*Zoological Studies* **34**(1): 59-61 (1995)

Zoological Studies

# Short Note

## Effect of Indomethacin on Survival of *Aeromonas hydrophila* Infected Tilapia, *Oreochromis mossambicus*

Ching-Lin Tsai* and Kuan-Hong Hoh

*Department of Marine Resources, National Sun Yat-sen University, Kaohsiung, Taiwan 804, R.O.C.*

*(Accepted June 15, 1994)*

**Ching-Lin Tsai and Kuan-Hong Hoh (1995)** Effect of indomethacin on survival of *Aeromonas hydrophila* infected tilapia, *Oreochromis mossambicus*. *Zoological Studies* **34**(1): 59-61. Fish perform behavioral fever when infected by pathogenic bacteria. Behavioral fever is a phenomenon in which body temperature is regulated by an elevated brain set-point, thus raising the preferred temperature of the animal. The mechanisms of how the set-point is regulated in fish has been little studied. In this study, tilapia *Oreochromis mossambicus* were treated, under differing experimental conditions, with *Aeromonas hydrophila*, bacteria pathogenic to bony fish, and with indomethacin, a highly potent inhibitor of prostaglandin synthesis. Results show that indomethacin decreased the survival of *A. hydrophila*-treated *O. mossambicus*, under febrile temperatures (35°C). Indomethacin- and *A. hydrophila*-treated *O. mossambicus* had higher survival rates at normal temperatures than at febrile temperature conditions. These results demonstrate that infection by *A. hydrophila* can elevate the behaviorally preferred temperature of *O. mossambicus*. This process is mediated by prostaglandin.

**Key words:** Prostaglandin, Behavioral fever, Fish.

Most vertebrates regulate their body temperature according to the set-point of the brains central thermostat. Endothermic animals maintain their body temperature by autonomic and behavioral thermoregulatory effectors. Ectothermic animals, such as fish, amphibians and reptiles, which have poorly developed autonomic thermoregulatory system, regulate their body temperatures largely through the utilization of behavioral thermoregulatory effectors (Bligh 1973, Matthew 1986).

Body temperature of both endotherms and ectotherms is determined and regulated by the brains central thermostat (Boulant et al. 1980). Liebermeister et al. (1887) suggested that fever results from central nervous regulation of body temperature due to an elevated set-point. This elevation of the set-point raises the behaviorally preferred temperature of the animal from normal to a febrile temperature. While the preferred temperature is elevated, endothermic animals are able to raise body temperature by increasing heat production and decreasing heat dissipation. Since ectothermic animals are unable to increase their body temperature by autonomic effectors, they will select warmer environmental conditions to raise their body temperature. The phenomenon of selecting a warmer environment to increase body temperature is known as behavioral fever.

Reynolds et al. (1976) and Covert et al. (1977) reported that injection of *Aeromonas hydrophila* induced behavioral fever in goldfish, elevating the preferred temperature from 1°C to 5°C. Selecting a febrile temperature will enhance survival rates of viral-infected fish, because the immunological responses of fish are significantly affected by temperature (Avtalion et al. 1973). Thus, behavioral fever is important for the survival of infected fish. In mammals, it has been suggested that prostaglandin acts directly on the brain thermoregulatory center to raise the set-point of thermoregulation (Watanabe et al. 1987). However, the physiological mechanism of behavioral fever in fish is not clear. In the present study, we investigated the effect of indomethacin (a potent inhibitor of prostaglandin synthesis) on *A. hydrophila*-infected tilapia.

**Materials and Methods**—Specimens of tilapia, *Oreochromis mossambicus*, were obtained from the Tainan Branch of the Taiwan Fisheries Research Institute. They were kept in aquaria at 25°C for one week prior to the experiments. Experiments were performed on fish whose body lengths were $8.6 \pm 0.1$ cm and body weights were $5.2 \pm 0.2$ g. They were kept at a density of ten fish per one 90×60×45 cm aquarium, feed with commercial pellet milk fish feed daily in a room of 10-14 light-dark cycle. Animals were divided into eight groups. In groups $A_{25}$ and $A_{35}$, animals were intraperitoneally injected with indomethacin and *A. hydrophila*, ($5 \times 10^5$ live *A. hydrophila* per gram body weight in 0.2 ml sterile pyrogen-free saline) and maintained at a temperature of 25°C, a normal temperature, and 35°C, a febrile temperature, respectively. These temperatures were controlled by automatic thermocontroller. In groups $B_{25}$ and $B_{35}$, animals were intraperitoneally injected with *A.*

*To whom all correspondence should be addressed.

*hydrophila* then maintained at a fixed temperatures of 25°C or 35°C, respectively. In groups $C_{25}$ and $C_{35}$, animals were injected with indomethacin (50 mg per kg in 0.2 ml sterile pyrogen-free saline), then maintained at fixed temperatures of 25°C or 35°C, respectively. In groups $D_{25}$ and $D_{35}$, animals were injected with sterile pyrogen-free saline, then maintained at fixed temperatures of 25°C or 35°C, respectively. The mortality of fish in each experimental group was monitored for five days after treatment. The bacterial strain, *A. hydrophila* ATCC 7966, was purchased from the Culture Collection and Research Center, Taiwan. The culture media, brain-heart infusion (BHI, Difco), was prepared according to the manufacturer's instructions.

**Results and Discussion**—The results of the present study reveal that five days after treatment indomethacin had decreased survival rates of *A. hydrophila*-infected fish which had been maintained at febrile temperature (Table 1). The Chi-square test was used for the statistical analysis. Survival rates between group $A_{25}$ (90%) and group $B_{25}$ (100%) are not significantly different. Whereas, group $A_{35}$ had a higher mortality (60%) than group $B_{35}$ (10%) ($\chi^2 = 5.924$, $p < 0.05$). On the other hand, the mortality rate of $A_{35}$ (60%) is significantly higher than $A_{25}$ (10%) ($\chi^2 = 5.49$, $p < 0.05$). The study revealed that the injection of indomethacin significantly increased the mortality rate of fish treated with *A. hydrophila* and maintained at febrile temperature (35°C). Table 1 also shows that groups C and D had 100% survival rate in either 25°C or 35°C. Indomethacin is a potent synthetic inhibitor of prostaglandin but not a lethal agent to fish. These results demonstrate that the inhibition of prostaglandin synthesis results in high mortality of infected fish at febrile temperature. Prostaglandin can increase the release of interleukin-1 by modulating the phospholipids of macrophage membranes (Ertel et al. 1993). Therefore, it is possible that prostaglandin is involved in increasing the immunological activity of fish. The survivors of wild largemouth bass infected with *A. hydrophila* have a high serum titre of an IgM-like antibody (Hazen et al. 1981). It was also discovered that the immunological responses of fish increased during moderate fever (Avtalion et al. 1973). Furthermore, fever increases the mobility and activity of leukocytes, enhances the production and function of interferon, and activates T-lymphocytes (Duff and Durum 1982). Thus, we suggest that prostaglandin plays an important role in the high survival rates of fish with bacterial infection at febrile temperatrure by increasing their immunological activity.

It is well known that prostaglandin can act on thermosensitive neurons of the preoptic area/anterior hypothalamus to induce fever (Watanabe et al. 1987). In the present study, *A. hydrophila*- and indomethacin-treated fish had higher survival rates at normal temperature than at febrile temperature. In other words, infected fish with indomethacin treatment at normal temperature survived better than at febrile temperature. Indomethacin is an inhibitor of prostaglandin synthesis. Therefore, when prostaglandin synthesis was inhibited (Groups $A_{25}$ and $A_{35}$), the infected fish survived better at normal temperature than at febrile temperature. This explains why there was a higher survival rate at normal temperature. These results further suggest that prostaglandin may be involved in the elevation of preferred temperature in bony fish. Goldfish with behavioral fever showed a higher survival rate when maintained at febrile temperature (Reynolds et al. 1976, Covert et al. 1977). In the present study, the mortality of tilapia was not found to be related to differences in water temperature between 25°C and 35°C. It has also been reported that *A. hydrophila*-induced mortality is not entirely mediated by water temperature (Groberg et al. 1978, Huizinga et al. 1979). The mechanism of thermoregulation through behavior of fish has been little studied. The results of our study suggest that prostaglandin can play an important role in the behavioral thermoregulation and immunological activity of fish trying to overcome problems of bacterial infections.

**Table 1.** The influence of indomethacin on the mortality of *Aeromonas hydrophila*-infected tilapia, *Oreochromis mossambicus*

| Group | $A_{25}$ | $A_{35}$ | $B_{25}$ | $B_{35}$ | $C_{25}$ | $C_{35}$ | $D_{25}$ | $D_{35}$ |
|---|---|---|---|---|---|---|---|---|
| no. surviving | 9 | 4 | 10 | 9 | 10 | 10 | 10 | 10 |
| no. dead | 1 | 6 | 0 | 1 | 0 | 0 | 0 | 0 |
| mortality (%) | 10 | 60 | 0 | 10 | 0 | 0 | 0 | 0 |
| | | a,* | | a,* | | | | |
| | b,* | b,* | | | c,- | c,- | c,- | c,- |

Fish treated with indomethacin plus *A. hydrophila*, kept at 25°C and 35°C are groups $A_{25}$ and $A_{35}$, respectively; treated with *A. hydrophila*, kept at 25°C and 35°C are groups $B_{25}$ and $B_{35}$, respectively; treated with indomethacin, kept at 25°C and 35°C are groups $C_{25}$ and $C_{35}$, respectively; treated with saline, kept at 25°C and 35°C are groups $D_{25}$ and $D_{35}$, respectively.

a,* significant difference between groups $A_{35}$ and $B_{35}$ ($p < 0.05$)

b,* significant difference between groups $A_{25}$ and $A_{35}$ ($p < 0.05$)

c,- no significant difference between groups $C_{25}$, $C_{35}$, $D_{25}$, and $D_{35}$ ($p > 0.05$)

**Acknowledgements**—We wish to thank Dr. C. H. Hsu, National Sun Yat-sen University, for suppling *A. hydrophila* and the Tainan Branch of the Taiwan Provincial Fisheries Research Institute for providing experimental fish. This work was supported by NSC grant (NSC 81-0209-B-110-506 to Dr. Ching-Lin Tsai).

## References

Avtalion RR, A Wojdani, Z Malik, R Shahrabani, M Duezyminer. 1973. Influence of environmental temperature on the immune response in fish. M. Curr. Top. Microbiol. Immunol. **61:** 1-35.

Bligh J. 1973. Temperature regulation in mammals and other vertebrates. Amsterdam: North-Holland Pub. Co.

Boulant JA. 1980. Hypothalamic control of thermoregulation: neurophysiological basis. *In* Behavioral Studies of the Hypothalamus, Handbook of the Hypothalamus, Vol. 3, part A, eds. PJ Morgane, J Panksepp. New York and Basel: Marcel Dekker Inc., pp. 1-82.

Covert JB, WW Reynolds. 1977. Survival value of fever in fish. Nature **267:** 43-45.

Duff GW, SK Durum. 1982. Fever and immunoregulation: hyperthermia, interleukins 1 and 2, and T-cell proliferation. Yale J. Biol. Med. **55:** 437-42.

Ertel W, MH Morrison, A Ayala, IH Chaudry. 1993. Modulation of macrophage membrane phospholipids by N-3 polyunsaturated fatty-acids increases interleukin-1 release and prevents suppression of cellular-immunity following hemorrhagic-shock. Arch. of Surg. **128:** 15-21.

Groberg WJ, RH McCoy, KS Pilcher, JL Fryer. 1978. Relation

of water temperature to infection of coho salmon (*Onchorhynchus kisutch*), chinook salmon (*O. tshawytscha*) and steelhead trout (*Salmo gairdneri*) with *Aeromonas salmonicida* and *A. hydrophila*. J. Fish. Res. Bd. Can. **35:** 1-7.

Hazen TC, GW Esch, ML Raker. 1981. Agglutinating antibody to *Aeromonas hydrophila* in wild largemouth bass. Trans. Am. Fish. Soc. **110:** 514-518.

Huizinga HW, GW Esch, TC Hazen. 1979. Histopathology of red sore disease (*Aeromonas hydrophila*) in naturally and experimentally infected largemouth bass *Micropterus salmoides* (Lacepede). J. Fish Dis. **2:** 263-277.

Kluger MJ. 1986. Fever: A hot topic. NIPS **1:** 25-27.

Liebermeister C. 1887. Vorlesungen uber specielle Pathologie und Therapie. Vogel, Leipzig. Verlag von F.C.W.

Reynolds WW, ME Casterlin, JB Covert. 1976. Behavioral fever in teleost fishes. Nature **259:** 41-42.

Watanabe T, A Morimoto, N Murakami. 1987. Effect of PGE2 on preoptic and anterior hypothalamic neurons using brain slice preparation. J. Appl. Physiol. **63:** 918-922.

# Indomethacin 對受*Aeromonas hydrophila* 感染吳郭魚存活率之影響

蔡 錦 玲[1] 侯 坤 鋐[1]

魚體受病菌感染時會產生行爲性發燒。行爲性發燒是因爲腦內體溫調節中樞的設定值(set point)提高，造成魚體嗜溫提高並游向高水溫區以增加體溫的現象。本研究以 indomethacin (前列腺素合成抑制劑)投予受 *Aeromonas hydrophila* 感染的吳郭魚，比較其在近常溫 25° 及高溫 35° 下之死亡率。結果顯示 indomethacin 減少受 *A. hydrophila* 感染的吳郭魚在高溫 35° 下的存活率。且，受 *A. hydrophila* 感染並投予 indomethacin 的吳郭魚在近常溫 25° 下比在高溫 35° 下有更高的存活率。此結果說明受 *A. hydrophila* 感染的吳郭魚嗜溫會增加，主要是經由前列腺素的作用。

**關鍵字**：前列腺素，行爲性發燒，魚。

[1] 國立中山大學海洋資源學系