Available online at www.elixirpublishers.com (Elixir International Journal)

Applied Zoology

Elixir Applied Zoology 89 (2015) 36709-36711

ISSN: 2229-712X

Histopathological Effects of Fluoxetine on Zebrafish (*Danio rerio*) Gills

Nazan Deniz Yön[1], Cansu Akbulut[1,*], Müge Alsaran[1], Burcu Öztürk[1], Figen Esin Kayhan[2], Güllü Kaymak[1] and Belgin Duman Süsleyici[2]

[1]Sakarya University, Science and Letters Faculty Department of Biology, Sakarya, Turkey.
[2]Marmara University, Science and Letters Faculty Department of Biology, İstanbul, Turkey.

ARTICLE INFO

Article history:
Received: 13 October 2015;
Received in revised form:
27 November 2015;
Accepted: 01 December 2015;

Keywords
Fluoxetine,
Zebrafish,
Gill,
Histopathology.

ABSTRACT

Fluoxetine is an antidepressant which is also known as the trade names Prozac, and Sarafem. It is used for the treatment of depressive disorders such as depression, obsessive–compulsive disorder, alcohol dependence and panic disorder. In this study, histopathological effects of fluoxetine on zebrafish gill tissue were investigated. 150 ng/L fluoxetine were exposed to adult individuals and after 15 minutes, 30 minutes, 60 minutes and 8 days fishes were dissected. In 15, 30 and 60 minutes experimental groups, minimal defects were defected. In the 8 day exposure group, thinning at secondary lamellae of gill tissues were observed. Growth were detected in the cell nuclei. Expansion were monitored at the central vena of gills.

Introduction

Fluoxetine is an antidepressant which is also known as the trade names Prozac, and Sarafem[1]. It is used for the treatment of depressive disorders such as depression, obsessive–compulsive disorder, bulimia nervosa, eating disorder, alcohol dependence and panic disorder [2][3]. The bioavailability of fluoxetine is relatively high and peak plasma concentrations are reached in 6 to 8 hours [4].

Fluoxetine is a potent psychotropic drug, acting as aselective serotonin reuptake inhibitor (SSRI) to block the plasmamembrane serotonin transporter, SERT [5] [6] [7] [8]. SSRIs arecurrently the most prescribed psychotropic medications, and fluoxetine is the most commonly used SSRI. Therefore, fluoxetine has rapidly become one of the most important drugs in biomedicine [9] [10] [11]. Paralleling clinical data, multiple experimental animal models, ranging from rodents [6] [10] [11] , to aquatic species [12] [13] , have been developed to address various aspects of SSRI antidepressant action and toxicity.

Fluoxetine is one of the most prescribed psychotropic medications, and is an agent of increasing interest for environmental toxicology. Fish and other aquatic organisms are excellent models to study neuroactive small molecules like fluoxetine [14]. In this study, investigation the histological effects of fluoxetine on zebrafish gill tissue were examined.

Zebrafish, *Danio rerio* (Hamilton-Buchanan, 1822) is a model organism which belongs to the Cyprinidae family. It naturally lives in oxygen-rich fresh waters in Pakistan and India. There are blue and silver stripes on the body of the fish [15]. Its hardiness, short generation time and survival skills to severe environmental changes make them excellent test model.

Material and Methods

Animal

Zebrafish were maintained under the standardized conditions at 28ºC ± 1 C. The light/dark cycle was 14h/10h. Fish system water was maintained pH between 7.2 and 7.7. test chambers were glass aquaria of about a 20-L capacity. The fish were fed once daily in the morning with flake food (Tetra, Germany) and once daily at dusk with frozen blood worm.

Exposure and Experimental Design

In the study, one dose group (150 ng / L fluoxetine) and one control group were determined. After 48 hours of adaptation, fluoxetine were added to the aquarium. For the histological analysis, at the end of 15 minutes, 30 minutes, 60 minutes and 8 days gill tissues were dissected. Tissues were fixed with 10% neutral buffered formalin fluid for 24 h. Tissues were dehydrated and embedded in the parafin wax and sectioned at 5 µm thickness and stained with Hematoxylin Eosin. The samples were evaluated by examining under the light microscope.

Results

In control group, normal gill histology were seen. Primary lamellae which supported by connective and cartilage tissue and secretory cells in secondary lamellae which are between the outer surfaces separated from primary lamellae were monitored clearly (Fig. 1).

In 15 minutes, 30 minutes and 60 minutes to 150 ng / L fluoxetine exposure group, minimal defects were observed. Deformations and openings were detected at central vena in zebrafish gill tissue. Fragmentations were monitored at secondary lamellae. Swelling was observed because of hypertrophy and hyperplasia at the edge of secondary lamellae (Fig. 2).

In the 8 day fluoxetine exposure group, severe degenerations were monitored. Thinning at secondary lamellae of gill tissues were observed. Growth were detected in the cell nuclei. Expansion were monitored at the central vena of gills (Fig. 3).

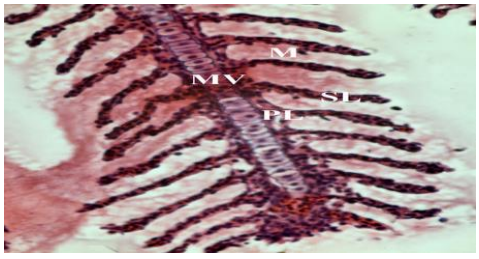

Figure 1. Control group gill tissue; MV: Central vena, PL: Primary lamellae, SL: Secondary lamellae, M: Mucosa cells. H&E X100

Tele: +902642956818
E-mail addresses: cansua@sakarya.edu.tr

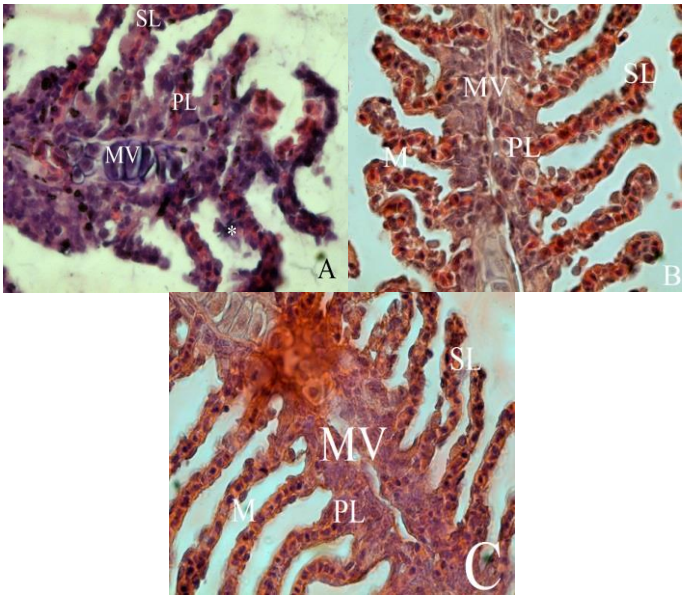

Figure 2. a) 15minutes b) 30 minutes c)60 minutes Experimental group gill tissue: MV: Central vena, PL: Primary lamellae, SL: Secondary lamellae, M: Mucus cell, *: Hyperplasia and Hypertrophy H&E X100

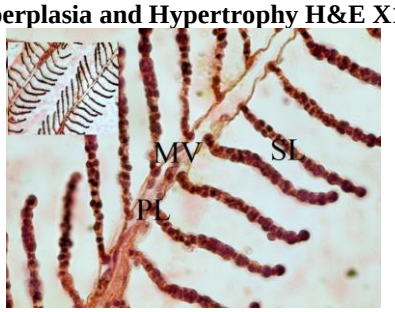

Figure 3. 8 days exposure group gill tissue; MV: Central vena, PL: Primary lamellae, SL: Secondary lamellae H&E X100

Discussion

Gills in direct contact with the external environment and breathing as well as osmoregulation, acid-base in the discharge of nitrogenous waste to the regulation of the balance of the officials and environmental changes and structures affected primarily with the skin of the water chemicals [16].

Saglam and Ural [17] determined to be healthy in the clinic of his work in rainbow trout (Oncorhynchus mykiss), copper sulfate (CuSO4) keeping the 1 ppm, 8 ppm, 16 ppm and 32 ppm concentration was examined the effect on fish. Macroscopically, accumulating CuSO4 on the operculum, tip of fins and gill flaments of fish, sticking each other of gill lamellae, and severe mucus secreting on the hole body surface were observed. Microscopically, pillar and epithelial cells degeneration in the gill lamellae, vacuoler degeneration, and hyperaemia and haemorrhage in the vessel were defined.

Gill tissues are in direct contact with the substance soluble in their environment. Hence the strength of the respiratory epithelium of the gills of fish is determined that no damage will occur to adversely affect overall health [18].

Fanta and colleagues [19] analysed lethal dose and water contamination effects of organophosphate methyl parathion on *Corydoras paleatus* gill and liver. Hyperplasia and edema were detected at gill epithelium. These results are consistent to our study. Similarly, in our study hyperplasia was detected.

Üçüncü and colleagues Dioctyl adipate (DOA) of ourselfs caeruleus, in them study on the impact gill histology the tissues were removed for histological examination. When compared to the untreated and 2.5 ppm acetone exposed controls, the gills of the experimental animals that exposed to 0.75 ppm DOA showed remarkable histopathological changes distinguished as hypertropy, severe hyperplasia, aneurysma, oedema, epithelial lifting, and striking fusion [20]

Lamella take place in epithelial hyperplasia and hypertrophy, increase the transition from defense purposes movement of chemicals in the aquatic environment creates a physical barrier. This barrier effect of narrowing the lamella structure to the support surface fusions. So many well-vascularised respiratory surface, which is thickened, narrowing of the blood tissue and undergo chemical can be prevented to some extent [21].

Hypertrophy and hyperplasia were monitored in fluoxetine exposure groups in our study. Hypertrophy, degenerations and hyperplasia were increased time dependent. Our results indicate that fluoxetine exposure cause degenerations at gill tissues so it can be harmful to aquatic organisms.

References

[1] Wong, D.T., Horng, J.S., Bymaster, F.P., Hauser, K.L., Molloy, B.B. (1974). A selective inhibitor of serotonin uptake: Lilly 110140, 3-(p Trifluoromethylphenoxy)-n-methyl-3-phenylpropylamine. Life Sciences, 15 (3), 471 9.

[2] Randi J. H. (16 September 1999). Neurodevelopmental Disorders: Diagnosis and Treatment. Oxford University Press. ISBN 019512314X. Dech and Budow (1991) were among the first to report the anecdotal use of fluoxetine in a case of PWS to control behavior problems, appetite, and trichotillomania.

[3]https://www.nlm.nih.gov/medlineplus/druginfo/meds/a689006.html (cited 12 October 2015)

[4] Fabbri C., Porcelli S., Serretti A. (2014). From pharmacogenetics to pharmacoge-nomics: the way toward the personalization of antidepressant treatment. Canad.J. Psychiatry, 59, 62–75.

[5] Kalueff A.V., Olivier J.D., Nonkes L.J., Homberg J.R. (2010). Conserved role for theserotonin transporter gene in rat and mouse neurobehavioral endophenotypes. Neurosci. Biobehav. Rev., 34, 373–386.

[6] Murphy D.L., Lesch K.P. (2008). Targeting the murine serotonin transporter: insights into human neurobiology. Nat. Rev. Neurosci., 9, 85–96.

[7] Stewart A.M., Cachat J., Gaikwad S., Robinson K.S., Gebhardt M., Kalueff A.V. (2013). Perspectives on experimental models of serotonin syndrome in zebrafish. Neurochem. Int., 62, 893–902.

[8] Bertorini T.E. (1997). Myoglobinuria, malignant hyperthermia, neuroleptic malignant syndrome and serotonin syndrome. Neurol. Clin., 15, 649–671.

[9] Haberzettl R., Bert B., Fink H., Fox M.A. (2013). Animal models of the serotoninsyndrome: a systematic review. Behav. Brain Res., 256, 328–345.

[10] Kalueff A.V., LaPorte J.L., Murphy D.L. (2008). Perspectives on genetic animal modelsof serotonin toxicity. Neurochem. Int., 52, 649–658.

[11] Egan R.J., Bergner C.L., Hart P.C., Cachat J.M., Canavello P.R., Elegante M.F., Elkhayat S.I., Bartels B.K., Tien A.K., Tien D.H., Mohnot S., Beeson E., Glasgow E., Amri H., Zukowska Z., Kalueff A.V. (2009). Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. Behav. Brain Res., 205, 38–44.

[12] Stewart A.M., Cachat J., Gaikwad S., Robinson K.S., Gebhardt M., Kalueff A.V. (2013). Perspectives on experimental models of serotonin syndrome in zebrafish. Neurochem. Int., 62, 893–902.

[13] Brooks B. W., Foran C. M., Richards S. M., Weston C., Turner P. K., Stanley J. K., Solomon K. R., Slattery M., La Point T. W. (2003) Aquatic ecotoxicology of fluoxetine. Toxicology Letters, 142, 169-183.
[14] Stewart A.M., Grossman L., Nguyen M., Maximino C., Rosemberg D.B., Echevarria D.J., Kalueff A.V. (2014). Aquatic toxicology of fluoxetine: Understanding the knowns and the unknowns. Aquatic Toxicology, 156, 269-273.
[15] Mills D. (1994). Akvaryum Bakımı, İnkılap Kitabevi, İstanbul, Türkiye.
[16] Üreten M., Üçüncü İşisağ S. (2013). Dioktil adipat (DOA) 'ın *Sparus aurata* (Çipura) karaciğer ve solungaç histolojisi üzerine etkileri. Su Ürünleri Dergisi Ege J Aqua Sci, 30(3), 115-122.
[17] Sağlam N., Ural M. (2003). Değişik Yoğunluktaki Bakır Sülfat (CuSO4) Solusyonunda Bırakılan Gökkuşağı Alabalıklarında (Oncorhynchus mykiss) Makroskobik ve Mikroskobik İncelemeler. F. Ü. Fen ve Mühendislik Bilimleri Dergisi, 15(1), 89-97.
[18] Lawrence A. J., Hemingway K. L. (2003). Effects of Pollution on Fish. UK. P: 144- 153 .
[19] Fanta E., Rios F.S., Romão S., Vianna A.C., Freiberger S. (2003). Histopathology of the fish Corydoras paleatus contaminated with sublethal levels of organophosphorus in water and food. Ecotoxicol Environ Saf., 54(2), 119–130.
[20] Üçüncü S.İ., Önen Ö., Ergen G., Üreten M., BOZ E., Seferoğlu K., Gökçe B. (2010). Dioktil Adipat'ın (DOA) Labidochromis caeruleus'un Fryer, 1956 (Cichlidae, Teleostei) Solungaç Histolojisi Üzerine Etkileri. Kafkas Univ Vet Fak Derg, 16 (2), 343-346.
[21] Moeller P.D.R., Morton S.L., Mitchell B.A., Sivertsen S.K., Fairey E.R., Mikulski T.M., Glasgow H., Deamer-Melia N.J., Burkholder J.M., Ramsdell J.S. (2001). Current progress in isolation and characterization of toxins isolated from Pfiesteria piscicida. Environ Health Perspect, 109, 739-743.