Study Comparing the effects of Different Anesthetic Drugs Used During Clinical Anesthesia in Koi Fish

[1*]Subasini Uthirapathy, [2]Israa Nabeel, [3]Sarmad Jabar, [4]Ibrahim Ahmed, [5]Twana Rzgar, [6]Pola Rzgar, and [7]Hassan Hadi.
[1]Assistant Professor, Faculty of Pharmacy, Department of Pharmacology and Toxicology, Tishk International University, Erbil, Kurdistan Region, Iraq 44001.
[2]Research Asst. Faculty of Pharmacy, Department of Pharmacology and Toxicology, Tishk International University, Erbil, Kurdistan Region, Iraq.
[3-7]Students, Faculty of Pharmacy, Department of Pharmacology and Toxicology, Tishk International University, Erbil, Kurdistan Region, Iraq 44001.
*subasini.uthirapathy@tiu.edu.iq

ABSTRACT

Fish have become a popular companion animal and experimental model, as well as being farmed and fished for food. Surgical and invasive techniques are therefore widespread in this animal group. The focus of this research will be on fish anesthesia. Fish are frequently immersed in a variety of anesthetic agents. Correct dose can lead to effective anesthetic for acute operations as well as loss of consciousness during surgical procedures. Anesthetic agent and dose differ amongst fish species, and physiological characteristics such as body weight, physiological stress, and ambient circumstances further complicate things (e.g., water temperature). Combined with general anesthesia, which involves the use of two anesthetic drugs, has been shown to be effective in fish, but it is not widely utilized due to a lack of experimental confirmation. Recent research has looked into local anesthetics to see how effective they are at reducing pain and discomfort. In Koi Fish, the local anesthetic lidocaine is quite effective at reducing pain-related reactions. Clove oil, on the other hand, is less useful in decreasing pain. In order to establish acceptable anesthetic procedures, researchers need investigate extensive range of anesthetic medication in a variety of fish species.

KEYWORDS: Anesthetic drugs, Fish, general anesthetics, Clove oil, Benzocaine
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INTRODUCTION

Historically, anesthetic drugs had been used on fish in aquaculture, research, and veterinary treatment. Their use decreases the pressure related to handling, mainly in the course of habitual upkeep along with tagging, weighing, blood samples, surgery and veterinary treatments. As a result, sedation or mild anesthetic can enhance the performance of speedy procedures (1-4). In invasive operations where tissue damage is likely, loss of consciousness, deep anesthesia is used to promote fish welfare and alleviate pain associated with surgical intervention. Anesthetics are usually administered through immersion, with the medicine dissolved in the fish's water or in an anesthetic vessel like a tank or large bucket, it also known as inhalation (5).

To avoid overdosing, keep an eye on the fish and monitor the extent of anesthesia when producing anesthesia through immersion. The gills and maybe the skin absorb the majority of these chemicals. Recording gill breathing rates, maintaining equilibrium (upright position), and reflex reactions (e.g., swimming response to tail pinch) are used to determine the extent of anesthesia (6).

Mechanisms of induction and recovery, sensitivity to external stimuli and handling, as well as the pharmacokinetics of these drugs, have all been studied in research investigations. On the other hand, these investigations usually concentrate on a small number of species, testing only one anesthetic agent or a small number of drugs on a single animal (7,8).

Because fish were originally thought to be impervious to anesthetics and pain, anesthesia received far less attention, especially before 2000. However, researchers are currently striving to show that anesthetic medication can reduce or eliminate any unfavorable behavioral changes and physiology produced by injury or unpleasant stimulation. This study will concentrate on what is known about anesthetics (mostly immersion) in fish in order to provide scientists and veterinarians with an up-to-date account. The utilization of the most effective means of decreasing stress and/or discomfort is crucial for the welfare of the animals being treated (9-11).

Different types of Anesthetic Agents

A number of anesthetic medicines are currently used in the laboratory, veterinary medicine, and aquaculture. The most commonly used anesthetic drugs in fish include “MS-222 (Tricaine), benzocaine, isoeugenol, metomidate, 2-phenoxyethanol, and quinaldine” (11). Experimental study has looked into induction, absorption rate, and pharmacokinetics, as well as unwanted or undesirable side effects. However, because these studies only looked at a small number of species, caution is required when using any of these drugs on a species that has not been evaluated (12).

Quinaldine

Quinolines are a class of compounds that have antimalarial, antibacterial and antipyretic properties and are used in a variety of disease. Quinaldine drug used to anesthetize fish for many years, although its mechanism of action is uncertain. At first, the fish exhibit tachycardia, but this is immediately followed by bradycardia and reduced breathing. Stress levels have also been discovered to be high (13, 14).

2-Phenoxyethanol

Burka et al., (1997) (15) analyzed that the 2-Phenoxyethanol is a preservative that is commonly found in vaccinations, cosmetics, and perfumes. This anesthetic's specific method of action in fish is unknown, however it could include the widening of neuronal cell membranes, which would diminish central nervous system activity. Lambooij et al., (2009) (16) established the Impaired breathing, slowed cardiovascular reactions, lower blood O_2, increased CO_2, and lower pH are all possible side effects, as is a possible stress reaction with higher plasma adrenaline and glucose levels.

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Metomidate

Non-barbiturate hypnotic metomidate hydrochloride stimulates and alters inhibitory gamma-aminobutyric acid type A (GABA A) receptors (17). This anesthetic works by working on the central nervous system. Metomidate is a medicinal and veterinary sedative that promotes drowsiness and hypnosis in humans and suppresses cortisol production by altering adrenal steroidogenesis, as observed in fish. In fish, metomidate causes reduced respiration and circulation, leading in hypoxemia and a reduction in blood pH (18).

Administration of Anesthetic Agents

Fish are normally given a single dose of the anesthetic medicines indicated above. Combining medications with distinct qualities offers a more thorough anesthetic in veterinary and therapeutic practice than delivering a single substance. Because of the medications' complementary effects, lower, safer doses may be possible. In some circumstances, induction and recovery durations can be shortened, and undesirable side effects can be minimized. Fish have been investigated under a combination anesthetic. “MS-222 and quinaldine, for example, reduced mortality and unpleasant side effects in rainbow trout (Oncorhynchus mykiss) and northern pike (Esox lucius)” (20, 21).

When gilthead sea bream (Sparus aurata) and European sea bass (Dicentrarchus labrax) were sedated with quinaldine and diazepam, the doses of both drugs were lowered (dose range from 0.5-1.2 ppm). This treatment combination also lowered the death rate and harmful effects of quinaldine in the two fish species investigated. During surgical procedures on koi carp (Cyprinus carpio), MS-222 immersion anesthetic was employed in conjunction with intraoperative injections of local analgesics (20). Butorphanol improved behavioral patterns and recovery, while ketoprofen reduced muscle atrophy, suggesting that their combined usage could be helpful. When Atlantic cod were administered a combination of metomidate and either benzocaine or MS-222, the results were comparable (21).

MATERIALS AND METHODS

Anesthesia in fish

The level of anesthetic required for a specific procedure will determine the degree of change desired. Fish are frequently monitored for behavior, eye movement, gill ventilation rate, posture, posture and reflex reactions. Induction and depth of anesthesia in fish, as in most other animals, are divided into ascending phases or planes. Behavior, swimming, eye movement, gill ventilation rate, posture, reflex reactions, and heart rate are all closely monitored in fish. The degree of alteration intended is determined by the level of anesthetic required for a certain surgery. It can be difficult to tell one step from the next when induction is done quickly. However, it is vital to use the correct dose to avoid overdose.

Light anesthesia can be sufficient for quick and noninvasive treatments like inspection, handling, weighing external tagging and gill crape. Surgical anesthesia is recommended for invasive and long-term surgeries, and it may be essential to supplement it with artificial gill breathing using a mouthpiece and pump to flush fresh or anesthetic-dosed water. It is vital that the water be aerated and kept at a temperature similar to that of the poikilotherms' native environment to prevent causing them undue stress. Hypoxia can generate stress in fish, making it more difficult for them to recover from anesthesia. The water used for anesthesia should ideally come from a home tank or aquarium system. All water quality parameters, such as pH and hardness, should be comparable to the fish's normal tank water.

The gill filaments collapse and become ischemic if the gills are not adequately hydrated, resulting in a hypoxic state. All water quality parameters, such as pH and hardness, should be comparable to the fish's normal tank water. To avoid stress, the water used for anesthesia should ideally come from a home tank or aquarium system. Other parameters such as bodyweight, temperature, and environment condition influence a fish's responsiveness to anesthesia.

Types of Anesthetic Agents used

MS-222 and Benzocaine

Topical analgesics of this family are commonly utilized in clinical and veterinary medicine. MS-222 and benzocaine are the two most commonly utilized anesthetics in fish research and food production. Several nations, notably the United States and Norway, have licensed both medications for use in aquaculture. By inhibiting voltage-gated sodium channels, these topical anesthetics prevent action potential initiation and propagation. MS-222 and benzocaine are commonly administered by the immersion method, in which they enter the body via gill absorption and produce anesthesia by inhibiting peripheral neuronal signal transmission to the central nervous system (22, 23). These medications' exact activity in fish is uncertain. Benzocaine has a molecular formula that is structurally similar to MS-222, however it must be dissolved in ethanol first, MS-222, on the other hand, is water soluble but has an acidic pH that necessitates the addition of a buffer, usually sodium bicarbonate (24).

Both drugs have different dosages based on the species. MS-222 and benzocaine are both administered to Atlantic salmon at 65 mg/L. However, MS-222 is given at 80 mg/L to halibut and benzocaine is given at 40 mg/L to halibut. Cod fish are administered 60 mg/L MS-222 and 25 mg/L benzocaine, respectively (25, 26). These medicines cause an increase in heart rate and respiration and hyperglycemia, in the first phase of anesthesia, which is followed by a decrease in heart rate and ventilation. Hypoxemia, hypoglycemia, and elevated lactic acid levels are all signs of anaerobic metabolism. "Other side effects include “increased hematocrit and hemoglobin levels, as well as erythrocyte enlargement. Several species' plasma catecholamine levels rise, indicating a probable stress response."

Clove oil (Isoeugenol):

Clove oil has isoeugenol, a chemically identical pain reliever to eugenol, which is used in dentistry. This anesthetic works by blocking NMDA receptors and potentiating GABA A receptors, as well as affecting sodium, potassium, and calcium channels. The active element of Aqui-S New Zealand, isoeugenol, has become a popular fish anesthetic. In some countries, it is also allowed for use in aquaculture (27). Isoeugenol causes undesirable side effects such as slowed heart rate, reduced cardiac output, and lower blood pressure, as well as impaired breathing and cardiovascular depression. Increased level of catecholamines and hematocrit may suggest that this anesthetic medication has triggered a stress response (28).

Administration of Anesthetic Agents

The medicine is dissolved in water, where the fish are held, or in some anesthetic vessels such as a tank or large bucket, and the anesthesia is administered via immersion or inhalation. When induction is carried out using the immersion method. Then there are numerous criteria to monitor the fish and a gauge of anesthetic depth to reduce the risk of overdosing. These compounds are largely absorbed by the gills and maybe the skin. Water was aerated and kept at a constant temperature. Recovery times were significantly faster when these agents were used combined than when they were used separately. Combination anesthesia may be safer since it allows for a lower dose, which leads to better recovery and lower mortality rates.

Combination anesthesia permitted the doses of each anesthetic agent to be lowered in the case of halibut. Induction and recovery rates improved with body size when only one agent was used, with smaller fish having higher induction periods but larger fish having a considerably faster recovery rate. As a result, combination anesthesia may be safer because it allows for a lower dose, resulting in improved recovery and decreased mortality rates, as well as less unpleasant side effects in some situations. More research on a wider range of fish species is needed to develop feasible combination methods.

Skin Scraping and Gill Biopsy

To evaluate the Koi fish, sedate it, and then put it through the many stages of anesthesia that a fish goes through in order for us to analyze it, as well as to recover the fish. When using a microscope to look for parasites (protozoa), costia, and flukes. Flagellate protozoa are single-

celled moving creatures, and Costia parasites are flagellate protozoa.

RESULT AND DISCUSSION

General anesthetics are the drugs that induce reversible loss of all sensations and unconsciousness. These drugs have analgesia and amnesia (loss of memory) and skeletal muscle relaxation and immobility and loss of reflexes. Stages of General anesthesia: These drugs are CNS depressant with increasing dose and time of exposure, the depth of central depression progressively increases. Stage 1 of Analgesia: Perception of pain is progressively abolished.

Amnesia and loss of consciousness develops by the end of this stage. Stage 2 of Delirium: Fish is delirious and excited. It may be struggle and hold his breath, muscle tone increases, heart rate may increase and blood pressure may increase. Stage 3 of Surgical Anesthesia: It has no pain reflexes. Stage 4 of Medullary paralysis: Final stage of course is respiratory and then cardiac arrest.

Anesthesia in fish

The stages or planes of anesthesia induction and depth in fish are generally separated into increasing stages or planes. Fish are frequently monitored for activity or swimming, posture, behavior, gill ventilation rate, eye movement, reflex reactions, and heart rate. Light anesthetic may be sufficient for quick and noninvasive treatments. Weighing, handling, examination, gill scraping, and external tagging are some examples. The amount of anesthesia and several parameters used to observe anesthesia in Koi Fish are listed in Table 1. A variety of operations are described as examples of what should be done to the fish while they are anesthetized at varying levels.

Adding tricaine with a nose sulfonate otherwise known as MS-222 at 100 mg/L. We notice that Fish are starting to feel the effects of anesthetic solution. They are sort of swimming aimlessly and crashing into the sides of the tank. We have noticed that when as Fish become anesthetized, they lose their ability to maintain the level in the water column and they will sink to the floor of the tank. Here, we entered stage 1 (t = 00.01.35- Fig 1) of anesthesia and within 15 seconds. They will jump into stage 2 anesthesia (t = 00.01.46 – Fig 2) Stage1: Fish are slightly sedated (t= 00.01.46), they slight loss of reactivity. To stimuli and slight decrease respiratory rate. Stage 2: There is total loss of reactivity to external stimuli (strong pressure) and there is a slight decrease in respiratory rate, so here they are more deeply sedated and then Stage 3: Now we will move to stage 3 of anesthesia (t= 00.02.12 – Fig 3). This stage, partial loss of equilibrium and muscle tone (Erratic swimming) and increased respiratory rate and the reactive illness to strong stimuli. (Different fish may react differently). This stage Fish shows that excited phase by spinal reflexes at this stage. Now all fish going to Stage 4 of anesthesia. Stage 4: Now we can provide some pressure onto its tail of Fish, we observed that there is no reaction to slight noxious stimuli applying (t= 00.04. 51- 00.14.00 – Fig 4, less than 10 mts). Now Fish have stage 5 (Fig 5) of anesthesia where the respiratory rate is slow and irregular. This stage only keeps for surgery, or samples taken from fish. This stage we have to collect the skin craping and taken a gill for biopsy. Stage 5: Stage 4 and 5 not too far into stage 5 otherwise we will run into the danger of respiratory and cardiac arrest. Very carefully, time to time, we can just check whether the spinal card reflexes are still there and keep an eye on their particular movement. Now we have to put the Fish back into fresh water, we have observed that respiratory rate is slowly, reversal of anesthesia and get it attic respiratory rates and shaking the head side to side that's mainly because of their particular movements Fish slowly regain consciousness and they take off and start swimming. Most of the Fish much recovered fully (29).

When these agents were used together, recovery times were substantially faster than when they were used alone. In the case of halibut fish, combined anesthesia allowed for a reduction in the dose of each anesthetic agent utilized. Induction and recovery rates improved with body size when only one agent was used, with smaller fish having higher induction periods but larger fish having a considerably faster recovery rate (30). As a result, combination anesthesia may be safe because it allows for a lesser dose, resulting in faster recovery and decreased fatality rates, as well as less unpleasant side effects in some cases. To establish effective combination strategies, more research on a larger range of fish species is required (31, 32).

Table 1: Different Anesthesia Stages and the Parameters Monitored in Koi Fish

Stage	Level of anesthesia	General Behavior	Activity	Equilibrium	Gill ventilation rate	Heart rate	Muscle tone	Reflex response
0	N	N	N	N	N	N	N	N
I	Lightly sedated	D	Reduced	N	N	N	N	N
II	E	A	Increased	Difficulty	Increased	Increased	N	Increased
III	Mild	Sedation	None	Loss	Decreased	Regular	Decreased	Reflex response + (Visual Inspection)
	Moderate	Sedation	None	Loss	Shallow (*Gill biopsy)	Reduced	Decreased	Reflex response +(Visual Inspection)
	Deep	Sedation	None	Loss	Shallow	Reduced	Relaxed	None
IV	Very Deep	Anesthetized	None	Loss	Rare movements	Cardiac arrest	None	None
V	Over dose	Apparently dead	None	Loss	None	Cardiac arrest	None	None

+ - reflex response is the fish swimming in response to a tail pinch
* Gills biopsy with fresh or anesthetic dosed water
Adapted from summerfelt and smith, 1990
N- Normal, D- Disoriented, E-Excitation, A- Agitated,

Types of Anesthetic agents

Changes in water quality and environmental conditions can have a significant impact on anesthetic efficacy in fish since they are so diverse. As a result, start with modest doses and moving up until you achieve the most effective dose (Table 2). Given that this study focuses on immersion anesthesia, injectable anesthetics are commonly used in larger fish species. Changes in water quality and environmental demands can have a significant impact on the effectiveness of anesthetic agents in fish (33). As a result, start with low doses and gradually increase until you reach the most effective dose. Injectable anesthetics are frequently utilized in larger fish species, despite the fact that this study focuses on immersion anesthesia. Table 2 lists the various anesthetic medications used in fish, as well as their dose ranges.

Table 2: Side effects of various anesthetic drugs used in Koi fish

Anesthetic drugs	Dose mg/L	Unwanted effects
MS-222	100-500	Increased respiratory and Tachycardia
Benzocaine	25-150	Tachycardia and increased respiratory rate
Clove oil	5 to 100	Mild sedations
Lidocaine	0.2 mg/kg	None
Benzocaine with clove oil	0.6-1.2 (ppm)	When these agents were used together, recovery times were substantially faster than when they were used alone.
MS-222 anesthesia with Tetracaine	0.6-1.2 (ppm)	When these agents were used together, recovery times were substantially faster than when they were used alone.

These doses are not suitable for all species or circumstances (e.g., temperature, body size, and physiological state must be investigated before use). Use the smallest doses and the smallest number of fish to test anesthetic efficacy when working with new species or substances.

Skin Scraping and Gill Biopsy

Gill breathing rates, maintenance of equilibrium (upright position), and reflex reactions are all used to measure anesthetic levels (swimming response to tail pinch). Rates of induction and recovery, response to external stimuli and handling, as well as the pharmacokinetics of various induction agents have all been evaluated in these experiments. Fig (8 & 9) shows that the skin scraping of Koi fish. Fig (10&11) shows that the gill biopsy. Under the microscope evaluation of skin scraping and gill biopsy, it very clearly showed that no parasites present in mucous area of skin, very healthy fish and regarding gill biopsy showed that x-mas tree like structure with bright yellowish red in color.

Fig 1 to 7: Different stages of Anesthesia

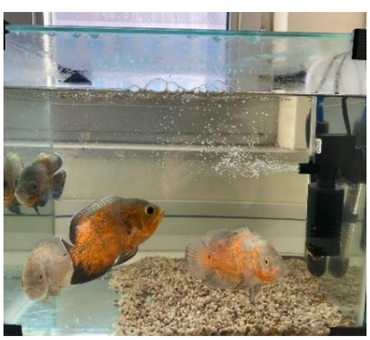


Fig 1: Normal-healthy Koi Fish

Fig 2: Stage-1 (mild sedation)

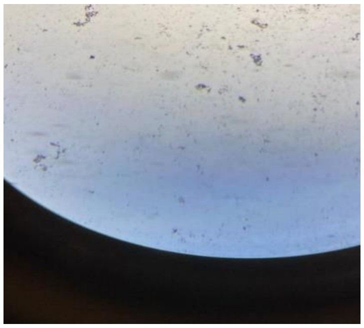
Fig 3: Stage 1 to 2 (mild)

Fig 4: Stage 3 (moderate sedation with Reflux activity)

Fig 5: Stage 4 (Irregular breath)

Fig 6: Stage 5 (Deep sedation without reflux)

Fig 7: Recovery Phase of Fish



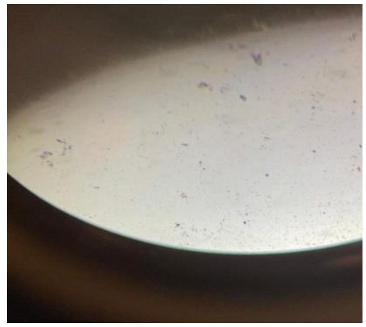
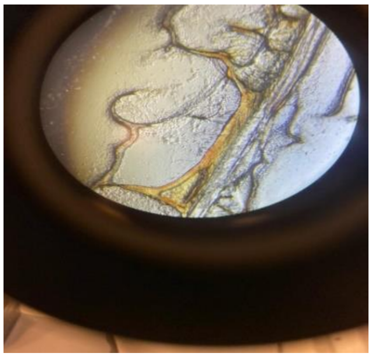


Fig 8: Skin scraping- not present any fungus   Fig 9: Skin scraping- no parasite present



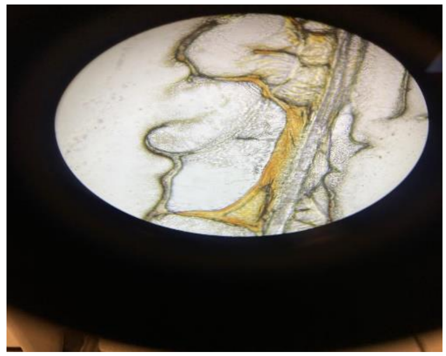


Fig (10 & 11): Gill Biopsy -shows normal structure (Fresh water)

Anesthesia-Related Factors

Biological parameters such as age, sex, health status and weight, growth conditions, maturation and immune condition, health, and reproductive condition all influence the efficacy of fish anesthetics. Abiotic parameters such as water quality, temperature, and oxygenation are all important. Fish have been researched to see how body health, water temperature, and physical stress effect anesthesia.

Body weight

Because drug dose is generally proportional to an animal's weight, research has focused on body weight. However, some fish experimental investigations suggest that weight has no effect on induction and recovery, while others find the opposite. Induction times were observed to be shorter in whitefish (Coregonus lavaretus) with larger bodies. Larger rainbow trout, on the other hand, had longer induction periods, while Atlantic salmon and brown trout showed no effect (Salmo trutta) (34, 35).

Isoeugenol, 2-phenoxyethanol, and metomidate induction increased with increasing body weight in Senegalese sole, but not for MS-222. In Atlantic cod, however, body weight had an effect on MS-222 and benzocaine activity; larger fish had longer induction and recovery durations. When utilizing metomidate in Atlantic cod, only recovery was affected by body size, but the properties of 2-phenoxyethanol anesthesia were unaffected by size. As a result, these drugs appear to behave differently depending on the species, and more research into aspects like lipophilicity and fatty acid profile in fish is needed to better understand the mechanisms behind these body weight connections (36, 37).

Water Temperature

Fish are poikilothermic, which means their physiology and metabolic rate are influenced by the temperature of the surrounding water. The effect of water temperature on the efficacy of anesthetic drugs has been studied. Induction and recovery times are often quickened when temperatures are raised (38). At warmer temperatures, MS-222 anesthetic is more effective. However, there is no consistent straightforward link between induction and recovery and water temperature. At higher temperatures, for example, halibut induction was significantly faster, but recovery took longer. The relationship between water temperature and anesthesia should be treated cautiously. Moreover, abrupt temperature fluctuations might stress fish, influencing their metabolic rate, circulation, and anesthetic drug uptake (39).

Physiological Stress

Stress has a significant impact on anesthesia in fish. Stress increases cardiovascular reactions and gill blood flow, resulting in greater immersion anesthetic drug diffusion. As a result, it's critical to keep stress to a minimum both before and during the anesthetic procedure. Many anesthetic medications, as previously stated, cause hormonal stress reactions as a side effect. Acute stress preceding anesthesia with MS-222 resulted in a shorter induction time and a longer recovery period in Koi fish. Following an acute stressor, these fish developed a deeper plane of anesthesia, requiring the dose of MS-222 to be lowered to minimize fatality (22, 40). However, the advantages of utilizing anesthetic medications to render the fish asleep during potentially stressful treatments are critical to minimizing any harmful impacts on their wellbeing. Several studies have demonstrated that anesthetizing fish reduces handling stress significantly. Cortisol levels in fish are enhanced during handling, but cortisol release is suppressed when they are sedated with MS-222. As a result, before anesthetizing the fish, its physiological status should be assessed in order to establish the most appropriate anesthetic drug and dose (41, 42-44).

Local Anesthetic Agents

Local anesthetics stop action potentials from propagating by blocking sodium channels and altering membrane function. As a result, topical anesthetics reduce pain by inhibiting nociceptive transmission. Few research has looked into local anesthetics; however, novocaine has been shown to inhibit reflex responses in cod fish. This isn't recognized enough evidence to suggest it for usage in animals. Lidocaine has had a lot of success in rainbow fish and Koi fish research (Table 2) A dose of 1 mg/kg lidocaine proved effective in lowering all of the unfavorable physiological and cognitive reactions to pain in this species. To design acceptable analgesic regimes, more research is needed to test a wide range of local anesthetic medications on a variety of fish species (7, 45- 47).

CONCLUSION

Several anesthetic medications have been tested to determine if they might give an effective anesthetic for fish. Given the vast number of fish species and their corresponding environmental and physiological needs. When choosing an anesthetic medicine, it's best to be cautious. Because a variety of parameters, such as body size, water temperature, and physiological status, influence anesthetic activity. It is critical to conduct a preliminary assessment of the optimal dose. Based on the positive results of a number of experiments that show reduced death rates and lower doses, as well as fewer side effects and faster recovery, combination anesthesia warrants additional investigation. In order to provide humane care, anesthetic agents must be used to alleviate pain and discomfort in fish who have suffered tissue injury.

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