2

A Cholesterol and Actinide Dependent Shadow Biosphere of Archaea and Viroids in Systemic Lupus Erythematosis, Multiple Sclerosis and Rheumatoid Arthritis

Introduction

Actinides like rutile, endogenous digoxin as well as organisms like phytoplasmas and viroids have been implicated in the etiology of systemic lupus erythematosis, multiple sclerosis and rheumatoid arthritis.[1-4] Endogenous digoxin has been related to the pathogenesis of systemic lupus erythematosis, multiple sclerosis and rheumatoid arthritis.[4] The possibility of endogenous digoxin synthesis by actinide based primitive organism like archaea with a mevalonate pathway and cholesterol catabolism was considered.[5-8] An actinide dependent shadow biosphere of archaea and viroids in the above mentioned disease states is described.[7, 9] Metal actinides in beach sands have been postulated to play a role in abiogenesis.[7] A hypothesis of cholesterol as the primal prebiotic molecule synthesized on actinide surfaces with all other biomolecules arising from it and a self replicating cholesterol lipid organism as the initial life form is presented.

Global warming can lead to osmotic stress consequent to dehydration. The increase in actinidic archaeal growth leads to cholesterol catabolism and digoxin synthesis. Digoxin produces membrane sodium potassium ATPase inhibition and increase in intracellular calcium producing mitochondrial dysfunction. This results in oxidative stress. The oxidative stress and osmotic stress can induce the enzyme aldose reductase which converts glucose to fructose. Fructose has got a low km value for ketokinase as compared to glucose. Therefore fructose gets phosphorylated more to fructose phosphate and the cell is depleted of ATP. The cell depletion of ATP leads to oxidative stress and chronic inflammation consequent to induction of NFKB. The fructose phosphate can enter the pentose phosphate pathway synthesizing ribose and nucleic acid. The depletion of cellular ATP results in generation of AMP and ADP which are acted upon by deaminases causing hyperuricemia. Uric acid can also produce mitochondrial

dysfunction. The fructose phosphate can enter the glucosamine pathway synthesizing GAG and producing mucopolysaccharide accumulation. Fructose can fructosylate proteins making them antigenic and producing an autoimmune response. This can lead to global warming related autoimmune disease.

Materials and Methods

The following groups were included in the study:- systemic lupus erythematosis, multiple sclerosis and rheumatoid arthritis. 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.[10] 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, dopamine, serotonin, pyruvate, ammonia, glutamate, cytochrome C, hexokinase, ATP synthase, HMG CoA reductase, digoxin and bile acids.[11-13] 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. Informed consent of the subjects and the approval of the ethics committee were obtained for the study. The statistical analysis was done by ANOVA.

Results

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 1-7 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 F420 and PAH.*

Group	CYT F420 % (Increase with Rutile)		CYT F420 % (Decrease with Doxy+Cipro)		PAH % change (Increase with Rutile)		PAH % change (Decrease with Doxy+Cipro)	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Normal	4.48	0.15	18.24	0.66	4.45	0.14	18.25	0.72
MS	22.12	1.81	61.33	9.82	22.83	1.78	59.84	7.62
SLE	22.06	1.61	57.81	6.04	23.46	1.91	61.56	4.61
RA	22.70	1.87	60.46	8.06	23.73	1.38	65.20	6.20
F value	306.749		130.054		391.318		257.996	
P value	< 0.001		< 0.001		< 0.001		< 0.001	

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

Group	DNA % change (Increase with Rutile)		DNA % change (Decrease with Doxy+Cipro)		RNA % change (Increase with Rutile)		RNA % change (Decrease with Doxy+Cipro)	
	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
MS	22.62	1.38	63.82	5.53	23.29	1.98	67.46	3.96
SLE	23.30	1.42	65.07	4.95	23.11	1.52	66.68	3.97
RA	22.29	2.05	58.70	7.34	22.29	2.05	67.03	5.97
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 ATP synthase.*

Group	HMG CoA R % change (Increase with Rutile)		HMG CoA R % change (Decrease with Doxy+Cipro)		ATP synthase % (Increase with Rutile)		ATP synthase % (Decrease with Doxy+Cipro)	
	Mean	±SD	Mean	± SD	Mean	± SD	Mean	± SD
Normal	4.30	0.20	18.35	0.35	4.40	0.11	18.78	0.11
MS	23.14	1.85	59.76	4.82	23.52	1.76	67.05	3.00
SLE	22.38	2.38	60.65	5.27	23.00	1.64	66.67	4.21
RA	22.92	1.48	61.91	7.56	23.37	1.31	63.97	3.62
F value	319.332		199.553		449.503		673.081	
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+Cipro)	
	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
MS	0.52	0.03	0.214	0.032	21.95	2.11	65.46	5.79
SLE	0.53	0.06	0.212	0.045	23.30	1.88	62.49	7.26
RA	0.51	0.05	0.213	0.033	23.41	1.41	58.70	7.34
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+Cipro)		Hexokinase % change (Increase with Rutile)		Hexokinase % change (Decrease with Doxy+Cipro)	
	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
MS	21.59	1.23	60.28	9.22	22.81	1.91	63.47	5.81
SLE	21.07	1.79	63.90	7.13	22.47	2.17	65.97	4.62
RA	22.29	2.05	62.37	5.05	21.66	1.94	67.03	5.97
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 delta amino levulinic acid.*

Group	H_2O_2 % (Increase with Rutile)		H_2O_2 % (Decrease with Doxy+Cipro)		ALA % (Increase with Rutile)		ALA % (Decrease with Doxy+Cipro)	
	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
MS	21.14	1.20	60.53	4.70	22.38	1.79	67.10	3.82
SLE	23.32	1.71	63.15	7.62	23.45	1.79	66.32	3.63
RA	22.86	1.91	63.66	6.88	23.17	1.88	68.53	2.65
F value	380.721		171.228		372.716		556.411	
P value	< 0.001		< 0.001		< 0.001		< 0.001	

Table 7. *Effect of rutile and antibiotics on dopamine and serotonin.*

Group	DOPAMINE % (Increase with Rutile)		DOPAMINE % (Decrease with Doxy+Cipro)		5 HT % change (Increase with Rutile)		5 HT % change (Decrease with Doxy+Cipro)	
	Mean	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**
Normal	4.41	0.15	18.63	0.12	4.34	0.15	18.24	0.37
MS	22.92	2.14	67.54	3.65	21.93	2.29	63.70	5.63
SLE	23.43	1.57	66.30	3.57	22.98	1.50	65.13	4.87
RA	23.70	1.75	68.06	3.52	23.81	1.49	64.89	6.01
F value	403.394		680.284		348.867		364.999	
P value	< 0.001		< 0.001		< 0.001		< 0.001	

Discussion

Archaeal Cholesterol Catabolism in Relation to Autoimmune Disease

The archaeaon steroidelle DXP pathway and the upregulated pentose phosphate pathway contributes to digoxin synthesis. There was increase in cytochrome F420 indicating archaeal growth. The archaea can synthesize and use cholesterol as a carbon and energy source.[6, 14] 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.[15] 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.[8] The archaeal cholesterol oxidase activity was increased resulting in generation of pyruvate and hydrogen peroxide.[14] The pyruvate gets converted to glutamate and ammonia by the GABA shunt pathway. The archaeal aromatization of cholesterol generating PAH, serotonin and dopamine was also detected.[16] 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.[17]

Archaeal-Viroidal Human Genomic Sequences and Autoimmunity

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.[18] 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 noncoding DNA using HERV integrase as has been described for borna and ebola viruses.[19] 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.[20] The integrated viroids and archaea can undergo vertical transmission and can exist as genomic parasites.[19, 20] This increases the length and alters the grammar of the noncoding region producing memes or memory of acquired characters as well as eukaryotic speciation and individuality.[21] 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.[18] 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. This can lead to the pathogenesis of systemic lupus erythematosis, multiple sclerosis and rheumatoid arthritis.

Archaeal Digoxin and Autoimmune Disease

NMDA receptors can be modulated by digoxin induced calcium oscillations, PAH increasing NMDA activity and viroid induced RNA interference.[4] The cholesterol ring oxidase generated pyruvate can be converted by the GABA shunt pathway to glutamate. NMDA excitotoxicity can lead to systemic lupus erythematosis, multiple sclerosis and rheumatoid arthritis. The dipolar PAH and

archaeal magnetite in the setting of digoxin induced sodium potassium ATPase inhibition can produce a pumped phonon system mediated Frohlich model superconducting state [22] inducing quantal perception with nanoarchaeal sensed gravity producing the orchestrated reduction of the quantal possibilities to the macroscopic world.[4, 22] The perception of low level EMF can lead to systemic lupus erythematosis, multiple sclerosis and rheumatoid arthritis.[16] The higher degree of integration of the archaea into the genome produces increased digoxin synthesis producing right hemispheric dominance and lesser degree producing left hemispheric dominance.[4] Right hemispheric dominance can lead to systemic lupus erythematosis, multiple sclerosis and rheumatoid arthritis. Archaea and RNA viroid can bind the TLR receptor induce NFKB producing immune activation and cytokine TNF alpha secretion. The archaeal DXP and mevalonate pathway metabolites can bind γδ TCR and digoxin induced calcium signaling can activate NFKB producing chronic immune activation.[4, 23] The archaea and viroid induced chronic immune activation and generation of superantigens can lead on to autoimmune disease. Archaea, viroids and digoxin can induce the host AKT PI3K, AMPK, HIF alpha and NFKB producing the Warburg metabolic phenotype.[24] The increased glycolytic hexokinase activity, decrease in blood ATP, leakage of cytochrome C, increase in serum pyruvate and decrease in acetyl CoA indicates the generation of the Warburg phenotype. There is induction of glycolysis, inhibition of PDH activity and mitochondrial dysfunction resulting in inefficient energetics and insulin resistance. The archaea and viroid generated cytokines can lead to TNF alpha induced insulin resistance. Insulin resistance can lead to systemic lupus erythematosis, multiple sclerosis and rheumatoid arthritis. The accumulated pyruvate enters the GABA shunt pathway and is converted to citrate which is acted upon by citrate lyase and converted to acetyl CoA, used for cholesterol synthesis.[24] The pyruvate can be converted to glutamate and ammonia which is oxidised by archaea for energy

needs. The increased cholesterol substrate leads to increased archaeal growth and digoxin synthesis leading to metabolic channeling to the mevalonate pathway. The archaeal bile acids are steroidal hormones which can bind GPCR and modulate D_2 regulating the conversion of T_4 to T_3 which activates uncoupling proteins, can activate NRF½ inducing NQO1, GST, HOI reducing redox stress, can bind FXR regulating insulin receptor sensitivity and bind PXR inducing the bile acid shunt pathway of cholesterol detoxification.[25] The Warburg phenotype induced increased mitochondrial dysfunction, archaeal PAH and viroid induced RNA interference can lead on to systemic lupus erythematosis, multiple sclerosis and rheumatoid arthritis. The RNA viroids can recombine with HERV sequences and get encapsulated in microvesicles contributing to the retroviral state. The prion protein conformation is modulated by RNA viroid binding producing prion disease. Prions and HERV sequences can lead to systemic lupus erythematosis, multiple sclerosis and rheumatoid arthritis.[4]

Abiotic Endosymbiotic Actinidic Archaea

The metal actinides provide radiolytic energy, catalysis for oligomer formation and provide a coordinating ion for metalloenzymes all important in abiogenesis.[7] The metal actinide surfaces would by surface metabolism generate acetate which could get converted to acetyl CoA and then to cholesterol which functions as the primal prebiotic molecule self organizing into self replicating supramolecular systems, the lipid organism.[9, 26, 27] Cholesterol by radiolysis by actinides would have formed PAH generating PAH aromatic organism.[9] Cholesterol radiolysis would generate pyruvate which would get converted to amino acids, sugars, nucleotides, porphyrins, fatty acids and TCA acids. Anastase and rutile surfaces can produce polymerization of amino acids, isoprenyl residues, PAH and nucleotides to generate the initial lipid organism, PAH organism, prions and RNA viroids which would have symbiosed to

generate the archaeal protocell. The archaea evolved into gram negative and gram positive bacteria with a mevalonate pathway which had a evolutionary advantage and the symbiosis of archaea with gram negative organism generated the eukaryotic cell.[28] The data supports the persistence of an actinide and cholesterol based shadow biosphere which throws light on the actinide based origin of life and cholesterol as the premier prebiotic molecule. This shadow biosphere can mediate the pathogenesis of systemic lupus erythematosis, multiple sclerosis and rheumatoid arthritis.

References

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

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

[3] 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.

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

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

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

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

[9] 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.

[10] 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.

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

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

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

[14] 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.

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

[16] 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.

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

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

[19] 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.

[20] 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.

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

[22] Lockwood, M. (1989). *Mind, Brain and the Quantum*. Oxford: B. Blackwell.

[23] Eberl M., Hintz, M., Reichenberg, A., Kollas, A., Wiesner, J., Jomaa, H. (2010). Microbial isoprenoid biosynthesis and human γδ T cell activation, *FEBS Letters*, 544(1), 4-10.

[24] Wallace D. C. (2005). Mitochondria and Cancer: Warburg Addressed, *Cold Spring Harbor Symposia on Quantitative Biology*, 70, 363-374.

[25] Lefebvre P., Cariou, B., Lien, F., Kuipers, F., Staels, B. (2009). Role of Bile Acids and Bile Acid Receptors in Metabolic Regulation, *Physiol Rev*, 89(1), 147-191.

[26] Wächtershäuser, G. (1988). Before enzymes and templates: theory of surface metabolism. *Microbiol Rev*, 52(4), 452-84.

[27] Russell, M. J., Martin W. (2004). The rocky roots of the acetyl-CoA Pathway. *Trends in Biochemical Sciences*, 29(7).

[28] Margulis, L. (1996). Archaeal-eubacterial mergers in the origin of Eukarya: phylogenetic classification of life. *Proc Natl Acad Sci USA*, 93, 1071-1076.