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NALTREXONE AS A PROMISING TREATMENT FOR CLINICAL SIGNS OF LATERAL LINE DEPIGMENTATION IN PALETTE SURGEONFISH (*PARACANTHURUS HEPATUS*)

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Abstract: Lateral line depigmentation (LLD) is a common condition in managed tropical saltwater fish, and treatment is somewhat elusive. Naltrexone, an opioid receptor antagonist, enhances epithelial cell replication, cytokine production, and angiogenesis to stimulate wound healing in mice. A treatment trial with 11 palette surgeonfish with LLD was performed. Seven fish underwent a single topical treatment of a mixture of 4 mg naltrexone and 10 g iLEX petroleum paste applied topically to LLD lesions. Four additional fish served as controls: two received only topical iLEX and two received no treatment. Severity of disease was scored on a 0–3 scale. Inflammatory response was gauged on a separate 0–3 scale for 5 d after treatment based on severity of erythema, as seen in a clinical case performed prior to this study. After 11 days, four affected animals that lacked an inflammatory response after naltrexone topical treatment were administered a single dose of intralesional 0.04% naltrexone (4 mg diluted into 10 ml saline). Lesions on all fish were photographed and measured at day 33. Clinical improvements in lesion size and pigmentation were apparent following topical naltrexone therapy in fish with severe lesions. Although these cases are promising, more data are needed to further evaluate the effectiveness of naltrexone 0.04% in treating LLD lesions in palette surgeonfish.

INTRODUCTION

Lateral line depigmentation (LLD) is a common presentation in managed fish that is described as focally depigmented to ulcerated skin along the lateral line of the head and flank, rendering some fish unfit for display.[5–7,15] Saltwater surgeonfish and tangs (*Acanthuridae*) and angelfish (*Pomacanthidae*) are particularly susceptible.[7,15] Fish are typically systemically unaffected but can become lethargic and anorexic if lesions become secondarily infected.[7,15] Postulated etiologies for LLD lesions include environmental causes such as the use of activated carbon or ozone, high copper levels, poor water quality, stray electrical currents, or overcrowding; nutritional inadequacies such as vitamin A and C deficiency in surgeonfish; and infectious agents such as diplomonad flagellates or reoviruses.[2,4,5,7,8,15]

Ultimate treatment for LLD depends on correction of the underlying cause; attempts to resolve LLD by controlling inciting parameters have been reported, with mixed reproducibility and success.[5,6,15,18] A topical platelet-derived growth factor, 0.01% becaplermin (Regranex, Ortho-McNeil Pharmaceutical, Inc. Raritan, NJ 08869, USA), has been effective for treatment of LLD lesions but is prohibitively expensive, prompting evaluation of other treatment options for aquarium fish.[6]

Naltrexone is an opioid receptor antagonist that is inexpensive and readily available for veterinary use. Preliminary studies in rodents and poultry indicate that topical low-dose naltrexone enhances wound healing via temporary intermittent blockade of the opioid growth factor (OGF)-OGFr receptor axis to promote angiogenesis, and stimulates fibroblasts, epithelial cells, mast cells, and collagen to create a granulation layer.[1,3,9,10] It may also improve keratinocyte differentiation and has anti-inflammatory effects on B and T immune cells.[11] It has been extensively studied in humans for the use of chronic dermatologic conditions such as nonhealing diabetic ulcers and Hailey-Hailey disease, as well as cancer and autoimmune diseases.[11,13,14,16,19,20] As LLD is generally considered to be a nonhealing and progressive skin condition, treatment with topical naltrexone has been suggested as a potential

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treatment option.[7] This study aimed to further investigate the use of naltrexone as a practical option for treatment of the clinical appearance of LLD.

MATERIALS AND METHODS

Eleven palette surgeonfish (*Paracanthurus hepatus*) with LLD living in a 1,022,060-L saltwater multispecies system, with life support of sand and drum filters, ozone, denitrification, and foam fractionators, were selected for the study. Water quality parameters were maintained within set ranges: temperature 76–78°F (24–25.5°C), salinity 30–34 g/L, pH 7.8–8.4, alkalinity 120–200 mg/L, ammonium ion 0–0.03 mg/L, nitrite 0–0.1 mg/L, and nitrate < 25 mg/L. Diet offered on exhibit and in the holding tank consisted of a broadcast mixture of Mazuri LS Aquatic Carni-blend pellets (Formula code 5E4S, PMI Nutrition International, LLC, Arden Hills, MN 55126, USA), Mazuri LS Aquatic Herbi-Blend pellets (Formula code 5E4R), Mazuri Omnivore Aquatic Gel Diet with High Vitamin C and Thiamin (Formula code 5M4K), mix of small cut pieces of shrimp and capelin, and krill (*Euphorbia pacifica*) (Hiromatrsu Kyu Fishery Co., Ltd., Fukuoka 813-0018, Japan). As the feed is broadcast to a variety and large number of fish, it is unknown what these animals precisely consume on exhibit. All animals had lived in this system for more than 7 yr and were in good body condition. One palette surgeonfish (hereafter referred to as the pilot case) had previously received naltrexone treatment for LLD 6 mon prior and been returned to exhibit.

During the study, the fish were housed in a marine system comprised of a 45,460-L total habitat with three 1,514-L, 5-ft round saddle tanks with a sand filter, protein skimmers, and degas filters. Three to four individuals were randomly assigned using the Microsoft Excel 365 random number tool to one saddle tank. Water quality parameters were maintained within set ranges: temperature 77–80°F (25.0–26.6°C), salinity 28–32 g/L, pH 7.8–8.4, alkalinity 120–200 mg/L, ammonium ion 0.00–0.03 mg/L, nitrite 0.0–0.1 mg/L, and nitrate < 25 mg/L. Fish acclimated to the new surroundings for 7 d prior to the experiment and were offered the same diet, although the animals ate exclusively krill during this time. After treatment, animals were separated by individual partitions within each tank and viewing windows were covered for the first 5 d. Aquarium décor was removed for the first 24 h to prevent rubbing. Two individuals, hereafter referred to as the "control group," were chosen randomly and served as negative controls. Two additional individuals, also chosen randomly and hereafter referred to as the "iLEX group," were treated with only the administration vehicle (iLEX paste). Each of the tanks contained zero to one "control group" fish, zero to one "iLEX only" fish, and two to three naltrexone-treated individuals.

The treatment group ($n = 7$) fish were individually anesthetized using tricaine methanesulfonate (MS-222, 90 mg/L, Syndel, Ferndale, WA 98248, USA) and sodium bicarbonate (180 mg/L) as a buffer. Fish were Passive Integrated Transponder (PIT) tagged in the left caudal epaxial muscles for identification and photographed out of water with a dry erase board and an attached ruler for scale. Severity of LLD lesions at onset of the study was evaluated subjectively by one veterinarian on a 0–3 scale (Supplemental Fig. 1). A score of "0" denoted no signs of LLD; score of "1" included focal circular lesions encompassing <25% of the head bilaterally; score of "2" typically included the occasional deep pitted lesion with 25%–50% of the head affected; and a score of "3" included the presence of deep erosive pitting affecting >50% of the head. Some individuals were scored with a half score (i.e., 0.5, 1.5, 2.5).

Naltrexone hydrochloride 4 mg (ZooPharm, Inc., Windsor, CO 80550, USA) and iLEX skin protectant paste 10 g (iLEX Health Products, Grafton, MA 01519, USA) were mixed using a wood tongue depressor to create a dosed paste immediately prior to application.[13,19] A concentration of 0.04% naltrexone, within the human dosing range for low-dose naltrexone, was chosen for ease of compounding.[13,19] The petroleum-based product iLEX was chosen due to its adherence to the skin of teleosts in water per author experience. Mucus was removed from the treatment areas using gauze, and the area was dried thoroughly. The paste was applied using a tongue depressor and/or gloved fingertips to clinically affected regions in an approximately 1- to 3-mm-thick layer.

Animals were placed into a separate container with the same dose of immersion anesthetic for 10 min after application and then placed directly into a partitioned holding space for recovery. The "iLEX group" ($n = 2$) was treated identically, except that the treatment paste did not contain naltrexone. The "control group" ($n = 2$) was anesthetized only for PIT-tagging and photographs.

The fish were monitored once daily for the first 5 d. Inflammatory response was graded on a 0–3 scale each day by a veterinarian blinded to the treatment groups (Supplemental Fig. 2). A score of "0" meant no inflammation was noted; a score of "1" included mild inflammation characterized by pink or red focal regions; a score of "2" denoted that much of the treatment area was affected and mostly pink colored with occasional red colored regions; and a score of "3" noted deep red color to most of the treated area. Anti-inflammatory therapeutics were not administered.

After 11 d, six animals treated with naltrexone that had a day 5 inflammation score less than a "2" met the criterion for an additional treatment with injectable naltrexone. Inflammation was expected after treatment as observed in the pilot case; therefore, fish without signs of inflammation were considered to have received an inadequate dose in the first round of treatment. The 11-d waiting period was based on veterinarian availability. Animals were anesthetized and photographed as in the original trial. Naltrexone (4.0 mg) was diluted into 10 ml sterile water (Sterile Water for Injection, USP, Hospira, Inc, Lake Forest, IL 60045, USA). Affected regions of LLD were then multifocally injected intradermally intralesionally using a 22-G, 1-in. needle (Monoject Veterinary Needle, Covidien, New Haven, CT 06511, USA). Considerable pressure was needed to inject 0.01–0.02 ml of the naltrexone dilution intralesionally, and the first subject's operculum was inadvertently punctured in the process. Due to this complication, two fish with LLD lesions over the operculum or cranium were excluded from this part of the study. The four treated fish were monitored and isolated as in the original trial, except that the scoring veterinarian was no longer blinded to the treatment groups. Thirty-three days after the topical treatments and 22 d after naltrexone injections, all fish were anesthetized and photographed as above.

Data recorded included animal PIT-tag number, location, treatment group, severity of LLD lesions (0–3 scale), visible paste duration in water after treatment, inflammation scores (0–3 scale) for days 1–5 after each treatment, and total polygonal area of LLD lesions at each assessment. The total area of LLD lesions per side (left and right separately) of each fish on day 0 (day of topical treatment) and on day 33 was measured using ImageJ (National Institutes of Health, Bethesda, MD 20814, USA).[17] Lesions were measured in millimeters squared (mm^2) using the polygon measurement feature, with the scale based on the in-photograph ruler. Lesions were considered resolved if they were normally pigmented. Percentage change in lesion size per side of the fish's head (left and right separately) was calculated after treatment. Descriptive statistics (mean, standard deviation, data charts including line of best fit) were calculated using Excel.

Table 1. Severity of LLD lesions in palette surgeonfish (*P. hepatus*) before treatment compared with the percentage changes in the LLD lesion sizes (% Δ) per side (left or right) after treatment by day 33.

Treatment group	Starting severity of LLD	% Δ left side	% Δ right side
Naltrexone	2.5	−75.7	−96.9
Naltrexone*	1.5	−100.0	N/A
Naltrexone*	1.5	−56.8	−4.3
Naltrexone*	1.5	0.9	−54.5
Naltrexone*	1	−12.5	−28.2
Naltrexone	1	−23.9	−15.9
Naltrexone	0.5	−5.2	3.9
Ilex	3	−18.8	−19.1
Ilex	1	−16.4	−39
Control	2	7.4	−21.4
Control	1	−16.6	5.3

Animals with more severe initial LLD lesions seemed to have the greatest percentage of improvement overall after naltrexone treatment. A negative % Δ number represents a decrease in size, whereas a positive number represents an increase in size.
*Animals treated with injectable naltrexone.

RESULTS

Lateral line depigmentation severity scores for all animals at the onset of the study varied from 0.5 to 3.0 and were represented across all treatment groups (Table 1). The iLEX paste was visually noted to remain on all treated regions for at least 4 h and then sloughed off in pieces; some pieces remained 12 h after application.

The average of the inflammation scores from the "treatment group" over the first 5 d after topical treatment was 0.0–1.6; the two fish in the "iLEX only" group showed mean scores of 0.4 and 1.0, and the two "control group" fish showed scores of 0.0 and 0.4. The four animals that met the criterion for intradermal naltrexone treatment all had scores of 0.0 each of the first 5 d after intradermal treatment.

The study compared starting severity of LLD lesions before treatments and calculated the percentage changes in the LLD lesion sizes, measured per side, after treatment (day 33), where a negative number represents a decrease in the overall size of the lesion and vice versa (Table 1).

Table 2. Response to treatment for LLD in palette surgeonfish (*P. hepatus*) using naltrexone.

Group	Sample size, measured per side of face (*N*)	Mean % Δ in lesion size	SD % Δ in lesion size
Naltrexone (topical ± intradermal)	13	−34.4	±35.8
Ilex	4	−23.3	±10.5
Control	4	−6.3	±14.8

Mean percentage change (% Δ) represents the change in size of the LLD lesion between day 1 and day 33 after treatment, followed by the standard deviation (SD % Δ) of that change. A negative number represents a decrease in size, whereas a positive number represents an increase in size. The number of animals in the "naltrexone" group is odd because one animal had no lesions on half of its head and was untreated.

The percentage change in the size of the initial LLD lesions 33 d after topical naltrexone treatment ranged from +3.9% to −100.0%, with a mean of −34.4% and a large standard deviation of ±35.8%, compared with those fish in the "iLEX only" group with a range of −16.4% to −39.0%, with a mean of −23.3% and a standard deviation of ±10.5%, and the fish in the "control group" with a range of +7.4% to −21.0%, with a mean of −6.3% and a standard deviation of ±14.8% (Table 2). Animals with more severe initial LLD lesions seemed to have the greatest percentage of improvement overall after naltrexone treatment, with >54.5% improvement on the more significantly affected sides of the fish (Table 1).

An inversely proportional relationship was found between the mean inflammation score (scale 0–3, average score per individual over days 0–5 after topical treatment) and the percent change in LLD lesion size per side of the head after treatment by day 33 (Supplemental Fig. 3). All inflammation scores and percentage changes were included in this evaluation, exclusive of treatment methodology. The dotted line represents the line of best fit ($y = -0.1808x^2 - 0.144x - 0.1375$).

DISCUSSION

Surgeonfish, particularly palette surgeonfish, were selected for this study due to their documented susceptibility to LLD lesions in managed care. [7,15] Surgeonfish with lateral line depigmentation displayed greater improvement 33 d after naltrexone treatment compared with the "iLEX only" and "control group" fish; this distinction was more pronounced in fish with higher LLD lesion scores. The difference in clinical response amongst animals with varying disease severities may represent dose-dependent systemic absorption of naltrexone, with higher doses of medication received in animals with a larger treated surface area, or a larger total area available for epidermal recruitment of mast cells, fibroblasts, and collagen. Systemic absorption of naltrexone applied topically to the cornea has previously been noted in dogs (*Canis lupus familiaris*).[1] Further studies are required to evaluate the systemic response of teleosts to treatment with topical naltrexone.

It was assumed that the red coloration on the skin represented inflammation rather than angiogenesis due to clinical experience. Inflammation was expected after topical treatment as observed in the pilot case as an initial effect of the treatment; therefore, fish without signs of inflammation were considered to have received an inadequate dose in the first round of treatment and were thusly redosed with an injectable form. The role of inflammation in this study as presented in Supplemental Figure 3 is unclear. Although fish with higher inflammatory scores appeared to have more notable clinical improvement, those fish were also typically more significantly affected by LLD and may have had more obvious visible changes in the 5 d after treatment. It is possible that the removal of the mucus layer using gauze may have induced trauma to the region, causing the noted response. This is supported by the "iLEX only" fish that also demonstrated a profound inflammatory response (Table 1). Although the authors' clinical experience using topical naltrexone for treatment of LLD supports the need for removing the mucus layer, further clinical trials using a less traumatic mechanical removal are warranted. Low-dose naltrexone (1–4 mg/d) is suspected to be anti-inflammatory in other species due to stimulation on B and T cells via β-endorphin and enkephalin release, and previous publications in dogs have not noted the presence of inflammation following topical naltrexone.[1,11] As such, it is unlikely that naltrexone induced the inflammatory response in these animals. Further investigation into the histopathologic differences in inflammatory response between these groups in correlation with and without mucus removal is warranted to further discern the underlying pathophysiology of this observation. Inflammatory signs typically resolved within 1 wk; anti-inflammatory drug administration can be considered for these cases to attempt to ameliorate discomfort.

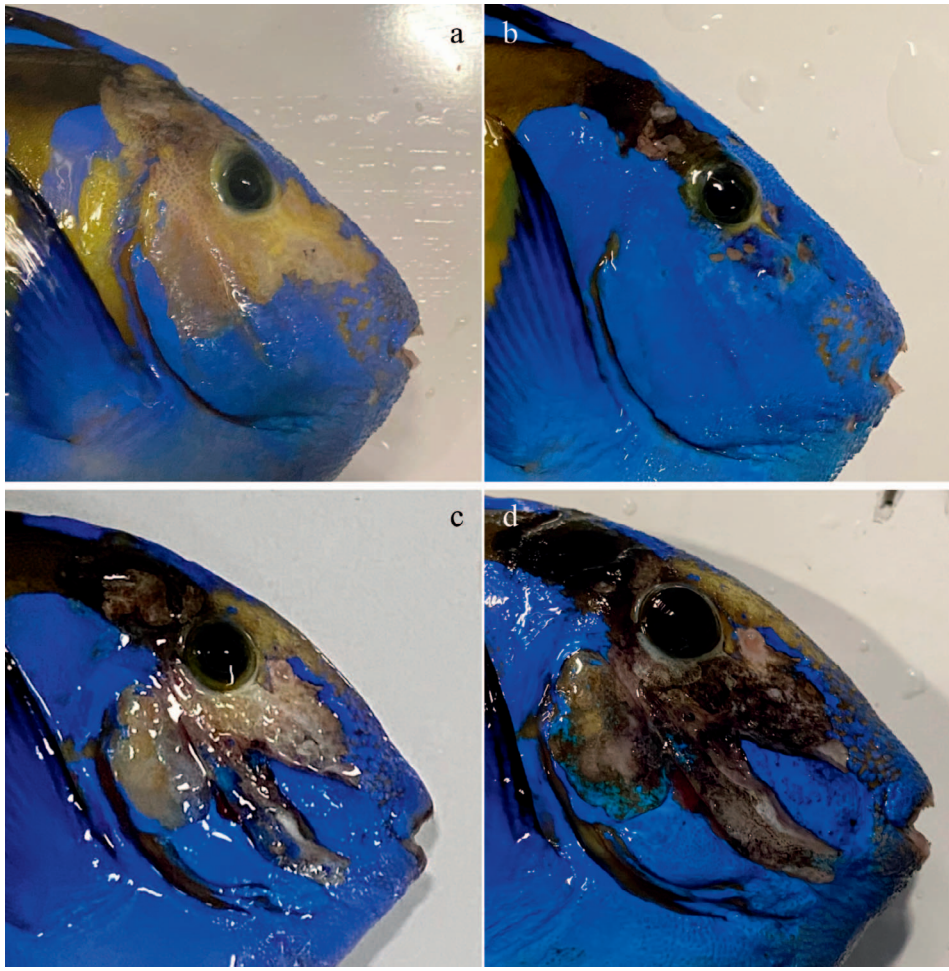

Figure 1. Pretreatment (a and c) and posttreatment (b and d) photographs of one palette surgeonfish (*P. hepatus)* (a and b) treated topically with one dose of 4 mg naltrexone and 10 g iLEX with an initial severity score of 2.5 and another palette surgeonfish (c and d) with an initial severity score of 3 treated topically with only one dose of 10 g iLEX.

Individuals given naltrexone displayed normal blue pigmentation of the treated skin by day 33, whereas one individual from the "iLEX" group displayed an irregular black pigmentation in the treated region at day 33 (Fig. 1). Melanization of teleost skin in response to inflammation has previously been documented.[12] The distinction in clinical appearance in fish treated with naltrexone versus just iLEX may lie in low-dose naltrexone's suspected anti-inflammatory properties. Histopathology of the epidermal and dermal tissues are required to make further distinctions, which was outside the scope of this study.

The authors do not recommend the injectable naltrexone application. Although the naltrexone treatments were coupled together in evaluation of this study, the authors believe that naltrexone injections carried an additional risk of iatrogenic complication and appeared not to improve efficacy.

This study was limited by low statistical power and the lack of histopathologic evaluation. Variations in measurements using the ImageJ software were possible due to minor differences in photography angle or movement of the fish during image capture.

Dermatologic lesion size reduction in response to treatment with naltrexone in teleosts was evaluated in this study. It is important to state that without identifying and treating the underlying cause of LLD, lesions will likely return in fish that undergo topical treatments while they remain in a suboptimal environment.[6] Topical treatment

is intended to improve the appearance of the individual animal and reduce chances for secondary infection while aquarium personnel attempt to determine and correct potential underlying causes of LLD in a given system. The mechanism by which these fish responded to the topical treatment is unknown. Research into the physiologic response of teleost species to treatment with topical naltrexone, including skin and muscle biopsies and histopathology, is warranted to further understand the findings in this report. Studies are also warranted to evaluate the use of naltrexone for acute and other chronic dermatologic lesions in teleosts.

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