Chapter 18

Global Warming Induced Endosymbiotic Actinidic Archaea and Viroids - Role in Genomic Regulation

Introduction

Climate change and related stress leads to increased porphyrin synthesis leading onto porphyrinuria and porphyria. The stimulus for porphyrin synthesis comes from heme deficiency. Heme suppresses ALA synthase. Stress induces heme oxygenase which converts heme to carbon monoxide and bilirubin. Thus heme is depleted from the system. Heme oxygenase is induced by environmental stress. Climatic changes of global warming and ice age can induce heme oxygenase. Heme oxygenase is also induced by EMF pollution of the environment. Thus there is increased porphyrin synthesis from succinyl CoA and glycine. The porphyrins can self organize to form macromolecular structures which can self replicate to form a porphyrin organism.[1] The photon induced transfer of electrons along the macromolecule can lead to light induced ATP synthesis. The porphyrins can form a template on which RNA and DNA can form generating viroids. The porphyrins can also form a template on which prions can form. They all can join together - RNA viroids, DNA viroids, prions - to form primitive archaea. Thus the archaea are capable of self replication on porphyrin templates. The self replicating archaea can sense gravity which gives rise to consciousness. They can also sense the anti-gravity fields which gives rise to the unconscious brain. Thus there can be both self replicating archaea and anti-archaea regulating the conscious and unconscious brain. Thus the climate change stress mediated increased porphyrin synthesis leads to prefrontal cortex atrophy, cerebellar dominance, cerebellar cognitive affective disorder, quantal perception and Neanderthalisation of the population. The porphyrions are self replicating supramolecular organisms which forms the precursor template on which the viroids, prions and nanoarchaea originate. Stress induced template directed abiogenesis of porphyrions, prions, viroids and archaea is a

continuous process and can contribute to changes in brain structure and behavior as well as disease process.

A hypothesis regarding the role of endosymbiotic actinidic archaea and viroids in genomic regulation is put forward. Endomyocardial fibrosis (EMF) along with the root wilt disease of coconut is also endemic to Kerala with its radioactive actinide beach sands. Actinides like rutile producing intracellular magnesium deficiency due to rutile-magnesium exchange sites in the cell membrane has been implicated in the etiology of EMF.[1] Endogenous digoxin, a steroidal glycoside which functions as a membrane sodium-potassium ATPase inhibitor has also been related to its etiology due to the intracellular magnesium deficiency it produces.[2] Organisms like phytoplasmas and viroids have also been demonstrated to play a role in the etiology of these diseases.[3, 4] Endogenous digoxin has been related to the pathogenesis of Huntington's disease and trisomy 21.[2] The possibility of endogenous digoxin synthesis by actinide based primitive organism like archaea with a mevalonate pathway and cholesterol catabolism was considered.[5-8] The role of archaeal viroids was also studied. An actinide dependent shadow biosphere of archaea and viroids in the above mentioned disease states is described.[6]

Materials and Methods

Informed consent of the subjects and the approval of the ethics committee were obtained for the study. The following groups were included in the study: - Huntington's disease and trisomy 21. There were 10 patients in each group and each patient had an age and sex matched healthy control selected randomly from the general population. The blood samples were drawn in the fasting state before treatment was initiated. Plasma from fasting heparinised blood was used and the experimental protocol was as follows (I) Plasma+phosphate buffered

saline, (II) same as I+cholesterol substrate, (III) same as II+rutile 0.1 mg/ml, (IV) same as II+ciprofloxacine and doxycycline each in a concentration of 1 mg/ml. Cholesterol substrate was prepared as described by Richmond.[9] Aliquots were withdrawn at zero time immediately after mixing and after incubation at 37℃ for 1 hour. The following estimations were carried out: - Cytochrome F420, free RNA, free DNA, polycyclic aromatic hydrocarbon, hydrogen peroxide, pyruvate, glutamate, hexokinase, ATP synthase, HMG CoA reductase, digoxin and bile acids.[10-13] Cytochrome F420 was estimated flourimetrically (excitation wavelength 420 nm and emission wavelength 520 nm). Polycyclic aromatic hydrocarbon was estimated by measuring hydrogen peroxide liberated by using glucose reagent. The statistical analysis was done by ANOVA.

Results

The parameters checked as indicated above were: - cytochrome F420, free RNA, free DNA, polycyclic aromatic hydrocarbon, hydrogen peroxide, pyruvate, glutamate, cytochrome C, hexokinase, ATP synthase, HMG CoA reductase, digoxin and bile acids. Plasma of control subjects showed increased levels of the above mentioned parameters with after incubation for 1 hour and addition of cholesterol substrate resulted in still further significant increase in these parameters. The plasma of patients showed similar results but the extent of increase was more. The addition of antibiotics to the control plasma caused a decrease in all the parameters while addition of rutile increased their levels. The addition of antibiotics to the patient's plasma caused a decrease in all the parameters while addition of rutile increased their levels but the extent of change was more in patient's sera as compared to controls. The results are

expressed in tables 1-6 as percentage change in the parameters after 1 hour incubation as compared to the values at zero time.

Table 1 *Effect of rutile and antibiotics on cytochrome F 420 and ATP synthase.*

Group	CYT F420 % (Increase with Rutile)		CYT F420 % (Decrease with Doxy)		ATP synthase % (Increase with Rutile)		ATP synthase % (Decrease with Doxy)	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Normal	4.48	0.15	18.24	0.66	4.40	0.11	18.78	0.11
HD	21.68	1.90	57.93	9.64	22.60	1.64	66.86	4.21
Trisomy 21	22.78	2.19	58.97	8.84	23.34	1.58	65.76	3.91
F value	306.749		130.054		449.503		673.081	
P value	< 0.001		< 0.001		< 0.001		< 0.001	

Table 2 *Effect of rutile and antibiotics on free DNA and RNA.*

Group	DNA % change (Increase with Rutile)		DNA % change (Decrease with Doxy)		RNA % change (Increase with Rutile)		RNA % change (Decrease with Doxy)	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Normal	4.37	0.15	18.39	0.38	4.37	0.13	18.38	0.48
HD	22.12	2.44	63.69	5.14	23.33	1.35	66.83	3.27
Trisomy 21	23.49	1.19	64.63	6.58	23.22	1.35	66.42	4.21
F value	337.577		356.621		427.828		654.453	
P value	< 0.001		< 0.001		< 0.001		< 0.001	

Table 3 *Effect of rutile and antibiotics on HMG CoA reductase and PAH.*

Group	HMG CoA R % change (Increase with Rutile)		HMG CoA R % change (Decrease with Doxy)		PAH % change (Increase with Rutile)		PAH % change (Decrease with Doxy)	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Normal	4.30	0.20	18.35	0.35	4.45	0.14	18.25	0.72
HD	22.72	1.89	64.51	5.73	22.61	1.42	64.48	6.90
Trisomy 21	23.82	1.78	61.63	7.96	24.06	1.50	63.01	7.76
F value	319.332		199.553		391.318		257.996	
P value	< 0.001		< 0.001		< 0.001		< 0.001	

Table 4 *Effect of rutile and antibiotics on digoxin and bile acids.*

Group	Digoxin (ng/ml) (Increase with Rutile)		Digoxin (ng/ml) (Decrease with Doxy+Cipro)		Bile acids % change (Increase with Rutile)		Bile acids % change (Decrease with Doxy)	
	Mean	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**
Normal	0.11	0.00	0.054	0.003	4.29	0.18	18.15	0.58
HD	0.53	0.08	0.205	0.041	22.21	2.04	63.84	6.16
Trisomy 21	0.52	0.08	0.208	0.031	21.65	2.37	65.91	4.82
F value	135.116		71.706		290.441		203.651	
P value	< 0.001		< 0.001		< 0.001		< 0.001	

Table 5 *Effect of rutile and antibiotics on pyruvate and hexokinase.*

Group	Pyruvate % change (Increase with Rutile)		Pyruvate % change (Decrease with Doxy)		Hexokinase % change (Increase with Rutile)		Hexokinase % change (Decrease with Doxy)	
	Mean	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**
Normal	4.34	0.21	18.43	0.82	4.21	0.16	18.56	0.76
HD	21.91	1.71	58.45	6.66	22.88	1.87	65.45	5.08
Trisomy 21	20.95	1.35	60.24	7.16	22.88	1.98	65.45	5.49
F value	321.255		115.242		292.065		317.966	
P value	< 0.001		< 0.001		< 0.001		< 0.001	

Table 6 *Effect of rutile and antibiotics on hydrogen peroxide and glutamate.*

Group	H_2O_2 % change (Increase with Rutile)		H_2O_2 % change (Decrease with Doxy)		Glutamate % change (Increase with Rutile)		Glutamate % change (Decrease with Doxy)	
	Mean	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**
Normal	4.43	0.19	18.13	0.63	4.40	0.10	18.48	0.39
HD	23.52	1.49	63.24	7.36	23.20	1.57	66.65	4.26
Trisomy 21	23.76	1.48	62.14	6.76	23.41	1.55	66.36	4.31
F value	380.721		171.228		372.716		556.411	
P value	< 0.001		< 0.001		< 0.001		< 0.001	

Abbreviation

HD - Huntington's disease

Discussion

There was increase in cytochrome F420 indicating archaeal growth. The archaea can synthesize and use cholesterol as a carbon and energy source.[14, 15] The archaeal origin of the enzyme activities was indicated by antibiotic induced suppression. The study indicates the presence of actinide based archaea with an alternate actinide based enzymes or metalloenzymes in the system as indicated by rutile induced increase in enzyme activities.[16] There was also an increase in archaeal HMG CoA reductase activity indicating increased cholesterol synthesis by the archaeal mevalonate pathway. The archaeal beta hydroxyl steroid dehydrogenase activity indicating digoxin synthesis and archaeal cholesterol hydroxylase activity indicating bile acid synthesis were increased.[7] The archaeal cholesterol oxidase activity was increased resulting in generation of pyruvate and hydrogen peroxide.[15] The pyruvate gets converted to glutamate and ammonia by the GABA shunt pathway. The archaeal aromatization of cholesterol generating PAH was also detected.[17] The archaeal glycolytic hexokinase activity and archaeal extracellular ATP synthase activity were increased. The archaea can undergo magnetite and calcium carbonate mineralization and can exist as calcified nanoforms.[18]

There was an increase in free RNA indicating self replicating RNA viroids and free DNA indicating generation of viroid complementary DNA strands by archaeal reverse transcriptase activity. The actinides modulate RNA folding and catalyse its ribozymal action. Digoxin can cut and paste the viroidal strands by modulating RNA splicing generating RNA viroidal diversity. The viroids are

evolutionarily escaped archaeal group I introns which have retrotransposition and self splicing qualities.[19] Archaeal pyruvate can produce histone deacetylase inhibition resulting in endogenous retroviral (HERV) reverse transcriptase and integrase expression. This can integrate the RNA viroidal complementary DNA into the noncoding region of eukaryotic non coding DNA using HERV integrase as has been described for borna and ebola viruses.[20] The noncoding DNA is lengthened by integrating RNA viroidal complementary DNA with the integration going on as a continuing event. The archaea genome can also get integrated into human genome using integrase as has been described for trypanosomes.[21] The integrated viroids and archaea can undergo vertical transmission and can exist as genomic parasites.[20, 21] This increases the length and alters the grammar of the noncoding region producing memes or memory of acquired characters.[22] The viroidal complementary DNA can function as jumping genes producing a dynamic genome important in storage of synaptic information, HLA gene expression and developmental gene expression. The RNA viroids can regulate mrna function by RNA interference.[19] The phenomena of RNA interference can modulate T cell and B cell function, insulin signaling lipid metabolism, cell growth and differentiation, apoptosis, neuronal transmission and euchromatin/ heterochromatin expression.

The integration of archaea and RNA viroid complementary DNA can modulate the genomic structure. The integration of nanoarchaea and viroids in to the eukaryotic and human genome produces a chimera which can multiply producing biofilm like multicellular structures having a mixed archaeal, viroidal, prokaryotic and eukaryotic characters which is a regression from the multicellular eukaryotic tissue. This results in a new genotype leading to human diseases like Huntington's disease and trisomy 21. The microchimeras formed can lead to polyploidy and trisomy 21. The microchimera develops proof reading errors leading to the formation of trinucleotide repeats. DNA

polymerase is the proof reading enzyme of the DNA. In the presence of endogenous digoxin there is sodium potassium ATPase inhibition and intracellular magnesium depletion leading on to defects in proof reading function of DNA polymerase in the nucleus during DNA replication. Defective function of DNA polymerase and the proof reading defects leads to generation of trinucleotide repeats. Intracellular magnesium depletion results in defective phosphorylation of MAP (microtubule associated protein). This results in defective microtubule related spindle fiber function and chromosomal non disjunction contributing to trisomy 21. The integrated viroidal RNA complementary DNA can form jumping genes producing defects in gene coding for different proteins and genetic disease. The HERV genes can also function as jumping genes producing defects coding genes as seen in Lesch nyhan syndrome, neurofibromatosis and genetic hyperlipidemias. Thus the integrated actinidic archaea and viroidal RNA complementary DNA can regulate genomic DNA and RNA function.

References

[1] Valiathan M. S., Somers, K., Kartha, C. C. (1993). *Endomyocardial Fibrosis.* Delhi: Oxford University Press.

[2] Kurup R., Kurup, P. A. (2009). *Hypothalamic digoxin, cerebral dominance and brain function in health and diseases*. New York: Nova Science Publishers.

[3] Hanold D., Randies, J.W. (1991). Coconut cadang-cadang disease and its viroid agent, *Plant Disease,* 75, 330-335.

[4] Edwin B. T., Mohankumaran, C. (2007). Kerala wilt disease phytoplasma: Phylogenetic analysis and identification of a vector, *Proutista moesta*, *Physiological and Molecular Plant Pathology,* 71(1-3), 41-47.

[5] Eckburg P. B., Lepp, P. W., Relman, D. A. (2003). Archaea and their potential role in human disease, *Infect Immun,* 71, 591-596.

[6] Adam Z. (2007). Actinides and Life's Origins, *Astrobiology*, 7, 6-10.

[7] Schoner W. (2002). Endogenous cardiac glycosides, a new class of steroid hormones, *Eur J Biochem*, 269, 2440-2448.

[8] Davies P. C. W., Benner, S. A., Cleland, C. E., Lineweaver, C. H., McKay, C. P., Wolfe-Simon, F. (2009). Signatures of a Shadow Biosphere, *Astrobiology*, 10, 241-249.

[9] Richmond W. (1973). Preparation and properties of a cholesterol oxidase from nocardia species and its application to the enzymatic assay of total cholesterol in serum, *Clin Chem*, 19, 1350-1356.

[10] Snell E. D., Snell, C. T. (1961). *Colorimetric Methods of Analysis.* Vol 3A. New York: Van Nostrand.

[11] Glick D. (1971). *Methods of Biochemical Analysis.* Vol 5. New York: Interscience Publishers.

[12] Colowick, Kaplan, N. O. (1955). *Methods in Enzymology.* Vol 2. New York: Academic Press.

[13] Maarten A. H., Marie-Jose, M., Cornelia, G., van Helden-Meewsen, Fritz, E., Marten, P.H. (1995). Detection of muramic acid in human spleen, *Infection and Immunity*, 63(5), 1652-1657.

[14] Smit A., Mushegian, A. (2000). Biosynthesis of isoprenoids via mevalonate in Archaea: the lost pathway, *Genome Res*, 10(10), 1468-84.

[15] Van der Geize R., Yam, K., Heuser, T., Wilbrink, M. H., Hara, H., Anderton, M. C. (2007). A gene cluster encoding cholesterol catabolism in a soil actinomycete provides insight into Mycobacterium tuberculosis survival in macrophages, *Proc Natl Acad Sci USA*, 104(6), 1947-52.

[16] Francis A. J. (1998). Biotransformation of uranium and other actinides in radioactive wastes, *Journal of Alloys and Compounds*, 271(273), 78-84.

[17] Probian C., Wülfing, A., Harder, J. (2003). Anaerobic mineralization of quaternary carbon atoms: Isolation of denitrifying bacteria on pivalic acid (2,2-Dimethylpropionic acid), *Applied and Environmental Microbiology*, 69(3), 1866-1870.

[18] Vainshtein M., Suzina, N., Kudryashova, E., Ariskina, E. (2002). New Magnet-Sensitive Structures in Bacterial and Archaeal Cells, *Biol Cell*, 94(1), 29-35.

[19] Tsagris E. M., de Alba, A. E., Gozmanova, M., Kalantidis, K. (2008). Viroids, *Cell Microbiol*, 10, 2168.

[20] Horie M., Honda, T., Suzuki, Y., Kobayashi, Y., Daito, T., Oshida, T. (2010). Endogenous non-retroviral RNA virus elements in mammalian genomes, *Nature*, 463, 84-87.

[21] Hecht M., Nitz, N., Araujo, P., Sousa, A., Rosa, A., Gomes, D. (2010). Genes from Chagas parasite can transfer to humans and be passed on to children. Inheritance of DNA Transferred from American Trypanosomes to Human Hosts, PLoS ONE, 5, 2-10.

[22] Flam F. (1994). Hints of a language in junk DNA, *Science*, 266, 1320.