

Similarities in Oxidative Damage from AMD and Retinal Light Damage

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ABSTRACT

Purpose: To screen for similarities in oxidative stress mechanisms caused by retinal light damage and age-related macular degeneration (AMD) using rats and *in vivo* blue light exposure.

Methods: Albino rats were dark adapted for 24 hrs. Control rats (N=10) remained in the dark while experimental rats (N=10) were exposed to blue light (220 fc) for 6 hours. Rats were sacrificed immediately following light treatment. Retinas were isolated, immediately washed with antioxidants and frozen at -80°C until analysis. Lipids were extracted with chloroform/methanol and proteins were solubilized with SDS. Western analysis was used to screen for oxidative protein modifications.

Results: Anti-carboxyethylpyrrole and anti-nitrotyrosine immunoreactivities were significantly greater after 6h light exposure compared with control animals maintained in the dark. Carboxyethylpyrrole (CEP) adducts are derived from oxidation of docosahexaenoyl lipids (DHA-PC) and nitrotyrosine is generated from reactive nitrogen oxide species.

Conclusions: Current results are consistent with our observations that green light stimulates *in vivo* protein nitration (2002 Mol. & Cell. Proteomics 1, 293) and CEP modifications in rat retina (2003 ARVO abstract 5129). Notably, AMD drusen/Bruch's membrane and light damaged rat retina both contain increased amounts of CEP adducts (2002 Proc Natl Acad Sci USA 99, 14682) and crystallin (2003 Exp Eye Res 76, 131). Rodent light damage models may be useful for validating therapies for AMD.

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INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of visual impairment among the elderly population in developed countries. AMD appears to involve oxidative damage and crystallin appears to be more common in drusen from AMD donor eyes than from normal human eyes. For example, carboxyethylpyrrole (CEP) adducts, a protein modification uniquely derived from oxidation of docosahexaenoate-containing lipids, are more abundant in Bruch's membrane/RPE/choroid from AMD donor eyes (Crabb et al., 2002). Our recent proteomic analyses of rat retina after green light exposure (1500 lux / 3h) have shown that light modulates tyrosine nitration (Miyagi et al., 2002), that crystallin 2D gel components increase 2-3 fold relative to unexposed retinas (Sakaguchi et al., 2003) and that CEP and argpyrimidine protein adducts increase 4-8 fold (Renganathan et al., 2003). These results support similarities between retinal light damage and AMD and suggest that rodent light damage models may be useful for validating therapies for AMD. This poster presents preliminary analyses of oxidative damage in rat retina caused by *in vivo* blue-light exposure.

METHODS

Animal Procedures and Retinal Preparations: Male Sprague-Dawley rats were maintained in a dark environment for 24 hours prior to exposure to blue light at 220 fc (half-amplitude band pass = 435-475 nm) for 6 hours. Control rats were maintained under normal cyclic light. Animals were sacrificed immediately following light exposure, retinas excised within 2 min of death, rinsed in PBS containing 2 mM diethylenetriaminepentaacetic acid (DTPA) and 100 μM butylated hydroxytoluene (BHT) and frozen in liquid nitrogen. The following day retinas were thawed and lipids extracted with chloroform/methanol and dried under nitrogen. Protein was then extracted with Laemmli SDS sample buffer. Extractions were performed under dim red illumination.

ERG evaluation of retinal function: Rats were allowed to recover for 5 days in darkness prior to ERG evaluation of retinal function. Rats were then returned to their home-cage and allowed to recover for three additional weeks. Before ERG measurement, rats were anesthetized by IP injection with Ketamine-HCl (75 mg/Kg) and Xylazine (6 mg/Kg). Flash ERGs were recorded from a platinum-iridium wire loop electrode positioned on the cornea and were elicited by viewing a Ganzfeld. Electrical responses to a series of light flashes increasing in intensity were digitized to analyze temporal characteristics of the waveform and response voltage-log intensity (VlogI) relationship.

Assessment of Retinal Morphology: Ocular tissues were fixed, dehydrated in an ascending ethanol series, embedded in JB-4 plastic resin, and 1 to 1.5-micron thick sections were analyzed using a quantitative computer image analysis system attached to a Nikon research microscope.

2D-Gel Electrophoresis and Western Analysis: Retinal protein was precipitated using two volumes of acetone, redissolved in solution 7 M urea, 2 M thiourea, 4% CHAPS, 0.5% Triton X-100, 2% IPG-ampholytes pH 3-10 NL, 1% DTT, and subjected to 2D PAGE. First dimension IEF was performed with the Pharmacia IPGphor system and non-linear pH 3-10 immobilized pH gradients (7 cm). Second dimension electrophoresis was according to Laemmli on 10% acrylamide gels with a 4% stacking gel (10 x 8 cm). Image analysis utilized PDQuest software (Bio-Rad). Protein was quantified using the Bradford assay. One and two dimensional electrophoresis and Western blotting methods were as described (West et al., 2001; Miyagi et al., 2002). Antibodies utilized in Western blots include anti-nitrotyrosine mAb (Upstate USA), anti-CEP mAb (Crabb et al., 2002), and anti-argpyrimidine mAb, obtained as a gift from Dr. Koji Uchida (Oya et al., 1999).

Protein Identification: Gel bands/spots were excised, digested with trypsin and proteins identified by LC MS/MS using a CapLC system and a quadrupole time-of-flight mass spectrometer, ProteinLynxTM Global Server and MassLynxTM software (Waters), and the Swiss-Prot and NCBI protein sequence databases (Crabb et al., 2002).

FIGURE 1

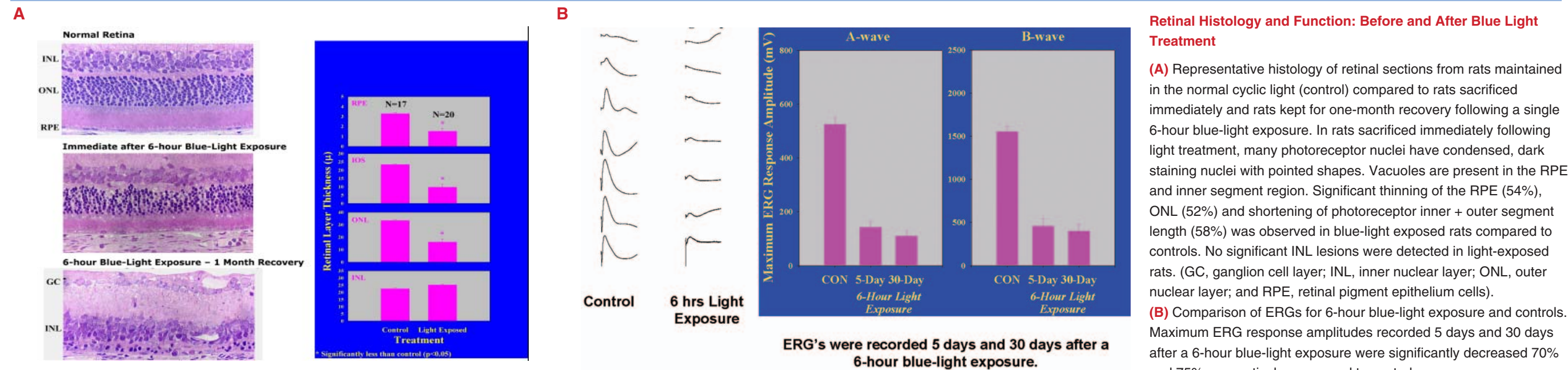
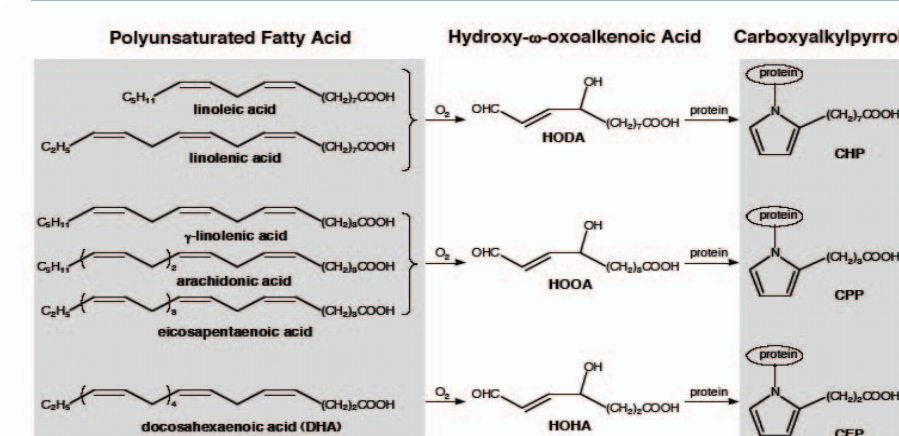


FIGURE 2



Generation of 2-(Carboxyethyl)pyrrole Epitopes Oxidative fragmentation of polyunsaturated fatty acids generates a host of oxidation products, including the hydroxy-oxoalkenoic acids HOEA, HOHA and HOHA which give rise to a host of protein modifications, including the family of carboxyethylpyrrole protein adducts CEP, CPP and CEP. Only DHA gives rise to CEP adducts, by oxidative cleavage to HOHA and reaction of the latter with protein amino groups. We have shown that HOHA-PC is an oxidative product from DHA-PC and that it can generate CEP peptide adducts (Gu et al., 2003b). Although DHA is rare in most human tissues, DHA is present in approximately 80 mol% of the polyunsaturated lipids in photoreceptor outer segments. The abundance of DHA in photoreceptors, the high photooxidative stress in retina, and the fact that DHA is the most oxidizable fatty acid in humans, all contribute to higher levels of CEP adducts in AMD Bruch's membrane/RPE/choroid (Crabb et al., 2002) [Abbreviations: DHA, docosahexaenoic acid; HOEA, 9-hydroxy-12-oxo-dec-10-enoic acid; HOHA, 4-hydroxy-7-oxohept-5-enoic acid; HOEA, 5-hydroxy-8-oxo-oct-6-enoic acid; CEP, 2-(carboxyethyl)pyrrole; CHP, 2-(carboxyheptyl)pyrrole; CPP, 2-(carboxypropyl)pyrrole; PC, phosphatidyl choline.]

FIGURE 3

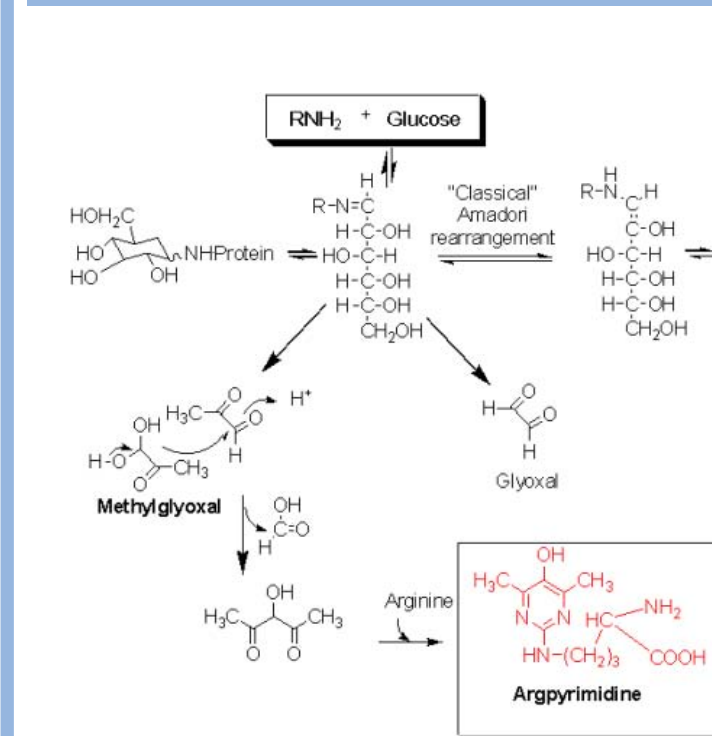


FIGURE 4

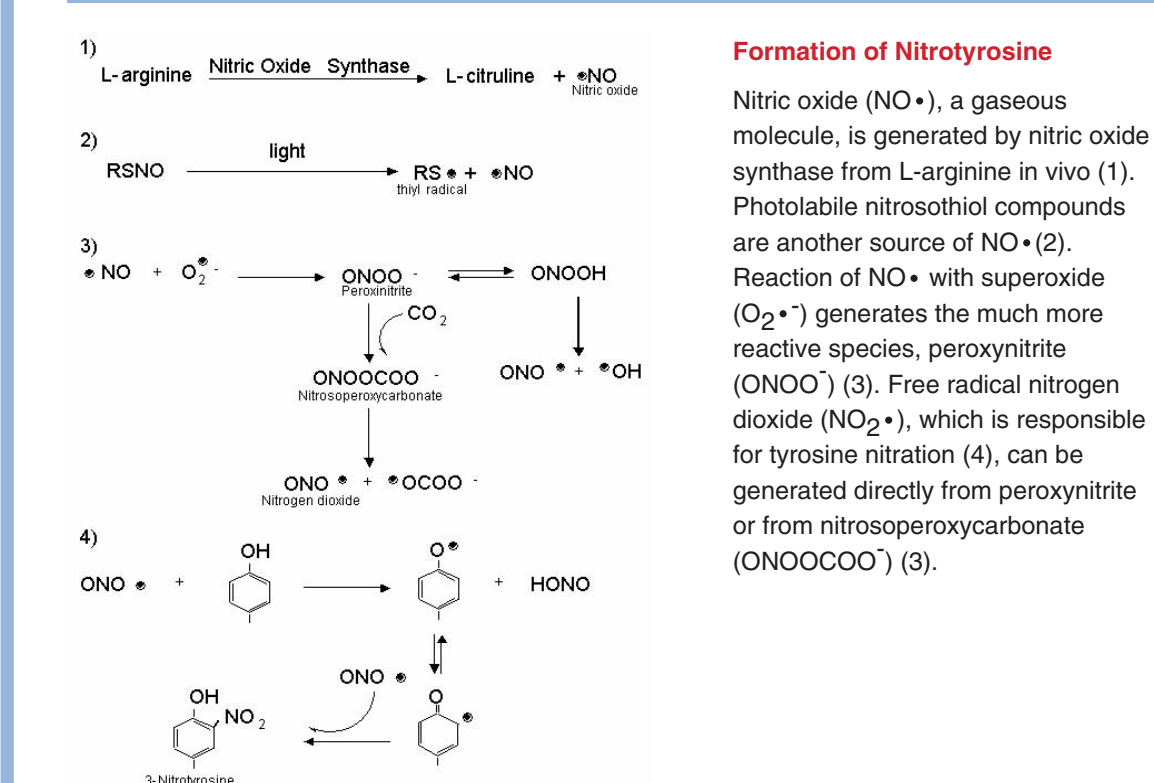


FIGURE 5

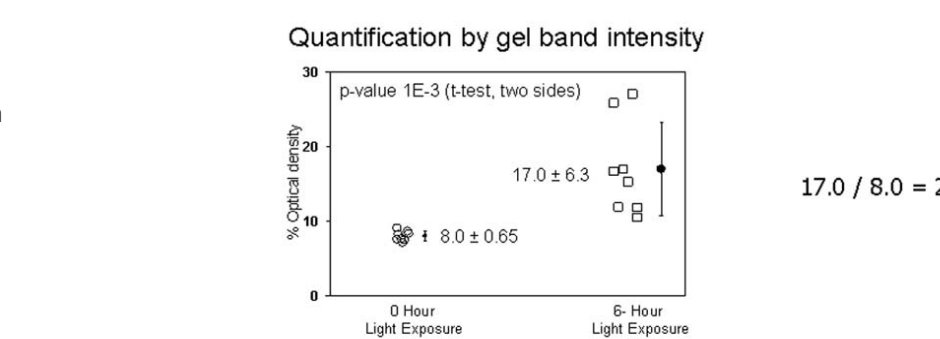
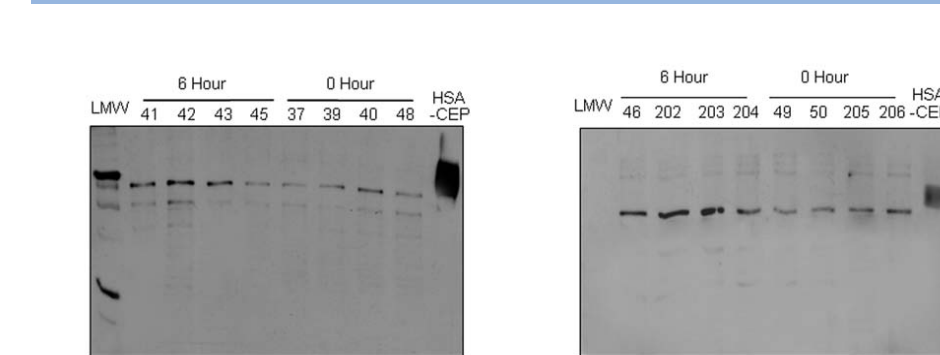


FIGURE 6

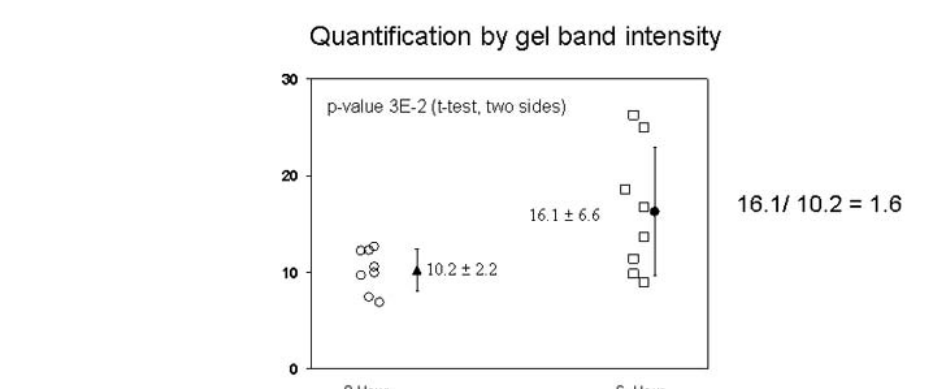
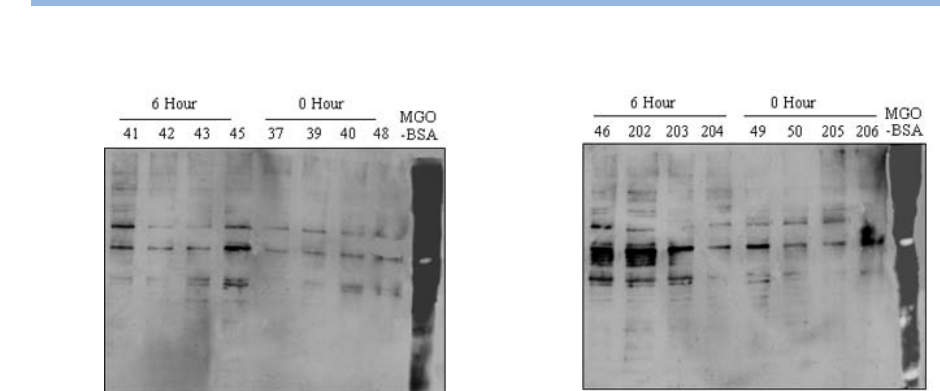


FIGURE 7

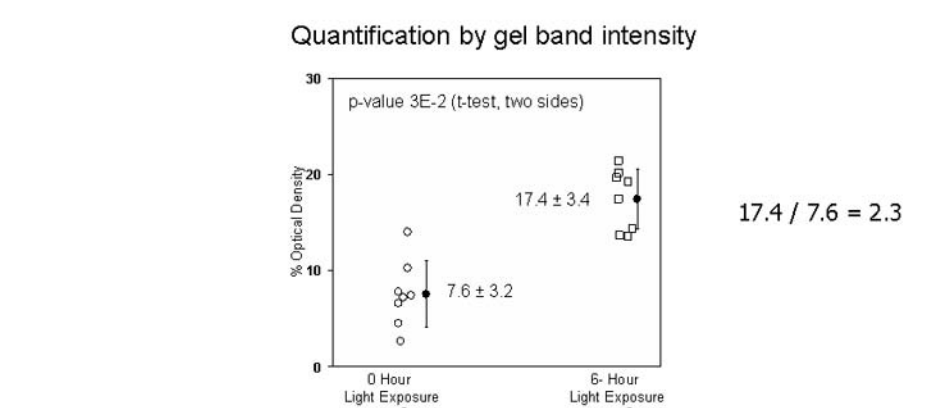
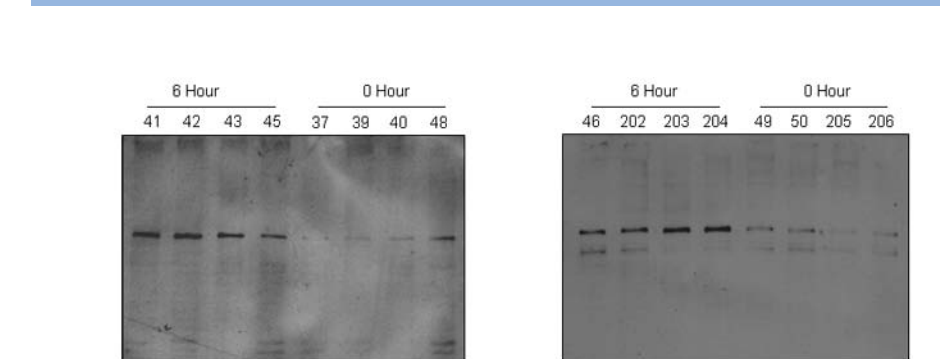


FIGURE 8

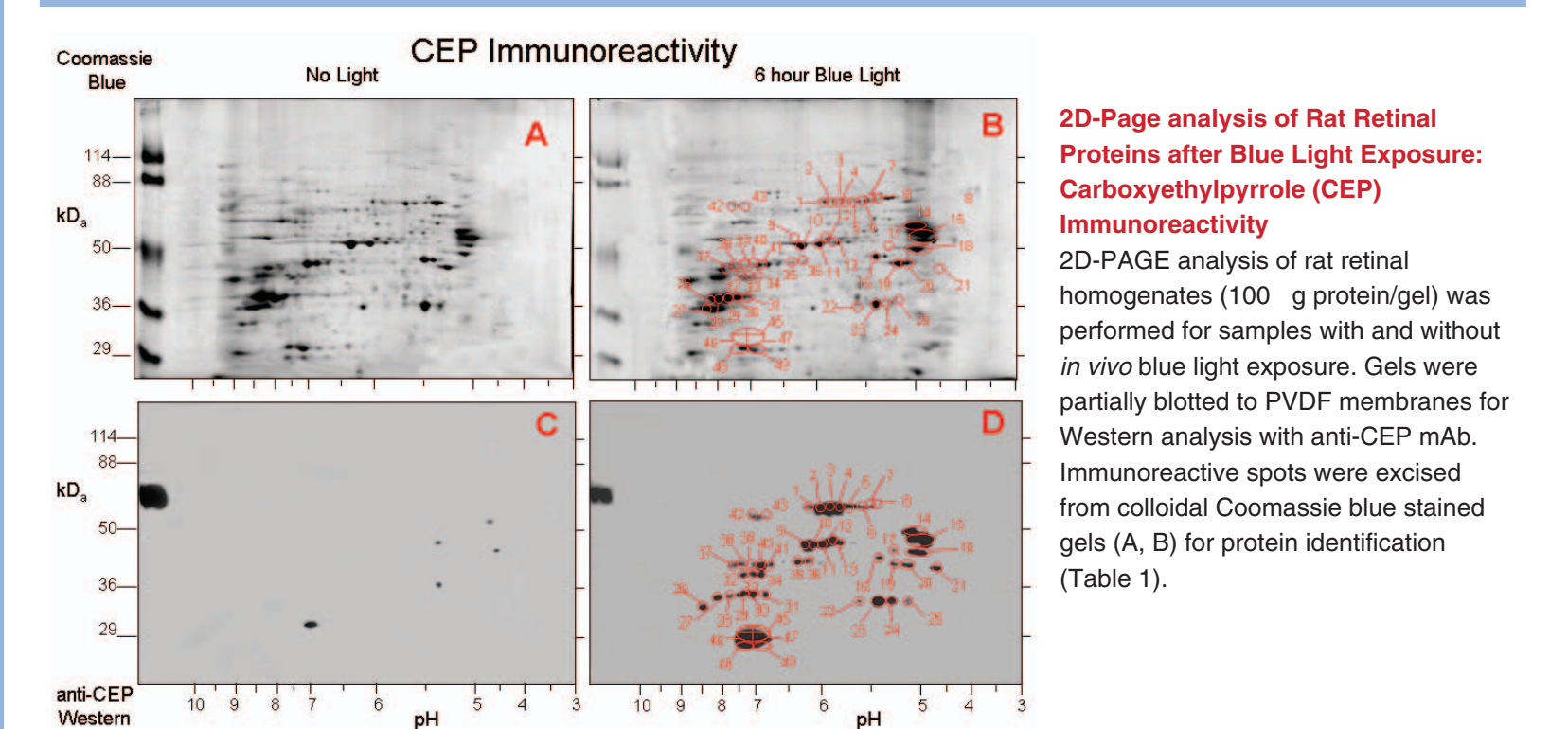


FIGURE 9

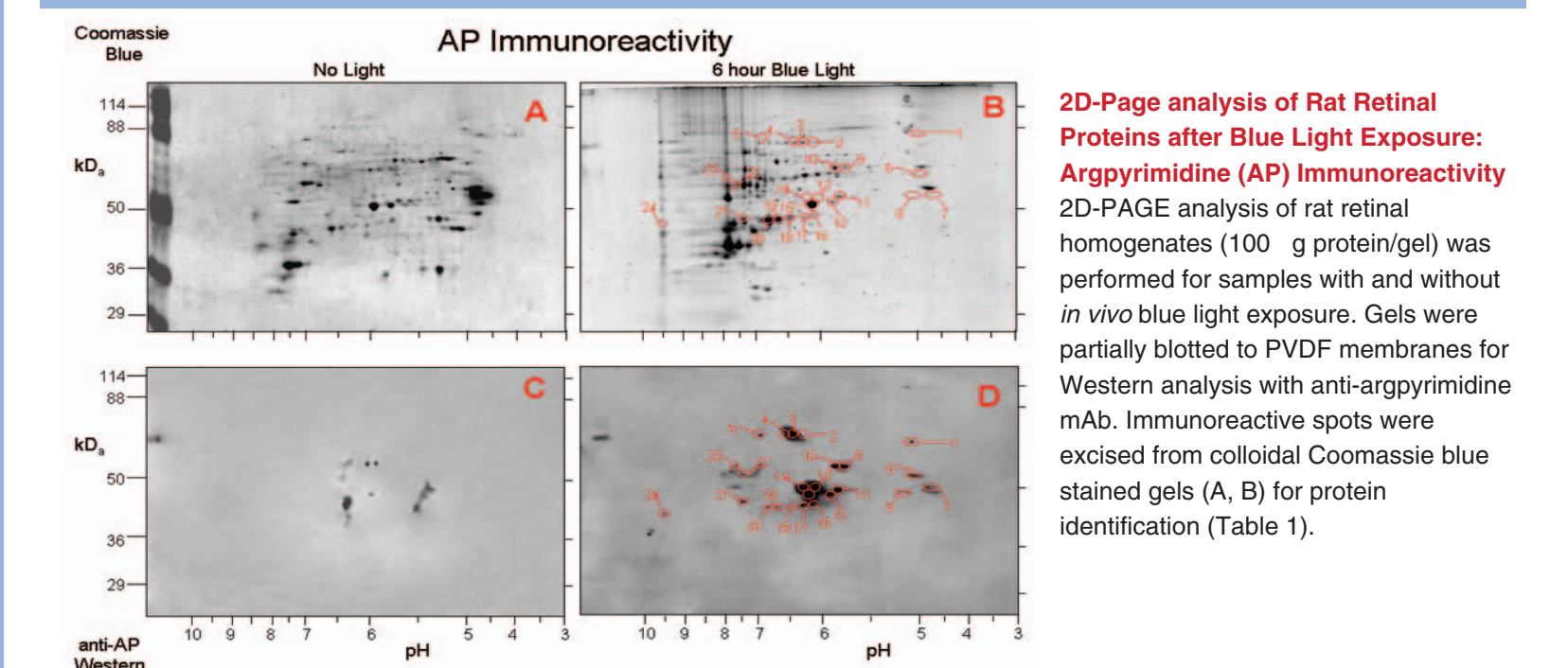


TABLE 1

Proteins Identified from Immunoreactive Gel Spots					
Spot No. (Fig 8)	Carboxyethylpyrrole Immunoreactive Proteins	Peptide Matches	Molecular Mass (kDa) pI	Accession Number*	
2	Serum albumin	4	70 / 6.0	68 / 6.4	P02770
12	S-arrestin (Retinal S-arrestin)	2	50 / 6.0	44.9 / 6.0	P15857
18	ATP synthase beta chain, mitochondrial	9	55 / 5.0	56.3 / 5.3	P10719
23	Guanine nucleotide-binding protein	4	36 / 5.5	37.4 / 5.9	P04801
35	Adenosylsuccinylase	3	50 / 6.4	47.4 / 6.4	P10789
35.37	Creatine kinase, ubiquitous mitochondrial	2	50 / 6.4	46.9 / 6.7	P30275
10, 11, 13	Alpha enolase	1-4	50 / 6.5	47.0 / 6.1	P04784
14, 15, 48	Vimentin	6-26	53 / 5.1	53.5 / 5.1	P20152
17	Glia fibrillary acidic protein, astrocyte	5	50 / 5.4	49.9 / 5.4	P47819
26-31	Glyceroldehyde 3-phosphate dehydrogenase	3-10	36 / 8.5	35.7 / 8.6	P16856
42	Malate dehydrogenase, mitochondrial*	4	38 / 8.5	35.6 / 9.1	P04636
37	Phosphoglycerate kinase 1	3	40 / 7.5	24.4 / 7.4	P00761
39, 40, 41	Glutamine synthetase (Glutamate-ammonia ligase)	2-8	42 / 7.0	42.3 / 7.1	P06806
42	Transferrin	5	67 / 7.7	67.6 / 7.7	P40142
47, 48	Carbonic anhydrase II	2	29 / 7.5	28.9 / 7.4	P21339
14, 48, 49	Tubulin alpha-1 chain (Alpha-tubulin 1)	2, 2	30 / 7.5	50.1 / 5.0	P02029

Argpyrimidine Immunoreactive Proteins					
Spot No. (Fig 8)	Argpyrimidine Immunoreactive Proteins	Peptide Matches	Molecular Mass (kDa) pI	Accession Number*	
15, 28	Alpha enolase	6-14	50 / 6.1	47.0 / 6.1	P04784
20	Glutamine synthetase (Glutamate-ammonia ligase)	6	42 / 7.0	42.2 / 7.1	P06806

* Swiss Protein database accession numbers shown use EXPASY server at <http://us.expasy.org/spdb/>
 * Nitroated after green light exposure (Miyagi et al., 2002)

CONCLUSIONS

Lipid oxidation products and oxidative protein modifications significantly increase in rat retina following intense blue-light exposure, suggesting that they may serve as mediators in the mechanism of retinal light-damage. Similar results were observed in rat retina after green-light exposure (Renganathan et al., 2003, Miyagi et al., 2002).

Several rat retinal proteins possibly containing carboxyethylpyrrole and/or argpyrimidine modifications were identified after blue-light damage. The majority of the proteins were similar to proteins obtained after green-light damage (Renganathan et al., 2003). Some of these same proteins were nitrated following green-light exposure (Miyagi et al., 2002).

AMD drusen and light-damaged rat retina both appear to contain increased amounts of crystallin and carboxyethylpyrrole adducts. We hypothesize that AMD retina also contains increased amounts of oxidative protein modifications. The rat retinal light-damage model may prove useful for testing the efficacy of various antioxidants and scavengers in reducing retinal oxidative protein damage.

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