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Anesthetic effects of lidocaine-hydrochloride on water parameters in simulated transport experiment of juvenile winter flounder, *Pleuronectes americanus*

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ABSTRACT

Simulated transportation of winter flounder, *Pleuronectes americanus* fingerlings was carried out to study the effects of lidocaine-hydrochloride on water parameters. Dissolved oxygen, ventilation rate, ammonia nitrogen and pH of control group, sham control group and lidocaine-hydrochloride treated groups of 5 ppm, 10 ppm, 20 ppm were tested at 1 h, 2 h, 3 h, 4 h and 5 h treatment duration. Lidocaine-hydrochloride treated groups, followed by sham control and control, were most effective in decreasing oxygen consumption and excretion of ammonia by fish. Lidocaine-hydrochloride dose-related decrease in oxygen consumption and excretion of ammonia were also established. pH declines in lidocaine-hydrochloride and sham control groups were more rapid compared to the control group. The results of this study reveal that lidocaine-hydrochloride is an effective sedative as a transportation mixture for winter flounder.

1. Introduction

In order to minimize stress and reduce the chance of fish mass-mortality (Ferreira et al., 1984) from long periods of transportation and handling, an effective high density transporting method is essential to reduce expenses and avoid losses (Ferreira et al., 1984; Staurnes et al., 1994). Increasingly, production of new equipments and transport procedures are having positive effects on the transportation of live fish. For example, the use of non-poisonous salt has been shown to alleviate stress and result in higher survival rates (Tomasso et al., 1980; Carmichael et al., 1984; Carmichael and Tomasso, 1988). The use of low concentrated calcium chloride is also a cheap and effective method in handling and transporting live fish (Grizzle et al., 1985; Carmichael and Tomasso, 1988).

Anesthetics are often used during transportation to reduce agitation and stress in fish. For example, Carmichael et al. (1984) showed that largemouth black bass, *Micropterus salmoides* treated with MS-222 (Tricaine methanesulfonate) exhibited enhanced rate of survival during transportation. The use of sodium amytal and benzocaine-hydrochloride on Java tilapia, *Oreochromis mossambicus*, reduced oxygen consumption to 1/3 while also decreasing ammonia and CO_2 excretion (Nemoto, 1957; Ferreira et al., 1984). The platyfish, *Xiphophorus maculatus* was reported to demonstrate a decrease in excretion of metabolic substances due to 2-phenoxyethanol, quinaldine sulfate and the ammonia in the presence of MS-222 (Guo et al., 1995).

Winter flounder, *Pleuronectes americanus* Walbaum, 1792 is the right eyed flounder in the Pleuronectidae family. Its main habitats extend from Georgia in North America to the Atlantic ocean of Newfoundland and Labrador (Liem and Scott, 1966; Witherell and Burnett, 1993; Park and Johnson, 2002; Park et al., 2004). This species is now recognized as commercially viable for aquaculture, based on their popularity as attractive game and food fish (Litvak, 1999; Park et al., 2003). The winter flounder is also acknowledged as one of the first ichthyological test specimens (Douglas et al., 1999; Litvak, 1999; Park and Johnson, 2002; Park et al., 2003).

Human anesthetic compound lidocaine-HCl [2-(diethylamino)-N-(2, 6-dimethylphenyl) acetimide hydrochloride] is a white powder soluble in water, and was first administered to fish by Carrasco et al. (1984). A more effective and risk-free anesthetic, such as lidocaine-HCl, which has been safely used in the dentistry industry, has been proven as a safe substitute for application on some freshwater and marine fishes in Korea (Park et al., 1988, 1998a, b; Hur et al., 2005). To date, a number of studies have investigated its effectiveness, economic viability, reusability, toxicity and side-effects to ascertain its appropriateness as a fish anesthetic (e.g., Park et al., 1988; Summerfelt and Smith, 1990). Park et al. (1998a,b), have administered lidocaine-HCl to *Rhynchocypris steindachneri* as a case-study experiment to investigate its sedative

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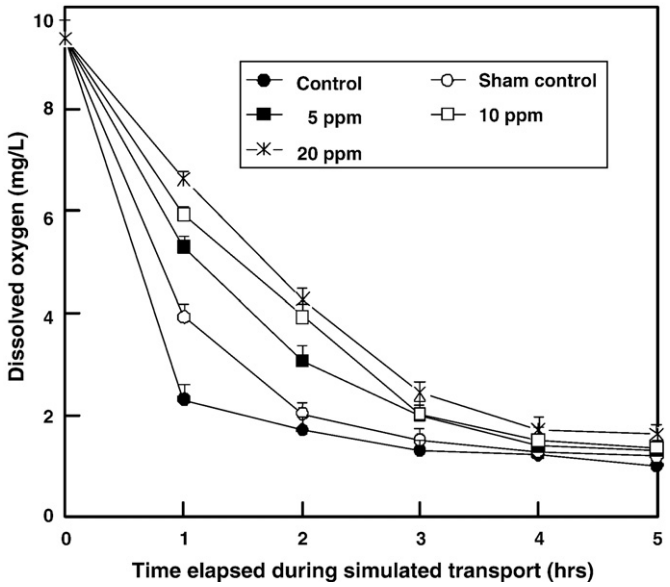

Fig. 1. Time-line analysis of dissolved oxygen (Means ± SE) in winter flounder, *Pleuronectes americanus*, at various doses of anesthetic lidocaine-HCl/1000 ppm $NaHCO_3$.

abilities on fish. Results indicated positive effects during transportation based on ventilation rate and various water quality parameters relative to the variety of lidocaine-HCl concentrations and applied duration. In this study, the anesthetic effects of lidocaine-HCl on winter flounder were investigated in a simulation experiment by analysis of various water and physiological parameters.

2. Materials and methods

Specimen winter flounders of 17.2 ± 0.1 cm (Mean ± SE) average total length and 16.3 ± 0.2 g average body weight were selected from the Aquaculture Research Station of the National Research Council (NRC), Sandy Cove, Nova Scotia, Canada. Culture water (salinity 31.5–36.5 ppt) after filtration was kept around 9.0 ± 0.5 °C. In order to neutralize the anesthetic solution and to amplify its effect (Carrasco et al., 1984; Park et al., 1998a), 1000 ppm $NaHCO_3$ (Sigma, USA) was prepared as the total concentration. The series of lidocaine-HCl (Chinwhoa Chemical Co., Korea) concentration samples were prepared at 5 ppm, 10 ppm and 20 ppm, based on the results of preliminary experiments. Diluted water with no other substances was prepared as the control group, while 1000 ppm of $NaHCO_3$ concentration sample was set up as the Sham control group.

Each specific sample was set up in a circular shaped glass aquarium (Ø 60×80 cm) filled with 170 l of diluted water with appropriate amounts of lidocaine-HCl followed by the admission of 50 healthy winter flounders. Each aquarium was sealed with Laboratory sealing film (Whatman, USA). Controllable 5 mm diameter siphon was systematically set up to collect 100 ml of the sample for quality measurement in 1 h intervals for 5 h. Furthermore, opercular movement, indicating the ventilation rate, and mortality were simultaneously monitored. The experiments were conducted in triplicate without the presence of light. Data were analyzed by ANOVA with the SPSS statistical package (SPSS 9.0, SPSS Inc., USA). Means were separated by using Duncan's multiple range test and were considered significantly different at $P<0.01$ (Duncan, 1955).

All experimental groups were measured for DO (dissolved oxygen), Ammonia (NH_4^+-N) and pH at 1 h intervals. Sample quality analysis followed standard methods (APHA et al., 1992). Ammonia was measured by the phenate method where indophenol blue, produced from the phenol reacting with ammonium ions and the hypochlorous acid were examined in a spectrophotometer (630 nm). Dissolved oxygen was measured by a DO meter (YSI model 57, Yellow Spring Instrument Co. Inc., USA) and pH was measured using a pH meter (Orion 290A, Orion Research Inc., USA).

3. Results and discussion

There were no signs of mortality in the control group, the sham control group, and lidocaine-HCl groups. Initial DO was 9.39 ± 0.45 ppm (Mean ± SE) for all experimental groups, declining precipitously in the control and sham control groups at 1 h, and more gradually so for the lidocaine-HCl groups (Fig. 1). DO at 1 h was 2.31 ± 0.29 ppm for the control group, 3.94 ± 0.22 ppm for the sham control group, 5.28 ± 0.19 ppm for 5 ppm lidocaine-HCl group, 5.93 ± 0.11 ppm for 10 ppm lidocaine-HCl group, and 6.63 ± 0.10 for 20 ppm lidocaine-HCl group. Clearly, the experimental group with the highest dose of anesthetic experienced lower and more gradual decline of DO, potentially reducing mortality during transportation.

At 2 h, the pattern of DO decline in the control group, the sham control group and the lidocaine-HCl administered groups, was consistent with reported trends in studies on *R. steindachneri* (Park et al., 1998a) and *Paralichthys olivaceus* (Ko et al., 1995). Based on these results, we can assume that large oxygen consumption during early stage of transportation may be due to high stress induced by handling or netting.

After 4 h, DO concentrations for all experimental groups were similar and did not change significantly thereafter (Fig. 1). DO concentrations at the end of the experiment were 1.01 ± 0.05 mg l^{-1} for the control group, 1.18 ± 0.06 mg l^{-1} for the sham control group, 1.31 ± 0.07 mg l^{-1} for 5 ppm lidocaine-HCl group, 1.36 ± 0.07 mg l^{-1} for 10 ppm lidocaine-HCl group, and 1.62 ± 0.11 mg l^{-1} for 20 ppm lidocaine-HCl group.

The results of this study were similar with a transportation experiment by Ferreira et al. (1984) where benzocaine-HCl was used as an anesthetic on Java tilapia, *O. mossambicus*. In general, both studies witnessed reduction in fish metabolism post anesthetic application, which is an indication of declining oxygen consumption. Here, there seemed to be a positive relationship between the concentration of lidocaine-HCl and DO, where the group with the highest concentration of anesthetic exhibited the lowest decline of DO.

DO concentration of the sham control group was notably different compared to the control group, which may be due to the anesthetic effect amplified by CO_2 production from $NaHCO_3$ (Booke et al., 1978; Carrasco et al., 1984).

As shown in Table 1, initial ventilation rate for the control group, the sham control group, and the lidocaine-HCl groups was 67.6 ± 3.1

Table 1
Time-line analysis of ventilation rate (min^{-1}) in winter flounder, *Pleuronectes americanus*, at various doses of anesthetic lidocaine-HCl/1000 ppm $NaHCO_3$.

Anesthetic dose (ppm)	Time elapsed from treatment (h)					
	0	1	2	3	4	5
Control	67.6 ± 3.1^{a}	102.3 ± 9.6^{a}	120.6 ± 11.9^{aa}	126.8 ± 10.9^{a}	124.6 ± 09.8^{a}	124.8 ± 08.8^{a}
Sham control	67.6 ± 3.1^{a}	83.9 ± 6.6^{b}	109.7 ± 09.2^{b}	119.7 ± 11.1^{b}	123.3 ± 11.0^{a}	123.9 ± 10.9^{a}
5	67.6 ± 3.1^{a}	81.7 ± 8.1^{b}	106.6 ± 10.0^{bc}	119.0 ± 08.9^{b}	120.6 ± 08.7^{a}	120.4 ± 09.1^{a}
10	67.6 ± 3.1^{a}	80.9 ± 5.1^{b}	104.1 ± 05.3^{c}	116.8 ± 09.1^{b}	119.8 ± 09.0^{a}	120.9 ± 10.2^{a}
20	67.6 ± 3.1^{a}	78.3 ± 3.9^{b}	98.4 ± 04.7^{db}	115.5 ± 08.3^{b}	117.2 ± 06.8^{a}	116.9 ± 08.6^{a}

Values (Mean ± SE of three replications) with different superscripts in each column indicate significant difference ($P<0.01$).

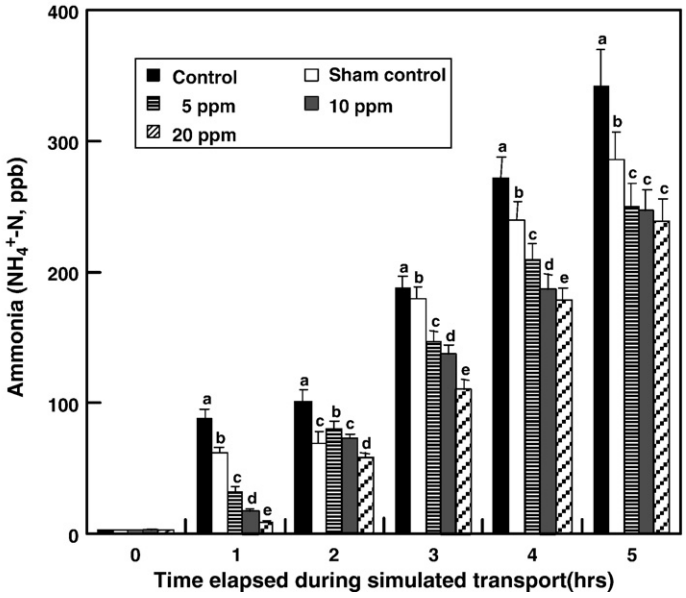

Fig. 2. Time-line analysis of ammonia (NH_4^+-N) in winter flounder, *Pleuronectes americanus*, at various doses of anesthetic lidocaine-HCl/1000 ppm $NaHCO_3$. Error bar represents the Mean ± SE of triplicate experiments. Different letters on the bars indicate statistical significance ($P<0.05$).

(Mean ± SE). Ventilation rate of the control group was still significantly higher compared to other groups at the 3 h mark ($P<0.01$), with the 20 ppm lidocaine-HCl group exhibiting the lowest value (Table 1).

By the 4 h mark, ventilation rates of all groups reached a plateau, not changing significantly thereafter. The detection of lower ventilation rates of both the sham control group and the lidocaine-HCl groups compared to that of the control group might have been reflected from the DO concentrations and the consideration of ventilation rate being relative to the metabolism decrease due to the anesthetic effects.

A similar designed experiment by Park et al. (1998a), with *R. steindachneri* as test subjects, showed that trends in DO change and ventilation rates exhibited the same patterns for 2 h, as was portrayed here for 3 h. Park et al. (1998a) used the same lidocaine-HCl dilution settings, but at a higher temperature setting (18 °C compared to our 9 °C for this experiment), which ultimately produced near identical trends. This is an important comparison, as it indicates the wide ranging effects of lidocain–HCL over a range of temperatures.

Fig. 2, displays the ammonia (NH_4^+-N) concentration of all groups during the 5 h experimental period. Initial ammonia concentration for all groups was 2.5 ± 0.01 ppb (Mean ± SE), markedly increasing thereafter across the board, with the 20 ppm lidocaine-HCl group exhibiting the slowest incline (Fig. 2). Ammonia concentration of the control group at the first hour was 87.6 ± 7.9 ppb, significantly higher than the sham control group and the lidocaine-HCl groups ($P<0.01$), eventually being measured at 342.5 ± 26.9 ppb at the completion of the experiment.

Ammonia concentration at the fifth hour mark was 342.5 ± 26.9 ppb for the control group, 285.4 ± 20.6 ppb for the sham control group, 248.6 ± 18.7 ppb for the 5 ppm lidocaine-HCl group, 246.1 ± 16.9 ppb for the 10 ppm lidocaine-HCl group, and 238.0 ± 17.0 ppb for the 20 ppm lidocaine-HCl group. At the completion of the experiment, relative difference in ammonia concentration between the 20 ppm lidocaine-HCl group was 43.9% with the control group, 19.9% with the sham control group, 4.5% with the 5 ppm lidocaine-HCl group, and 3.4% with the 10 ppm lidocaine-HCl group.

Guo et al. (1995) carried out a transportation experiment on the playfish, *X. maculatus* (Günther) treated with three different anesthetics; 2-phenoxyethanol (200 ppm), quinaldine sulfate (10 ppm) and MS-222 (30 ppm). At 16 h post-administration, the 2-phenoxyethanol had only 65% ammonia concentration of the control group, 20% ammonia concentration of the quinaldine sulfate, and relatively lower concentration compared to MS-222.

Trends in ammonia concentration exhibited by the five experimental groups are in line with reported trends by Park et al. (1998a) and Guo et al. (1995). Our conclusions are in agreement with Park et al. (1998a) where overall reduction in ammonia excretion was determined to be directly related to lower anesthetic induced metabolism. We have to take note of the fact that ammonia concentration for the sham control group was lower than the lidocaine-HCl groups at the 2 h mark (Fig. 2). However, this may due to the production of CO_2 from $NaHCO_3$ in the sham control group (Carrasco et al., 1984), as already highlighted in the DO experiment.

Fig. 3, displays the pH status of all groups throughout the 5 h experimental period. Initial pH was 7.12 ± 0.36 for all groups. The pH of the control group gradually declines throughout the experiment, finally reaching 6.20 ± 0.31 at the fifth hour. However, the sham control group and the lidocaine-HCl groups exhibited similar pH

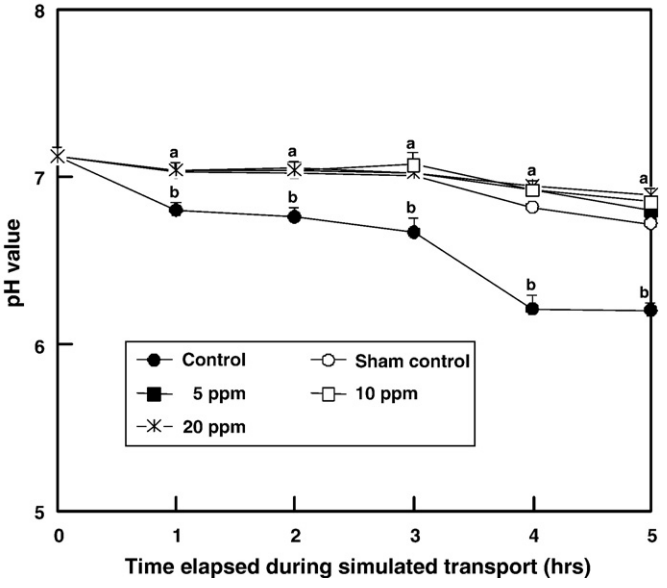

Fig. 3. Time-line analysis of pH values (Means ± SE) in winter flounder, *Pleuronectes americanus*, at various doses of anesthetic lidocaine-HCl/1000 ppm $NaHCO_3$. Points identified by the same letter are not significantly different ($P>0.01$). Each point represents the means of triplicate experiments.

throughout the experiment, ranging from 6.82 to 6.94 at the completion of the experiment. There was significant statistical difference in pH between the control and other groups ($P<0.01$), which may be due to more CO_2 excretion (Ferreira et al., 1979; Smit, 1980). The relatively higher pH observed in the sham control group and the lidocaine-HCl groups could be due to CO_2 from 1000 ppm $NaHCO_3$ exercising a buffering reaction and the anesthetic effect by lidocaine-HCl.

Lidocaine-HCl induced expected anesthetic effects on the winter flounder in terms of increased sedation, which is associated with reduced metabolic rates and oxygen consumption, along with reduced production of NH_3 and CO_2 (McFaland, 1959; Park et al., 1998a, 2004). Currently, MS-222 remains the only fish anesthetic the Food and Drug Administration (FDA) has approved in the United States (Schnick and Meyer, 1978). However, fish treated with MS-222 are not edible until 21 days post-administration, the withdrawal period required for traces of the chemical to disappear from the flesh (Carmichael and Tomasso, 1988). This obviously raises operational issues, as fish cannot be harvested for three weeks.

The results of this experiment suggest that lidocaine-HCl decreased metabolic activity of the winter flounder which induced reduction of ammonia excretion, stable pH, and lower oxygen consumption. In conclusion, lidocaine-HCl has shown to be an effective anesthetic, having also improved the transportation of *R. steindachneri* (Park et al., 1998a). In order to address other aspects of lidocaine-HCl effectiveness, anesthetic effects relative to body size and weight of winter flounder, an additional series of evaluations of stress reduction should be considered.

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