INTRASACCULAR INJECTION OF AMINOGLYCOSIDES:

A NOVEL METHOD FOR TEMPORARY DAMAGING FISH INNER EAR HAIR CELLS

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INTRODUCTION: In contrast to mammals, many fish species have the ability to regenerate their inner ear hair cells after damage (Lombarte *et al.*, 1993; Scholik & Yan, 2001). Given this capacity, fish inner ear hair cells are nowadays a suitable model for investigations on hair cell regeneration and hearing function in animals. In this scope, it has been common to use aminoglycoside antibiotics (gentamicin, streptomycin or kanamycin) to damage fish inner ear hair cells and then observe the regenerative process (Matsuura *et al.*, 1971; Lombarte *et al.*, 1993). These antibiotics are known to displace calcium ions from their receptors, thereby blocking the cation channels that are located at the apices of hair cell stereocilia (Hudspeth, 1983; Kroese *et al.*, 1989). Until now, intramuscular injections of aminoglycosides have led to damage of inner ear hair cells but adverse health disorders associated with osmoregulatory problems were reported leading to death of study animals (Yan *et al.*, 1991; Lombarte *et al.*, 1993). For the study of inner ear hair cell regeneration in fish using ototoxic drugs, it was important to develop a protocol that minimized adverse effects, but produced acute hair cell damage. The present study examined two different methods for the delivery of an ototoxic drug to the Atlantic cod (*Gadus morhua*): i) systemic (intravenous) and ii) local (intrasaccular) gentamicin injection and compared these methods with regard to adverse effects and efficacy of inner ear hair cell damage.

MATERIAL & METHODS:

- Anaesthesia with 55 mg l^{-1} MS-222
- Fish immobilized in a polystyrene gutter

SYSTEMIC TREATMENT
Intravenous injection of gentamicin

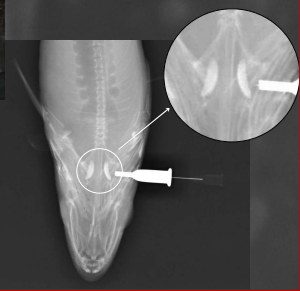
N = 160 individuals (~ 500 g)

- No injection for control fish
- Injection of gentamicin at 5, 10, 20, 40, 60 and 80 mg kg^{-1}
- Injection of saline solution (10 g l^{-1} NaCl) for sham fish
- Volume injected: 1 ml kg^{-1}
- Injection in the caudal vein

LOCAL TREATMENT
Intrasaccular injection of gentamicin

N = 75 individuals (~ 500 g)

- No injection for control fish
- Injection of gentamicin at 10, 20 and 40 mg ml^{-1}
- Injection of saline solution (10 g l^{-1} NaCl) for sham fish
- Volume injected: 0.05 ml

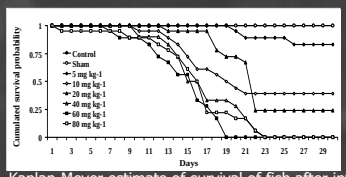


- Injection into both inner ear sacculi after removing endolymph
- Correct positioning of the needle ascertained using X-Ray imaging
- Trephination through the pterotic bone using a metal device

- Inner ear SEM observations
- Fish survival examination
- Histopathology (liver, intestine and kidney)

RESULTS*:

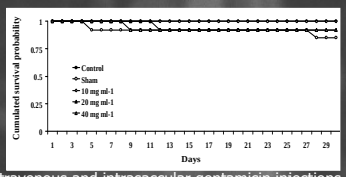
SYSTEMIC TREATMENT
Intravenous injection



➢ Intravenous gentamicin led to dose-dependent mortality caused by nephrotoxicity.

➢ Acute kidney tubuli necrosis was observed.

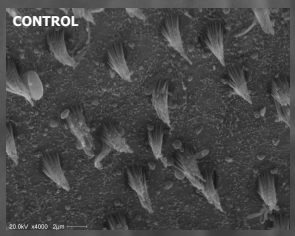
LOCAL TREATMENT
Intrasaccular injection



➢ Intrasaccular gentamicin led to negligible fish mortality

Kaplan-Meyer estimate of survival of fish after intravenous and intrasaccular gentamicin injections

➢ Intact sensory hair cells



➢ Intact sensory hair cells

➢ If hair cell density was significantly decreased after injection at 5, 20 and 80 mg kg^{-1}, no significant effect was seen after 10, 40 and 60 mg kg^{-1} injection.

➢ High prevalence of immature hair cells and significant shorter cilia were also observed.

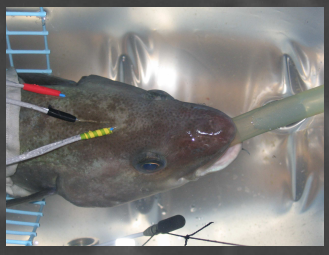
➢ Hair cells were damaged regardless of dose: decreased hair cell density, high prevalence of immature hair cells and significant shortened cilia

SEM observations of the inner ear sacculi maculae after intravenous and intrasaccular gentamicin injections

* Faucher *et al.*, 2008

CONCLUSION: intrasaccular administration of aminoglycosides should be the preferred delivery route for studies of ototoxic effects of drugs in fish. This method is more efficient in producing damage of inner ear hair cells and has a higher degree of organ specificity thus improving animal welfare through significant reduction in fish mortality.

PERSPECTIVES: this novel method for damaging fish inner ear hair cells will allow us to study the alteration as well as the regeneration of this sensory organ after damage. In this scope, ABR (Auditory Braisntem Response) may be used.

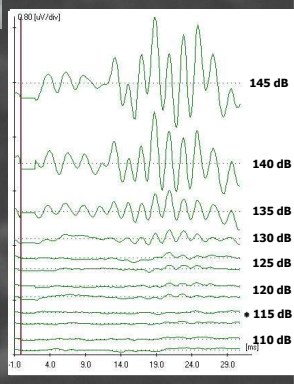


→ ABR are electrical potentials generated by acoustic stimuli and recorded using electrodes inserted under the skin akin to nervous tissues.
→ This technique is fast, non-invasive (alive animals) and reproducible.

→ ABR waveforms consisted of a series of narrow waves with an onset latency of the major complex around 11 ms at higher stimulus levels.

→ When the response threshold approached, the ABR was recorded twice.

→ The asterisk shows the response thresholds. Stimulus levels are expressed in dB (re 1 µPa).



ABR recording obtained in the cod in response to tone bursts at 250 Hz

References: Hudspeth, 1983, *Ann. Rev. Neurosci.*; Kroese *et al.*, 1989, *Hear. Res.*; Lombarte *et al.*, 1993, *Hear. Res.*; Matsuura *et al.*, 1971, *Jap. J. Physiol.*; Scholik & Yan, 2001, *Hear. Res.*; Yan *et al.*, 1991, *Proc. R. Soc. Lond. B.*