"Pain" and analgesia in fish:
What we know, what we do not know, and what we need to know, before using analgesics in fish

Professor E. Don Stevens

Department of Biomedical Sciences
Atlantic Veterinary College
University of Prince Edward Island
Charlottetown, PE Canada C1A 4P3

Abstract

There is considerable pressure for Animal Care and Ethics Councils to recommend using analgesics in experiments that involve fish. Although we do have some data regarding this issue, I argue that we do not know enough to recommend any being used. For example, the doses of morphine used in fish experiments range from 10 to 3000 mg/kg relative to doses used in mammals that typically range from 2 to 5 mg/kg. I recommend that we continue to use anaesthetics that are commonly used with fish (tricaine, benzocaine, and eugenol) because these also probably act as analgesics in fish as they do in mammals, and thus, may have some analgesic properties post-operatively. There are no adequate tests that would allow us to recommend using analgesics for farmed fish that may be consumed by humans or pets.

Introduction

Recently, there has been considerable pressure applied on Local Animal Care and Ethics Committees to recommend using analgesics in experiments that involve fish. I know this because I receive many emails and phone calls from concerned committee members. I receive these because I was a member of the subcommittee of the Canadian Council on Animal Care that wrote the "CCAC Guidelines on: The Care and Use of Fish in research, Teaching, and Testing" (CCAC 2005). This pressure has arisen for two reasons. First, there have been a few studies that used analgesics in fish that caught the attention of the media and resulted in many articles in the grey literature and popular press. Second, more and more fish are used in research programmes. For example, in 2003, CCAC reported that 988,784 fish were used by researchers—more than the number of mice (789,061) or rats (314,871) (Griffin 2005). However, as argued by Horsberg (1994), before any animal care agency in any jurisdiction recommends that any drug be administered to any animal, its metabolism and metabolites should be studied. The purpose of the present review is to discuss what is known and what is not known about analgesics in fish. Before discussing what we know about analgesics in fish, I will briefly address the question: "can fish feel pain?" borrowing from the approach used by Bateson (1992) in his consideration of the problem. Then I will discuss analgesics in fish, and I will conclude with a consideration of what procedures might be potentially painful in fish and thus warrant the use of an analgesic.

Pain in fish

Can fish feel pain?

"Do you believe that fish can feel pain?" This is the way that the question usually is asked, and there are two serious problems with this way of looking at the problem. The first problem concerns the word "believe". Belief is the acceptance as true of any statement in the absence of evidence. I am a proponent of evidence-based medicine, and most nations

have legislation that requires that there be evidence concerning efficacy of drugs before their approval for use in humans or in animals. The second problem concerns the word "feel". This word is used because of the way that pain is defined by the International Association for the Study of Pain (IASP). They define pain as "An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage]. Note: The inability to communicate verbally does not negate the possibility that an individual is experiencing pain and is in need of appropriate pain-relieving treatment. Pain is always subjective. Each individual learns the application of the word through experiences related to injury in early life. Biologists recognise that those stimuli which cause pain are liable to damage tissue. Accordingly, pain is that experience we associate with actual or potential tissue damage. It is unquestionably a sensation in a part or parts of the body, but it is also always unpleasant and therefore also an emotional experience."

"Experiences which resemble pain but are not unpleasant, e.g., pricking, should not be called pain. Unpleasant abnormal experiences (dysesthesias) may also be pain but are not necessarily so because, subjectively, they may not have the usual sensory qualities of pain". They go on to state that "Many people report pain in the absence of tissue damage or any likely pathophysiological cause; usually this happens for psychological reasons. There is usually no way to distinguish their experience from that due to tissue damage if we take the subjective report. If they regard their experience as pain and if they report it in the same ways as pain caused by tissue damage, it should be accepted as pain. This definition avoids tying pain to the stimulus. Activity induced in the nociceptor and nociceptive pathways by a noxious stimulus is not pain, which is always a psychological state, even though we may well appreciate that pain most often has a proximate physical cause." (IASP 2008). A noxious stimulus is one that is damaging to normal tissues, or potentially damaging and a nociceptor is a receptor preferentially sensitive to a noxious stimulus or to a stimulus that would become noxious if prolonged. Given this definition of pain, it is very difficult to answer the question "do fish feel pain?".

This difficulty in dealing with the question has prompted a number of articles in the popular press. Some examples of these are: "Fish 'capable of experiencing pain' (Randerson 2003); "Do fish feel pain? (Gregory 1999). Others have pointed out difficulties with some of the studies—for example faults in Sneddon's study are presented in "A critique of the paper 'Do fish have nociceptors: evidence for the evolution of a vertebrate sensory system' (Rose 2008). Consequently, those of us working in this area of pain in non-human animals have devised working definitions that are different from those given by IASP.

The definition that we use in our laboratory, and that adopted by the CCAC, is "Pain is a response to a noxious stimulus that results in a change in behaviour or physiology of the fish, AND, the same noxious stimulus would be painful to humans". The important notion in this definition is that both criteria must be met. That is, first, there must be a reliable change in behaviour or physiology that we can use to measure the response to the application of a noxious stimulus. And, second, the same stimulus would be painful to humans. In summary, in my view we want to avoid using the words "feel" and "pain" in the same sentence when looking at this problem in fish. We need to focus on repeatable and measurable responses to specific stimuli with explicit properties. It also is important to appreciate that very few of the papers on this topic actually contain any original data, most are hand-waving speculation—a search will reveal that for every paper with original data, there are 100 papers that are reviews of this minute amount of data. What is needed is data, not speculation. I especially like the view expressed by Derbyshire (2003) on this issue: "We may feel sorry for the trout, but does the trout feel sorry for itself?"

Two types of pain—the mammalian perspective

It is important to keep in mind that there are two types of pain. First there is acute or escapable pain, for example the jab of a needle or touching a hot object. The response to this type of stimulus is an immediate very rapid motor response—withdrawal of the arm from the needle or the hot objects and vocalise (scream "ouch!"). The other type of pain is chronic or inescapable pain, for example lower back pain, head ache, or the pain associated with a burn, days after touching the hot object that caused an acute pain response. There is no rapid motor response to this type of pain, rather there tends to be a reduction in motor activity and the vocalisation tends to

be moaning and groaning rather that a scream. These two types of pain in mammals are associated with clearly distinct pathways in the spinal cord to the central nervous system. Acute pain is rapidly conducted along larger A-delta axons that are lightly myelinated, have medium to large diameter cell bodies and mediate a pricking quality of pain. Chronic pain is slowly conducted along smaller C axons that are unmyelinated, have smaller cell bodies and mediate the slower, burning quality of pain. In humans C-fibres make up around 70% of all nociceptors.

This distinction in the types of receptors and fibres is important to the present discussion for two reasons. First, essentially all of the fish studies done to date have concerned acute pain, whereas in mammals by far most of the work concerns chronic pain. Thus, we know something about responses to acute pain in fish, but nothing about responses to chronic pain. Second, whereas most (70%) of the fibres in humans are C-fibres involved in slow chronic pain, only 4% are C-fibres in fish (Sneddon 2002). Given this difference, we would predict that, in fish, the detection and response to acute pain is much more important to the animal than the detection and response to chronic pain.

Can fish sense a noxious stimulus?

Sneddon did an initial study that clearly showed that trout have nociceptors (Sneddon et al. 2003a). Later, she asked a post-doctoral student, Paul Ashley, to follow up her initial study with two further studies that provide excellent evidence that rainbow trout have receptors in the head region and on the cornea of the eye (Ashley et al. 2006; 2007). These receptors have properties very similar to receptors in mammals that are termed nociceptors. Ashley also provided convincing evidence that fish do not have cold nociceptors whereas mammals do. This has important welfare implications in that it implies that the practice of live-chilling or icing used by commercial fishers would not stimulate nociceptors and thus not be nociceptive or "painful". This implication also is important to many researchers who use live-chilling with zebrafish. Thus, we know that fish have nociceptors with properties very similar to mammals, and that fish have more receptors of the acute type than mammals, but that they do not have cold nociceptors.

Much is known about the nature and location of the proteins involved in detection of noxious stimuli in mammals. For example, there are five receptor proteins involved in temperature perception; some are involved in temperature regulation and some involved in nociception of noxious cold or noxious hot stimuli (McKenna 2005). These receptors differ between different animals. For example, in mammals the noxious hot receptor binds to the active component in chilli peppers (capsaicin) whereas that in birds does not. Hence, birds can eat chilli peppers, whereas mammals (except humans) do not eat them. All are in the TRP (transient receptor potential) family. The presence of these proteins has been demonstrated in fish, but little is known about their function or how they are modulated *in vivo*.

This is in contrast to the situation in mammals—any textbook on pain will list about 25 modulators of pain, the location of these modulators, and their mechanism of action. This is important because many analgesics act by interacting with modulators, either by blocking or stimulating them. For example, aspirin irreversibly inactivates a cyclooxygenase enzyme and thus suppresses prostaglandin synthesis. Prostaglandins do not cause pain themselves, but rather they enhance the pain-producing effect of other modulators and they produce second messengers that facilitate the opening of channels associated with nociceptors. This area of research is a large unknown in the fish literature and is essential to an understanding of how analgesics might work in fish.

Do fish have "pain" brain structures analogous to ours, and does the signal from the nociceptor go to these structures?

This is a controversial area of research with some suggesting that fish do have analogous structures, for example, Bateson (1992) and others arguing that fish do not because they lack a cortex (Rose 2002). Rose argues that "Awareness of pain in humans depends on functions of the neocortex. Fish lack these essential regions or any functional equivalent making it untenable that they can experience pain." In my view, the question is still open and it will require much more neurophysiology and elaboration of pathways within the nervous system in order to be resolved.

There are two studies that show that stimulation of a nociceptor in fish will result in a signal in the brain (Dunlop & Laming 2005; Nordgreen et al. 2007). Both these studies show that the signal goes to the brain. This is in contrast to many thousands

of studies on mammals that have elucidated the exact pathways from the nociceptor to and from the brain, the neurotransmitters, and the modulators that operate at each synapse along the way. This is important because the action of many analgesics is on these specific pathways and transmitters within the pathways. For example we know that there are opioid receptors on both the pre-synaptic and post-synaptic neurons at the first synapse in the spinal cord in the pathway from the nociceptor to the brain in mammals.

Even more importantly, we know that there is a descending pathway from the brain to the spinal cord in mammals that can inhibit pain and that this is one important action of endogenous opioids. The existence of this efferent pathway has not been demonstrated in fish. This is important because synapses within the pathway are a locus of action of many analgesics, especially those that interact with opioid receptors.

Do fish have opioid receptors and endogenous opioids?

Opioid receptors are the proteins present on neurons that bind to morphine and other similar compounds, including the endogenous opioids involved in natural analgesia. In mammals, we know that there are at least four different types of opioid receptors that have different binding affinities for the different opioids, including morphine, and that these receptor proteins are found in different amounts in different parts of the nervous system. Mammalian opioid receptors are located in the periphery associated with nociceptors, in the spinal cord, and in the brain—in all of these locations it is known that the receptors are involved in attenuation of the responses to a noxious stimulus. In fish, we know that these receptors are present and we also know that fish produce compounds very similar to the endogenous opioids produced by mammals. However, the function of the receptors in fish is much less clear. In the periphery, morphine causes a colour changed by stimulating chromatophores (Dwivedi 1978) and there are no studies of opioid receptors in the spinal cord. Essentially all of the studies of opioid receptors in the brain of fish concern their role in controlling appetite; none concerns their role in pain attenuation. Research in these areas would be important, because a main analgesic action of morphine in mammals is in binding to receptors in the spinal cord.

Analgesics in fish

Which analgesic to study?

In the studies in our laboratory, we have used morphine as the test substance. The reason for this is that morphine is the gold standard against which all other analgesics are compared, and we hold the view that we need this background information before we launch into studies of other potential analgesics. Before commencing our studies, we did a literature search to see what was known about morphine and fish. We turned up about 30 studies of morphine and fish. A similar search of the mammalian literature yields thousands of studies. However, most of the 30 good hits of morphine AND fish are not relevant to the issue at hand, that is, the analgesic action of morphine in fish. There are a number of studies looking at the effect of morphine on inflammation in the gut, the effect of morphine or release of other hormones, and the effect of morphine on behaviour, etc.

When we eliminate these other studies not relevant to morphine's analgesic action in fish, then we are left with the studies of Jansen and Greene (1970), Ehrensing et al. (1982), Sneddon (2003a, 2003b) and our studies. Chervova (1994, 1997) also has done some important studies, but not with morphine per se. I will discuss what we know using the framework: pharmacodynamics and efficacy, then pharmacokinetics, and then side effects and safety of morphine.

What is known about pharmacodynamics and efficacy of morphine in fish?

Pharmacodynamics is the study of the efficacy of a drug and how it changes over the time interval after taking different doses of the drug. Efficacy is the capacity of the drug to produce the desired effect—in our case for the administration of morphine to decrease the pain, or more exactly, to decrease the response to a noxious stimulus. Figure 1 illustrates what is meant by pharmacodynamics. Shortly after the administration of the analgesic (taking the aspirin to alleviate your headache), the pain decreases and then returns over time. It takes some time for the drug to act because it must be absorbed. Its effect does not last forever because the drug is metabolised and eliminated from the body.

What do we know about the pharmacodynamics of morphine in fish? Nothing—there are no published studies that relate the change in efficacy over

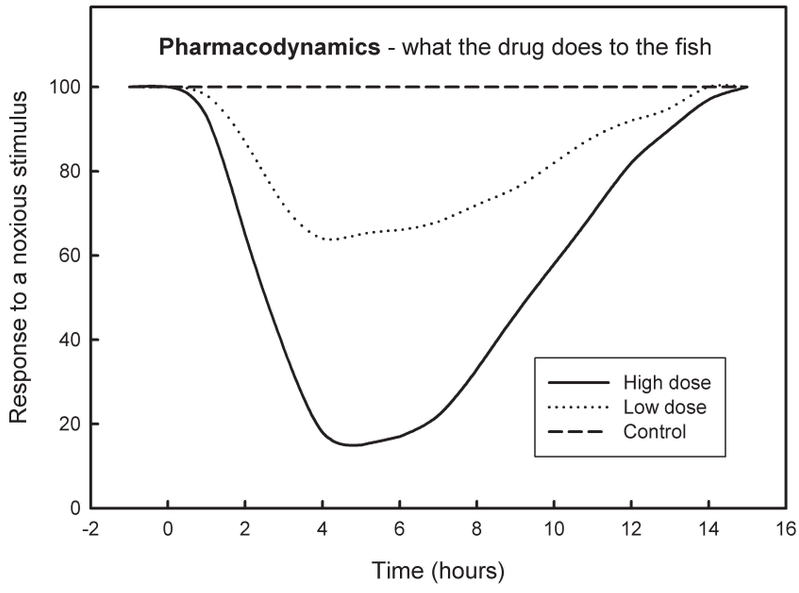

Fig. 1 An exemplary sketch to illustrate a pharmacodynamic study of the effect of morphine on attenuating the response to a noxious stimulus.

time to morphine dose in fish. This is the main reason that I argue that it is premature to recommend using analgesics in fish. Nothing is known about the dose-response relationship of morphine. On the other hand, Chervova has published some studies of the dose response relationship of some other analgesics in fish and presented these at the American Fisheries Meeting in Newfoundland in 2006.

Even though there are no pharmacodynamic studies of morphine in fish, there are a few studies that suggest it is efficacious. Jensen & Greene (1970) reported that morphine administered to the water (10 mg/L) attenuated the response to an electric shock in goldfish. However, we have not been able to replicate their results. The most convincing results are those of Ehernsing et al. (1982). They showed that morphine (30 mg/kg) administered into the cranial space over the optic tectum attenuated the response to an electric shock in restrained goldfish. More importantly, they showed that this analgesic effect of morphine was blocked by the prior administration of naloxone, the blocker used in mammalian studies. Both these studies used movement of the tail as the metric of a response to the noxious stimulus. It is interesting that this important study by Ehernsing et al. (1982) did not arouse the interest of the popular press—it seems that the time was not right to raise flags about the "fish pain" problem.

However, the time was right in 2003 when Sneddon published her study on trout "The evidence for pain in fish: the use of morphine as an analgesic" because it was extensively reported in the popular press and reviewed in some important scientific journals as well. In my view, it is important to appreciate that she used an unreasonable dose of morphine. She states "morphine sulfate (0.3 g/1mL sterile saline) was injected intramuscularly (0.1mL/10g fish weight)". This works out to be 3000 mg/kg—many, many times the lethal dose for any mammal or bird. It is interesting that none of the reviewers of the original article, none of the authors of the many articles in the popular press, or even the authors of the articles in *Nature* and the *New Scientist* noticed this important aspect of this study. When Sneddon was questioned about this by email asking if it was a typographical error, she responded that it was indeed, and that the actual dose was only 300 mg/kg. This is still well over the lethal dose in mammals—the LD50 in mice is 200 mg/kg (Votava & Horakova 1952). Figure 2 shows the dose used by Sneddon relative to doses recommended in mammals by CCAC. It is interesting that Sneddon reported that there were no changes in respiration rate or changes in swimming behaviour with this very large (lethal in mammals) dose of morphine. Clearly the action and pathways must be different in fish from those in mammals.

In summary, there are no studies of the dose-response relation for morphine in fishes, but there is some evidence of efficacy.

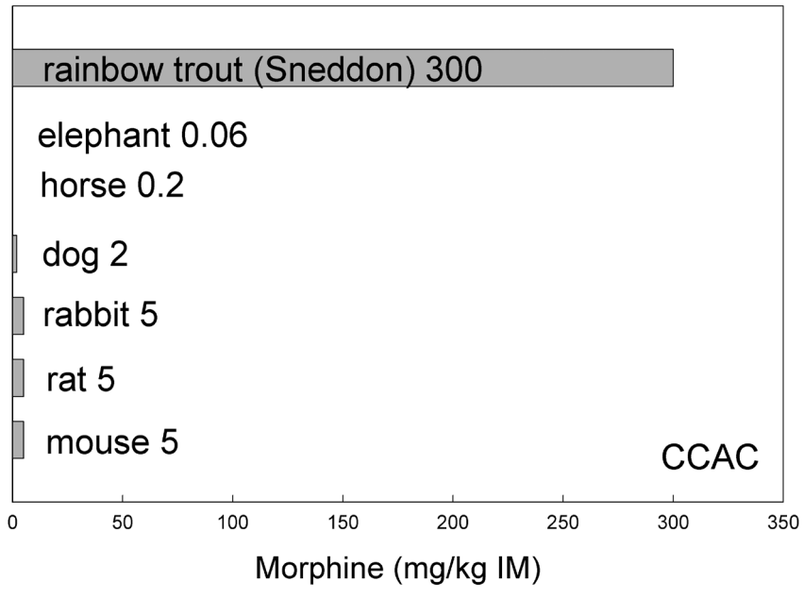

Fig. 2 A comparison of the dose used by Sneddon in her oft-cited 2003 study relative to recommended doses of morphine in mammals. (Sneddon 2003b).

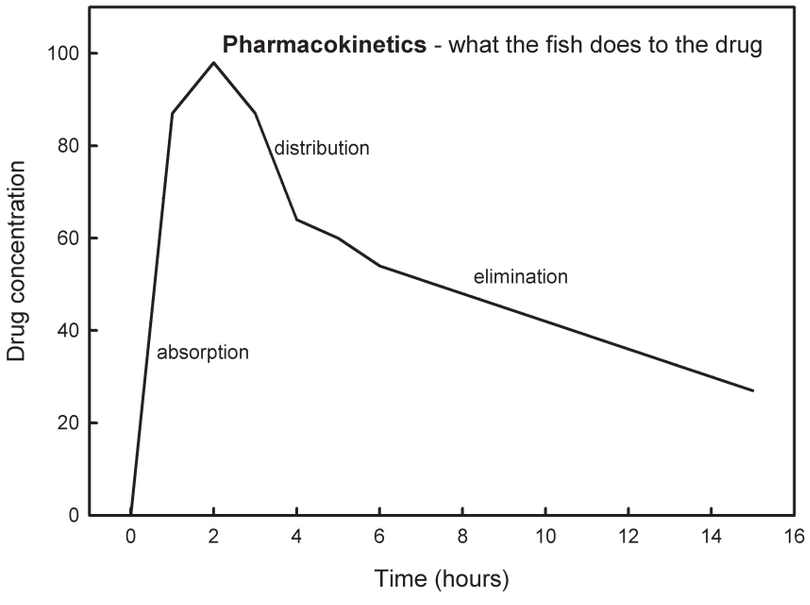

Fig. 3 An exemplary sketch to illustrate the results from a typical pharmacokinetic study of the changes in concentration of a drug after an oral dose. There are three phases to the response: absorption, distribution, and elimination. Usually the distribution and elimination look linear when the drug concentration is plotted on a logarithmic scale.

What is known about pharmacokinetics of morphine in fish?

Whereas pharmacodynamics concerns what the drug does to the animal, pharmacokinetics concerns what the animal does to the drug. Pharmacokinetics (pk) is the study of the changes in the concentration of the drug in the body (usually the blood) with time. Figure 3 shows an exemplary trace of the expected changes in blood concentration. The course of concentration usually shows three phases: absorption as the drug is absorbed into the blood; distribution as the drug is distributed to all the tissues of the body; and then elimination as the body metabolises the drug and eliminates it from the body. Distribution and elimina-

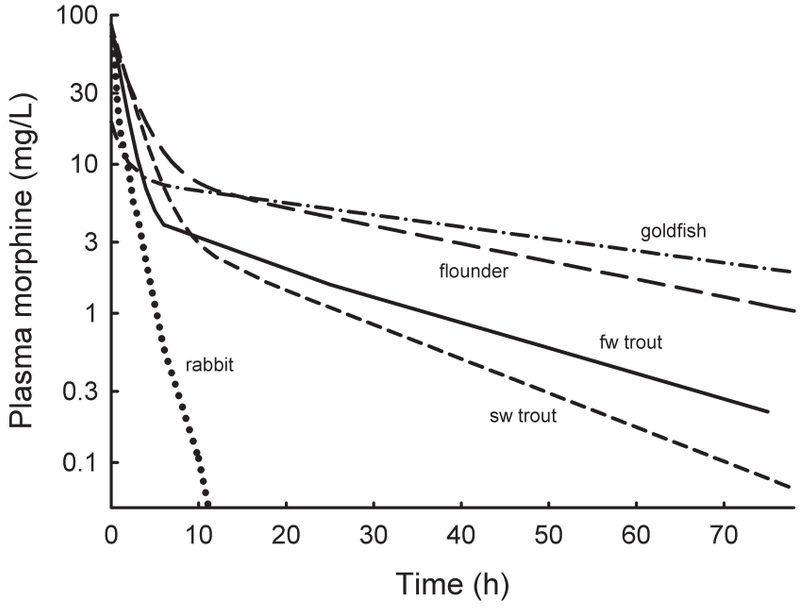

Fig. 4 Actual pharmacokinetic curves in fish after an IP injection of morphine. Data from Newby et al. 2006; Newby et al. 2008; and Newby et al. unpublished data. Flounder and trout data at 10°C and goldfish data at 18°C.

tion phases are usually referred to as the disposition of the drug.

Before our studies, no pharmacokinetic data of morphine or any other analgesics in fish were available. Our initial studies were done in winter flounder and rainbow trout adapted to seawater at 10°C. A blood vessel was cannulated, morphine administered either IP (intraperitoneal injection) or IV (intravenous injection), and the same fish repeatedly sampled via the indwelling cannula. Morphine was measured either by ELISA or by LC/MS-MS. Because we injected the morphine IP or IV, the absorption phase was too fast for us to measure; maximum values in the blood were recorded with the first blood sample at 15 minutes. These experiments were done in the same room, at the same time, with the same input water, at the same temperature, the same procedures, and the same dose of morphine (Newby et al. 2006). Results from these initial experiments and subsequent ones are shown in Figure 4. The second experiment compared the results with rainbow trout adapted to seawater with those adapted to freshwater and showed that the differences in this case were very small. We also did a large number of the reverse experiments, looking at the uptake of morphine from the water, but only in goldfish.

The results of these experiments are important for three reasons. First, because they are discordant with those of Jansen & Greene (1970) who reported that "morphine moved across the fish gills to equilibrate with the water within 15 minutes". Jansen and Greene did not actually measure the morphine in the fish, but rather they estimated it from changes in the concentration in the water, whereas in our experiments we actually measured blood morphine concentrations. We showed that the half-time for uptake from water in goldfish is greater than 700 hours, not 15 minutes. Jansen and Greene also reported that all morphine was eliminated from the fish within 2 hours, whereas our actual measurements showed that the half-time for elimination was about 36 hours. Thus, application of morphine via the water route is very slow and would require large amounts of the drug.

Second, our experiments showed that there were species differences. For example, the elimination half-time in flounder (34 hours) was more than twice that of seawater adapted rainbow trout (14 hours), even though the experiments were done under identical experimental conditions at the same time in the same room with identical procedures. This is important because it implies that we should measure the kinetics in every species in which we use the drug.

Third, the disposition of morphine is about 10 times faster in mammals than it is in fish. Thus, if we used the dose used by Sneddon, and if morphine is effective at blood concentrations similar to that

in mammals, then the single dose used by Sneddon would result in plasma levels greater than the effective dose for 32 days—much longer than the usual repeat dose time in humans of 4–6 hours. Our results also showed that the only metabolite of morphine in rainbow trout was morphine-3-glucuronide (Newby et al. 2008).

In summary, the disposition of morphine differs between fish species and is an order of magnitude slower in fish than in similarly sized mammals. The slow elimination of morphine by fish implies that one would have to be cautious when giving repeated doses over time.

What are the side-effects of morphine in fish?

Before any drug is used in any animal we should know something about its side-effects and its safety. It is important to learn from the lessons of thalidimide and vioxx. Sneddon reported that the large dose of morphine that she used did not result in any noticeable change in swimming behaviour, rate of respiration, or in the latency to feed (Sneddon et al. 2003). This is interesting, because doses much smaller than she used result in respiratory depression and death in mammals. Our experiments with smaller doses also showed no change in swimming behaviour, but we did see a slow increase in heart rate and cardiac output that persisted for a few days after a single IP dose of 40 mg/kg (Newby et al. 2007; Newby & Stevens 2008). Side-effects like these would be important to researchers who are doing an experiment in which the variables being measured are influenced by heart rate or cardiac output.

Some psychophysics experiments have shown that if morphine is added to the water, and if fish have a choice to be in the water that contains morphine relative to control water, the fish will chose to be in the water that contains morphine (Shelford 1918; Lau et al. 2006). The mechanism involved in fish choosing the side with morphine is not known. These experiments are important because they show that fish can detect and respond to morphine and provide convincing evidence that fish have functional morphine receptors.

Although Chervova has not studied the effects of morphine in fish, some of her experiments are important to the present discussion because she showed that some analgesics used in veterinary medicine in other animals are lethal in fish—these include sidnophen and analgin.

Also relevant to the present discussion is the fact that little is known about tolerance or dependence of fish to morphine. Tolerance (the increase in dose required to achieve the same effect with repeated administration) is extremely important in human and veterinary medicine. Dependence may be more of academic interest in fish research, but has little practical importance. We know that fish prefer water that contains morphine over control water, but we do not know if they become addicted or dependent on it.

What procedures are potentially "painful" to fish?

The last topic I want to discuss concerns potentially "painful" procedures. It is especially important for persons on Local Animal Care or Ethics Committees to constantly think about what sorts of experiments involving fish might require the use of an analgesic. Because these persons constantly review research protocols, they are in a good position to ask this question. Some Europeans are testing the idea that analgesics should be used during vaccination of farmed fish.

We have tested only one procedure with this in mind—the injection of PIT tags. PIT tags are passive integrated transponders; they are the same microchips used by veterinarians in pets. About one million PIT tags are injected into juvenile salmonids each year to monitor their behaviour during downstream migration and many are used in many other experiments. We did three separate experiments looking at the effects of PIT tags on three separate batches of juvenile rainbow trout. We chose variables that should be potential indicators of "pain". First, we looked at the latency to feed because Sneddon (2003) used this as an indicator of pain and because we reasoned that it is important for recently released fish to eat. Second, we looked at the daily amount of feed eaten because, as any fish farmer knows, going off feed is the first sign of fish being stressed. Third, we did a swimming test because we reasoned that swim performance would be important to recently released fish in escaping predators and in capturing prey. In all three experiments there was no significant difference between the control fish and fish that had just been injected with a PIT tag. Based on the results

of these three experiments, we concluded that an analgesic would not be necessary during the tagging procedure.

Conclusions

In this review, I have tried to summarise what we know, do not know, and what we need to know, about analgesics, particularly morphine, in fish.

We need some dose-response curves for analgesics in fish, and we need these for different species of fish. We need a reliable metric of "pain" in fish. We know that there are important differences between different mammalian species in their response to morphine, for example between dogs and cats. Similarly, we predict that there will be important differences between dogfish and catfish. We also need to know the time course of the effect and how the response is influenced by ambient temperature and other environmental variables.

We need to know safety margins in different species and we need to know more about side effects of analgesics.

We need to know more about the fundamental neurophysiology of fishes—in particular we need to know if there is an efferent pathway from the brain to the spinal cord that influences the response to noxious stimuli. This is extremely important, because it is the *modus operandi* of many analgesics used in mammals.

Horsberg (1994) has argued that it is essential to know the pharmacokinetics and metabolites of any drug before we recommend it being routinely used in fish or in any other animal for that matter.

There are some other things that would be nice to know, but are more of academic interest than essential to our question of using analgesics in fish. For example, it would be good to know the location of "pain" centre(s) in the brain and the pathways to and from these centres. It would also be good to know the location of morphine receptors in the periphery and in the spinal cord and to have an understanding of their function.

Thus, based on our lack of knowledge, especially regarding the nature of the dose-response relation and of safety factors, I argue that, at present, it is NOT appropriate for any Animal Care or Ethics Organisation in any jurisdiction to recommend using analgesics. There is too much that we do not know.

On the other hand, most of the anaesthetics commonly used in fish (tricaine methane sulfonate, benzocaine, and eugenol) act as analgesics in mammals. In fact, benzocaine is used as an analgesic or local anaesthetic and not as a general anaesthetic in mammals. It is likely that these drugs have similar analgesic actions in fish at low doses, they are well-studied, and much is known about their metabolism and pharmacokinetics. Thus, the use of these anaesthetics likely affords some analgesia in fish.

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