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RESEARCH ARTICLE

Finding Blue Tracks in *Gephyrocharax melanocheir* Fish Similar to the Locations of Acupuncture Meridians after Injecting Alcian Blue

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Abstract
This study investigated whether a meridian-like distribution of Alcian blue (AB) existed after it was injected into a fish's body and suggested a new animal model for meridian study. Twenty *Gephyrocharax melanocheir* fish with translucent bodies were injected with AB at a point near the spinal column or the dorsal fin. Distribution of AB was observed using a digital camera and a stereomicroscope. Three or more obvious blue tracks were found: one along the spinal column, another along the posterior margin of the abdomen extending to the superior margin of the anal fin, and a third along both sides of the dorsal fin. They were similar to the locations of the governor, conceptual vessel, and urinary bladder meridians, respectively, on the human body according to the classic theory of traditional Chinese medicine. A few other blue tracks were also found, which apparently did not correspond to any known meridians. The results show that the tracks of AB share important similarities with the locations of classically described meridians and that they are mainly distributed in the interstitial space around bones and blood vessels and inside muscular interstices. This study may provide a new experimental animal model for exploring acupuncture meridians.

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1. Introduction

Meridians can be defined as special lines distributed longitudinally on the human body that include sites where acupuncture needling or other forms of stimulation are applied for therapeutic purposes. Their distribution does not coincide, to any significant degree, with any known structures such as blood vessels or nerves. Finding anatomical structures that match the distribution of meridians is, therefore, one of the primary questions in meridian research. For many years, scientists have tried, through various techniques, to prove the existence of the meridians or meridian channels that are described in the classical literature as conduits of "Qi" and blood. In 1963, Bong Han Kim [1] claimed to have found meridian channels and described them as tubal structures, which he named the "Bonghan duct". However, such structures were not confirmed in the research from other laboratories, including those from China [2]. It was not until 2002 that the Korean scientists Shin and Soh [3] and Jiang et al [4] began to study Kim's Bonghan ducts and, using Alcian blue (AB), found novel threadlike structures, seemingly similar to Bonghan ducts, in blood vessels, in lymph vessels, and on the surface of internal organs. They carried out a series of experiments, eventually naming this set of structures the primo vascular system (PVS) [5,6]. However, PVS has not been found on peripheral tissue along the meridians using AB.

Zhang's [7] work has shown that meridians and collaterals are composed of two components: vessels and interstices. Vessels circulate blood, whereas interstices circulate Qi. The latter seems to correspond to interstitial fluid (IF) flow. IF flows in interstitial spaces under the condition of a low hydraulic resistance. Zhang et al [8] have undertaken multiple experiments to prove the existence of low hydraulic resistance channels along classically described meridian pathways. If these are indeed one of the essential characteristics of meridian channels, then this model can also well explain meridian phenomena such as propagated sensation along channels [9], low impedance, and high conduction of sound [10]. These experiments were mostly carried out in mini-pigs, which have been shown to be successful animal models for meridian study. However, channels cannot be observed directly in the body of a live mini-pig, making identification of the underlying structures difficult.

Gephyrocharax melanocheir fish are translucent and small in size. We chose this new animal model to see if it would be possible to observe the AB dye tracks directly inside the body of the fish, to determine whether it would diffuse along the routes of meridians and/or the branch collaterals.

2. Materials and methods

2.1. Animals and reagent preparation

Twenty *G. melanocheir* fish, 4.5 ± 0.5 cm in length and 2.4 ± 0.4 cm in width, were purchased from Beijing Laiguangying fish market and used as experimental animals. They were kept in a fish bowl in water at a temperature of 24°C and given full access to food and oxygen. AB dye (8 GX; Sigma Co., USA) was diluted to 1% and filtered through 0.22-μm pore-sized filter paper. Tricain, a widely used fish anesthetic, was diluted to 0.03 g/L to keep the fish in a quiet state.

2.2. Experimental procedure

The *G. melanocheir* fish was put into 0.03 g/L tricain solution. When the fish body became inclined, it was taken out and fixed on a piece of sponge in a Petri dish. A point near the spinal column or dorsal fin (Fig. 1) was injected using an insulin syringe needle connected to a microinjector at a 45° angle with the fish body to a depth of 1 mm. We injected 20—25 μL AB dye at a speed of 1.2 μL/min, controlled by a microinjection pump (KDS-310-PLUS; KD Scientific, USA). During the injection, the narcosis of the fish was adjusted as necessary with a bottle of tricain solution, which was combined with a bottle of water by a three-way valve. The needle was pulled out after the AB dye diffused completely, and the residual dye was washed away with water. Pictures were taken using a digital camera (D5000; Nikon, Japan) and a stereomicroscope (SMZ1000; Nikon) with the help of a cold light illuminator (Fig. 2).

3. Results

Three or more obvious blue tracks were observed after AB was injected. When AB was injected near the spinal column, a blue track appeared along the spinal column, similar to the location of the governor meridian. In eight fish specimens, blue tracks appeared along the spinal column (Figs. 3A and 3B). The differences in some specimens led us to conclude that AB should be injected close to the spinal column to an appropriate depth. If not, distribution of blue tracks would be short and thick, diffusing in many directions (Figs. 3C and 3D). Note that sometimes the color of the tracks looks green because the fish have a reddish colored body.

When AB was injected near the spinal column, another blue track appeared along the posterior margin of the abdomen, extending to the superior margin of the anal fin. This was similar to the location of the conceptual vessel meridian in humans. Five fish displayed such tracks, which were thin and clear (Figs. 4A and 4B). Using a microscope to observe a small segment of the track, we could see that AB was distributed around bones and it was denser on condyles.

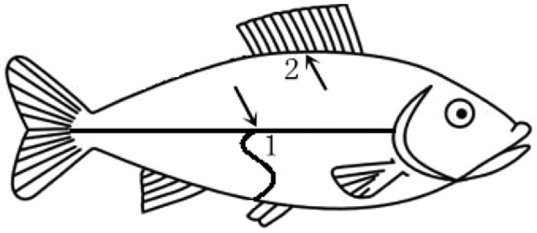

Figure 1 Positions of injection points, as indicated by the arrows.