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RESEARCH ARTICLE

APPROACH FOR LENS MOLECULAR STRUCTURE CHANGES IN ATP RAT'S MODEL OF RETINAL DEGENERATION

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ABSTRACT

Intravitreal administration of adenosine triphosphate (ATP) has been found to be effective for inducing photoreceptor death and loss of retinal function. Due to lack of studies that investigate the changes consumed the lens during early steps of retinal degeneration, the aim of the present search is to give an approach for lens molecular structure changes in ATP rats model of retinal degeneration by using fourior transformer infrared technique. Fifty healthy albino Wistar rats were divided into 4 groups: Group I served as the control group and received intravitrial injection of saline. All other groups were received a single intravitreal injection of 2µL of 1 M ATP and decapitated after 1, 3 and 6 days, respectively. The results indicated that NHOH region was found to be dramatically affected in particular after one day of ATP injection where more vibrational mode was observed relative to control. These reflex more conformational changes which involve moiety of lens fiber. The detection of asymNH after 3 and 6 days of ATP injection indicated the increase of disorder of lens fiber. CH region shows that the symmetric vibration mode ($symCH_3$) is sensitive to ATP injection as indicated by the fluctuation change in its band frequency and this band is associated with lens lipid molecule. The phosphate lipids were found to be sensitive by the ATP injection and this represented by the detection of $symPO_2$vibrational mode in all ATP injected groups relative to control group. We concluded that ATP injection dramatically affect both protein and lipid moieties of lens fiber. Recommendation of a regular examination for whole eye especially lens in case of retinal degeneration not retina only.

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INTRODUCTION

Retinal degeneration mainly from photoreceptor death, as occurs in age-related macular degeneration (AMD), accounts for 50% of blindness in the Western world (Taylor *et al.,* 2005). These diseases are characterized by loss of photoreceptors, followed by inner retinal neuronal cell death and progressive remodeling (Gargini *et al.,* 2007). There are few treatment procedures for these diseases, but those that exist involve therapies to slow photoreceptor death using pharmacological agents and, more recently, electronic implants (O'Brien *et al.,* 2012; Stingl *et al.,* 2013). In particular, investigation into the efficacy of retinal implants has fuelled the need for the development of inducible animal models of retinal degeneration that mimic the human retinal disease phenotype and are experimentally tractable. Thus, although there are many transgenic or natural genetic mutations in small animals, including genetic models of retinitis pigmentosa (Fletcher *et al.,* 2011), few inducible models of retinal degeneration are available for which the timing can be regulated. Among the available inducible models, acute light damage has recently become popular and has been extensively researched (Hunter *et al.,* 2012, Soliño *et al.,* 2018). However, this method is limited in application, because, although it is very effective in albino animals, pigmented animals are relatively resistant to light-induced damage (LaVail and Gorrin, 1987). Also, other aspects of the genetic background, even between albino strains, can moderate the effectiveness of this method (Danciger *et al.,* 2007). Pharmacological treatments to induce retinal degeneration have also been developed, but many of these have their drawbacks. Systemic administration of N-methyl-Nnitrosourea (MNU) has been found to induce tumor formation in addition to photoreceptor death (Tsubura *et al.,* 2011), which makes it a poor choice for long term studies of retinal implants. Similarly, methanol administration has detrimental systemic effects on the central nervous system and affects both inner andouter retinal neurons to induce blindness in rodents and humans (Eells *et al.,* 1996). Systemic administration of iodoacetate has also been used as a model of retinal degeneration in rodents but induces bilateral vision loss (Wang *et al.,* 2011). Other novel inducible models of retinal degeneration that can be administered to one eye only without systemic side effects are desirable. Recently, intravitreal administration of adenosine triphosphate (ATP) has been found to be effective for inducing photoreceptor death and loss of retinal function in the injected

eye, suggesting it as a potential candidate for an inducible model of retinal degeneration (Notomi *et al.,* 2011, 2013; Felix *et al.,* 2014; 2016). Due to lack of studies that investigate the changes consumed the lens during early steps of retinal degeneration, the aim of the present search is to give an approach for lens molecular structure changes in ATP rats model of retinal degeneration by using fourior transformer infrared technique.

MATERIALS AND METHODS

Chemicals Supply: All chemicals used in the experiments were obtained from Sigma Company (St. Louis, MO, USA) with the highest purity grad available.

Animals and Grouping: Fifty healthy albino Wistar rats of both sexes weighing 50±10 g (20 days old) were used in this study. The rats were randomly selected from the animal house facility at the Research Institute of Ophthalmology (RIO), Giza, Egypt. They were housed in special designed cages and maintained under constant air flow and illumination during the experimental periods, the rats were fed with balanced diet (protein 21%, starch 70%, fat 3.5%, fiber 3.5%, minerals and vitamins 2%) and drink water ad libitum. The animal was handled according to The Association for Research in Vision and Ophthalmology (ARVO) statements and regulations for the use of animals in research. Rats will be divided into 4 groups: Group I served as the control group and were received intravitrial injection of saline. All other groups were received a single intravitreal injection of 2µL of ATP and decapitated after 1, 3 and 6 days, respectively.

Intraocular Injections of ATP in Rats: Rats were anesthetized by an intramuscular administration of a mixture of ketamine (60 mg/kg; Provet, Victoria, Australia) and xylazine (5 mg/kg; Provet). In addition, the corneal reflex was anesthetized with topical administration of Alcaine (0.5%; Alcon Laboratories, Victoria, Australia). Using a 30-G needle, a single intravitreal injection of 2 µL of 1 M ATP prepared in sterile saline vehicle (0.9%) was injected into the vitreous of treated groups. The control rats received 2 µL saline vehicles (Dureau *et al.,* 2001).

Transforms Infrared Spectroscopy (FTIR): Lenses were freeze-dried separately for 1 h, and then mixed with KBr powder (2 mg lens: 98 mg KBr) to prepare the KBr disks that will be used for the FTIR investigation. FTIR measurements were carried out using Nicolet-iS5 infrared spectrometer (Thermo Fisher Scientific Inc, Madison, USA) with effective resolution of 2 cm^{-1}. Each spectrum was taken from 100 sample interferograms. The spectrometer was subject to a continuous dry N_2 gas purge to remove interference from atmospheric CO_2 and H_2O vapor. The spectra were baseline corrected, then smoothed with Savitsky–Golay filter to remove the noise before Fourier transformation. Three spectra from each sample were obtained and averaged using OriginPro8 software (Origin Lab Corporation, Northampton, MA, USA) to obtain the final average group spectrum which was normalized according to certain peaks and used in the figures.

Statistical Evaluation: Data was represented as the mean±SD. For comparison between multiple groups the analysis of variance (ANOVA) procedure was used, where a commercially available software package (SPSS-11, for windows) was used and the significance level was set at $P<0.05$.

RESULTS AND DISCUSSION

FTIR spectroscopy is a technique provides quantitative biophysical information about biological samples. It is high sensitivity in detecting changes in the molecular constituents of tissues and also about the function of the organ. The FTIR a spectrum of lens is complex and contain several bands arise from the different functional groups belonging to lipids, proteins, and others. Figure (1) panel (a) shows the vibrational frequency range corresponding to lens tissue of control rats group , the spectral can be described in three distinct frequency ranges; 4000–3000 cm^{-1} (NH-OH region), 3000–2800 cm^{-1} (CH region), and 1800–1000 cm^{-1} (fingerprint region). Panel (b) of figure (1) shows the CH region in detail to appear underlined peaks. The infrared absorption pattern of control rat lens was characterized by existence of eight absorption bands. Two of them related to (1) strOH and (3) strOHsym in NHOH region, two bands related to (6)asymCH_2 and (7)symCH_3 in CH region and the rest of bands in fingerprint region that related to (9) amide I, (10) amide II (12) COOsym, (13) asymPO_2. The numbers of bands for facility to their assignment and the missing numbers are function groups appeared in others groups. Figures (2), (3) and (4) illustrated all vibrational frequency range corresponding to the lens tissue for all groups of rats injected intravitreal with ATP and decapitated after 1,3 and 6 days respectively in panel (a) and CH region in detailed for all the same groups in panel (b).

Table (1) shows NH-OH region assignment for control group and injected groups with ATP and decapitated after 1, 3 and 6 days, respectively. the data indicated shifting of the strOH mode band to the lower wave numbers with significant decrease ($p<0.05$) in band width for groups II and III that injected by ATP for 1 and 3 days. Shifting of the strOH mode band to the higher wave numbers with significant increase ($p<0.05$) in band width for 6 day group was observed and splitting of the band after1day, 3day to 4, and 2 peaks respectively. Appeared of new band around 3317 cm-1 that related to strNHasym for 3day group and also 3338 cm-1 for 6 day group as indicated in table (1).

The strOHsym band was also affected after 1, 6 days. The strOHsym band had the lower wavenumbers with significant decrease ($p<0.05$) in band width after 1day and 6 days then disappear after 3days. The final observation was the appeared of new band around 3154 cm-1 that related to strNHsym and 3071 cm-1 that related to C-Hring after 1day of intravitreal injection of ATP. NHOH region was found to be dramatically affected in particular after one day of ATP injection where more vibrational mode was observed relative to control. These reflex more conformational changes which involve moiety of lens fiber. The detection of asymNH after 3 and 6 days of ATP injection indicated the increase of disorder of lens fiber (Paluszkiewicz *et al.,* 2018) . Table(2) shows CH region for all the studied groups control, ATP 1day, ATP 3days and ATP 6 days respectively, we observed the shifting of the symCH_3 mode band to the higher wavenumbers with significant increase ($p<0.05$) in band width for 1day and 6 day only, and observed the shifting of the symCH_3 mode band to the lower wavenumbers with significant decrease ($p<0.05$) in band width in day 3 group. The careful analysis for CH region (table 2) shows that the symmetric vibration mode (symCH_3) is sensitive to ATP injection as indicated by the fluctuation change in its band frequency and this band is associated with lens lipid molecule (Berterame *et al.,* 2016).

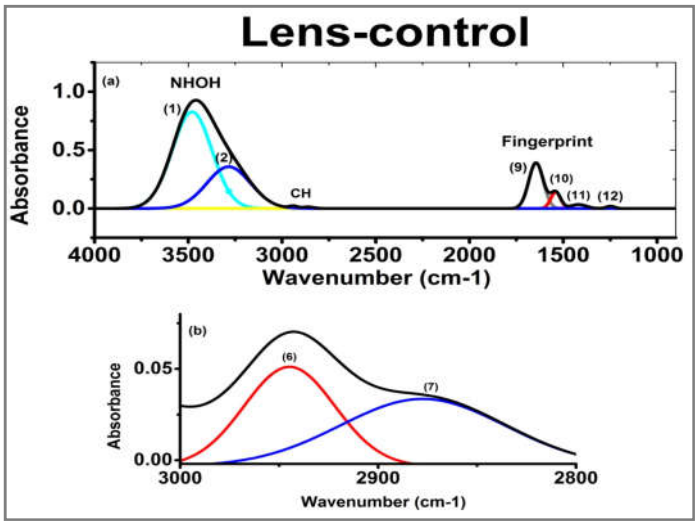

Fig. 1. FTIR spectra (4000-1000 cm^{-1}) shows NHOH region and fingerprint region in panel (a)and CH region in detailed in panel (b) for lenses of control rats

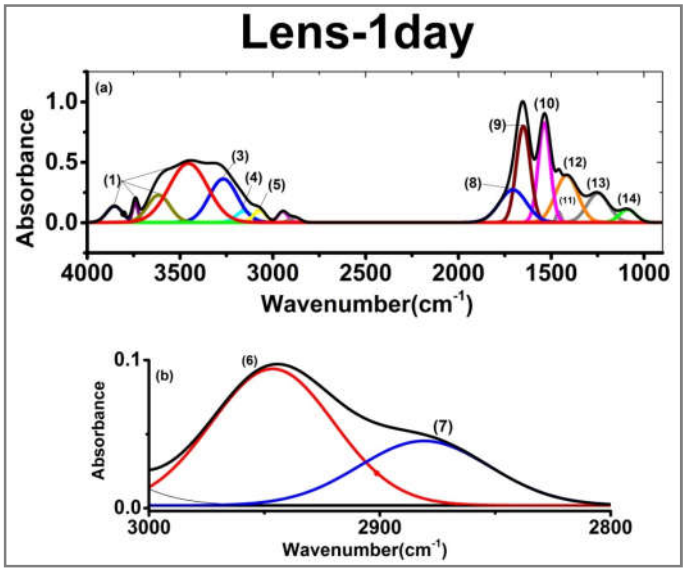

Fig. 2. FTIR spectra (4000-1000 cm^{-1}) shows NHOH region and fingerprint region in panel (a) and CH region in detailed in panel (b) for lenses of rats intravitreal injected with ATP and decapitated after 1 day

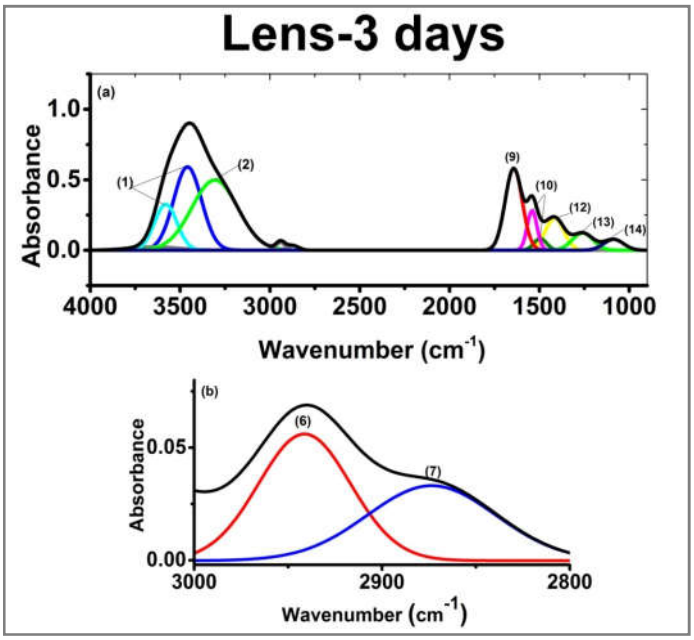

Fig. 3. FTIR spectra (4000-1000 cm^{-1}) shows NHOH region and fingerprint region in panel (a) and CH region in detailed in panel (b) for lenses of rats intravitreal injected with ATP and decapitated after 3 days

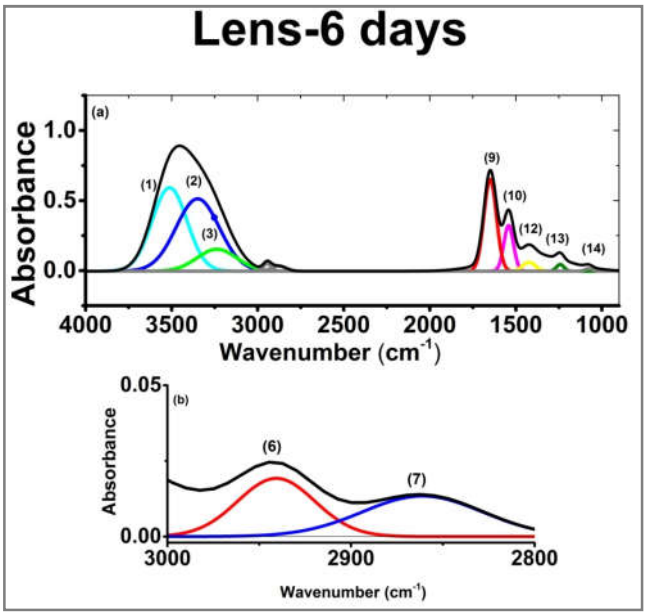

Fig. 4. FTIR spectra (4000-1000 cm^{-1}) shows NHOH region and fingerprint region in panel (a) and CH region in detailed in panel (b) for lenses of rats intravitreal injected with ATP and decapitated after 6 days.

Table 1. NH-OH region (4000-3000cm^{-1}) of rat's lens for control group and rats injected with ATP after 1,3 and 6 days

	(1) strOH					(2) strNHasym	(3) strOHsym	(4) strNHsym	(5) CHring
ontrol					3480±4 284±3		3284±5 274±1		
1 day-ATP	3853±2 100± 2	3741±3 35 ± 5	3615±5 141 ±2		3455±2* 244 ±4*		3267±3* 178±2*	3154±4 103 ±1	3071±5 80±4
3 day-ATP				3579±2 147±1	3458±3* 182±2*	3317±1 300±4			
6 day-ATP					3500±3* 238±1	3338±1 294±1	3246±1* 270±1		

*Statistical significant p<0.05 The first line is the absorbance and the second is the width.

Table 2. CH Region (3000-2800 cm^{-1}) of rat lens for all groups injected with ATP compared to control

	(6) $AsymCH_2$	(7) $SymCH_3$
Control	2940±1 49± 4	2861±1 77 ±2
Day 1-ATP	2946± 2 62 ± 1	2880±1* 66 ± 1*
Day 3-ATP	2941±1 57 ± 1	2837±2* 80 ± 2
Day 6-ATP	2944± 2 54 ± 1	2877±1* 98 ± 1*

*Statistical significant p<0.05 The first line is the absorbance and the second is the width.

Table 3. Fingerprint region (1800-900 cm^{-1}) to lens of control rats and intravitrous injected rats with ATP after 1,3 and 6 days

	(8) Ester C=O	(9) Amide I	(10) Amide II	(11) CH_2 bend	(12) COOsym	(13) $AsymPO_2$	(14) $SymPO_2$
Control		1645±1 90± 2	1540± 2 61± 4		1418± 1 99± 5	1247± 1 54± 2	
Day 1-ATP	1706± 2 172± 1	1650± 1 88± 1	1537± 2 76± 1	1460± 1 28± 2	1417± 2 142±3c	1248± 2 138± 1*	1088± 1 104 ± 2
Day 3-ATP		1643± 1 111± 2	1540± 2 64± 2	1495± 1 83± 2	1415± 1 121± 2*	1259± 1* 137± 2*	1087± 1 130 ± 2
Day 6-ATP		1649± 1* 85± 2	1541± 2 65± 1		1423± 2 86± 3	1242± 2 51± 2	1076± 2 51.9± 1

*Statistical significant p<0.05
The first line is the absorbance and the second is the width.

Table (3) shows fingerprint region for all the studied groups that covers the range 1700-900 cm^{-1} We observed increased the number of bands estimated after 1day group to seven bands compared to control (four bands). Also appeared of three new band around 1706±2 cm^{-1} that related to Ester C=O ,1460± 1 cm^{-1} that related to CH_2bend and 1088± 1 cm^{-1} that related to $symPO_2$.

After ATP 3days group the split of amide II to two bands 1540±2 and 1495 ± 1, significant increase ($p < 0.05$) of asym $PO2^{-}$ and appear of new band at 1087 ± 1 cm^{-1}related to $symPO_2$. After ATP 6 day's group, appeared a new band at 1076±2 cm^{-1} that related to $symPO_2$. Regarding the protein moiety of lens fibers, the band position was shifted from 1645 for the control to 1649 after 6 days for injection. Amide II bands splitting supporting to protein changes.

The detection of CH2 bending band 1460±1 after 1 day of ATP injection conform the changes in lens lipid. The phosphate lipids were found to be sensitive (affected) by the ATP injection and this represented by the detection of symPO_2vibrational mode in all ATP injected groups relative to control group. Grossniklaus *et al.,* (2013) stated that retinal vessels hyalinized and loss of rods in macula due to age is associated with lens changes.

Conclusion

At day 1 of ATP injection changes target is lipid but at day 3 and 6 changes target protein part of lens fiber. All together can lead to the conclusion that ATP injection dramatically affect both protein and lipid moieties of lens fiber. Recommendation of a regular examination for whole eye especially lens in case of retinal degeneration not retina only.

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