Trends in Biotechnology Research
An International Peer-Reviewed Journal
Online and Print Version
www.sciencejournal.in

PLASMA CIRCULATING NUCLEIC ACIDS (CNAs) OF FISH AS POTENTIAL BIOMARKER OF TOXICITY

Reddy P.B., Shehla Ishaque and V. Subhashini
*PG Department of Zoology, Govt.PG Arts and Science College, Ratlam.M.P, India.
PG Department of Zoology, Govt.Madhav Science College, Ujjain, India.
Department of Zoology,KBN Autonomous College,Vijayawada, A.P., India.
(E-mail: reddysir@yahoo.co.in)

ABSTRACT
Unlike mammalian red blood cells, fish erythrocytes contain nuclei (and therefore DNA). It is therefore easy to obtain DNA samples from very small amounts of fish blood. Since many of the contaminants found in the study sites are known mutagens, an index that measures genetic damage could be a useful biomarker. The present investigation is aimed to study the changes in circulating nucleic acids (CNA) and total protein in a catfish Mystus tengara as biomarkers in combination with chemical analysis of the water from different sites along the whole course of the river Chambal at Nagda (M.P). The effluents were collected from non point source of River Chambal in different research stations in different seasons of the year (2010-11) to reflect seasonal influence. Physico chemical parameters such as COD, BOD, TSS, TS and TDS were assessed. They were found to be much higher than the standard quality. Fish were sampled in all seasons and from all sampling stations during 2010 and 2011. Blood was assayed for circulating, DNA, RNA and total plasma protein. Results clearly indicate that total protein, DNA and RNA content were minimum in control group (station 1) than experimental groups. These DNA, RNA and small RNA molecules are thought to come from dying cells that release their contents into the blood as they break down. Circulating nucleic acids (CNA) are present in small amounts in the plasma of healthy individuals. Therefore large amount of CNA was found in fish from station 2 and 3.It was concluded that the content of plasma CNA can be used as sensitive tool as molecular diagnosis.

KEY WORDS: Biomarkers, Circulating nucleic acids, Effluent, *Mystus tengara*

INTRODUCTION

The majority of environmental pollutants are threatening initially human and environmental health but also the integrity and function of ecosystems. (Newman, 1998, Walker et al, 2004).The pollutants can become a major threat to the health of the aquatic ecosystem due to their accumulation in the tissues of various species. Also, the pollutants disperse through the biomagnifications into higher trophic levels across the food chains reaching areas of significant human activity (Reddy and Baghel, 2010, 2012, Reddy and Renu singh, 2011, Clark, 2006, Galloway et al, 2002). In order to evaluate the environmental impact of these pollutants on the ecosystem it has become important for a rapid assessment of their toxic effects on the aquatic organisms.

The blood indices, changes in circulating nucleic acids, total protein, due to exposure to mutagenic inorganic and organic xenobiotics, liver function tests and kidney function tests etc. are commonly used in pollution assessment studies. Such eco toxicological studies are using biomarkers in order to establish the risk of environmental pollution to key components in the ecosystem. Measurements of toxicity in sensitive species (sentinel species) like fish can be used as an early warning of population decline and as an ecologically relevant endpoint (Carlson, 2007).

Unlike mammalian red blood cells, fish erythrocytes contain nuclei (and therefore DNA). It is therefore easy to obtain DNA samples from very small amounts of fish blood. Since many of the contaminants found in the study sites are known mutagens, an index that measures genetic damage could be a useful biomarker. Furthermore, genetic damage indicators are direct measurements of the effects of contaminants, and would not be affected by sampling methods or handing stress. These DNA, RNA and small RNA molecules are thought to come from dying cells that release their contents into the blood as they break down. Circulating nucleic acids (CNA) are present in small amounts in the plasma of healthy individuals. However, increased levels of plasma CNA have been reported in a number of clinical disorders. CNA has received special attention because of its potential application as a non-invasive, rapid and sensitive tool for molecular diagnosis. The origin, nature and the precise mechanism(s) as to how nucleic acids end up extracellularly are not fully understood. Accumulating evidence suggests that these molecules are preferentially released in circulation in the form of nucleosomes through apoptosis and necrosis. In addition, other types of nucleic acids have been detected in the circulation that includes DNA, RNA, mitochondrial DNA and microRNA. Although CNAs are shown to have promising diagnostic utility as biochemical and genetic biomarkers for a variety of pathologies especially cancer, there is deficiency in our knowledge about the functional significance of CNAs. Unlike mammalian red blood cells, fish erythrocytes contain nuclei (and therefore DNA). It is therefore easy to obtain DNA samples from very small amounts

of fish blood. Since many of the contaminants found in the study sites are known mutagens, an index that measures genetic damage could be a useful biomarker. Furthermore, genetic damage indicators are direct measurements of the effects of contaminants, and would not be affected by sampling methods or handing stress. Therefore, an attempt has been made to estimate serum nucleic acids in catfish Mystus tengara at different sites of Chambal River, in different seasons of the year at Nagda.

MATERIAL AND METHODS

Study Area

Chambal River in Nagda is very close to tropic of cancer at 23'27 N and 75'25 and 517 meters above MSL. More than one lakh of residents in and around the Nagda rely on water from Chambal River for public use, industrial supplies, power plant cooling and waste water treatment. The river receives water from different units of Industrial complex .Waste after coming from the factory complex runs in a channel for about 3 km and joins River Chambal near Juna Nagda.

Sampling

Effluents were collected in sterilized phosphate free cleaned polythene bottles, near Mukteswar temple at Juna Nagda where discharges of industrial complex and domestic waste are drained into this station. The samples after collection were immediately placed in dark boxes and processed for physico chemical analysis like pH, electrical conductivity (EC), total dissolved solids (TDS), total hardness (TH) dissolved oxygen (DO), Biochemical oxygen demand (BOD) and chemical oxygen demand (COD).The procedure for analysis followed 'Standard methods of analysis of water and waste water (APHA).

Test organism

Mystus tengara (Ham.) is a common Indian catfish. Living healthy specimens of fishes of approximately same size (irrespective sex) were collected during 2009-10, from local fresh water sources from different research stations along with river.

Collection of blood sample

Blood was collected from the caudal region by puncturing the peduncle with a sharp knife. The blood sample collected in the watch glass was gently stirred with a clean, dry, thin, blunt glass rod and covered with a petridish and kept in a dry place away from light.

Plasma Nucleic acids

DNA was estimated by diphenylamine method as described by Plummer. The estimation of RNA was done by orcinol method described by Plummer. Plasma total protein was estimated according to Lowry et al, (1951).

RESULTS

Water analysis: A summary of physico chemical parameters obtained in Chambal River for all the different stations are shown in Table. Results clearly indicate that the physico chemical parameters monitored in station 2 and 3 showed high levels of BOD, COD, TDS, TSS, EC and low DO in all seasons and exceeds the limits WHO standards. This must have been as a result of the nature of effluents discharged from the industries. However, pH and temperature did not show much variation in all sampling stations in all seasons.

Total protein

The total plasma protein content values of fish of Station 2 and 3 were decreased ($P < 0.05$) in all seasons.

Plasma DNA and RNA

CNA (circulating DNA, RNA) content of fish was minimum from station 1 when compared to other research stations ($P > 0.001$) and it was significantly higher in the fish of station 3 in all seasons of the year.

Table 1. Annual Changes in circulating DNA and RNA and total plasma protein content in Mystus tengara of Chambal River in different segments and different seasons of the year. Season Rainy Winter Summer

Parameter	S 1	S 2	S3	S1	S2	S3	S1	S2	S3
DNA (mg/L)	2.1±0.3	3.1±0.2	4.7±0.1*	1.8±0.1	8.1±2.1**	9.1±0.3*	2.2±0.1	12.1±0.4*	13.9±0.5
RNA (mg/L)	22.1±1.1	26±1.8	24.1±0.2NS	16.1±1.1	29.3±1.1**	44.1±2.1*	21.1±0.6	34.1±3.1*	46.1±4.1**
Total proteinmg/L	3.4±0.32	2.95±0.11*	1.89±0.37**	3.5±0.34	3.0±0.28NS	2.4±0.22*	3.40.22±	2.8±0.41*	2.9±0.44*

DISCUSSION

Most of the DNA and RNA in the body are located within cells, but a small amount of nucleic acids can also be found circulating freely in the blood. These DNA, RNA and small RNA molecules are thought to come from dying cells that release their contents into the blood as they break down. Circulating DNAs offer a non-invasive approach to a wide range in diagnostics of clinical disorders that will allow the basic information necessary not only for use in predictive medicine but also for direct use in acute medicine. Further free CNAs offer unique opportunities for early diagnosis of clinical conditions. The recent interest in nucleic acids in plasma and serum has opened up numerous new areas of investigation and new possibilities for molecular diagnosis. These findings have important implications for the detection, monitoring, and prognostication of many types of malignancies caused by the secondary infections in fish due to pollution in water. Although blood indices have proven useful for numerous diagnostic applications, the molecular diagnosis of diseases requires advance technology. For these reasons, the finding of circulating cell-free DNA in the blood of healthy and diseased individuals has gained increasing attention during the last few years.

Our results clearly indicate that DNA and RNA content in the fish from polluted stations of Chambal River (2 and 3) significantly increased ($P>0.01$) in different seasons of the year. Circulating nucleic acids (CNA) are present in small amounts in the plasma of healthy individuals. However, increased levels of plasma CNA have been reported in a number of clinical disorders like cancer, stroke, trauma, myocardial infarction, autoimmune disorders, and spawning – associated complications. (Vishnu Swaroop and Rajeswari, 2007). It is known that the products of DNA/RNA in plasma actually arise from cell death or by lysis of tumor cells which was also evidenced by histopatholoigcal alterations in various organs (Reddy and Baghel, 2012). Therefore, the increased levels of circulating nucleic acids (DNA and RNA) in blood of fish can be used as a non-invasive, rapid, sensitive and accurate method of diagnosis of several diseases of fish including human. The presence of DNA and RNA in the plasma of Mystus tengara in the present investigation may be due to break down of blood cells, brake down of any pathogens, e.g. bacteria or viruses, leukocyte surface DNA, apoptosis or necrosis of various organs (Holdenrieder *et al.*, 2005). The nucleic acid in question can be RNA, mitochondrial DNA or genomic DNA, but DNA is generally used as it is less labile than RNA. Necrosis is clearly an option for the origin of Circulating Nucleic Acids in Plasma and Serum (CNAPS). Apoptosis is confirmed as a major DNA source especially since nucleosomes are present in the blood. Naked DNA fragments are also found in serum, possibly due to apoptosis. RNA is only recently of importance through its exploitation in clinical diagnosis and prognosis. The stability of RNA or microRNA in the bloodstream is due to the availability and type of the RNAs and RNAs present. However, a newly synthesised RNA is released spontaneously from cells together with the DNA-lipoprotein complex. In consequence, RNA is primarily released by apoptosis and through the DNA/RNA-lipoprotein complex. Some RNA may also be derived by necrosis e.g. some mRNAs.

Plasma proteins

Plasma proteins largely consist of albumin and globulins such as immunoglobulins, carrier proteins, and acute phase reactants. Elevated globulin levels are concerning. Elevated proteins is whether there is an increase in multiple immunoglobulins (i.e. polyclonal gammopathy like HIV, viral hepatitis, liver disease, connective tissue disease or anything that stimulates a generalized immune response) or in one specific 'clone' (i.e. monoclonal, produced by a malignant plasma cell or other B-cell malignancy). Blood plasma is not only the most studied among biological fluids, but also the primary material for disease diagnosis. Blood plasma contains a very high concentration of proteins, typically in the range of 60-80 mg/of protein per ml. The elevated level of plasma Total protein in experimental fish may be due to increase in immunoglobulins to fight with various variety of xenobiotics and diseases. Each protein has a potential for a variety of post-translational and metabolic modifications, both in normal and diseased cells.

CONCLUSIONS

The work presented here only threatened the chemical quality of the effluent, but it is equally important to extend the study to include a) measurement of a range of biological as well as physicochemical properties of soil which receive this polluted water for irrigation purposes b) identification and chemical analysis of plants grown on soils receiving this water and c) microbial analysis of soil (Sail et al, 2006).From the data obtained in this research must have been as a result of the nature of effluents discharged from the industries. Accordingly, water from these sampling stations is not free from the pollution and cannot be used for domestic purposes, drinking and even for agriculture.It was concluded that the content of plasma circulating DNA and circulating RNA can be used as sensitive tool as molecular diagnosis.

REFERENCES

APHA. (1985). Standard methods of for the examination of water and waste water. American Public Health Association, Washington DC, pp1244.

Carlsson, Gunnar (2007). *Fish and amphibians as test organisms for evaluation of effects caused by chemicals.* Sveriges lantbruksuniv., Acta Univ. Agricul.Sueciae. 1652-6880.

Clark R.B. (2006). Marine Pollution, 5th edition. Oxford University Press, Oxford .

Dick P.T. and Dixon D.G. (1985). Changes in circulating blood cell levels of rainbow trout, *Salmo gairdneri* Richardson, following acute and chronic exposure to copper. *J. Fish Biol.* 26:475–481.

Galloway T.S. (2002). Biomarkers in environmental and human health risk assessment. Mar Poll Bullet 53:606-613,

Goetz W. (1980). Diagnostik. Von Lebererkran- Kungen (In German). G.l.T. Verlag Ernst Giebeler, Darmstedt. Germany.

Holdenrieder S., Stieber P., Chan L.Y., Geiger S., Kremer A., Nagel D. and Lo Y.M. (2005). Cell-free DNA in serum and plasma: comparison of ELISA and quantitative PCR. Clin Chem. 2005 51(8): 1544-1546.

Hutchinson T.H., Field M.D.R. and Manning M.J. (1999). Evaluation of immune function in juvenile turbot (*Scopthalmus maximus* L.) exposed to sediments contaminated with polychlorinated biphenyls. *Fish. Shellfish Immunol.* 9:457–472.

Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.I. (1951). Protein measurement with folin phenol reagent. *J. Biol. Chem.* 193: 265-275.

Newman M.C. (1998). Fundamentals of Ecotoxicology. Ann Arbor Press, Chelsea, MI, persistent organic pollutant risk to human and wildlife. *Human Ecol. Risk Assess.* 9:303-324.

Plummer D. (1962). In 'Practical biochemistry'. Academic press. London,

Reddy P.B. and Baghel B.S. (2010). Impact of the industrial effluent on physico chemical characteristics of Chambal River at Nagda, M.P. India. *Nature. Pollu. Tech.* 3: 519-526.

Reddy P.B. and Renusingh. (2011). Biomarker responses of industrial effluent in cat fish. In' Proc. Int. Conference. Green Tech. *Envi.Conserv.* 265-269.

Reddy P.B. and Baghel B.S. (2012). Impact of Industrial waste water on the Chambal River and Biomarker responses in fish due to pollution at Nagda.M.P.India. *DAV Int. J. Sci.* 1(1): 86-91

Vishnu Swarup, M.R. Rajeswari. (2007). Circulating (cell-free) nucleic acids– A promising, non-invasive tool for early detection of several human diseases. *FEBS Lett.* 581:795–799.

Walker C.H., Hopkin S.P., Sibly R.M., Peakall D.M. (2004). Principles of Ecotoxicology, 3rd edition. Taylor and Francis, New York.

