

EXOGENOUS AND ENDOGENOUS PHOTSENSITIZERS AND THE OPTICAL PROPERTIES OF PISCINE SKIN

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Abstract

Quinolines are a class of compounds that are effective therapeutic agents and are useful as antibiotics. These compounds have been previously used in non-food animal aquaculture and are known to produce photobiological effects under certain circumstances because they can function as photosensitizers.

Photosensitization (i.e., the interaction of a photosensitizer with adjacent molecules) reaction require three components: (1) the photosensitizer, (2) a receptor cell or organism, and (3) light of the proper energy. A hazard assessment of the potential photosensitizer, copper-8-hydroxyquinolate (CHQ), involved preliminary studies of its photochemistry and the photodynamics of fathead minnow (*Pimephales promelas*) and mummichog (*Fundulus heteroclitus*) at a range of light energies.

A spectrophotometric analysis of CHQ identified it as a potential photosensitizer at 360 to 420-nm (ultraviolet-A to visible light). While the optical properties of dorsal and ventral piscine integument (i.e., receptor skin) varied depending on species/culture conditions, piscine skin absorbed less light between 360 and 420-nm regardless of species/culture, than did CHQ (2-fold greater light-absorbing potential). It was hypothesized that biological effects might occur in fish if they were exposed to a combination of CHQ and visible, blue-violet (actinic) light.

Fish cultured indoors under diffuse fluorescent lighting had skin that was less light-absorbing, relative to wild-caught fish. Fish exposed to CHQ and actinic light demonstrated effects that included dermatitis (acute lymphocytic), hyperpigmentation, hypertrophy and hyperplasia of the branchial epithelium, and hematological effects. No effects were observed in "sham" or "vehicle" control fish.

Introduction

Copper-8-hydroxyquinolate (CHQ) is a nitrogen heterocyclic hydrocarbon that is formed from the chelation of copper by two molecules of quinoline (**Figure 1**). Photochemical dissociation of CHQ results in the formation of 8-quinolinol (8-Q) and 8-Q plus monovalent copper (1:1 Chelate). Quinolines are considered effective antibiotics (MacMillian, 1995; Andriole, 1988; Von Oettingen, 1933) that occur naturally in the environment. CHQ is an especially potent agent (Gershon et al., 1989; Dacre, 1984) which has been linked to the production and quenching of free radical molecules (Cherton et al., 1983). Compounds in the quinonoid group commonly act as photosensitizers

(Grossweiner, 1969; Patai, 1974) and can cause photosensitization in fish (Smith, 1989). The adverse effects of photosensitization in fish have been previously described (Roberts, 1989; McCloskey and Oris, 1991).

Light approximately >360-nm is commonly involved in aquatic photochemistry and photobiology because solar light at these energies has potential to penetrate clear freshwater and seawater in excess of 30-m depth (Jerlov, 1976). In addition, compared to ultraviolet light, actinic light can readily pass through biological tissue (Smith, 1977).

The objective of this study was to compare the absorbance spectra of piscine skin and CHQ, and to evaluate the effects of exposing fish to a combination of CHQ and actinic light.

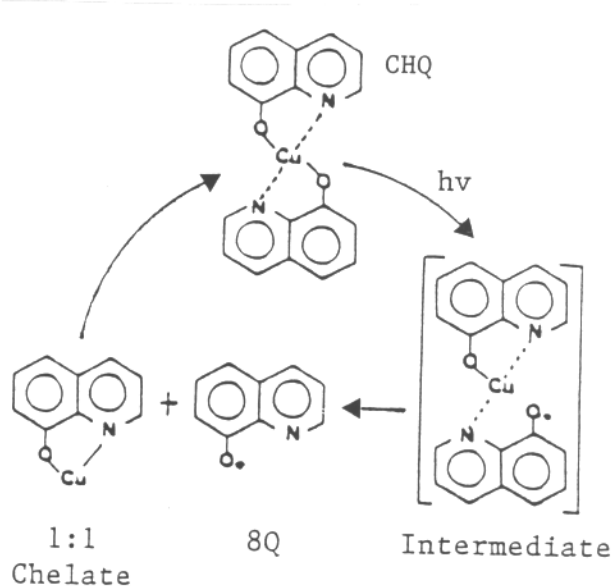


Figure 1. Products of CHQ photodissociation (Cherton et al., 1983).

Materials and Methods

Nine-month old, female fathead minnow were cultured and tested under laboratory conditions that included a freshwater temperature of 23±1 °C, 136 mg/l hardness (as CaCO₃), dissolved oxygen (DO) at saturation, and pH 7.6 to 7.8. Larval and juvenile life-stages were fed live, newly hatched *Artemia* and dry *Spirulina* twice per day. Fingerling and adult fish were fed a diet of TetraMin™ flakes (Tetrawerke, Inc., FRG) ad lib. twice per day. The light cycle was approximately 10:14 (light:dark) using diffuse, fluorescent lighting throughout the culture period. The mean length of the subject fish was 4.6±0.7-cm.

Two-year old, female mummichog were wild-caught from salt-marsh waters and were acclimated to laboratory test conditions within 600-L circular, fiberglass tanks receiving filtered natural seawater at 21±1 °C, 32 to 34-ppt salinity, and DO at saturation for two-months. During acclimation, fish were fed a diet of Standard flakes (Tetrawerke, Inc., FRG), ad. lib. once per day, and fresh frozen krill twice per week. The light cycle was approximately 12:12 (light:dark) using diffuse, fluorescent lighting throughout the acclimation period. The mean length of the subject fish was 7.8±0.5 cm (range).

Fish were euthanized by cervical spinal transection immediately before physiological preparations of the skin and integument were made to evaluate their optical properties. The mid-section of each fish was bisected on the lateral mid-line and resulted in dorsal and ventral body-halves from each species. Head and tail portions, and viscera were discarded. Dorsal or ventral body-halves were mounted individually in vertical orientation, within 2-mL quartz cuvettes containing phosphate buffered saline (PBS). The tissues were mounted so that a beam of light from a Lambda-3 UV/VIS, model 6180900 spectrophotometer (Perkin-Elmer, Inc., FRG) would intercept the preparations at the epidermal surface on a perpendicular. Physiological PBS (only) was added to the reference cuvette. Preparations were scanned twice from 700 to 200-nm at a rate of 1-nm/second. Absorbance data was collected every 10-nm interval.

Two-mLs of a solution of 1.44-mg/L CHQ (Cat.#C25380, Pfaltz & Bauer, Inc., Waterbury, CT) in 95% reagent grade ethanol was placed in a quartz cuvette and scanned at a rate of 1-nm/second. The reference cuvette contained 95% ethanol only. Data was collected at 10-nm intervals.

Methods for evaluating the toxicology and clinical pathology and histopathology of fish exposed to CHQ and actinic light using Actinic^R 03 emission (between 400 and 425-nm) are presented in detail elsewhere (Rosiu, 1994).

Results

CHQ had peak absorbance at approximately 260-nm, and lesser absorbance between 360 and 420-nm (Figure 2) which includes the band of Actinic^R 03 emission that was later used in the hazard assessment (Rosiu, 1994). Absorbance profiles of dorsal and ventral preparations demonstrated significant light-absorbing activity in mummichog only. Between 315 and 500-nm, absorbance was similar between species (absorbance <0.5), but between 200 and 315-nm, both dorsal and ventral preparations of mummichog were 2-fold more light-absorbing than fathead minnow. The general pattern of absorbance differed significantly between these species.

Exposure of live fathead minnow and mummichog to combinations of waterborne CHQ and actinic light produced major effects that were similar between species and included (1) dermatitis (acute lymphocytic) with erythema, edema, and hyperpigmentation, (2) hypertrophy and hyperplasia of secondary lamellar branchial epithelium, and (3) intravascular hemolysis (Rosiu, 1994). Lowest Observed Adverse Effect Levels (LOAELs) were 5.5- $\mu\text{g/L}$ (fathead minnow) or 70- $\mu\text{g/L}$ CHQ (mummichog). No effects were observed in "sham" or "vehicle" control fish.

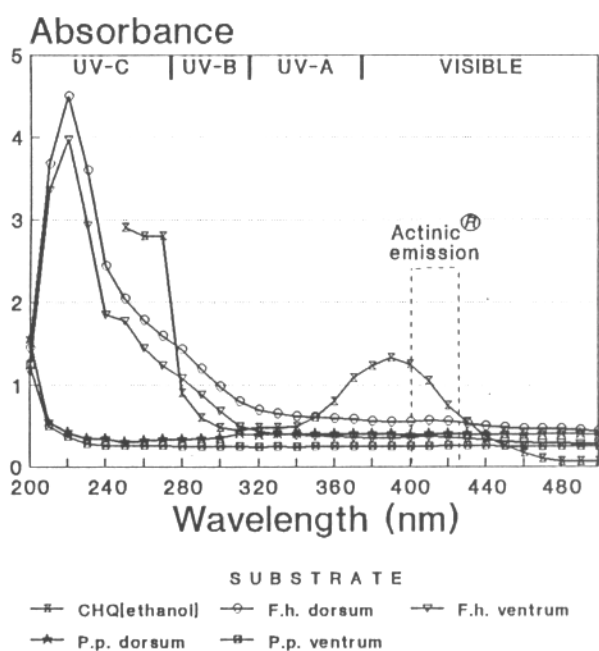


Figure 2. Absorbance spectra of CHQ and tissue preparations of fathead minnow (P.p.) and mummichog (F.h.).

Discussion and Conclusions

Photosensitizers participate in photobiology, energy transfer, and free radical chemistry as either exogenous or endogenous compounds (Stegemann et al., 1992; Seis, 1985). Cells naturally contain endogenous photosensitizers such as heme proteins (Kawanishi et al., 1986), carotenoids and flavins (Smith, 1989), or melanin pigments (Edelstein, 1971). Endogenous compounds are particularly effective in absorbing actinic light (Smith, 1989), however, actinic light can readily pass through biological tissue (Smith, 1977). For example, cells have exhibited photosensitivity to visible light when the cells are incubated in vitro with flavins, and when hydrogen peroxide was produced as a result of flavin-sensitized photochemical degradation of cysteine, histidine, methionine, tryptophan, or tyrosine in culture (Smith, 1989).

The structure of quinonoids make them especially effective in electron transfer and oxidation reactions because they are centrally involved in many routine biological processes (Seis, 1985; Hanzlik, 1991). For example, biosynthesis of indole-5,6-quinone within melanocytes provides substrate for polymerization of melanin pigment. The resulting melanin biopolymer molecule possesses electrophilic properties (Riley, 1980), reduction-oxidation cycling capability (Gan et al., 1977), but is rarely a photosensitizer because melanin is typically stabilized by electrophilic

resonance within the biopolymer (Riley, 1980). This stability property of melanin makes it particularly effective at trapping, and ameliorating the damaging effects of, free radical molecules within its molecular lattice structure. This protective role of melanin might influence its occurrence within piscine dermis, the Central Nervous System, peritoneal cavity, retina, and aggregations of piscine macrophages (Wolke, 1992). Cytological evidence exists to suggest that macrophages become susceptible to photochemical damage in actinic light after they are loaded with melanin pigment (Johnson et al., 1972).

The types of expected effects in photosensitized cells can be predicted by estimating the location of the photosensitizer compound (Ito, 1978). For example, cell membranes contain enzymes, structural proteins, and unsaturated lipids, all of which can be photodegraded. Thus, sensitizers located in the cell membrane could alter catalytic and transport processes and change membrane permeability. Photosensitizers located in various regions of the cytoplasm could mediate the photodegradation of enzymes, tRNA, and the components of various organelles. Finally, activation of photosensitizers located in the nuclear region of cells could result in guanine destruction in DNA, nucleic acid strand breaks, and formation of DNA-protein adducts, all of which can interfere with normal cell function and survival.

The spectrophotometric data from the present study supports a conclusion that CHQ may act as a photosensitizer in fish exposed to actinic light. This conclusion was supported by experimental evidence of photosensitization and biological effects following exposure of fathead minnow and mummichog to CHQ and actinic light (Rosiu, 1994). Effects were seen on cells and cell membranes. The spectrophotometric data also identified wild-caught fish as having more light-absorbing potential relative to cultured fish. This conclusion seems reasonable assuming that natural solar radiation promotes the development of melanophores and chromatophores in fish. Actinic light may have special relevance to aquatic photochemistry and photobiology because it occurs to a significant extent in aquatic environments.

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