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Degradation of Oxytetracycline Hydrochloride in Fresh- and Seawater

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Abstract

The degradation of oxytetracycline hydrochloride in fresh- and seawater in plastic tanks placed in a hatchery was studied. Reverse phase HPLC technique was used for the detection of oxytetracycline. The half-life of oxytetracycline hydrochloride in freshwater (average pH 7.3, average temperature 27°C, under natural lighting) was 58 h, and in seawater (average pH 7.9, average temperature 27°C, under natural lighting) 298 h.

Introduction

Oxytetracycline (OTC) is a widely used antibiotic in aquaculture and is also sometimes added to prawn/fish feed as a growth promoter. In the USA, OTC is one of two antibiotics registered by the US Food and Drug Administration for the treatment of bacterial disease in cultured catfish (McPhearson et al. 1991). OTC was the most frequently prescribed drug in Norwegian salmonid farms during 1980-88 (Grave et al. 1990). Ackefors et al. (1990) reported that to produce a tonne of farmed fish in 1988, Norway used 0.21 kg of OTC, Finland 0.115 kg, and British Columbia, Canada 0.48 kg. This drug is also commonly used for fish and prawn culture in Malaysia. Since the use of antibiotics is not regulated and the sale need not be officially recorded in Malaysia, the amount used for aquaculture is not known.

In many countries, especially those in the tropics, there is no legislation governing use of antibiotics for aquaculture, although in the more developed countries, legislation to control their use is available (Macmillan 1985; Howarth 1990). With the expansion of aquaculture worldwide, there is increasing concern about antibiotic abuse. There is also concern that its persistence in the aquatic environment may cause adverse effects on the ecosystem (Anon. 1988; Jacobsen and Berglind 1988; Björklund et al. 1991).

The degradation of OTC in seawater is light- and temperature-dependent (Samuelson 1989) and an aqueous solution of OTC is pH-dependent (The Merck Index 1983). Although much work on OTC degradation in the temperate countries have been reported, little information on its degradation in fresh- and seawater in tropical conditions is available. Hence this experiment attempts to examine the degradation of OTC in fresh- and seawater under tropical conditions.

Materials and Methods

Chemicals

Methanol (HPLC Grade) and acetonitrile (HPLC Grade) were obtained from Merck (Darmstadt, Germany), oxalic acid (Analar Grade) from BDH (Poole, England), oxytetracycline hydrochloride from Sigma Chemical Co. (St. Louis, USA) and sodium hydroxide from Riedel-de-Haen (Hannover, Germany).

Apparatus

A High Performance Liquid Chromatography (HPLC) Pump Model Waters 600E (Millipore Corporation, USA) connected to an auto sampler Model Waters 715 Ultra WISP was used. The column (Novapak steel column 3.9 x 150 mm, with spherical packing material of 4 um) was connected to a Guard-Pak™ u Bondapak ™ C_{18} HPLC precolumn insert. The UV detector Model Waters 486 was used, and the peak integrations and concentrations were worked out using the Waters Maxima 825 Chromatography Software and Workstation.

Chromatography

Reverse phase chromatography was used. The mobile phase was prepared from methanol, acetonitrile and 0.01M aqueous oxalic acid (pH=3.5 adjusted with 6N NaOH) at a ratio of 1:1.5:7.5. The mobile phase was vacuum filtered using an FH 0.5 μm Millipore filter and degassed with a stream of helium (He) gas at 100 $ml{\cdot}min^{-1}$ for 15 minutes. A small stream of He (20 $ml{\cdot}min^{-1}$) was passed through the mobile phase throughout the analyses. The detection of OTC was made at a wavelength of 360 nm, a flow rate of 1.0 $ml{\cdot}min^{-1}$, a pressure of about 1,400 psi and a sensitivity of 0.5 AUF.

Experiment

The experiment was carried out in 20-l plastic tanks of 37.5 x 21.0 x 27.0 cm. The tanks were filled with 10 l of fresh- or seawater and placed indoor in the mollusc hatchery of the Fisheries Research Institute, Penang, where natural lighting (approximately 12 h light and 12 h darkness) was maintained. One tank was used as the freshwater control and another as the seawater control where no OTC was added. Three tanks were filled with freshwater and another three with seawater, and OTC was added so that the concentration in the tanks measured 1 ppm. The tanks were topped up with fresh- or seawater to compensate for evaporation whenever the volume of the tanks fell below the 10-l mark. All the tanks were provided with gentle aeration. However, it was difficult to adjust the aeration in all the eight tanks to provide exactly the same amount of air, and some tanks had slightly stronger aeration than others. The pH (measured with a Corning pH meter 220), salinity (measured with an Atago refractometer) and temperature (measured with a Yoshino Mercury Thermometer, range -6-36°C) were monitored daily. A luxmeter was not available, so an exposure meter for photography was used to estimate light intensity in the hatchery. About 1 ml of water was taken from each tank daily, and the water samples were filtered through a Millex -HV_{13} Millipore filter and 50 µl injected into the column.

Two sets of OTC standard curves were prepared, one set with reverse-osmosis water and another set with seawater. The reverse-osmosis and seawater were filtered through vacuum using a Millipore HA filter of 0.45 µm pore size before being used for standard preparation. The concentrations prepared were 0.1, 0.5, 1.0 and 2.0 ppm.

Results

The lighting in the hatchery where the tanks were placed was not very bright. With the exposure meter set at ASA 100, the reading taken during the experiment (29 March - 13 April 1993) at 1000 h daily usually fell between 2.8 and 5.6. Two readings taken at 1200 h on two different occasions during the course of the study were 4.0 and 5.6. The average pH of the freshwater was 7.32, while that of the seawater was 7.85. The average salinity of the seawater was 22 ppt, and the average water temperature in the fresh- and seawater tanks was 27°C.

In both the fresh- and seawater controls, no peaks were recorded from the HPLC runs in the region where the OTC peak was detected from the standard solutions. Retention time of OTC in the standards prepared from freshwater was 2.77 ± 0.04 minutes, and in seawater 2.78 ± 0.04 minutes.

The OTC standard curves in freshwater have a high degree of linearity (average $r = 0.998$), with an average slope (concentration of OTC/area of peak) of $(5.77 \pm 0.39)10^{-4}$. The average recovery of OTC was 98.6%. The half-life of OTC in freshwater (intersection of the horizontal line drawn through 50% OTC concentration with the OTC degradation freshwater curve) was 58 h (Fig. 1). The OTC standard curves in seawater also have a high linearity (average $r = 0.998$), with an average slope of $(5.88 \pm 0.14)10^{-4}$. The average recovery of OTC was 98.7%. The half-life of OTC in seawater was 298 h (Fig. 1).

A student's t test (Steel and Torrie 1980) was carried out to determine whether there was any difference between the slopes of the fresh- and seawater OTC standard curves. No significant difference was found between the two curves at 1% significance level.

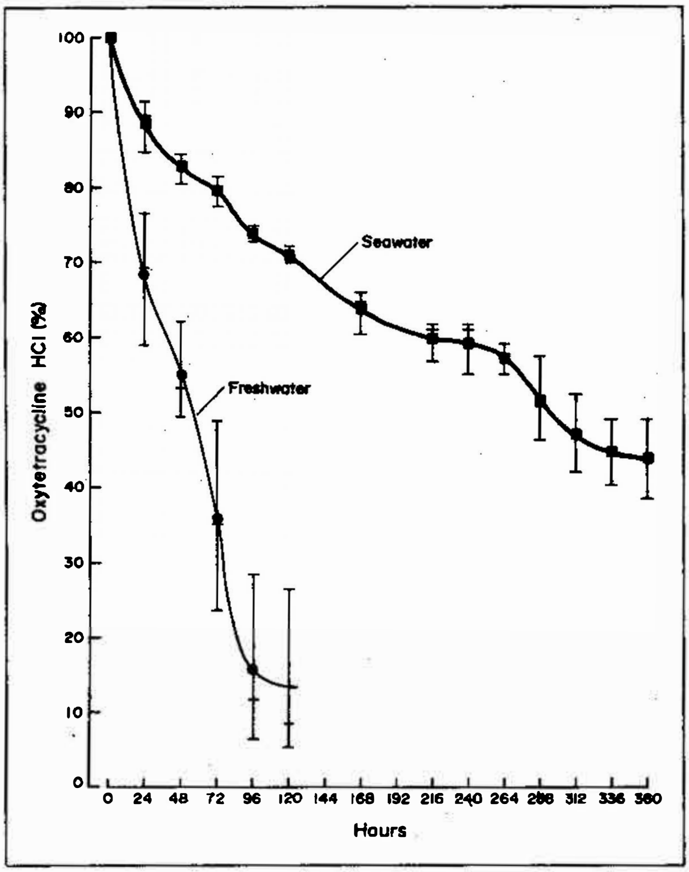

Fig. 1. Degradation of oxytetracycline HCl in fresh- and seawater.

Discussion

The retention time of OTC in fresh- and seawater, as well as the slopes of the standard OTC curves in fresh- and seawater were not significantly different. Therefore, in future work, one set of OTC standard curves prepared in either fresh- or seawater could be used for the simultaneous determination of OTC in fresh- and seawater.

The results obtained from this study indicated that degradation of OTC was slower in seawater compared to freshwater. The results, however, differed from those reported by Lunestad (1991) who calculated the half-life of OTC solutions illuminated with a 20W fluorescent tube to be 30 d in a freshwater solution (pH = 7) and 30 h in seawater.

The half-life of OTC in freshwater (average pH 7.3, average temperature 27°C, with natural lighting) was around 58 h and compares favorably with the half-life of aqueous OTC solution reported by the Merck Index (1983). The half-life of aqueous OTC at 37°C at pH 7.0 was 26 h, and 33 h at pH 8.5. Lighting conditions, however, were not mentioned.

The half-life of OTC in seawater (average pH 7.9, average temperature 27°C, with natural lighting) was 298 h. Samuelsen (1989) reported a half-life of OTC at 15°C under 24-h illumination with a 40-W fluorescent tube to be 128 h and 168 h at 4°C. In total darkness, the half-life at 4°C was 390 h, and at 15°C was 234 h. Samuelsen's results showed that degradation of OTC in seawater was slower at 4°C when compared to 15°C when illuminated, and in total darkness the degradation was also slower at the lower temperature.

A loss of antibacterial activity of OTC in seawater has been reported, due to the complexion of OTC by divalent ions (Peterson et al. 1993). Lunestad and Goksoyr (1990) reported that calcium and magnesium in seawater have been shown to reduce the concentration of free OTC in seawater by 95%.

Due to its short half-life in water, dilution from the aquatic environment, and the loss of antibacterial activity in seawater, the danger posed by OTC in water is probably negligible. Peterson et al. (1993) reported that OTC cannot be considered persistent in the aquatic environment as epimerization and photolysis rapidly degrade OTC. Samuelsen (1989) suggested that OTC is probably most effectively spread over long distances in the aquatic environment in concentrations high enough to affect the fauna when it is bound to particles. Although OTC does not persist in water, it has been found to persist in sediments and could pose adverse effects to the environment (Jacobsen and Berglind 1988; Samuelsen 1989; Björklund et al. 1990, 1991).

In most cases in fish farming, OTC is given to the fish through medicated feeds. Uneaten pellets normally fall through fish cages and settle on bottom sediments where they persist especially under anoxic conditions. Bacteria resistance to OTC in freshwater and marine fish, and sediments have been reported (Aoki et al. 1985; Lewis and Plumb 1985; Björklund et al. 1991; McPhearson et al. 1991; Nygaard et al. 1992). Nygaard et al. (1992) also reported that exposure to OTC would initiate bacterial resistance to other drugs. Moster (1986) found detectable concentrations of OTC in blue mussels 80 m from a farm using this antibiotic. Björklund et al. (1990) reported the presence of OTC in wild fish caught in the vicinity of two farms where medication was used.

Contamination of wild fish could take place when they feed on waste pellets from fish farms. Contamination of filter and detrital feeders such as molluscs could occur when they consume particles bound with OTC. The use of OTC in aquaculture should therefore be regulated because of the danger posed by uneaten medicated pellets and the persistence of OTC in the sediments.

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