

The efficacy of an ionophoric polyether, salinomycin, against the dinoflagellate *Piscinoodinium pillulare* Lom, 1981, the causative agent of „velvet disease“ in fresh water fish species

Zur Wirkung des ionophoren Polyethers Salinomycin gegen den Dinoflagellaten *Piscinoodinium pillulare* Lom, 1981, den Erreger der Pillularis-Erkrankung bei Süßwasserfischen

Günter Schmahl¹, Sabine Brudler² und Wolfgang Raether³

¹ Institut für Zoomorphologie, Zellbiologie und Parasitologie der Heinrich-Heine-Universität Düsseldorf, Universitätsstr. 1, D-40225 Düsseldorf, Germany; schmahl@alphabiocare.de

² Lehrstuhl für Spezielle Zoologie der Ruhr-Universität Bochum, Universitätsstr. 150, D-44780 Bochum, Germany

³Freigasse 3, D-63303 Dreieich, Germany

Summary: *Piscinoodinium pillulare* Lom, 1981 is the causative agent of “velvet disease” in fresh water fishes. Under laboratory conditions, the ionophoric polyether salinomycin given in the fish diet has been proven to be effective against the skin-inhabiting trophozoites of *P. pillulare*. Adult experimentally infested swordtails (*Xiphophorus hellerii*) were fed once a day ad libitum food pellets containing either 60 or 90 ppm salinomycin for a 16 or 19 days period. The efficacy of the treatment was monitored by counting the numbers of trophozoites of each fish at day 0, 3, 6, 9, 12, 16 and 19, respectively. As revealed by transmission electron microscope investigations, the damages in the trophozoites caused by the treatment consisted in malformation of the trophozoites, aggregation of droplets within the cytoplasm, and the formation of electron dense bodies along the limiting membrane. Following a prolonged treatment period, ruptures in the trophozoites limiting membranes were seen and the rhizocysts were no more detectable. Under the experimental conditions described, fishes showed no signs for adverse effects.

Key words: fish parasites, *Piscinoodinium pillulare*, chemotherapy, salinomycin.

Zusammenfassung: Auf der Suche nach neuen Möglichkeiten der Chemotherapie gegen *Piscinoodinium pillulare* Lom, 1981, dem Erreger der „Samtkrankheit“ bei Süßwasserfischen, erwies sich in Laborversuchen der ionophore Polyether Salinomycin als wirksam. Als Wirtsfische dienten Schwertträger (*Xiphophorus hellerii*) eigener Aufzucht. Im Vergleich zu den unbehandelten Kontrollen bewirkte Salinomycin-haltiges Medizinalfutter – 60 oder 90 mg Salinomycin pro kg Futter – die Eliminierung der hautständigen Trophozystenstadien nach 16 bzw. 19 Tagen bei jeweils 1x täglicher Verfütterung ad libitum. Der Verlauf der Infestation bei den unbehandelten Kontrollen und den behandelten Fischen wurde im Abstand von 3, 6, 9, 12, 16 und 19 Tagen nach Beginn der Behandlung durch das Auszählen der Parasiten dokumentiert. Licht- und elektronenmikroskopische Untersuchungen an sukzessive während der Behandlung isolierten Trophozysten ergaben als eindeutig durch die Behandlung hervorgerufene Schädigungen die Deformation der äußeren Gestalt, eine Aggregation von Lipidtröpfchen im Cytoplasma, sowie ein Auftreten von elektronendichten Extrusionskörpern an der Außenseite des Plasmalemmas. Im weiteren Verlauf der Behandlung traten zusätzlich Rupturen des Plasmalemmas auf, und die Rhizocystenläufer waren nicht mehr nach-

weisbar. Unter den hier gewählten Versuchsbedingungen wurden keine Anzeichen einer medikamentbedingten Unverträglichkeit bei den behandelten Fischen beobachtet.

Schlüsselwörter: Fischparasiten, *Piscinoodinium pillulare*, Chemotherapie, Salinomycin.

1. Introduction

Piscinoodinium pillulare (Schäperclaus, 1954) Lom, 1981, which belongs to the class Dinoflagellida Bütschli 1885, is a dangerous ectoparasite in public display aquaria and in fish culture in tropical as well as in temperate zones (Lom 1992). The parasite appears to be non-specific and indiscriminately infects various fish species invading skin, fins and gills. Casually, the occurrence of trophonts is reported from the oesophageal epithelium and intestine, and even in the subcutis (Lom 1992).

This ectoparasitic stage, the trophont, has a subspherical shape reaching up to 160 µm in length and is brown-yellowish in colour. A variety of cell organelles and inclusions is found in the cytoplasm such as chloroplasts, mucocysts, starch grains, fat reserves and rhizocysts. The nucleus contains large chromosomes.

After grown-up the mature trophont drops off from the host's surface and sinks to the bottom to become a tomont. Then, successive divisions take place resulting in 128 or 256 tomites which differentiate into gymnospores. These gymnospores swim spirally forward in search for a proper host fish. After contact with the host, the gymnospore adheres to its surface by the sulcal flagellum. Then, in the bottom sulcal region a protuberance is formed which later on develops into the attachment disc (Lom 1992). Heavily infected fish have a golden, velvety hue, the opercula are wide open, and the fins are folded. Locally, the skin may exert petechiae and reddish spots of inflammation. Especially in high density fish culture mass mortalities can occur (Shararom-Harrison and Anderson 1990).

So far, therapeutic measurements consist in medicinal baths containing dissolutions of sodium chloride, copper sulphate, or ethacridine (Kabata 1985). Some of the chemicals, especially the salts and compounds with a fixative effect, not only affect the parasites but are harmful to the hosts. The often non-satisfying results of these more general treatments led to trials with different chemotherapeutics against a wider range of parasites (Schmahl 1998).

Among different classes of compounds against parasites, the ionophoric polyethers represent very effective agents against various parasites, i.e. coccidians in poultry, different *Plasmodium* species and *Henneyuya* sp. (Myxozoa, Myxosporaea) in tapir fish (Mehlhorn et al. 1983, 1984, Raether et al. 1985, Dohle et al. 2002). In addition, the ionophoric polyethers, when given at therapeutic concentrations, are well tolerated by animals and excreted completely in short periods (Gerhard et al. 1995). Their mode of action consists in disturbing the ion balance between the inner and the outer milieu of cells.

In pilot trials, and under laboratory conditions, a ionophoric polyether, salinomycin (α -ethyl-6-[5-ethyl-9-(2-furanyl)-2,6-dihydroxy-1,3,7-trimethyl-4-oxodecyl]-tetrahydro-5-methyl-2H-pyran-2-acetic acid) incorporated in medicinal flakes for treatment trials was tested against the skin-inhabiting trophozoite stages of *P. pillulare*. Salinomycin has been described as a fermentation product from *Streptomyces albus* (Streptomycetaceae; Prokaryota). It reveals a strong activity against Gram-positive bacteria, mycobacteria, fungi and against protozoans, especially coccidia (Kinashi et al. 1973, Miyazaki et