Best anesthesia for zebrafish

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Duration: 6 – 12 months

Short introduction:

There is a wide array of different teleost species used in scientific research world-wide [1]. These fish are frequently anaesthetised for various procedures and previous studies suggest that there is a divergence in the response to different anaesthetics among species [2]. The use and efficiency, from a practical point of view, of anaesthetics in different species have been studied and reviewed [3]. Whereas, the fish welfare aspect and the in depth understanding of the pharmacological mechanism of action and effect in different species are largely unknown. In view of our increased awareness of animal welfare, also regarding fish along with an increased use of fish as experimental animals, standardised anaesthetic protocols that are species specific is a necessity to assure best practises with focus on fish welfare.

Zebrafish (Danio rerio) is increasingly used in different kinds of research, as a model organism in biomedical research, studies in developmental biology, screening of effects of various substances, behavioural studies and neuroscience. In 2016 more zebrafish than rats were used in research in Sweden, underlining the importance for good anaesthetics for this species.

We will focus on four different commonly used anaesthetic, and cooling:

1. Rapid cooling (for zebrafish only). Some studies claim that gradual cooling is useful for sedation and immobilization of zebrafish for non-painful procedures [13]. This means immersion of the fish in water at 4° C which will reduce motor activity [14]. The anaesthetic effect is moderate and includes decreased movement and decreased sensitivity. The mechanism of action is not clear. However, in some studies carried out using in vitro models, it is proposed that the effect of hypothermia would be related to GABA activity and modulation of the calcium signal in the nervous system. This allows raising of a possible GABAergic effect, an increase in inhibitory activity in various neural circuits, as a potential explanation to the possible neuroprotective effect of hypothermia in some cases [15]. However, more studies are needed to confirm this theory. The method of hypothermia to induce anaesthesia in fish has limitations, especially to perform studies related to the cardiovascular system because cold exposure has a direct impact on the regulation of the activity of this system. In addition, even if the fish become motionless, stress levels might be high. This needs to be investigated.

2. Metomidate. This anaesthetic agent is a centrally acting drug. It produces sedation and hypnosis in humans, and affects adrenal steroidogenesis inhibiting production of cortisol. Deleterious effects of metomidate in fish are reduced respiration and heart rate, subsequently leading to hypoxemia and reduced pH of the blood. However, it has been claimed that it does not induce general anaesthesia, as is evident in the maintenance of opercular respiration for twice as long as with other anaesthetics at
the effective concentration [16] and in muscle fasciculations at low doses [17]. Thus, metomidate is probably a poor analgesic and should not be used alone for major surgical or noxious procedures [18].

3. MS-222 (tricaine methanesulphonate) is the most commonly used anaesthetic for fish worldwide. One advantage over other anaesthetic agents is that it is very water-soluble. It is a reversible sodium channel blocker that has rapid effects on muscle activity and is regarded as a good analgesic. However, because the effect of the substance may be remaining, although declining, after as long time as one hour, it might have negative effects when handling wild fish, like the brown trout. Furthermore, several studies show that MS222 may cause release of cortisol, which might be a disadvantage [19,20,21]. In addition, it has been shown to affect carotenoid pigmentation, i.e. number of pigment spots, over time compared to no-analgesic treatment and the results indicate that the anaesthetic MS-222 affects number of pigment spots as well as increasing dopaminergic activity in the telencephalon [22].

4. Benzocaine has a low water solubility and needs to be dissolved in ethanol before use in water. One advantage is that in solution benzocaine have a neutral pH. Thus, when handling wild fish, the substance can be dissolved in the same water as the fish are kept in, and no concern about effects on pH are needed. The effect is similar to those of MS-222 and benzocaine is also regarded as a good analgesic. However, some species differences have been reported; for example, eels are problematic to sedate in MS-222 [23], whereas benzocaine and eugenol seem to be more efficient [24].

5. Eugenol and its derivate isoeugenol. Compared to MS-222 it is characterised by rapid induction, prolonged recovery, and the narrow margin of safety. The long recovery time might be detrimental if used for wild fish. However, the fact that eugenol is a natural product and is rapidly metabolised by fungus and bacteria makes it a good candidate for field research. Ventilator failure may occur rapidly, especially when using high doses. It is a commonly used anaesthetic for farmed fish but it is debated whether it can be used or not for fish destined for human consumption. Its function as analgesic agent is also debated.

Fish will be exposed to different doses of the different substances. We will monitor induction time and recovery. In addition, effects on stress responses, e.g. cortisol, plasma ions and brain monoamines will be analysed. Long-term behavioural effects will also be monitored in fish subjected to different aesthetics.

References


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