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Brief Report

Embryonic Growth and Hatching Implications of Developmental 670-nm Phototherapy and Dioxin Co-exposure

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ABSTRACT

Objective: **We assessed the effect of 670-nm light therapy on growth and hatching kinetics in chickens (*Gallus gallus*) exposed to dioxin.** ***Background Data:*** **Photobiomodulation has been shown to stimulate signaling pathways resulting in improved energy metabolism, antioxidant production, and cell survival.** ***In ovo*** **treatment with 670-nm light-emitting diode (LED) arrays improves hatching success and increases hatchling size in control chickens. Under conditions where developmental dioxin exposure is above the lethality threshold (100 ppt), phototherapy attenuates dioxin-induced early embryonic death. We hypothesized that 670-nm LED therapy would attenuate dioxin-induced developmental anomalies and increase hatching success.** ***Methods:*** **Fertile chicken eggs were injected with control oil, 2, 20, or 200 ppt dioxin, or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) prior to the start of incubation. Half of the eggs in each dose group were treated once per day from embryonic days 0–20 with 670-nm LED light at a fluence of 4 J/cm^2. Hatchling size, organ weights, and energy parameters were compared between dose groups and LED treatment.** ***Results:*** **LED therapy resulted in earlier pip times (small hole created 12–24 h prior to hatch), and increased hatchling size and weight in the 200 ppt dose groups. However, there appears to be an LED–oil interaction within the oil-treated controls that results in longer hatch times and decreased liver weight within the LED control dose groups in comparison to the non-LED control dose groups.** ***Conclusion:*** **Size and hatching times suggest that the hatching success and preparedness of chicks developmentally exposed to dioxin concentrations above the lethality threshold is improved by 670-nm LED treatment administered throughout the gestation period, but the relationship may be complicated by an LED–oil interaction.**

INTRODUCTION

LOW-ENERGY PHOTON IRRADIATION by light in the far red to near infrared spectral range (630–1000 nm) using low-energy lasers or light-emitting diode (LED) arrays modulates multiple biological processes *in vitro* and *in vivo*.[1–5] Photobiomodulation has been used to treat soft tissue injuries and has been shown to accelerate wound healing and tissue regeneration.[1,2,5–9] At the cellular level, photo-irradiation at low fluences induces cellular proliferation, the release of growth factors from cells, increased angiogenesis, and the generation of ATP.[10–14]

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Developmental exposure to dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin [TCDD]) and related chemicals has been shown to result in decreased embryonic survivorship, hatch and liver weight, pip and hatch success, and growth in chickens.[15] Mammals have experienced organ atrophy and decreased reproductive success as a result of developmental exposure to dioxin.[16]

Previous studies in our laboratory indicate that developmental 670-nm light treatment improves hatching success in no-inject control chickens by decreasing pip and hatch times, and increasing body weight and size.[17] Phototherapy also attenuates the dioxin-induced embryonic mortality observed when *in ovo* dioxin concentrations exceed 100 ppt (100 pg/g).[18] In this article, we evaluate the effect of 670-nm light treatment on dioxin-induced changes in hatchling growth parameters, liver weight, and hatching kinetics.

METHODS

Fertile, domestic chicken eggs (*Gallus gallus*) were collected from Purdue University Poultry Farm (West Lafayette, IN) and hand delivered to the laboratory. Three batches of eggs were used for this study. Eggs were equally distributed based upon weight into eight dose groups (LED treatment, sunflower oil vehicle control, 2 ppt dioxin, 20 ppt dioxin, and 200 ppt dioxin; non-LED, sunflower oil vehicle control, 2 ppt dioxin, 20 ppt dioxin, and 200 ppt dioxin). Dioxin stock was obtained at a specified concentration in sunflower oil (Ultra Scientific, Providence, RI) and diluted in fresh sunflower oil (Hain, Bloomingfoods, Bloomington, IN). The same fresh sunflower oil was used for the vehicle control. Half of the eggs in each dose group were treated with a far red LED array positioned directly above the air cell approximately 1 inch from the surface of the egg (640–690 nm; 670-nm peak; Quantum WARP-10; Quantum Devices, Barneveld, WI) *in ovo* once every 24 h for 80 sec. The radiant energy specifications for the WARP-10 are as follows: aperture area = 10 cm^2; LED chip population = 48; radiant power minimum = 500 mw; irradiance minimum = 50 mw/cm^2; dosimetry minimum = 4 J/cm^2. Eggs not treated with LED (non-LED) were at room temperature at the same time as paired LED-treated eggs. Egg cleaning, air sac injection, incubation, and LED treatment methods were adopted from Yeager et al.[17] and Henshel et al.[19]

Since chicks usually pip late on embryonic day 20 (ED 20), pip and hatch times were monitored beginning after ED 20 (480 h after incubation began) to assess hatching kinetics, which are indicative of embryonic energy and preparedness to hatch. Body weight and crown–rump (CR) length were measured prior to sacrifice, and liver and yolk weight were measured during necropsy. Yolk-corrected body weight (YCBW) and liver weight corrected YCBW (liver Somatic Index [SI]) were determined in order to normalize body and liver weight across all hatchlings. Animals were handled in accordance with the *Guide for the Care and Use of Laboratory Animals*, as adopted and promulgated by the National Institutes of Health. Animal care and handling protocols were approved by the Indiana University Bloomington Animal Care and Use Committee (BIACUC).

Endpoints were evaluated both graphically (Microsoft Excel) and statistically (SAS System, SAS Institute, Inc, Cary, NC).

Statistical analysis

Statistical analyses included regression (PROC REG), *t*-test (PROC TTEST), and analysis of variance (ANOVA; PROC GLM) using PDIFF (LSMEANS) and Gabriel's (MEANS) within the PROC GLM procedure to compare multiple endpoints for statistically significant differences. Statistical significance was indicated by an alpha (α) of 0.05. Marginal statistical significance was indicated by $0.05 < \alpha < 0.10$xx. Logarithmic regressions were generated by substituting 0.002 ppt for the vehicle control (=0 ppt) or by removing the vehicle control data from the regression analysis. Statistically significant logarithmic regressions were selected from the most significant relationship generated from each method.

RESULTS

Size and energy parameters

Dioxin decreased mean body weight and slightly decreased CR length in a dose-dependent manner (Table 1). The dioxin-related decrease in YCBW was more severe in the lethal (200 ppt) non-LED group compared to the 200 ppt LED-treated group. Within the non-LED dose groups, there was an overall 9.3% reduction in YCBW at 200 ppt dioxin versus the vehicle control, whereas within the LED-treated dose groups there was a 7.05% reduction in YCBW at 200 ppt dioxin versus the vehicle control. Moreover, CR length is increased (0.9–3.2%) with LED therapy at all dose groups.

LED treatment by itself in the vehicle control group (compared to non-LED) resulted in a *decrease* in liver weight (16.8%) and liver SI (17.2%). *In ovo* dioxin treatment, by itself (non-LED: 2–200 ppt), caused a decrease in liver weight (10.2–14.5%) and liver SI (5.6–13.3%) in the hatchling chicks. By comparison, LED co-treatment with dioxin induced an *increase* in liver weight and liver SI compared to the liver parameters in the LED-treated vehicle control group. This liver weight increase was statistically significant ($p = 0.0301$) in the 2 ppt dose group.

Dioxin by itself (non-LED) caused an *increase* of 6.3–17% in the total hatching time (ED 20 to pip + pip to hatch), with a maximal increase of 17% at 20 ppt. LED treatment across all dioxin dose groups resulted in a *decrease* of 1.4–4.1% in overall hatching time. When considering the dioxin treatment alone (non-LED), the largest *increase* in pip to hatch time is evident in the 2 ppt dose group, whereas LED co-treatment at the same dose results in a *decrease* in pip to hatch time.

Decreased pip times from ED 20 are evident within the 200 ppt dose groups when LED therapy is administered throughout development (Table 1). Within only the LED dose groups, there is a statistically significant ($p = 0.0524$) *decrease* in pip time in the 200 ppt dose group compared to the vehicle control. No equivalent decrease in pip time was seen in the non-LED dioxin-dosed groups. There is a net negative change in pip time in the difference between LED and non-LED treated groups as the dose increases (oil vehicle, 1.65% *increase*; 2 ppt, 0.35% *increase*; 20 ppt, 14.95% *decrease*; 200 ppt, 30.9% *decrease*).

TABLE 1. AVERAGE ± SEM (*N*) LED-TREATED AND NON-LED-TREATED DOSE GROUP ANALYSIS OF HATCHLING CORRECTED BODY WEIGHT, CROWN-RUMP LENGTH, LIVER WEIGHT, AND HATCHING TIMES

	VC (0 ppt)	*2 ppt*	*20 ppt*	*200 ppt*
LED-treated (L)				
YCBW (g)	34.43 ± 1.06 (9)	33.69 ± 0.77 (13)	33.49 ± 0.77 (11)	32.00 ± 1.18 (6)
CR length (mm)[A]	98.44 ± 2.21 (9)	96.85 ± 0.99 (13)	96.20 ± 0.91 (11)	94.83 ± 1.07 (6)
Liver wt. (g)[B]	0.817 ± 0.02 (10)	0.906 ± 0.03 (13)[1]	0.8287 ± 0.039 (11)	0.809 ± 0.045 (6)
Liver SI (g)	0.024 ± 0.0006 (9)[6]	0.027 ± 0.0007 (13)[1]	0.025 ± 0.001 (11)	0.026 ± 0.0022 (6)
ED 20 to pip (h)[C]	25.15 ± 2.14 (10)	23.49 ± 1.9 (12)[4]	24.05 ± 2.71 (11)	17.39 ± 3.11 (6)[2,4]
Pip to hatch (h)[D]	15.75 ± 1.7 (10)[6]	15.47 ± 2.26 (11)[3]	16.31 ± 2.67 (11)[4]	22.57 ± 1.45 (6)[1,3,4,5]
Total hatch time (h)	40.90 ± 2.36 (10)	39.24 ± 2.41 (11)	40.36 ± 2.51 (11)	39.96 ± 2.73 (6)
Non-LED-treated (NL)				
YCBW (g)[E]	34.46 ± 0.91 (10)	34.48 ± 1.15 (8)	33.88 ± 0.833 (10)	31.25 ± 1.53 (3)
CR length (mm)	95.40 ± 1.07 (10)	94.12 ± 1.48 (9)	94.70 ± 1.43 (10)	94.00 ± 2.65 (3)
Liver wt. (g)[F]	0.982 ± 0.077 (10)	0.882 ± 0.028 (9)	0.839 ± 0.017 (10)[2]	0.850 ± 0.018 (3)
Liver SI (g)	0.029 ± 0.0022 (10)[6]	0.026 ± 0.001 (8)	0.025 ± 0.0008 (10)	0.027 ± 0.0016 (3)
ED 20 to pip (h)	24.74 ± 2.29 (10)	23.41 ± 1.19 (10)[4]	28.27 ± 2.12 (10)[4]	25.17 ± 2.489 (3)
Pip to hatch (h)	12.17 ± 0.907 (10)[6]	18.07 ± 1.61 (9)[1]	14.93 ± 2 (10)	14.5 ± 3 (2)[5]
Total hatch time (h)[G]	36.91 ± 2.33 (10)	41.56 ± 1.86 (9)	43.20 ± 2.36 (10)[2]	39.25 ± 7.25 (2)

T-test:
[1]Statistically different from VC (within LED/non-LED treatment), where $p \leq 0.05$
[2]Marginally statistically different from VC (within LED/non-LED treatment), where $0.05 < p < 0.10$xx
[3]Statistically different across dose (within LED/non-LED treatment), where $p \leq 0.05$
[4]Marginally statistically different across dose (within LED/non-LED treatment), where $0.05 < p < 0.10$xx
[5]Statistically different from comparable dose (across LED/non-LED treatment), where $p \leq 0.05$
[6]Marginally statistically different from comparable dose (across LED/non-LED treatment), where $0.05 < p < 0.10$xx
Regression:
[A](L), CR length = −0.66903 * log(dose) + 96.79945 ($p = 0.0913$; $R^2 = 0.0751$)
[B](L), Liver wt. = −0.05234 * log(dose) + 0.91596 ($p = 0.0717$; $R^2 = 0.1112$)
[C](L), ED 20 to pip = −0.0347 * dose + 24.3282 ($p = 0.0464$; $R^2 = 0.1030$)
[D](L), Pip to hatch = 0.0354 * dose + 15.4411 ($p = 0.0312$; $R^2 = 0.1226$)
[E](NL), YCBW = −0.01603 * dose + 34.3926 ($p = 0.0756$; $R^2 = 0.1049$)
[F](NL), Liver wt. = −0.03234 * log(dose) + 0.89242 ($p = 0.0279$; $R^2 = 0.1511$)
[G](NL), Total hatch = 1.29097 * log(dose) + 40.7165 ($p = 0.0732$; $R^2 = 0.1065$)
SEM, standard error of the mean; LED, light-emitting diode; VC, vehicle control; YCBW, yolk-corrected body weight; CR, crown–rump; SI, Somatic Index; ED, embryonic day.

DISCUSSION

These studies confirm that dioxin decreases hatchling body weight, CR length, and liver weight,[15] and report for the first time that dioxin also delays hatching time. LED treatment (670 nm) reverses or prevents some of these deleterious effects, reducing, for example, the net (24.2%) decrease in body weight at the lethal dose (200 ppt). The transition point where LED–dioxin co-exposure results in higher YCBW in comparison to non-LED–dioxin is very similar to the lethality transition point (~70 ppt) described in Yeager et al.,[18] above which LED therapy clearly attenuated dioxin-induced early embryonic mortality. In addition, the extent of the dioxin-induced decrease in liver weight and liver SI across all doses was mitigated by LED co-treatment. Current studies in our laboratory are aimed at elucidating the biochemical mechanisms behind these effects, including how brain ATP content is related to hatch time and embryonic survivorship.

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