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ORIGINAL ARTICLE

Mathematical Evaluation of Thermal Retinal Damage after Laser Exposure in Chicken Eyes

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ABSTRACT

Laser photocoagulation therapy is widely employed for the treatment of many human–eye retinal diseases, yet the technique is not hazard-free and unfavorable results and/or variable success outcomes may pursue; depending on laser type, wavelength and method of application. The purpose of the present study was to evaluate mathematically the laser–induced retinal damage and recovery after laser exposure in chicken eyes. Thirty-five chickens were divided into 7 equal groups: a control unexposed group, an immediately decapitated group after exposure, and five decapitated groups on consecutive recovery days 1, 2, 3, 4, and 5; respectively. Dark adapted chicken eye was exposed to Argon laser (488 nm, 1 mW/cm^2; 0.12 sec duration) and the electrical conductivity was measured for chicken retina at temperatures 10, 20 and 30 °C. The retinal thermal damage induced by the ionic activation energy $\Omega_w(t)$ and by the relaxation activation energy $\Omega_r(t)$ was evaluated using an Arrhenius formula for all study groups. Results showed that, after thermal retinal damage by laser exposure in the first two days, recovery was attained in the next four days.

Keywords: Arrhenius; Dielectric Conductivity; Ionic Activation Energy; Relaxation Activation Energy.

INTRODUCTION

Laser photocoagulation therapy, in which a thermal laser is used to seal leaky retinal capillaries or to destroy tissue to slow the growth of new abnormal blood vessels, is widely employed for the treatment of many human–eye retinal diseases (e.g., recurrent vitreous hemorrhages, proliferative diabetic retinopathy, retinopathy of prematurity and retinal vein occlusion) (1–3). It has been shown that the technique is not hazard-free and that unfavorable results and/or variable success outcomes may arise; depending on laser type, wavelength and method of application (1, 4). To date, Argon green laser (514–532 nm), Argon ion blue-green laser (488–514.5 nm), orange dye laser (600 nm) and infrared diode laser (810 nm) are amongst those being employed for human–eye retinal treatment (1–9).

Laser treatment is often complicated by the immediate side effects caused by the unavoidable laser–induced destruction of normal tissue lying adjacent to the lesion and affected directly by the laser beam (10). For example, focal and scatter laser photocoagulation procedures have been shown to result in nonselective retinal damage leading to the permanent loss of vision (3, 5). Furthermore, studies have shown that laser photocoagulation also results in the excessive formation of reactive oxygen species in the surrounding tissue thus, promoting its inflammation (6, 7) and triggering the recurrence of neovascularization in the eye, by stimulating the proliferation of fibroblasts and retinal–pigment epithelial cells (11–15). Thus, to attain optimal eye recovery after specific laser treatment, diagnoses of laser–induced retinal damage should be evidence–based using specific measurable quantities. Measuring changes of arterial blood flow during and after laser photocoagulation treatment is considered a useful indicator of tissue thermal damage (2). In addition, measuring changes in the dielectric properties of ocular tissue membranes (e.g., counter–ion relaxation associated with intrinsic membrane charges, dipole relaxation in cell membrane, conductive transport in the ex-

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Table 1. Values of the critical frequency (*Ln* ω_p, sec^{-1}), ionic activation energy [$E_w(t)$, eV], and relative thermal damage $\Omega_w(t)$ for all study groups of dark adapted chicken eye at temperatures 10, 20, and 30 °C.*

Group	Ln ω_p			$E_w(t)$	$\Omega_w(t)$
	3.53	3.41	3.30		
Control	9.21	9.0	9.06	0.13	7.91×10^{-17}
Immediate Exposure	9.34	9.17	9.17	0.07	1.77×10^{-9}
Recovery Day 1	9.27	9.14	9.14	0.06	1.22×10^{-6}
Recovery Day 2	9.18	9.10	9.08	0.03	1.70×10^{-8}
Recovery Day 3	9.41	9.31	9.22	0.08	2.17×10^{-7}
Recovery Day 4	9.63	9.60	9.45	0.03	1.72×10^{-5}
Recovery Day 5	9.14	8.90	9.72	0.20	1.30×10^{-6}

*Inverse temperature was calculated as 1000/T (K^{-1}).

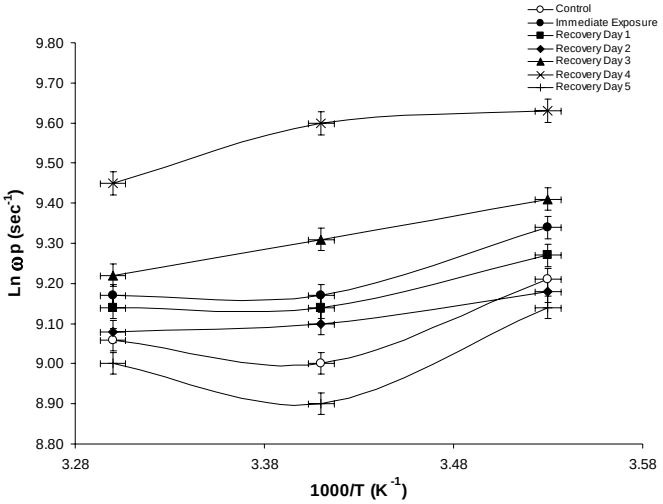

Figure 1. The relation between the critical frequency *Ln* ω_p (sec^{-1}) versus *1/T* (K^{-1}) at temperatures 10, 20, and 30 ºC for Control, Immediate Exposure, and 5 consecutive recovery days (1 through 5).

tracellular medium and through the membrane, and tissue water relaxation) are considered very useful for studying structural and functional variations in cornea, retina, choroid, iris, and the cortical and nuclear zones of the lens (16–18). The objective of the present study was to mathematically evaluate the argon laser–induced retinal damage and recovery after laser exposure in chicken eye.

MATERIAL AND METHODS

A. Specimens and Laser Irradiatiation

Thirty–five chickens with an age range 15–20 days old were used in the study protocol. Dark adapted eyes of 30 chickens were in vivo exposed to a blue Argon laser beam of wavelength 488 nm and intensity 1 mW/cm^2 for 0.12 sec. Chickens were divided into 7 groups of equal number as follows: a control unexposed group, an immediately decapitated group after exposure, and five decapitated groups on consecutive recovery days 1, 2, 3, 4, and 5; respectively. Chickens were housed in plastic boxes and were receiving the same diet during the whole study period. The experimental protocol and use of birds in the present study were in accordance with national and international legal requirements and institutional guidelines.

B. Dielectric Cell

After decapitation, chicken eyes were enucleated, moistened with Ringer's solution, and placed consecutively between two disc electrodes of a dielectric cell. An impedance meter (TESLA BM 507, Siemens, Germany) was used for measuring impedance and dielectric parameters for all chicken eyes in the frequency range 0.5–50 kHz at 10, 20 and 30 ºC, as previously described (18). Measurements were corrected for series impedance, as described earlier for this type of cell (18, 19). Measurements were carried out for each eye 5 consecutive times at any given frequency and were then averaged. The repair mechanism after thermal damage due to laser exposure was calculated on bases of simple Arrhenius relations for the activation energies due to temperature increase (E_w) and due to dielectric relaxation (E_r) in the biological system.

C. Theoretical Basis for Dielectric Parameters

The total conductivity σ_t of a sample measured in the dielectric cell can be given by the relation of Daivs and Mott (20):

$$\sigma_t = \sigma_o + A\ \omega_p^{\ s} \qquad \text{(Eq. 1)}$$

where σ_o is the D.C. conductivity component ($S\cdot m^{-1}$),

Table 2. Values of the relaxation frequency (*Ln F_s*, Hz), relaxation activation energy [*$E_r(t)$*, eV], and relative thermal damage *$\Omega_r(t)$* for all study groups of dark adapted chicken eye at temperatures 10, 20, and 30 °C.*

Group	Ln F_s			$E_r(t)$	$\Omega_r(t)$
	3.53	3.41	3.30		
Control	3.95	4.07	4.17	1.00	8.85×10^{-26}
Immediate Exposure	4.30	4.39	4.47	0.70	2.64×10^{-20}
Recovery Day 1	4.23	4.32	4.36	0.65	8.91×10^{-17}
Recovery Day 2	4.00	3.95	3.9	0.40	7.45×10^{-15}
Recovery Day 3	3.90	3.77	3.69	0.62	1.14×10^{-16}
Recovery Day 4	3.84	3.69	3.60	0.71	3.54×10^{-17}
Recovery Day 5	3.69	3.60	3.47	1.22	3.11×10^{-27}

*Inverse temperature was calculated as 1000/T (K^{-1}).

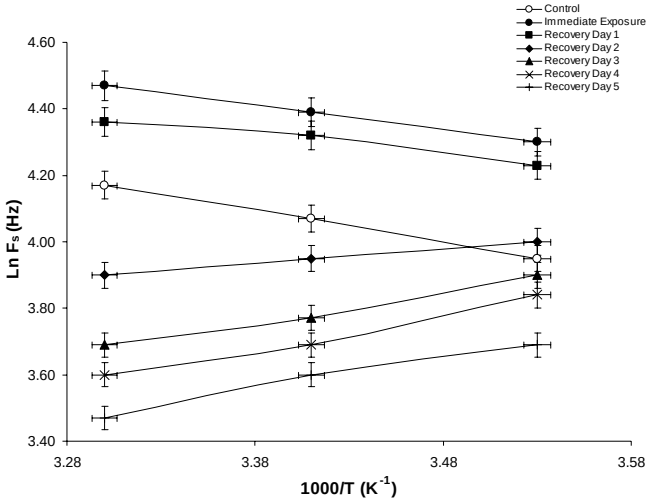

Figure 2. The relation between relaxation frequency *Ln F_s* (Hz) versus *1/T* (K^{-1}) at temperatures 10, 20, and 30 ºC for Control, Immediate Exposure, and 5 consecutive recovery days (1 through 5).

A and *s* are temperature–dependent factors, and ω_p is the angular frequency (= $2\pi f$, sec^{-1}) at which the charged ions diffuse to the other side of a living cell membrane. Values for σ_o, *A*, and *s* can be obtained using a Least Square Fitting to Eq. 1. At low temperatures, as in the case of our experiments carried out at temperatures from 10 to 30 °C, and if correlated barrier hopping of bipolarons is assumed, some bipolaron states convert into a single polaron state thus, $\sigma_t = 2\ \sigma_o$ (21, 22). After substitution in Eq. 1, it yields an expression for the critical frequency ω_p, which is defined as the hopping rate, that is:

$$\omega_p = (\sigma_o/2A)^{1/s} \qquad \text{(Eq. 2)}$$

The relation between *Ln* ω_p (sec^{-1}) and *1/T* (K^{-1}) has been shown to be linear within the observation temperatures and frequency ranges, as depicted in Fig. 1, thus we can assume that ω_p is given by a simple Arrhenius formula:

$$\omega_p = \omega_o\ Exp\ (-E_w/kT) \qquad \text{(Eq. 3)}$$

where E_w is the activation energy (eV) due to temperature increase *T* (K), ω_o is the initial frequency of polaron (Hz), and *k* is the Poltzman's constant (5.67 $\times\ 10^{-8}$ $W\cdot m^{-2}\cdot K^{-4}$). Values of E_w were calculated for samples of all studied groups and listed in Table 1. Moreover, the temperature–dependence of the cross-over frequency (F_s, Hz) of the peaks of dielectric relaxation (*Ln* F_s versus *1/T*) has been shown to be linear within the observation temperatures and frequency ranges, as depicted in Fig. 2, thus F_s can be given also by a simple Arrhenius formula:

$$F_s = F_o\ Exp\ (-E_r/kT) \qquad \text{(Eq. 4)}$$

where E_r is the activation energy due to dielectric relaxation (eV), F_o is the initial polaron frequency (Hz). Thomas *et al.* (23) derived an Arrhenius damage model for calculating the thermal damage during interstitial laser photocoagulation. In the same way, the activation energies $E_i(t)$ can be calculated using a second order fitting equation to Figures 1 and 2:

$$E_i(t) = at^2 + bt + c \qquad \text{(Eq. 5)}$$

where $E_i(t)$ is in eV and *t* is the recovery period in days. Fitting results showed constants values to be: *a* = 0.018 $eV\cdot s^{-2}$, *b* = – 0.071 $eV\cdot s^{-1}$, and *c* = 0.1155 eV. Eq. 5 is used to calculate the total thermal damage $\Omega_i(t)$ as:

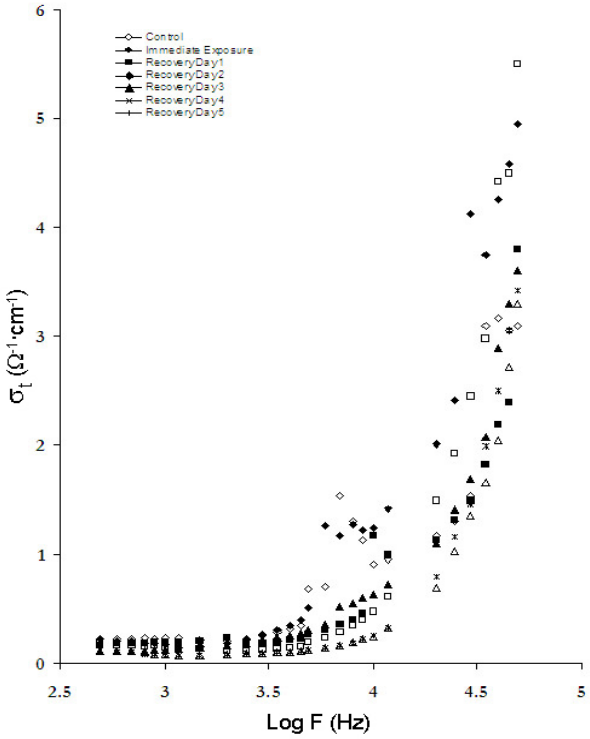

Figure 3. The relation between total conductivity σ ($\Omega^{-1}cm^{-1}$) and logarithmic frequency *Log F* (Hz) of dark adapted chicken eye exposed to argon laser of wavelength 488 nm for a duration of 0.12 sec at constant temperature 20 ºC for Control, Immediate Exposure, and 5 consecutive recovery days (1 through 5).

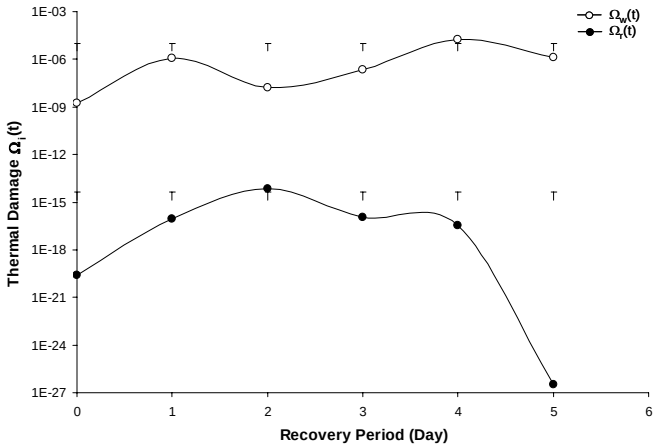

Figure 4. The relation between the thermal damage $\Omega_i(t)$ due to Argon laser exposure for 0.12 sec and recovery days (0 through 5) at constant temperature 20 ºC as induced by ionic activation energy $\Omega_w(t)$ and by relaxation activation energy $\Omega_r(t)$.

$$\Omega_i(t) = A\int_0^t Exp\left[-E_i(t)/RT\right]dt$$

$$= A\int_0^t Exp\left[-(at^2+bt+c)/RT\right]dt$$

$$= A/2\sqrt{\frac{\pi RT}{a}}\cdot Exp\left(\frac{b^2-4ac}{4aRT}\right)\cdot\left[-Erf\left(b/2\sqrt{aRT}\right)+Erf\left(b+2at/2\sqrt{aRT}\right)\right]$$

(Eq. 6)

where R is the universal gas constant (8.31 $J\cdot mol^{-1}\cdot K^{-1}$) and *Erf (x)* is an error function given by the relation:

$$Erf\,(x) = 1 - \frac{Exp\,(-x^2)}{x\sqrt{\pi}}\left[1 - \frac{2!}{1!(2x)^2} + \frac{4!}{2!(2x)^4} - \frac{6!}{3!(2x)^6} + \ldots\right]$$

(Eq. 7)

RESULTS AND DISCUSSION

Argon laser of fixed intensity 1 mw/m^2 and duration 0.12 sec was used to study thermal effects on the dielectric properties of dark adapted chicken eye at temperature 10, 20, and 30 °C through 5 recovery days after exposure. Figure 3 illustrates the frequency dependence of total conductivity σ_t of chicken eye after laser exposure at 20 °C for all study groups. It is evident that σ_t was frequency–independent till approximately 5 kHz then, it increased non–linearly with any subsequent increase in the applied frequency, as described mathematically by Eq. 1.

Figure 1 shows a semi–logarithmic plot of *Ln* ω_p versus *1/T*, a straight line relation the slope of which determines the value of the activation energy

due to temperature increase E_w for each study group, as shown in Table 1. ω_p decreases as temperature increases, following the Arrhenius formula in Eq. 3. ω_p defines the nonlinear hopping rate at which charged ions diffuse through a living cell membrane. Thus, E_w is a measure of the ionic charge diffusion through cell membrane.

The energy of a living cell is mainly produced by mitochondria thus; any change in structure or membrane content of mitochondria changes the cellular activation energy. Fedorenko and Uzdenky (24) showed that Helium–Cadmium laser micro–irradiation of neuron cytoplasm affected mitochondria more significantly than other organelles and that mitochondria swelling, cristae disruption, and partial or complete loss of matrix may occur in the irradiated cytoplasm. The degree of mitochondria lesion depended on the phase of neuron response to laser irradiation (24, 25). Thus, the observed increase in E_w during recovery days (Table 1 and Figure 1) is maybe due to developing cristae and moderately dense matrix as well as the formation of myelin–like bodies, which are probably the product of mitochondria degeneration (26).

Figure 2 shows also a semi–logarithmic plot of $Ln\ F_s$ versus $1/T$, a straight line relation the slope of which determines value of the activation energy due to dielectric relaxation E_r for each study group, as shown in Table 2. Values of E_r for all studied groups ranged from 0.40 to 1.22 eV, which were approximately one order of magnitude higher than those for E_w. This may suggest the existence of a different type of dipole contributing to the dielectric relaxation process. Moreover, this difference can be thought of by intrinsic structural alterations in the living cell membrane and the ions content, which may affect the activation energy for ionic charge diffusion through a certain barrier (E_w). The most significant laser damage is caused by rupturing Bruch's membrane between the deepest retinal layer and the underlying vessels in the choroids (27). In other words, laser exposure may have induced thermal damage to the living cell membrane in the first two days, which was followed by the regeneration of cell membrane in the next 4 recovery days approaching values for the Control group.

Figure 4 shows the relation between the thermal damage $\Omega_i(t)$ and recovery days 0 through 5 at constant temperature 20 ºC. The retinal thermal damage induced by ionic activation energy $\Omega_w(t)$ was about 10 orders of magnitude higher than that induced by the relaxation activation energy $\Omega_r(t)$ during recovery days. Birngruber *et al.* (28) reported that the maximum temperature increases in neuroretina are 3 ºC for every 1 mW of laser power entering the eye. Thus, the results of the present report suggest that the increase in dielectric conductivity of chicken eye may explain that the relaxation processes return to normal function after recovery days, whereas this condition is difficult with the mechanism of membrane permeability.

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