Efficacy of, and toxicity associated with, the use of levamisole in seawater to treat amoebic gill disease

B L Munday¹ and D Zilberg²

¹School of Human Life Sciences, University of Tasmania, Launceston, Tasmania, Australia. ²The Jacob Blaustein Institute for Desert Research, Ben-Gurion University of the Negev, Beer Sheva, Israel

Abstract
Atlantic salmon smolts with severe amoebic gill disease (AGD) and bathed in 50 ppm levamisole in seawater showed resolution of lesions and survival comparable with that of cohorts treated by a freshwater bath. However, high mortality occurred in smolts with extreme AGD when they were bathed in 50, 25 and 12.5 ppm levamisole in seawater. The possible mechanisms involved in relation to these observations are discussed.

Introduction
Within two years of amoebic gill disease (AGD) being recognised in Tasmanian salmonids cultured in the sea (Munday 1986), freshwater bathing had been introduced as an efficacious and environmentally-friendly method of treatment for the disease (Foster and Percival, 1988). Because freshwater bathing is time-consuming and stresses the fish a search has continued for agents which are efficacious (as assessed by fewer mortalities and/or gross gill lesions) when used in seawater or the feed. To date, the only products found to have any apparent effects by these routes have been oral narasin (Cameron 1992) and glucans (Lee et al. 1994). Unfortunately, narasin renders the feed unpalatable and the response to glucans is insufficient to warrant its use.

Because Howard and Carson (1994) reported that that ≥10 ppm levamisole was lethal to Neoparamoeba pemaquidensis (the causative agent of AGD) it was decided to assess the efficacy of this compound for the treatment of AGD. Dose rates between 12.5 and 50 ppm in seawater for two hours was chosen based on the above information and the recommended dose rate of 50 ppm for two hours for trematodes (Stoskopf 1992).

Materials and methods

Experiment 1
Fish were 100g Atlantic salmon with severe experimental AGD (mean 7.5 patches on the second gill arches) induced by cohabitation with infected fish (Findlay, 2001). Ninety of these fish were subjected to treatments as documented below and then were placed in a four cubic metre Rathbun tank containing seawater which was recirculated through a biofilter.

Treatments were undertaken in one cubic metre bins and consisted of a freshwater bath for two hours (30 fish, freshwater group), 50 ppm levamisole in seawater adjusted to pH 7.0 with citric acid (levamisole is hydrolysed in alkaline solutions, Symoens and Rosenthal, 1977) for two hours (30 fish, levamisole group) and unmedicated seawater for two hours (30 fish, control group). Because the fish were all sourced from the same cohort and also, because of the stress involved in the treatments, gill examinations were not undertaken at that time but commenced 24 hours later. The fish were examined daily and mortalities recorded. At 24 hours and seven days after treatment the number of gill lesions in the form of pale patches
on the second gill arches were counted by the method of Alexander (1991). At 14 days after treatment the surviving fish were euthanased and gill patches counted using a modification of Alexander’s (1991) method described by Zilberg et al. (2001). The third gill arches were fixed in seawater Davidson’s solution, embedded in paraffin wax and processed by routine methods for haematoxylin and eosin stained sections.

**Experiment 2**

This was virtually a repeat of Experiment 1 except that there were only two groups of 20 fish with extreme AGD (mean 20 patches on the second gill arches) and the treatments were levamisole 50 ppm in seawater and a freshwater bath.

**Experiment 3**

This was similar to Experiment 2, using the same cohort of fish, and two levels of levamisole, 12.5 and 25 ppm as well as a freshwater bath.

The number of gill lesions in the different treatments, were compared using one way analysis of variance (ANOVA, Sigma Stat, 1992-1994, Jandel Corporation). Mortality curves were compared by Kaplan-Meier survival analysis (Kaplan and Meier 1958) and the log rank test (Petö and Petö 1972) with software of SPSS (Advanced Statistics 8.0, Chicago, Illinois). Results were considered significantly different at 5% level (p < 0.05).

**Results**

**Experiment 1**

At 37% of these fish had died in contrast to 7% of freshwater bathed fish and no levamisole treated fish. At 14 days after treatment the patch counts had increased slightly in all groups and cumulative mortality in the control group was 43% whereas there had been no more mortalities in the other groups.

Histological examination of gill sections revealed typical AGD lesions in the control fish. Very few areas of hyperplastic epithelium with attached amoebae were found in the gills of the fish treated with freshwater but there were moderate numbers of small hyperplastic lesions on the gills of the fish treated with levamisole and these had numerous amoebae attached to them.

**Experiment 2**

By the end of the bathing period 14 of the 20 fish treated with levamisole had died and the others were euthanased on humane grounds thus terminating the experiment.

**Experiment 3**

All the fish bathed in levamisole had died by seven days after treatment compared with only two of the fish bathed in freshwater. As for Experiment 2, this experiment was prematurely terminated before the seven day gill check.

**Discussion**

These preliminary results indicate that levamisole in seawater can be almost as efficacious as freshwater in curing AGD. The results obtained were due to the effects of levamisole and not pH change as Clark et al. (2001) have shown no deleterious effects on *N. pemaquidensis* over a pH range of 6-9. However, unlike bathing in freshwater, bathing in levamisole mixed in seawater is associated with obvious gill damage and mortalities. It is interesting that there appears to be a relationship between the severity of the AGD lesions, the concentration
of levamisole, and the onset of mortalities. This suggest that it may be possible to use levamisole in seawater baths to treat fish with mild or even, perhaps, moderate AGD but close attention would need to be paid to the level of gill damage (patch count) and the concentration of levamisole.

Of particular scientific interest is the mechanism by which levamisole produced a resolution of the AGD lesions. While it is probable that levamisole killed a proportion of the amoebae on the gills that, in itself, would not be sufficient to lead to such resolution of lesions, especially in the presence of infected control fish. The result of such “cleansing” would only be a short “holiday” from infection with the presence of significantly more gill lesions than were actually observed at two weeks after treatment (Findlay et al. 1995). We suggest that not only with levamisole in seawater, but also with freshwater bathing, there is a stimulation of a cellular response which leads to enhanced resolution of the hyperplastic/inflammatory lesions of AGD. In the instance of levamisole treatment it was striking that numerous foci of hyperplastic epithelium remained in comparison to the freshwater treated group and these were heavily parasitised by amoebae. It could be argued that the effects of levamisole were due to its immunostimulatory properties, but such effects are usually only seen at low concen-

Figure 1. The severity of AGD, measured by numbers of gill patches and cumulative mortality, following a levamisole bath (50ppm), a fresh water bath and a non-bathed control group (n=30).
trations of the drug and 50 ppm has been reported to suppress a range of immune parameters (Siwicki et al. 1990). It may be that there are compounds which are capable of normalising the hyperplastic epithelium, which is the preferred site of attachment for *N. pemaquidensis* (Adams et al. 2002), without the serious side-effects noted with levamisole.

**References**


