chondrocytes express the receptor for advanced glycation end products: Potential role in osteoarthritis. Arthritis Rheum (2005) 52: 2376-2385.
17. Steenvoorden MM, Huizinga TW, Verzijl N, Bank RA, Ronday HK, Luning HA, Lafeber FP, Toes RE and DeGroot J: Activation of receptor for advanced glycation end products in osteoarthritis leads to increased stimulation of chondrocytes and synoviocytes. Arthritis Rheum (2006) 54: 253-263.
18. Ulloa L, Batliwalla FM, Andersson U, Gregersen PK and Tracey KJ: High mobility group box chromosomal protein 1 as a nuclear protein, cytokine, and potential therapeutic target in arthritis. Arthritis Rheum (2003) 48: 876-881.
19. Attur M, Dave M, Akamatsu M, Nakagawa N, Miki J, Yang H, Katoh M, Wisniewski J, Tracey K and Amin A: Differential expression of high mobility group protein in human normal and arthritic cartilage; Functional genomic analysis; in 49th Annual Meeting of the Orthopaedic Reseach Society, LA, USA (2003): 18.
20. Sado Y and Okigaki T: A novel method for production of monoclonal antibodies. Evaluation and expectation of the rat lymph node method in cell and molecular biology. Cell Biol Int (1996) 20: 7-14.
21. Pritker KP, Gay S, Jimenez SA, Ostergaard K, Pelletier JP, Revell PA, Salter D and van den Berg WB: Osteoarthritis cartilage histopathology: grading and staging. Osteoarthritis Cartilage (2006) 14: 13-29.
22. Bruckner P, Horler I, Mendler M, Houze Y, Winterhalter KH, Eich-Bender SG and Spycher MA: Induction and prevention of chondrocyte hypertrophy in culture. J Cell Biol (1989) 109: 2537-2545.
23. Heinola T, Kouri VP, Clarijs P, Sukura A, Salo J and Konttinen Y: High mobility group box-1 (HMGB-1) in osteoarthritic cartilage. Clin Exp Rheumatol (2010) 28: 511-518.
24. Wake H, Mori S, Liu K, Takahashi HK and Nishibori M: High mobility group box 1 complexed with heparin induced angiogenesis in a matrigel plug assay. Acta Med Okayama (2009) 63: 249-262.
25. Liu-Bryan R and Terkeltaub R: Chondrocyte innate immune myeloid differentiation factor 88-dependent signaling drives procatabolic effects of the endogenous Toll-like receptor 2/Toll-like receptor 4 ligands low molecular weight hyaluronan and high mobility group box chromosomal protein 1 in mice. Arthritis Rheum (2010) 62: 2004-2012.

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ORIGINAL ARTICLE

Deleterious Role of Anti-high Mobility Group Box 1 Monoclonal Antibody in Retinal Ischemia-reperfusion Injury

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ABSTRACT

Purpose: To investigate the effect of anti-high mobility group box 1 (HMGB1) monoclonal antibody (mAb) against ischemia-reperfusion injury in the rat retina.

Materials and Methods: Retinal ischemia was induced by increasing and then maintaining intraocular pressure at 130 mmHg for 45 min. An intraperitoneal injection of anti-HMGB1 mAb was administered 30 min before ischemia. Retinal damage was evaluated at 7 days after the ischemia. Immunohistochemistry and image analysis were used to measure changes in the levels of reactive oxygen species (ROS) and the localization of anti-HMGB1 mAb. Dark-adapted full-field electroretinography (ERG) was also performed.

Results: Pretreatment with anti-HMGB1 mAb significantly enhanced the ischemic injury of the retina. HMGB1 expression increased at 6–12 h after ischemia in the retina. After the ischemia, production of ROS was detected in retinal cells. However, pretreatment with anti-HMGB1 mAb increased the production of ROS. On the seventh postoperative day, the amplitudes of both the ERG a- and b-waves were significantly higher in the vehicle group than in the groups pretreated with anti-HMGB1 mAb.

Conclusions: The current *in vivo* model of retinal injury demonstrated that anti-HMGB1 mAb plays a large deleterious role in ischemia-reperfusion injury. In order to develop neuroprotective therapeutic strategies for acute retinal ischemic disorders, further studies on anti-HMGB1 mAb function are needed.

Keywords: Anti-HMGB1 mAb, Retinal ischemia, Reactive oxygen species, Electroretinogram, Retinal thickness

INTRODUCTION

Ischemic injury to the retina is a major cause of visual loss and morbidity. As these morbidities are difficult to treat, research into various potential treatments is currently ongoing.[1–5] Ischemia-reperfusion injury involves many signaling mechanisms that ultimately result in necrotic and apoptotic cell death.[6] Delayed neuronal cell death in the brain and retina secondary to transient ischemic injury occurs, in part, by apoptosis.[7,8] During or after ischemia, reactive oxygen species (ROS) can be produced in large quantities and act as cytotoxic metabolites.[9] ROS can provoke cell death by reacting with cell components that lead to necrosis, or by activating specific targets that trigger apoptosis.

High-mobility group box-1 (HMGB1) protein was originally described 30 years ago as a nonhistone DNA-binding protein with high-electrophoretic mobility.[10] HMGB1 is a nuclear protein involved in nucleosome stabilization and gene transcription.[11] It is known that these functions are essential for survival, as HMGB1-deficient mice have been shown to die of hypoglycemia within 24 h of birth.[12] HMGB1 is

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found in almost all eukaryotic cells, and its presence has been confirmed in the rodent retina.[13] HMGB1 has also been implicated in the mechanism of ischemic brain damage.[14–20] In a stroke model, short hairpin (sh) RNA-mediated HMGB1 down-regulation in the brain reduces the severity of lesions.[15] Intravenous injection of the anti-HMGB1 monoclonal antibody (mAb) causes a dramatic reduction in the infarct size in the stroke model.[17]

The purpose of the present study was to investigate the role of anti-HMGB1 and its specific expression in retinal ischemia-reperfusion injury.

MATERIAL AND METHODS

Animals

Male Sprague-Dawley rats weighing 200–250 g were obtained from Charles River Japan (Yokohama, Japan). Rats were permitted free access to standard rat food (Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water. Animal care and all experiments were conducted in accordance with the approved standard guidelines for animal experimentation of the Kagawa University Faculty of Medicine, and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Anti-HMGB mAb or IgG2a was injected by three different methods. Intraperitoneal injection of anti-HMGB1 monoclonal antibody (mAb) (200 μg)[17] or class-matched control mAb (IgG2a) (200 μg) against *Keyhole Limpet* hemocyanin was administered 30 min before ischemia. Anti-HMGB mAb (200 μg) or IgG2a (200 μg) was administered intravenously immediately and 6 h after reperfusion. The pupil was dilated with topical phenylephrine hydrochloride and tropicamide; anti-HMGB mAb (20 μg) or IgG2a (20 μg) was injected into the vitreous space 30 min before ischemia.

Ischemia

Rats were anesthetized by an intraperitoneal injection of 50 mg/kg pentobarbital sodium (Abbott, Abbott Park, IL) followed by a topical administration of 0.4% oxybuprocaine hydrochloride. The anterior chamber of the right eye was cannulated with a 27-gauge infusion needle connected to a reservoir containing normal saline. The intraocular pressure (IOP) was raised to 130 mmHg for 45 min by elevating the saline reservoir. Only the right eye of each rat was subjected to ischemia. Retinal ischemia was indicated by whitening of the iris and fundus. The left eyes served as the sham-treated controls, with these eyes undergoing a similar procedure that did not include elevation of the saline bag, thus maintaining normal ocular tension. Rectal and tympanic temperatures were maintained during the operation at approximately 37°C via the use of a feedback-controlled heating pad (BRC, Nagoya, Japan). After restoration of blood flow, temperature was maintained continuously at 37°C.

HISTOLOGICAL EXAMINATION

For histological examination, rats were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg) 7 days after ischemia. Eyes were enucleated and stored in a 4% paraformaldehyde solution for 24 h at room temperature. The retinas were removed and embedded in paraffin, and thin sections (5-μm thickness) were cut using a microtome. Each retina was mounted on a silane-coated glass slide and then stained with hematoxylin and eosin (HE).

Morphometric analysis was performed to quantify ischemic injury. These sections were selected randomly in each eye. Light microscopic examination was performed by a person with no prior knowledge of the treatments. A microscopic image of sections obtained within 0.5–1 mm of the optic disc was scanned. For each computer image, the number of cells in the ganglion cell layer (GCL) was counted. The thicknesses of the inner plexiform layer (IPL), inner nuclear layer (INL), outer nuclear layer (ONL), and outer plexiform layer (OPL) for the entire frame were measured. The number of cells in the GCL was normalized as linear cell density (cells per millimeter). Thicknesses of the IPL, INL, ONL, and OPL were obtained by calculating the mean value of seven measurements in each eye. Similarly, the linear cell density in the GCL was also determined by calculating the mean value of seven measurements. For each animal, the right eye parameter was normalized to that of the intact left eye and shown as a percentage.

ELECTRORETINOGRAMS (ERGS)

ERG responses were measured after overnight dark adaptation (at least 6 h) using a recording device (Mayo Corporation, Aichi, Japan) 7 days after ischemia. Rats were anesthetized by an intraperitoneal injection of 50 mg/kg pentobarbital sodium. Pupils were dilated with 0.5% tropicamide and 0.5% phenylephrine hydrochloride eye drops (Santen Pharmaceuticals, Osaka, Japan). All procedures were performed in dim red light, with all rats kept warm during the procedure. The LED corneal electrode was set vertical to the cornea center. A reference electrode was set subcutaneously on the forehead and the ground connection was set on the base of the tail. An LED stimulator LS-W controlled the stimulus duration and intensity during the 11-step intensity series, which ranged from 0.0003–30 cds/m^2. The ERG response was amplified using an AC amplifier ML135 (Bio Amplifier, AD Instruments, NSW, Australia) with a bandwidth of 0.3–500 Hz and amplification of 2,000 times. The ischemic damage to the retina was

evaluated as the percentage of the a- and b-wave amplitudes of the ischemic right eyes as compared to the non-ischemic left eyes.

IMMUNOHISTOCHEMISTRY

Eyes were enucleated at 6, 12 or 24 h after 45 min of ischemia. Eyes were then fixed in 4% paraformaldehyde in the PBS and embedded in paraffin. Retinal sections (5 μm) were rinsed in 100% ethanol twice for 5 min each, followed by a separate 95% ethanol and 90% ethanol rinse for 3 min each. The sections were then washed using PBS, pH 7.4, three times for 10 min each and treated with 0.3% Triton X-100 in PBS, pH 7.4, for 1 h. After further washing three times for 10 min each with PBS, pH 7.4, sections were then blocked in 3% normal horse serum and 1% bovine serum albumin (BSA) in PBS for 1 h in order to reduce nonspecific labeling. Sections were incubated overnight at 4°C in PBS with either 2.0 mg/mL of monoclonal antibody against HMGB1[17] which served as the primary antibody. After washing in PBS for 50 min, sections were then immersed in the second antibody conjugated to horseradish peroxidase for 1 h at room temperature. Images were acquired using 40× objective lenses (DXM 1200; Nikon, Tokyo, Japan). Adobe PhotoShop v. 5.0 was used to adjust the brightness and contrast of the images.

FLUORESCENT LABELING OF ROS

To investigate the production of ROS, we intraperitoneally injected 5 mg/kg dihydroethidium (DHE; Sigma-Aldrich, St. Louis, MO) in 5% dimethyl sulfoxide (DMSO) in PBS 15 min before ischemia. A 0.3-mL aliquot of distilled water, 200 μg anti-HMGB1 mAb, or 200 μg IgG2a was administered intraperitoneally 30 min before ischemia. Eyes were enucleated 15 min after ischemia and then embedded in OCT compound (Sakura Finetek, Torrance, CA), after which cryosections (20 μm) were prepared. Sections were examined with a microscope (Radiance 2100/Rainbow, Carl Zeiss, München, Germany) using a laser set (excitation laser 514 nm; emission laser >580 nm).

Statistical Analysis

Fluorescence was quantified by automated image analysis with Image-Pro Plus software (version 4.0, Media Cybernetics, The Imaging Expert, Bethesda, MD). For each section, mean fluorescence was calculated from five separate high-power fields per eye. A threshold was set to define positive labeling.

All data are presented as the mean ± SD. Data were analyzed using an independent Student's *t*-test and ANOVA followed by Tukey-Kramer post-hoc testing corrected for multiple comparisons. Statistical analysis was performed using SPSS for Windows (SPSS Inc, Chicago, IL). A p value of < 0.05 was considered statistically significant.

RESULTS

Histologic Changes in the Retina after Ischemia with Anti-HMGB1

Figure 1A shows a normal retina. Light microscopic photographs were taken 7 days after ischemia and treatment with IgG2a (Figure 1B) or anti-HMGB1 (Figure 1C). In animals pretreated with IgG2a, the GCL cell number was reduced to 73.9 ± 16.2% of the control; the IPL thickness was reduced to 67.7 ± 14.6% of the control; the INL thickness was reduced to 82.8 ± 13.5% of the control; the OPL thickness was reduced to 88.6 ± 30.8% of the control; and the ONL thickness was reduced to 88.1 ± 13.0% of the control ($n = 7$; Figure 1D). In animals pretreated with anti-HMGB1 mAb, the GCL cell numbers were 67.7 ± 16.8% of the control ($p = 0.50$); the IPL thickness was 51.6 ± 12.3% of the control ($p = 0.02$); the INL thickness was 69.0 ± 6.8% of the control ($p = 0.03$); the OPL thickness was 51.9 ± 10.8% of the control ($p = 0.01$); and the ONL thickness was 72.4 ± 13.7% of the control ($p = 0.049$) ($n = 7$; Figure 1D).

Treatment with intravenous injection of IgG2a or anti-HMGB1 mAb twice (immediately and 6 h after reperfusion) reduced the retinal thickness dramatically (n = 4, each group) (Figure 2).

Treatment with local administration of IgG2a or anti-HMGB1 mAb 30 min before ischemia was similar to the results with intraperitoneal injection of anti-HMGB1 mAb ($n = 4$, each group) (Figure 3).

EFFECT OF ANTI-HMGB1 ON NORMAL RETINA

Animals were killed at 7 days after the intraperitoneal injection of anti-HMGB1 mAb or IgG2a. Treatment with anti-HMGB1 did not affect the retinal thickness in normal rat (Figure 4) ($n = 4$, each group).

EFFECT OF ANTI-HMGB1 ON RETINAL FUNCTION

Scotopic ERG was recorded to evaluate anti-HMGB1 mAb effects on retinal function. A representative example of function is seen in Figure 5A. Mean amplitudes of the a- and b-wave are shown in Figure 5B. We observed a statistically significant difference between the three groups ($n = 5$, each group).

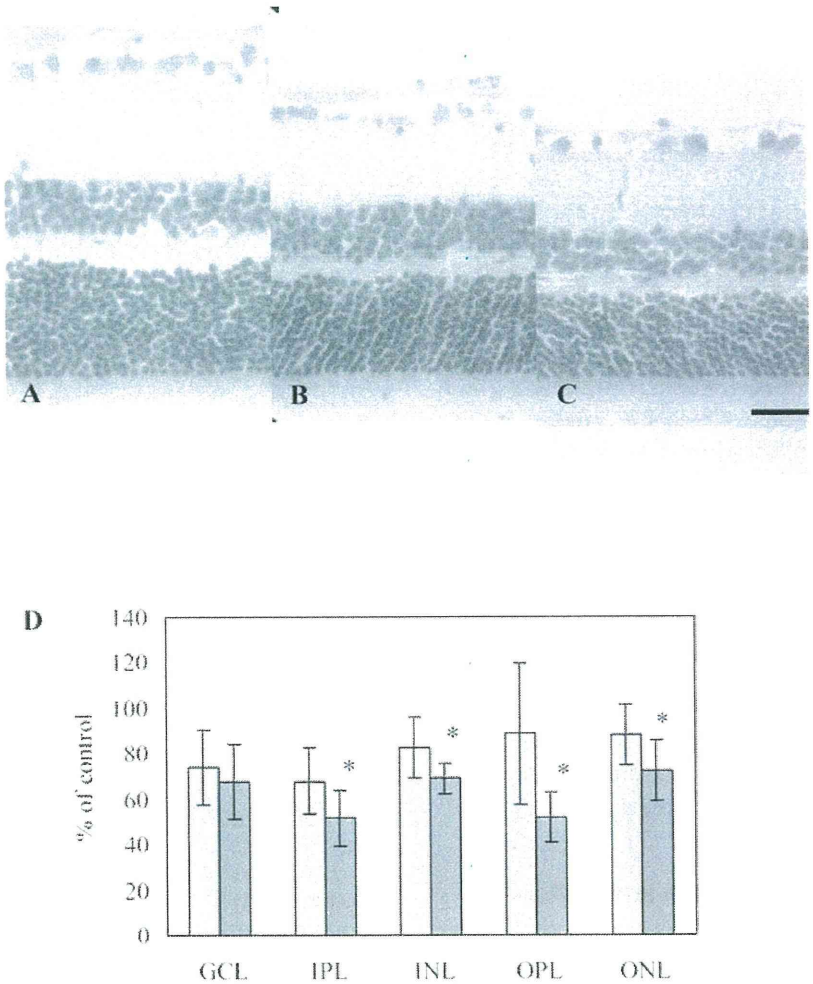

FIGURE 1 Light micrographs of a cross-section through normal rat retina (A) and at 7 days after ischemia when treated with control class-matched mAb (anti-*Keyhole Limpet* hemocyanin mAb, IgG2a) (B) or anti-HMGB1 mAb (C). Percentages indicate change relative to control values for the number of GCL cells and for the IPL, INL, ONL, and OPL thicknesses 7 days after ischemia when treated with IgG2a ( ) or anti-HMGB1 ( ). Results are expressed as the mean ± SD (*$p < 0.05$). Scale bar = 20 μm.

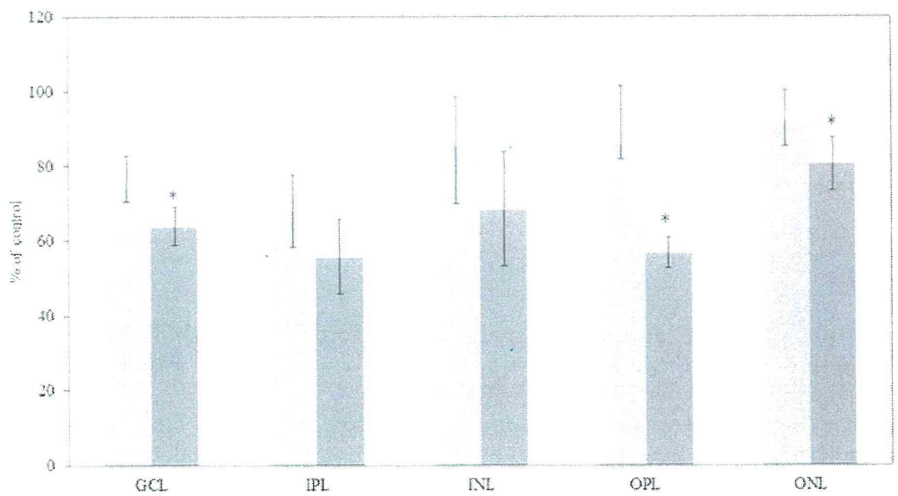

FIGURE 2 Percentages indicate change relative to control values for the number of RGC cells and for IPL, INL, ONL, and OPL thickness 7 days after ischemia when treated with intravenous injections of IgG2a ( ) or anti-HMGB1 ( ). Results are expressed as the mean ± SD (*$p < 0.05$).

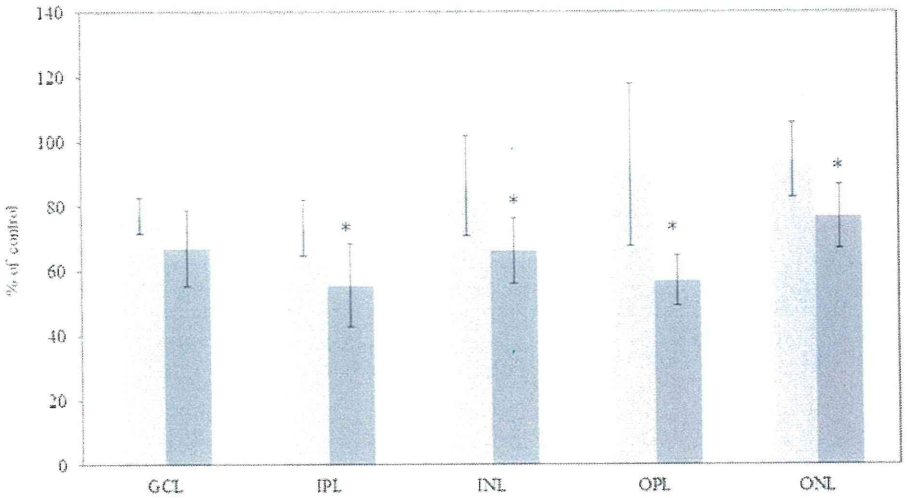

FIGURE 3 Percentages indicate change relative to control values for the number of RGC cells and for IPL, INL, ONL, and OPL thickness 7 days after ischemia when treated local administration of IgG2a ( ) or anti-HMGB1 ( ). Results are expressed as the mean ± SD (*$p < 0.05$).

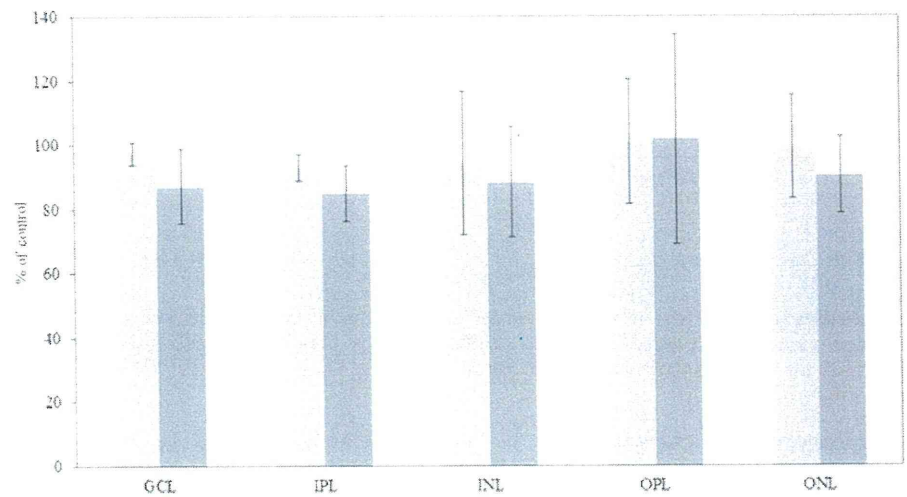

FIGURE 4 Percentages indicate change relative to control values for the number of RGC cells and for IPL, INL, ONL, and OPL thickness 7 days after treatment with IgG2a ( ) or anti-HMGB1 ( ). Results are expressed as the mean ± SD.

Scotopic ERG was measured 7 days after the intraperitoneal injection of anti-HMGB1 mAb. There was no significant difference between the two groups (Figure 6A, B) ($n = 5$, each group). Treatment with anti-HMGB1 did not affect the retinal function in normal rat.

IMMUNOHISTOCHEMISTRY

We examined the expression of HMGB1 in the retina at 6, 12, and 24 h after 45 min of ischemia (Figure 7). Figure 3A shows the localization of HMGB1 in the normal retina. Immunostaining for HMGB1 was noted in the ONL in the normal retina. However, immunostaining for HMGB1 in the post-ischemic retina (Figure 7B–D) was detected in not only the ONL but also in the INL and GCL. A high degree of edema was noted in the post-ischemic retina.

We examined the effect of anti-HMGB1 mAb on endogenous HMGB1 expression in the normal retina and the retina at 6, 12, and 24 h after 45 min of ischemia (Figure 8). Intraperitoneal injection of anti-HMGB1 mAb was administered 30 min before ischemia. Normal eye balls were enucleated after 30 min administration of anti-HMGB1. Administration of anti-HMGB1 suppressed the expression of endogenous HMGB1.

We also examined the direct effect of anti-HMGB1 mAb on retinal HMBG1 expression (Figure 9). Normal eye balls were enucleated at 12 h after intravitreous injection of anti-HMGB1. Immunostaining for HMGB1 was not detected.

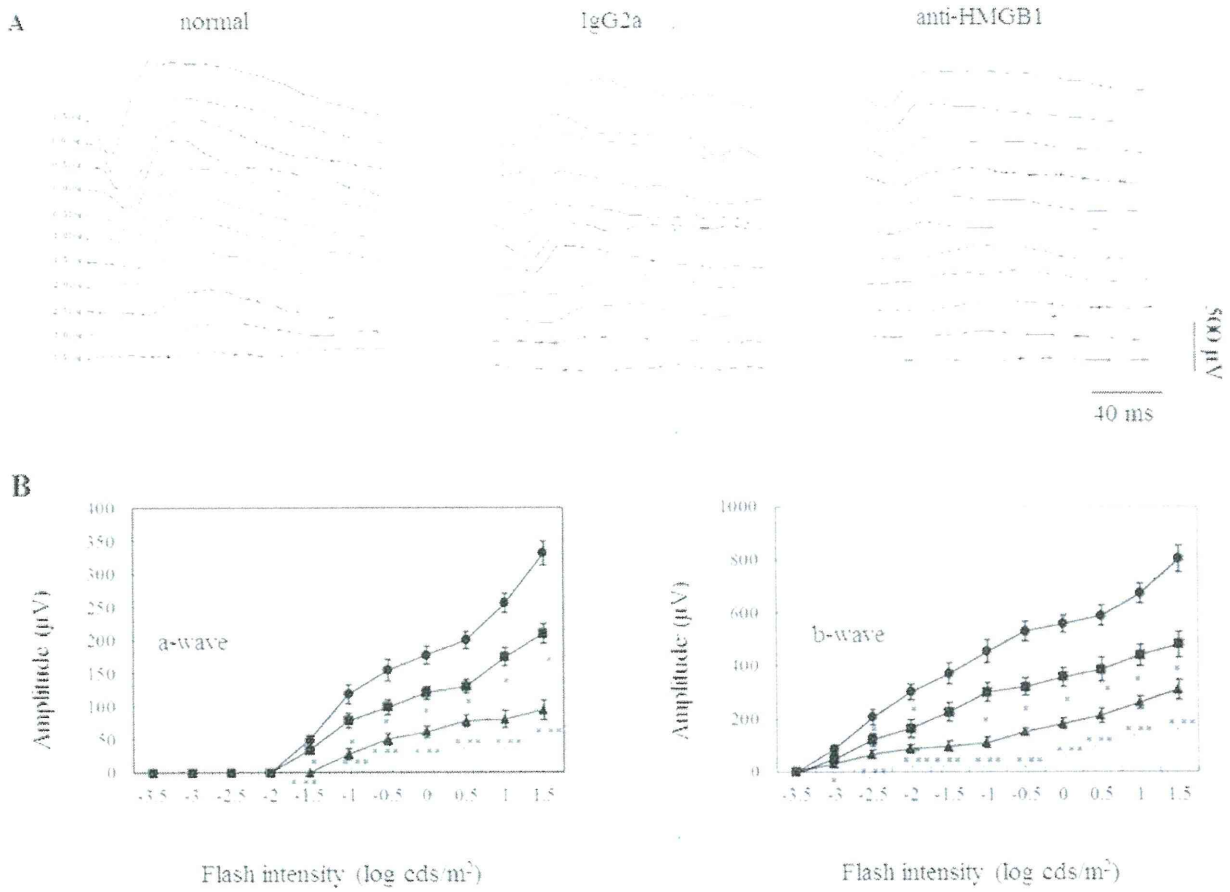

FIGURE 5 (A) Representative scotopic ERGs at baseline and at 7 days after ischemia when treated with control mAb or anti-HMGB1 mAb. (B) Amplitudes for a- and b-waves plotted as a function of flash intensity. Pretreatment with anti-HMGB1 mAb markedly reduced the amplitudes. Results are expressed as the mean ± SD. •: normal, ▪: IgG2a, ▲: anti-HMGB1. *$p<0.05$ versus normal retina. **$p<0.05$ versus ischemic retina with IgG2a.

ROS ACTIVATION BY ISCHEMIA

We used DHE staining to test whether ROS were enhanced by treatment with 200 μg anti-HMGB1 mAb. DHE specifically reacts with intracellular O_2^-, a ROS, and is converted to the red fluorescent compound ethidium in nuclei. In the post-ischemic retina, DHE fluorescence was clearly up-regulated in the retinal neuronal cells, and this up-regulation was efficiently enhanced by anti-HMGB1 mAb (Figure 10A–C). Figure 9D shows the quantified specific retinal DHE fluorescence. The mean ROS activation was significantly increased by treatment with anti-HMGB1 mAb ($n=4$, each group).

DISCUSSION

This study shows that, compared to the IgG2a treatment, pretreatment with anti-HMGB1 mAb significantly enhanced the ischemic injury of the retina. The results also showed that there was expression of HMGB1 mAb in the retina after ischemia-reperfusion injury.

A recent study showed that HMGB1 inhibited glial glutamate transport by GLAST in mouse gliosomes and suggested that HMGB1 can increase extracellular glutamate levels in ischemic brain.[21] We previously reported that anti-HMGB1 mAb suppressed ischemia-reperfusion-induced brain injury in a transient middle cerebral artery occlusion model in rats.[17] Based on these findings, we predicted that neutralizing mAb could be used to inhibit HMGB1 activity, and thus to significantly decrease the progression of the retinal ischemia-reperfusion injury. However, use of the neutralizing anti-HMGB1 mAb treatment in the present study remarkably increased the retinal damage following ischemia-reperfusion. This was due to an increased production of ROS caused by the anti-HMGB1 mAb treatment. Therefore, it might be possible that elevated levels of HMGB1 had neuroprotective effects against retinal ischemia- reperfusion injury. It has also been reported that treatment with anti-HMGB1 mAb increased ischemia-reperfusion

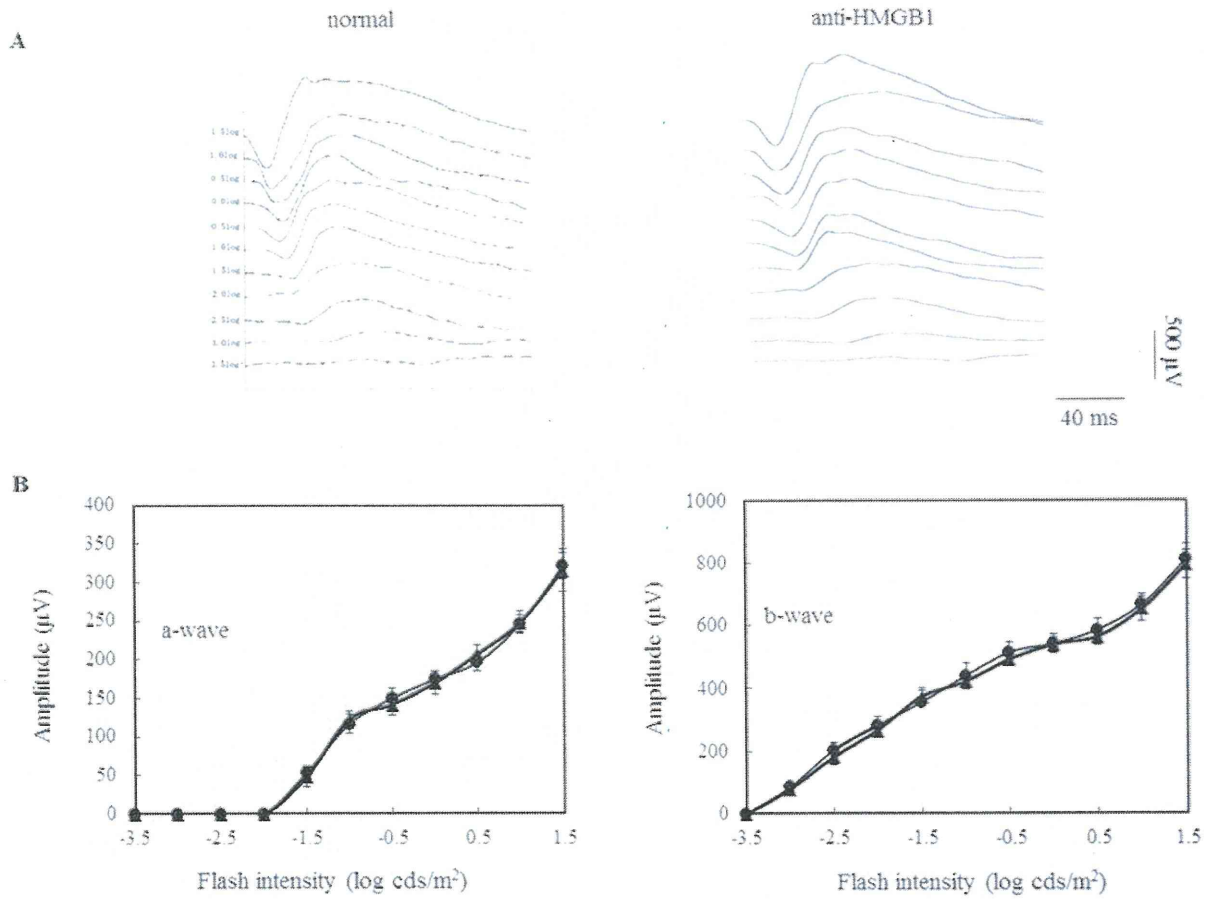

FIGURE 6 (A) Representative scotopic ERGs 7 days after treatment with anti-HMGB1. (B) Amplitudes for a- and b-waves plotted as a function of flash intensity. Results are expressed as the mean ± SD. •: normal, ▲: anti-HMGB1.

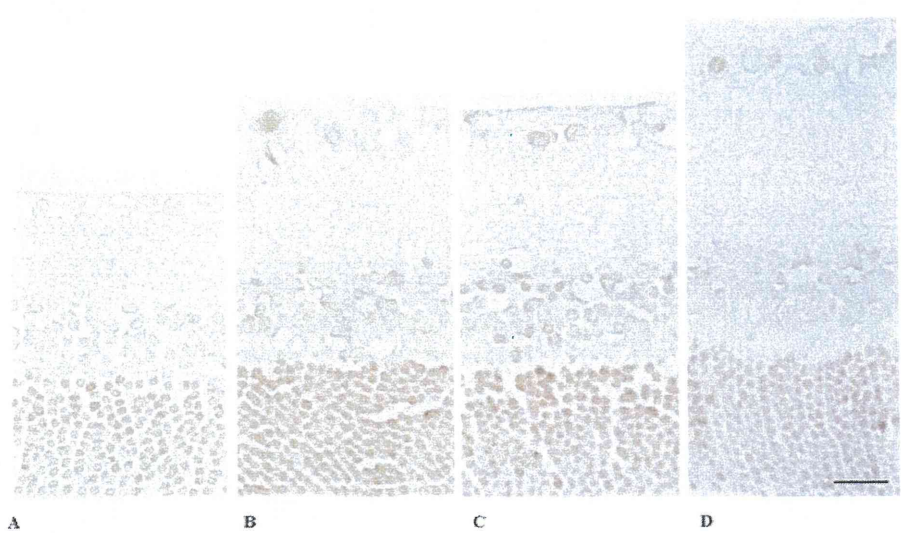

FIGURE 7 Immunohistochemical staining of HMGB1 expression in the retina. Retinal sections from normal animals (A) or 6 h (B), 12 h (C), or 24 h (D) after ischemia. Scale bar = 20 μm.

injury in the rat heart.[22] Therefore, it appears that the effect of anti-HMGB-1 mAb depends on the organ involved.

When the IOP is increased, glutamate is released from the retina during and after the ischemia.[3,23,24] The major causes of the cell death that occur after activation

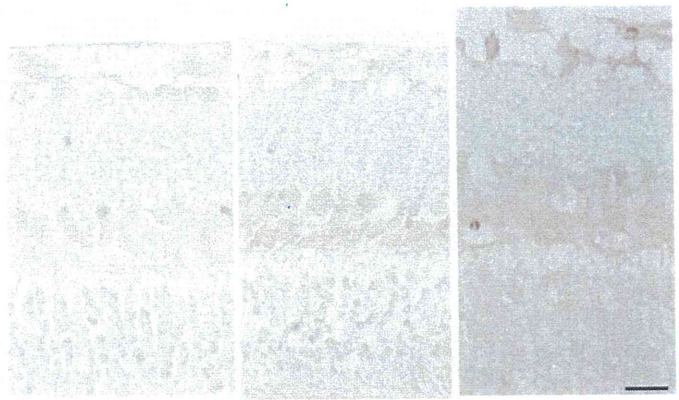

FIGURE 8 The effect of anti-HMGB1 mAb on endogenous HMGB1 expression. Intraperitoneal injection of anti-HMGB1 mAb was administered 30 min before ischemia. Retinal sections from normal animals (A) or 6 h (B), 12 h (C), or 24 h (D) after ischemia. Scale bar = 20 μm.

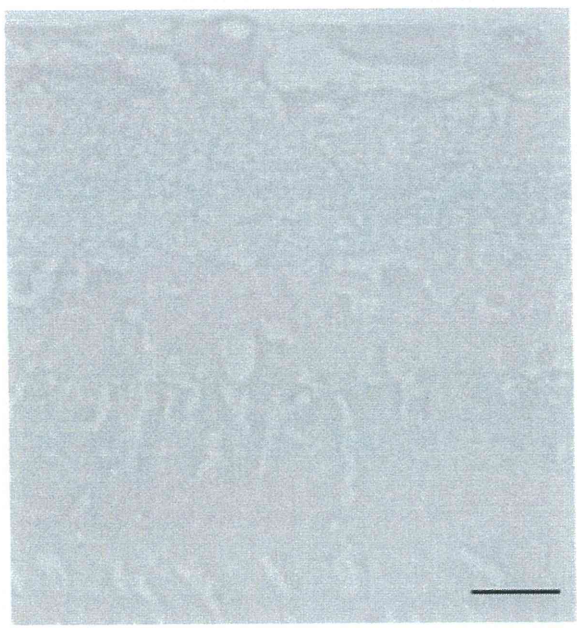

FIGURE 9 The direct effect of anti-HMGB1 mAb on endogenous HMGB1 expression in the normal retina. Scale bar = 20 μm.

of the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors are related to the influx of calcium into the cells and the generation of free radicals.[25] Excessive accumulation of intracellular free Ca^{2+} ($[Ca^{2+}]_i$) can have a wide range of detrimental effects, including inhibition of mitochondrial function, reduction of cellular ATP levels, enhancement of ROS production, and activation of cellular proteases and nitric oxide (NO) synthase.[26] Since production of ROS was increased by anti-HMGB1 mAb treatment in the current study, anti-HMGB1 mAb played a large deleterious role in the resultant ischemia-reperfusion injury. In the present study, there was an increase in the HMGB1 level in the retina after the ischemia-reperfusion injury. These results suggest that endogenous HMGB1 released from retinal cells may modulate ischemia-reperfusion injury in the retina. Therefore, the anti-HMGB1 mAb treatment increased the delayed neuronal death.

We evaluated the functional retinal damage after ischemia-reperfusion injury by measuring the ERG a- and b-wave amplitudes. The b-wave of the ERG has been identified as a particularly sensitive index of retinal ischemia both in humans[25] and in experimental models of retinal ischemia in vitro.[27] After the anti-HMGB1 mAb treatment, there was a decrease in the thickness of ONL following ischemia-reperfusion, with the a-wave of the ERG also lower than that noted in the eyes treated with IgG2a. There was a good correlation between the ERG for both a- and b-waves and the histological results. It has been reported that administration of pentobarbital enhance the a- and b-wave of the ERG.[28,29] Under the anesthesia, many factors indirectly affecting the retinal activity could not be completely excluded.

It has been reported in previous studies that HMGB1 is expressed in GCL, INL, ONL, the inner and outer segments of photoreceptors, and in the retinal pigment epithelial cells in normal retina.[30,31] However, the current immunohistochemical study showed that HMGB1 was present in the ONL in normal retinas, which may be due to the use of different antibodies in the various studies (monoclonal antibody vs. polyclonal antibody).

HMGB1 passively released from necrotic cells.[32] Cell death was frequently observed in both the GCL and the INL after 3 h of ischemia-reperfusion.[33] In the present study, we demonstrated that immunostaining for HMGB1 in the post-ischemic retina was detected in not only the ONL but also in the INL and GCL. HMGB1 may play a key role in the protection of retinal injury after ischemia-reperfusion.

The current study showed, for the first time, that treatment with anti-HMGB1 mAb increased ischemia-reperfusion injury in the rat retina. Further investigations are needed to clarify the mechanism of anti-HMGB1 mAb in retinal ischemia-reperfusion

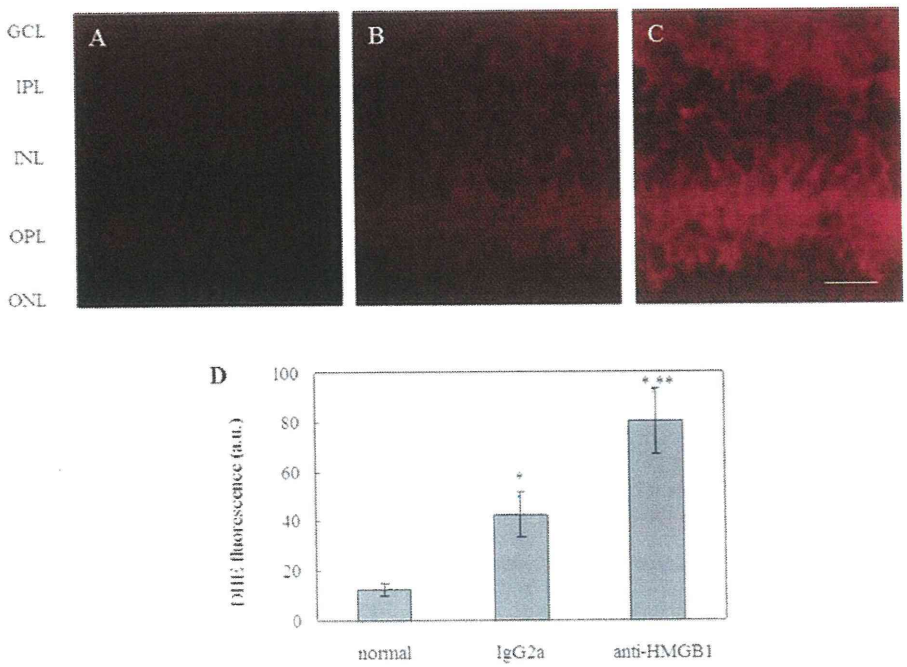

FIGURE 10 Effect of anti-HMGB1 mAb pretreatment on the release of ROS. The use of DHE to detect ROS indicated up-regulation of retinal neuronal cells in the retina after ischemia (IgG2a (B) as compared to normal retina (A)). Pretreatment with anti-HMGB1 mAb enhanced the level of ROS (C). (D) Quantified specific retinal DHE fluorescence is expressed for sections in arbitrary units (AU) for each group. Data express the mean ± SD; $*p < 0.05$ normal retina. $**p < 0.05$ versus ischemic retina with IgG2a. Scale bar = 20 μm.

injury. Additionally, anti-HMGB1 mAb function needs be further explored, as this could potentially lead to the development of neuroprotective therapeutic strategies for acute retinal ischemic disorders.

ACKNOWLEDGMENTS

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***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

[1] Tsujikawa A, Ogura Y, Hiroshiba N, Miyamoto K, Kiryu J, Honda Y. Tacrolimus (FK506) attenuates leukocyte accumulation after transient retinal ischemia. *Stroke*. 1998;29:1431–1437; discussion 1437–1438.

[2] Tsujikawa A, Ogura Y, Hiroshiba N, Miyamoto K, Kiryu J, Tojo SJ, Miyasaka M, Honda Y. Retinal ischemia-reperfusion injury attenuated by blocking of adhesion molecules of vascular endothelium. *Invest Ophthalmol Vis Sci*. 1999;40:1183–1190.

[3] Hirooka K, Miyamoto O, Jinming P, Du Y, Itano T, Baba T, Tokuda M, Shiraga F. Neuroprotective effects of D-allose against retinal ischemia-reperfusion injury. *Invest Ophthalmol Vis Sci*. 2006;47:1653–1657.

[4] Iwama D, Miyamoto K, Miyahara S, Tamura H, Tsujikawa A, Yamashiro K, Kiryu J, Yoshimura N. Neuroprotective effect of cilostazol against retinal ischemic damage via inhibition of leukocyte-endothelial cell interactions. *J Thromb Haemost*. 2007;5:818–825.

[5] Sakamoto K, Kawakami T, Shimada M, Yamaguchi A, Kuwagata M, Saito M, Nakahara T, Ishii K. Histological protection by cilnidipine, a dual L/N-type $Ca^{(2+)}$ channel blocker, against neurotoxicity induced by ischemia-reperfusion in rat retina. *Exp Eye Res*. 2009;88:974–982.

[6] Büchi ER. Cell death in the rat retina after a pressure-induced ischemia-reperfusion insult: An electron microscopic study. I: Ganglion cell layer and inner nuclear layer. *Exp Eye Res*. 1992;55:605–613.

[7] Nitatori T, Sato N, Waguri S, Karasawa Y, Araki H, Shibanai K, Kominami E, Uchiyama Y. Delayed neuronal death in the CA1 pyramidal cell layer of the gerbil hippocampus following transient ischemia is apoptosis. *J Neurosci*. 1995;15:1001–1011.

[8] Rosenbaum DM, Rosenbaum PS, Gupta A, Michaelson MD, Hall DH, Kessler JA. Retinal ischemia leads to apoptosis which is ameliorated by aurintricarboxylic acid. *Vision Res*. 1997;37:3445–3451.

[9] Halliwell B, Gutteridge JM. Free Radicals in Biology and Medicine. Oxford, UK: Clarendon Press; 1985.

[10] Goodwin GH, Sanders C, Johns EW. A new group of chromatin-associated proteins with a high content of acidic and basic amino acids. *Eur J Biochem*. 1973;38:14–19.

[11] Lotze MT, Tracey KJ. High-mobility group box 1 protein (HMGB1): Nuclear weapon in the immune arsenal. *Nat Rev Immunol*. 2005;5:331–342.

[12] Calogero S, Grassi F, Aguzzi A, Voigtländer T, Ferrier P, Ferrari S, Bianchi ME. The lack of chromosomal protein Hmgb1 does not disrupt cell growth but causes lethal hypoglycaemia in newborn mice. *Nat Genet*. 1999;22:276–280.

[13] Hoppe G, Rayborn ME, Sears JE. Diurnal rhythm of the chromatin protein Hmgb1 in rat photoreceptors is under circadian regulation. *J Comp Neurol*. 2007;501:219–230.
[14] Goldstein RS, Gallowitsch-Puerta M, Yang L, Rosas-Ballina M, Huston JM, Czura CJ, Lee DC, Ward MF, Bruchfeld AN. Elevated high-mobility group box 1 levels in patients with cerebral and myocardial ischemia. *Shock*. 2006;25:571–574.
[15] Kim JB, Sig Choi J, Yu YM, Nam K, Piao CS, Kim SW, Lee MH, Han PL, Park JS, Leem JK. HMGB1, a novel cytokine-like mediator linking acute neuronal death and delayed neuroinflammation in the postischemic brain. *J Neurosci*. 2006;26:6413– 6421.
[16] Faraco G, Fossati S, Bianchi ME, Patrone M, Pedrazzi M, Sparator B. Moroni F, Chiarugi A. High mobility group box 1 protein is released by neural cells upon different stresses and worsens ischemic neurodegeneration *in vitro and in vivo J Neurochem*. 2007;103:590–603.
[17] Liu K, Mori S, Takahashi HK, Tomono Y, Wake H, Kanke T, Sato Y, Hiraga N, Adachi N, Yoshino T, Nishibori M. Anti-high mobility group box 1 monoclonal antibody ameliorates brain infarction induced by transient ischemia in rats. *FASEB J*. 2007;21:3904–3916.
[18] Kim JB, Lim CM, Yu YK, Lee JK. Induction and subcellular localization of high-mobility group box-1 (HMGB1) in the postischemic rat brain. *J Neurosci Res*. 2008;86:1125–1131.
[19] Muhammad S, Barakat W, Stoyanov S, Murikinati S, Yang H, Tracey KJ, Bendszus M, Rossetti G., Nawroth PP, Bierhaus A, Schwaninger M. The HMGB1 receptor RAGE mediates ischemic brain damage. *J Neurosci*. 2008;28:12023–12031.
[20] Qiu J, Nishimura M, Wang Y, Sims JR, Qiu S, Savitz SI, Salamone S, Moskowitz MA. Early release of HMGB-1 from neurons after the onset of brain ischemia. *J Cereb Blood Flow Metab*. 2008;28:927–938.
[21] Pedrazzi M, Raiteri L, Bonanno G, et al. Stimulation of excitatory amino acid release from adult mouse brain glia subcellular particles by high mobility group box 1 protein. *J Neurochem* 2006;99:827–838.
[22] OozawaS, Mori S, Kanke T, Takahashi H, Liu K, Tomono Y, Asanima M, Miyazaki I, Nishibori M, Sano S. Effect of HMGB1 on ischemia-reperfusion injury in the rat heart. *Circ J*. 2008;72:1178–1184.
[23] Louzada-Junior P, Dias JJ, Santos WF, Lachat JJ, Bradford HF, Coutinho-Netto J. Glutamate release in experimental ischaemia of the retina: An approach using microdialysis. *J Neurochem*. 1992;59:358–363.
[24] Adachi K, Kashii S, Masai H, Ueda M, Morizane C, Kaneda K, Kume T, Akaike A, Honda Y. Mechanism of the pathogenesis of glutamate neurotoxicity in retinal ischemia. *Graefes Arch Clin Exp Ophthalmol*. 1998;236:766–774.
[25] Osborne NN, Ugarte M, Chao M, Chidlow G., Bae JH, Wood JP, Nash MS. Neuroprotection in relation to retinal ischemia and relevance of glaucoma. *Surv Ophthalmol*. 1999;43:S102–S128.
[26] Coleman K, Fitzgerald D, Eustace P, Bouchier-Hayes D. Electroretinography, retinal ischemia and carotid artery disease. *Eur J Vasc Surg*. 1990;4:569–573.
[27] Zager E, Ames A 3rd. Reduction of cellular energy requirements. Screening for agents that may protect against CNS ischemia. *J Neurosurg*. 1998;69:568–579
[28] Jacobson JH, Gestring GF. Centrifugal influence upon the electroretinogram. *AMA Arch Ophthalmol*. 1958;60:295–302.
[29] Noell WK. Differentiation, metabolic organization, and viability of the visual cell. *AMA Arch Ophthalmol*. 1958;60:702–733.
[30] Arimura N, KI-I Y, Hashiguchi T, Kawahara K, Biswas KK, Nakamura M, Sonoda Y, Yamakiri K, Okubo A, Sakamoto T, Maruyama I. Intraocular expression and release of high-mobility group box 1 protein in retinal detachment. *Lab Invest*. 2009;89: 278–289.
[31] Watanabe T, Keino H, Sato Y, Kudo A, Kawakami H, Okada AA. High mobility group box protein-1 in experimental autoimmune uveoretinitis. *Invest Ophthalmol Vis Sci*. 2009;50:2283–2290.
[32] Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature*. 2002;418:191–195.
[33] Büchi ER. Cell death in the rat retina after a pressure-induced ischemia-reperfusion insult: an electron microscopic study. I. ganglion cell layer and inner nuclear layer. *Exp Eye Res*. 1992;55:605–613.

岡山大学医学部の挑戦は続いています！

現代の魔法の弾丸
「分子標的治療」

- ●脳梗塞（抗体医薬）
- ●脳腫瘍（中性子捕捉）
- ●消化器癌（テロメラーゼ）

厚生労働省大型研究費
研究成果報告

ご挨拶

本日は、岡山大学医学部医学科のオープンキャンパスにおいでいただきありがとうございます。本パンフレットの表紙写真は、我々岡山大学医学部の前身である旧制第三高等学校医学部出身の秦佐八郎博士とドイツ留学時代の師、エールリッヒ教授のツーショットです。

二人は、当時怖れられていた梅毒の治療薬を独自の考えで開発しました。その考えとは、病原体に強く結合できる化合物の中に治療効果をもたらす「薬」の候補が存在するというものです。サルバルサン 606 号と命名された化学療法薬の開発は、当時「魔法の弾丸」と呼ばれました。それは今日の、特定の生体内分子に狙いを定めて治療する「標的治療」の先駆けとなりました。化学療法薬サルバルサン 606 号はその後の多くの薬物開発に道筋をつけるものとなったのです。

岡山大学医学部の研究者は、偉大な先輩に続くべく、日々研究に取り組んでいます。癌あるいは生活習慣病といった現代の医学上の課題に対し、新しい「魔法の弾丸」の観点から幾つかの成果を挙げることができました。それらの中から、本日、厚生労働省より大型の研究費補助を得て達成された4つの研究内容を、高校生の皆さんにわかりやすく紹介したいと思います。

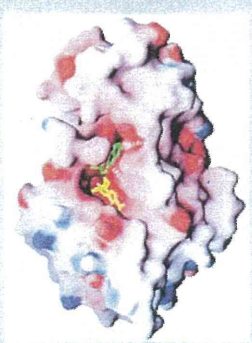
患者さんの診療と治療は、日々臨床の医師によって担われていますが、そこで用いられる治療法は、地道ではありますが挑戦的な研究に支えられていることをこの機会にぜひ知っていただけたらと思います。そして将来、皆さんの中から新しい発想と熱意を持った研究者が生まれることを強く期待しています。

平成 23 年 8 月 5 日

西堀　正洋

松井　秀樹

藤原　俊義



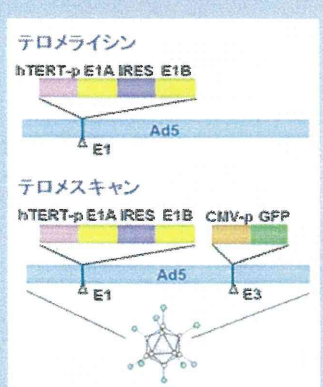
ウイルスで癌を見て治す！

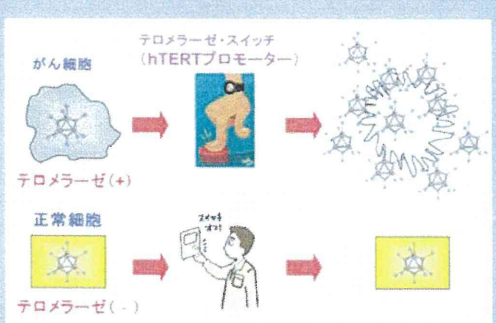
研究代表者　藤原　俊義

癌による死亡は年々増加傾向にあり、1986 年以降は日本人の主な死亡原因の第 1 位を占めています。その克服のために新しい技術開発が進んでいますが、私たちはウイルスを使って癌を診断したり、治療したりしようと試みています。

ウイルスは細胞に感染すると増殖して細胞を殺します。私たちは、遺伝子操作によって、癌細胞でのみ増えるスイッチがオンとなるウイルスを作成しました。このテロメライシンというウイルスは癌細胞では増えて細胞を殺しますが、正常細胞では増えないため安全性が保たれます。また、このウイルスにノーベル賞を受賞した下村博士が発見した GFP という蛍光遺伝子を組み込んだテロメスキャンというウイルスも作成しました。テロメスキャンは癌細胞でだけ緑色の蛍光を発するので、ナビゲーションのようにその部分を見ながら必要最小限の手術をすることができるようになります。

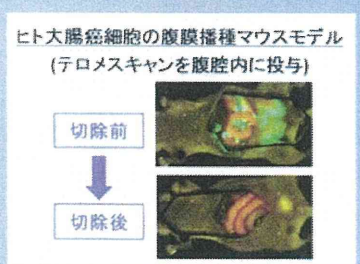
テロメライシンはすでにアメリカで患者さんに投与されたことがあり、さらに特殊な光で癌細胞を殺す工夫をしています。テロメスキャンも光学企業と共同で、臨床応用を目指しています。その実際を本日は紹介させていただきます。





テロメライシンで
癌を殺す

テロメスキャンで
癌を見る



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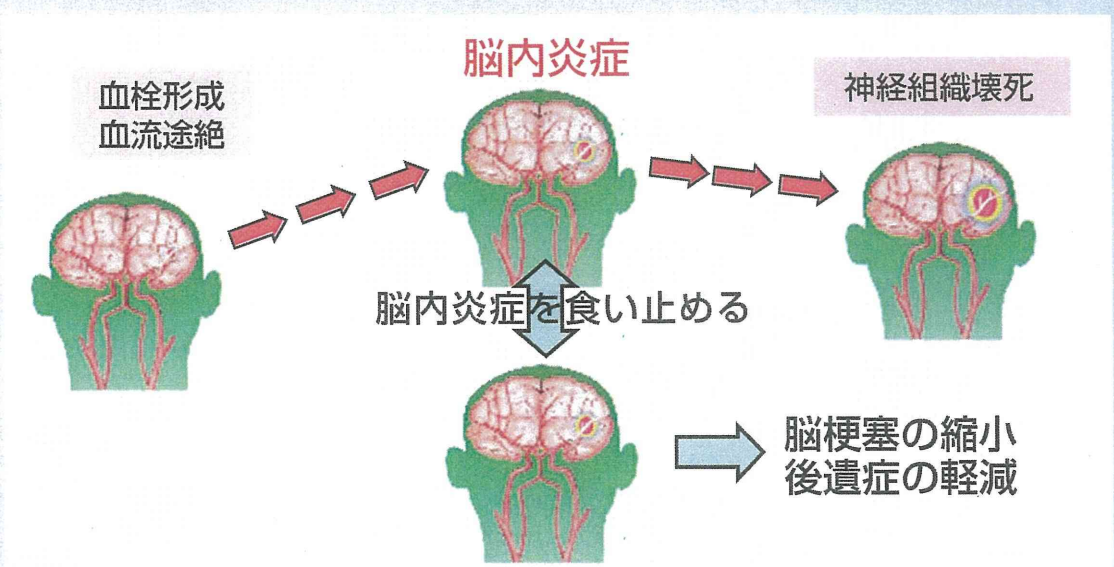
脳梗塞を抗体医薬で治療する！

研究代表者　西堀　正洋

野球の日本代表監督であった長嶋茂雄氏やサッカーのイビチャ・オシム氏が脳梗塞で倒れられたことは、皆さんの記憶にも新しいことと思います。脳梗塞は、脳血管内で血栓が形成され、血栓部位より先に血液が流れない状態が続くことで、血流の途絶した脳組織が壊死するという病気です。脳梗塞を始めとする脳血管障害は日本人の死因の第３位を占めていますし、運動麻痺のような神経後遺症が患者さんご本人と家族にとって重大な問題になります。

私たちは、血流が停止してから脳組織が壊死に陥るまでには一定の時間を要することに注目し、この間に「脳内炎症」が進行するとの仮説を立てました。さらに、「脳内炎症」を惹き起こす因子として「HMGB1」と呼ばれる蛋白質に着目しました。つまり、この因子の働きを抑えることで、脳梗塞の大きさをうんと小さくできないかと考えたわけです。その方法と結果についてご紹介します。



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趣味：　散歩　　座右の銘：　牛の如く

夢の粒子線治療を実現する！

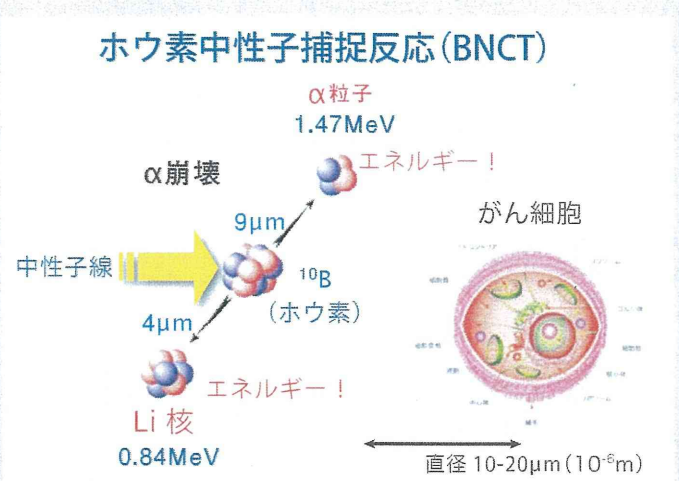
研究代表者　松井　秀樹

今でも日本人の死因の1位は「がん」です。3人に1人はがんで亡くなっています。ちなみに第2位は心疾患、第3位は脳血管疾患です。一方で、医学の進歩により治療可能な「がん」も増えています。効果的な手術、抗がん剤、抗体医薬、放射線治療などが開発され、特に早期発見・早期治療で完治するものは多くなりました。

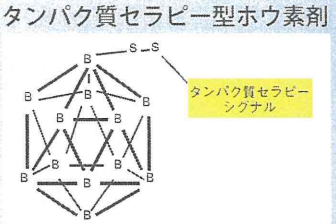
しかしながら、少しも治療成績が向上していないがんも多数あります。特に悪性度が強く、転移しやすいもの、正常な組織に浸潤するがん、例えば脳腫瘍などについてはお手上げの状態です。

私たちは「中性子捕捉療法（BNCT）」と呼ばれる全く新しい二段階方式の粒子線治療法により、周りの正常組織を傷つけることなく、がん細胞だけを一気に叩く、夢の治療法を開発しようとしています。この治療法の成功には私たちが作った「タンパク質セラピー法」そして「多機能型イムノリポソーム」という技術が必須です。これらの原理や開発の状況についてお話しします。

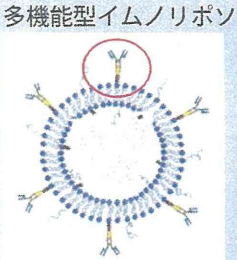
東日本大震災のあとの原子力発電所の事故で、放射能や放射線という言葉に不安を感じる人も多いと思います。しかし、私たちが「がん」と戦うためには、それらがとても重要な武器であり有用な技術であることも、あわせて理解していただければ幸いです。



タンパク質セラピー型ホウ素剤



多機能型イムノリポソーム



■研究者プロフィール

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1982-1984年	カナダ・カルガリー大学 医学部・医化学留学
1995年-	岡山大学医学部・生理学　教授
2006-2008年	岡山大学　医学部長
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アクセス

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■JR 岡山駅東口から岡電バス「労災病院」行き、「当新田・大東」行き、「東山」行き、または「岡南営業所」行きに乗車、「大学病院前」下車
■JR 岡山駅東口からタクシー7 ～ 10 分