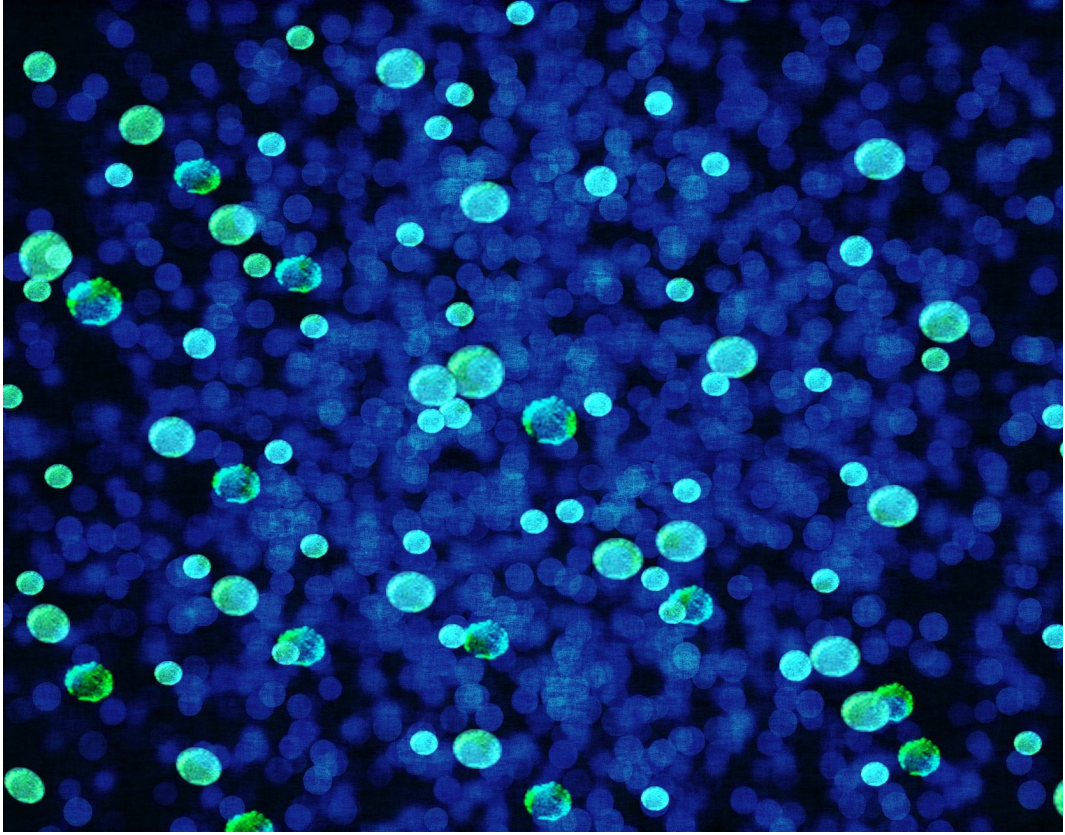

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Chapter 1

The Warburg Phenotype, Global Warming and Human Disease

The Neanderthals are symbiotic life form due to archaeal endosymbiosis. The archaea induces the Warburg phenotype with increased glycolysis and the blockade of the TCA cycle and mitochondrial oxidative phosphorylation. The Warburg phenotype is seen in autoimmune disease, schizophrenia, autism, cancer, degeneration and metabolic syndrome x. The Neanderthals ate a ketogenic diet of fat and protein to suppress the glycolytic pathway. The Neanderthal hybrids formed by homo sapien mating had a high carbohydrate diet due to grain cultivation in settled colonies. This tends to increased glycolysis and accentuates the Warburg phenotype and associated disorders. The glycolytic pathway is upregulated and the mitochondrial oxidative phosphorylation is inhibited. To counteract this certain disease patterns developed in the hybrid population as a adaptive mechanism. These group of disorders develop autoantibodies against glycolytic enzymes. The cell envelope is of archaeal origin and the glycolytic enzymes are cytosolic. This is opposed to the mitochondrial oxidative phosphorylation scheme which is rickettsial in origin. The primitive parts of the brain the cerebellum functions as a archaeal colony network and promotes the Warburg phenotype and glycolysis. The cerebellar brain is dominant in Neanderthals. The HLA genes are neanderthalic in origin and modulate lymphocytic function. The lymphocytes depend on glycolysis for its energy needs. The neocortex functions as a retroviral colony and promotes mitochondrial oxidative phosphorylation. The HERV genes functions as jumping genes and they can jump and insert themselves in between glycolytic enzyme genetic sequences producing mutations and mutated glycolytic enzymes. The glycolytic pathway becomes dysfunctional. Antibodies are formed against the mutated glycolytic proteins. Thus glycolysis and energy metabolism comes to a halt due to the inhibitory effect of the selfish HERV genes which needs mitochondrial function and ROS generation for its replicatory function and communicating with the cell. Disorders like autoimmune disease, schizophrenia, autism, cancer, degeneration

and metabolic syndrome x are disorders of glycolysis and have an autoimmune component against glycolytic enzymes. Glycolytic inhibition and ketogenic diet is one way to treat autoimmune disease, schizophrenia, autism, cancer, degeneration and metabolic syndrome x. All autoimmune diseases develop to suppress the Warburg phenotype in Neanderthal hybrids. The increased glycolysis contributes to oncogenesis via the mitochondrial PT pore hexokinase. The increased glycolysis produces nuclear cell death via the GAPDH pathway. The phophoglycerate gets converted to phosphoserine and glycine which can modulate NMDA. Fructose 1,6 diphosphate enters the pentose phosphate pathway generating NADPH which activates NOX modulating NMDA function. Thus the glycolytic pathway can modulate the NMDA pathway contributing to schizophrenia and autism due to dysfunction of consciousness. The PDH inhibition accumulates pyruvate which enters the GABA shunt generating succinyl CoA and glycine as well as GABA. Succinyl CoA and glycine are substrates for porphyrin synthesis and contributes to quantal perception important in schizophrenia and autism. The increased lymphocytic glycolysis and glycolytic antigens contribute to autoimmune disease. Glycolytic antigens also contribute to neurodegeneration, neuropsychiatric disorders and metabolic syndrome x. GAD antibodies are involved in metabolic syndrome x. Autoimmunity is a part of antibody mediated attempt to inhibit glycolysis and Warburg phenotype in Neanderthal hybrids who consume a high carbohydrate diet. This as a by-product generates neurodegeneration, autoimmune disease, schizophrenia, autism, cancer and civilisational disease. All these can be controlled by glycolytic inhibitors and ketogenic diet.

Global warming also as described leads to increase in endosymbiotic actinidic archaeal growth. Archaea are extremophiles. The actinidic archaea survive by catabolising cholesterol. The archaea and its antigens induce HIF alpha and activate the glycolytic pathway. The glycolytic pathway activation induces

increased conversion of glucose to fructose by activation of the sorbitol pathway. Glucose is converted to sorbitol by the enzyme aldose reductase and sorbitol is converted to fructose by the action of sorbitol dehydrogenase. Fructose is phosphorylated by hexokinase or fructokinase to fructose phosphate. Hexokinase has a low km value for fructose and minimal amounts of fructose will be converted to fructose phosphate depleting the cellular ATP. ATP is converted to AMP and by the action of AMP deaminase is converted to uric acid. Thus there is resultant hyperuricemia and the depletion of ATP also produces membrane sodium potassium ATPase inhibition. Inhibition of membrane sodium potassium ATPase increases intracellular calcium and depletes magnesium. This produces cell death by opening up the mitochondrial PT pore, NFKB activation and immune activation, glutamate excitotoxicity and oncogene activation leading to systemic disorders. The depletion of ATP finally inhibits hexokinase as such and glucose phosphorylation stops blocking the glycolytic pathway and its coupling to the mitochondrial oxidative phosphorylation by the action of PT pore hexokinase. The cell is depleted of energy by glycolysis and the oxidative phosphorylation scheme and dies. Thus global warming via induction of glycolysis and Warburg phenotype and the increased conversion of glucose to fructose and the resultant cellular depletion of ATP can produce systemic disorders and cell dysfunction as well as death. This can produce the global warming related renal, pulmonary, gastrointestinal, hepatic, endocrine and cardiovascular syndromes. This can lead to interstial lung disease, chronic obstructive pulmonary disease, coronary artery disease, cerebrovascular accidents, type 2 diabetes mellitus, chronic renal failure, cirrhosis liver, inflammatory bowel disease, degenerative joint disease, cancer syndromes, neurodegenerations, autoimmune diseases and neuropsychiatric diseases. These diseases have an increased incidence in recent epidemiological studies.

Chapter 2

Endosymbiotic Actinidic Archaeal Mediated Warburg Phenotype Mediates Human Disease State

Introduction

Endomyocardial fibrosis along with the root wilt disease of coconut is endemic to Kerala with its radioactive actinide beach sands. Actinides like rutile as well as organisms like phytoplasmas and viroids have been implicated in the etiology of these diseases.[1-4] The Warburg phenotype has been related to the pathogenesis of schizophrenia, malignancy, metabolic syndrome x, autoimmune disease and neuronal degeneration.[4] The possibility of Warburg phenotype induced by actinide based primitive organism like archaea with a mevalonate pathway and cholesterol catabolism was considered in this paper.[5-8] An actinide dependent shadow biosphere of archaea and viroids in the above mentioned disease states is described.[7, 9]

Materials and Methods

The following groups were included in the study: - endomyocardial fibrosis, Alzheimer's disease, multiple sclerosis, non-Hodgkin's lymphoma, metabolic syndrome x with cerebrovascular thrombosis and coronary artery disease, schizophrenia, autism, seizure disorder, Creutzfeldt Jakob disease and acquired immunodeficiency syndrome. 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 and hexokinase.[11-13] Cytochrome F420 was estimated flourimetrically (excitation wavelength 420 nm and emission wavelength 520 nm). 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 1-2 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.*

Group	CYT F420 % (Increase with Rutile)		CYT F420 % (Decrease with Doxy+Cipro)	
	Mean	**±SD**	**Mean**	**± SD**
Normal	4.48	0.15	18.24	0.66
Schizo	23.24	2.01	58.72	7.08
Seizure	23.46	1.87	59.27	8.86
AD	23.12	2.00	56.90	6.94
MS	22.12	1.81	61.33	9.82
NHL	22.79	2.13	55.90	7.29
DM	22.59	1.86	57.05	8.45
AIDS	22.29	1.66	59.02	7.50
CJD	22.06	1.61	57.81	6.04
Autism	21.68	1.90	57.93	9.64
EMF	22.70	1.87	60.46	8.06
F value	306.749		130.054	
P value	< 0.001		< 0.001	

Table 2. *Effect of rutile and antibiotics on hexokinase.*

Group	Hexokinase % change (Increase with Rutile)		Hexokinase % change (Decrease with Doxy+Cipro)	
	Mean	± SD	Mean	± SD
Normal	4.21	0.16	18.56	0.76
Schizo	23.01	2.61	65.87	5.27
Seizure	23.33	1.79	62.50	5.56
AD	22.96	2.12	65.11	5.91
MS	22.81	1.91	63.47	5.81
NHL	22.53	2.41	64.29	5.44
DM	23.23	1.88	65.11	5.14
AIDS	21.11	2.25	64.20	5.38
CJD	22.47	2.17	65.97	4.62
Autism	22.88	1.87	65.45	5.08
EMF	21.66	1.94	67.03	5.97
F value	292.065		317.966	
P value	< 0.001		< 0.001	

Discussion

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, 16] The archaeal glycolytic hexokinase activity were increased. The part of the increased glycolytic hexokinase activity detected is human. The archaea can undergo magnetite and calcium carbonate mineralization and can exist as calcified nanoforms.[17]

Archaea can induce the host AKT PI3K, AMPK, HIF alpha and NFKB producing the Warburg metabolic phenotype.[18] The increased glycolytic hexokinase activity indicates the generation of the Warburg phenotype. The generation of the Warburg phenotype is due to activation of HIF alpha. This stimulates anaerobic glycolysis, inhibits pyruvate dehydrogenase, inhibits mitochondrial oxidative phosphorylation, stimulates heme oxygenase, stimulates VEGF and activates nitric oxide synthase. This can lead to increased cell proliferation and malignant transformation. The mitochondrial PT pore hexokinase is increased leading onto cell proliferation. There is induction of glycolysis, inhibition of PDH activity and mitochondrial dysfunction resulting in inefficient energetics and metabolic syndrome. The archaea and viroid generated cytokines can lead to TNF alpha induced insulin resistance and metabolic syndrome x. The increase in glycolysis can activate glyceraldehyde 3 phosphate dehydrogenase which gets translocated to the nucleus after polyadenylation. The PARP enzyme is activated by glycolysis mediated redox stress. This can produce nuclear cell death and neuronal degeneration. The increase in the glycolytic enzyme fructose 1,6 diphosphatase increases the pentose phosphate pathway. This generates NADPH which activates NOX. NOX activation is related to NMDA activation and glutamate excitotoxicity. This leads onto neuronal degeneration.[18]

The increase in glycolysis activates the enzyme fructose 1,6 diphosphatase which activates the pentose phosphate pathway liberating NADPH. This increases NOX activity generating free radical stress and H_2O_2. Free radical stress is related to insulin resistance and metabolic syndrome x. Free radicals can activate NFKB producing immune activation and autoimmune disease. Free radicals can open the mitochondrial PT pore, produce release of cyto C and activate the caspase cascade. This produces cell death and neuronal degeneration. The free radicals can activate NMDA receptor and induce the

enzyme GAD generating GABA. This activates the NMDA/GABA thalamo-cortico-thalamic pathway mediating conscious perception. Increased free radical generation can also initiate schizophrenia. Free radicals can also produce oncogene activation and malignant transformation. Free radicals can produce HDAC inhibition and HERV generation. The encapsulation of HERV particles in phospholipids vesicles can mediate the generation of the acquired immunodeficiency syndrome. Free radicals can also promote atherogenesis.[18]

The lymphocytes depend on glycolysis for its energy needs. The increase in glycolysis owing to the induction of Warburg phenotype can lead to immune activation. Immune activation can lead to autoimmune disease. TNF alpha can activate the NMDA receptor leading to glutamate excitotoxicity and neuronal degeneration. TNF alpha activating NMDA receptor can contribute to schizophrenia. TNF alpha can induce expression of HERV particles contributing to generation of acquired immunodeficiency syndrome. Immune activation has also been related to malignant transformation mediated by NFKB. TNF alpha can also act upon the insulin receptor producing insulin resistance. NOX activation consequent to the generation of the Warburg phenotype also activates the insulin receptor. Thus there is a hyperinsulinemic state leading on to metabolic syndrome x.[18]

The inhibition of pyruvate dehydrogenase results in defective formation of acetyl CoA. This results in defective N acetylation of lysyl residues of proteins producing defective protein confirmation. There are nearly 3000 acetylated proteins and defects in protein acetylation can lead to defective function. Thus the inhibition of PDH can modulate proteonomic function. Acetate is also a HDAC inhibitor. Acetyl CoA is also required for histone acetylation. HDAC inhibition and histone acetylation can modulate chromatin structure and gene expression. Thus PDH inhibition can modulate genomic expression.

The Warburg phenotype results in upregulation of glycolysis and the immune system depends upon glycolysis for its energetics. The immune system is regulated by the HLA system and the HLA genes are of neanderthalic origin. The mitochondria is of homo sapiens origin and the cytosolic glycolytic pathway is of Neanderthal origin. This leads to autoimmunity against glycolytic enzymes. Antiglycolytic enzymes autoantibodies in the hybrid population are common in multiple sclerosis, lupus, autoimmune encephalitis and antibasal ganglia antibody disease.

Thus the induction of the Warburg phenotype can lead to malignancy, autoimmune disease, metabolic syndrome x, neuropsychiatric disease and neuronal degeneration. The Warburg phenotype leads to inhibition of pyruvate dehydrogenase and accumulation of pyruvate. 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. 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 further induction of the Warburg phenotype.[18]

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

Chapter 3

Endosymbiotic Actinidic Archaeal Cholesterol Depletion Syndrome

Introduction

Actinidic archaea have been implicated in the pathogenesis of schizophrenia, malignancy, metabolic syndrome x, autoimmune disease and neuronal degeneration.[1-9] Actinide based primitive organism like archaea have a mevalonate pathway and cholesterol catabolism. Cholesterol catabolism by actinidic archaea can lead to cholesterol depletion and a hypocholesterolemic state contributing to the pathogenesis of these disorders.[10-17]

Archaea can use cholesterol as a carbon and energy source. Archaeal cholesterol catabolism can lead to multiple systemic disease. Low cholesterol values in populations have been related to high mortality. The archaeal cholesterol catabolizing enzymes were studied and the results in presented in this paper. This can be described as the endosymbiotic actinidic archaeal cholesterol catabolic syndrome.[10-17]

The induction of the Warburg phenotype results in inhibition of pyruvate dehydrogenase and defective generation of acetyl CoA. Acetyl CoA is the substrate for cholesterol synthesis by the isoprenoid pathway. This results in defective cholesterol synthesis and cholesterol deficient state.

Materials and Methods

The following groups were included in the study: - meditation group, endomyocardial fibrosis, Alzheimer's disease, multiple sclerosis, non-Hodgkin's lymphoma, metabolic syndrome x with cerebrovascular thrombosis and coronary artery disease, schizophrenia, autism, seizure disorder, Creutzfeldt Jakob's disease and acquired immunodeficiency syndrome. 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.[18] 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, polycyclic aromatic hydrocarbon, digoxin, bile acid, cholesterol oxidase activity measured by hydrogen peroxide liberation, pyruvate, butyrate and propionate were estimated.[19-21] 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 1-4 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
Schizo	23.24	2.01	58.72	7.08	23.01	1.69	59.49	4.30
Seizure	23.46	1.87	59.27	8.86	22.67	2.29	57.69	5.29
AD	23.12	2.00	56.90	6.94	23.26	1.53	60.91	7.59
MS	22.12	1.81	61.33	9.82	22.83	1.78	59.84	7.62
NHL	22.79	2.13	55.90	7.29	22.84	1.42	66.07	3.78
DM	22.59	1.86	57.05	8.45	23.40	1.55	65.77	5.27
Meditation	22.29	1.66	59.02	7.50	23.23	1.97	65.89	5.05
CJD	22.06	1.61	57.81	6.04	23.46	1.91	61.56	4.61
Autism	21.68	1.90	57.93	9.64	22.61	1.42	64.48	6.90
EMF	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 butyrate and propionate generation from cholesterol.*

Group	Butyrate % change (Increase with Rutile)		Butyrate % change (Decrease with Doxy+Cipro)		Propionate % change (Increase with Rutile)		Propionate % change (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
Schizo	22.50	1.66	60.21	7.42	22.52	1.90	66.39	4.20
Seizure	23.81	1.19	61.08	7.38	22.83	1.90	67.23	3.45
AD	22.65	2.48	60.19	6.98	23.67	1.68	66.50	3.58
MS	21.14	1.20	60.53	4.70	22.38	1.79	67.10	3.82
NHL	23.35	1.76	59.17	3.33	23.34	1.75	66.80	3.43
DM	23.27	1.53	58.91	6.09	22.87	1.84	66.31	3.68
Meditation	23.32	1.71	63.15	7.62	23.45	1.79	66.32	3.63
CJD	22.86	1.91	63.66	6.88	23.17	1.88	68.53	2.65
Autism	23.52	1.49	63.24	7.36	23.20	1.57	66.65	4.26
EMF	23.29	1.67	60.52	5.38	22.29	2.05	61.91	7.56
F value	380.721		171.228		372.716		556.411	
P value	< 0.001		< 0.001		< 0.001		< 0.001	

Table 3. *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
Schizo	0.55	0.06	0.219	0.043	23.20	1.87	57.04	4.27
Seizure	0.51	0.05	0.199	0.027	22.61	2.22	66.62	4.99
AD	0.55	0.03	0.192	0.040	22.12	2.19	62.86	6.28
MS	0.52	0.03	0.214	0.032	21.95	2.11	65.46	5.79
NHL	0.54	0.04	0.210	0.042	22.98	2.19	64.96	5.64
DM	0.47	0.04	0.202	0.025	22.87	2.58	64.51	5.93
Meditation	0.56	0.05	0.220	0.052	22.29	1.47	64.35	5.58
CJD	0.53	0.06	0.212	0.045	23.30	1.88	62.49	7.26
Autism	0.53	0.08	0.205	0.041	22.21	2.04	63.84	6.16
EMF	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 4. *Effect of rutile and antibiotics on pyruvate and hydrogen peroxide.*

Group	Pyruvate % change (Increase with Rutile)		Pyruvate % change (Decrease with Doxy+Cipro)		H_2O_2 % (Increase with Rutile)		H_2O_2 % (Decrease with Doxy+Cipro)	
	Mean	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**
Normal	4.34	0.21	18.43	0.82	4.43	0.19	18.13	0.63
Schizo	20.99	1.46	61.23	9.73	22.50	1.66	60.21	7.42
Seizure	20.94	1.54	62.76	8.52	23.81	1.19	61.08	7.38
AD	22.63	0.88	56.40	8.59	22.65	2.48	60.19	6.98
MS	21.59	1.23	60.28	9.22	21.14	1.20	60.53	4.70
NHL	21.19	1.61	58.57	7.47	23.35	1.76	59.17	3.33
DM	20.67	1.38	58.75	8.12	23.27	1.53	58.91	6.09
Meditation	21.21	2.36	58.73	8.10	23.32	1.71	63.15	7.62
CJD	21.07	1.79	63.90	7.13	22.86	1.91	63.66	6.88
Autism	21.91	1.71	58.45	6.66	23.52	1.49	63.24	7.36
EMF	22.29	2.05	62.37	5.05	23.29	1.67	60.52	5.38
F value	321.255		115.242		380.721		171.228	
P value	< 0.001		< 0.001		< 0.001		< 0.001	

Discussion

There was increase in cytochrome F420 indicating archaeal growth. The archaea can synthesize and use cholesterol as a carbon and energy source.[22-24] 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.[22-24] The archaeal beta hydroxyl steroid dehydrogenase activity indicating digoxin synthesis and archaeal cholesterol hydroxylase activity indicating bile acid synthesis were increased.[22-24]

The archaeal cholesterol oxidase activity was increased resulting in generation of pyruvate and hydrogen peroxide.[22-24] The pyruvate gets converted to glutamate and ammonia by the GABA shunt pathway. The archaeal aromatisation of cholesterol generating PAH was also detected.[22-24] This indicates archaeal cholesterol aromatase activity. The archaeal cholesterol side chain oxidase activity generates butyrate and propionate. Thus archaeal cholesterol oxidase, cholesterol aromatase, cholesterol side chain oxidase, cholesterol hydroxylase and beta hydroxyl steroid dehydrogenase activity were detected in high levels in the patient population of endomyocardial fibrosis, Alzheimer's disease, multiple sclerosis, non-Hodgkin's lymphoma, metabolic syndrome x with cerebrovascular thrombosis and coronary artery disease, schizophrenia, autism, seizure disorder, Creutzfeldt Jakob's disease and acquired immunodeficiency syndrome. The archaeal cholesterol catabolizing enzymes were actinide dependent. The archaea can undergo magnetite and calcium carbonate mineralization and can exist as calcified nanoforms.[25] This leads to a cholesterol depleted state and hypocholesterolemic syndrome in patients with schizophrenia, malignancy, metabolic syndrome x, autoimmune disease and neuronal degeneration.

Low cholesterol has been related to multiple systemic diseases. Low cholesterol is detected in patients with autism and schizophrenia. Low cholesterol is also associated with neuronal degenerations like Alzheimer's disease and Parkinson's disease. Cholesterol is required for the formation of synaptic connectivity in neuronal cultures. Depletion of cholesterol from the brain results in loss of synaptic connectivity in multiple neuronal circuits contributing to neuropsychiatric disorders and neuronal degeneration. Low cholesterol has also been related to malignancy. Cholesterol is required for contact inhibition. Absence of cholesterol results in loss of contact inhibition

and uncontrolled cell proliferation. Low cholesterol has been related to autoimmune disease.[10-17]

The gut endotoxins and lipo-polysaccharides are absorbed along with fat producing the syndrome of metabolic endotoxaemia. The endotoxins and lipo-polysaccharides can combine with lipoproteins and are detoxified. Metabolic endotoxaemia produces chronic immune activation and generation of superantigens. This has been related to the genesis of autoimmune disease. Metabolic endotoxaemia results in immune activation and generation of TNF alpha which modulates the insulin receptor producing insulin resistance. Insulin resistance is related to metabolic syndrome x and vascular thrombosis. Metabolic endotoxaemia has been related to neuronal degenerations like Alzheimer's disease and Parkinson's disease. Metabolic endotoxaemia related chronic immune activation drives the retroviral state. Metabolic endotoxaemia can induce NFKB which can drive malignant cell transformation. Thus hypocholesterolemia leads to non-detoxification of endotoxins and lipo-polysaccharides resulting in metabolic syndrome x, neuronal degnerations and autoimmune disease.[10-17]

Infections have been related to schizophrenia, malignancy, metabolic syndrome x, autoimmune disease and neuronal degeneration. *H. pylori* infection and nocardiosis has been related to Parkinson's disease. Chlamydial infection and actinomycosis has been related to Alzheimer's disease. Clostridial infection has been related to motor neuron disease. Atypical mycobacterial infection had been related to malignancy like lymphoma. Staphylococcal infections have been related to carcinoma of the breast. Gut bacterial infections had been related to rheumatoid disease. Toxoplasmosis has been related to schizophrenia. Gut bacteria with increase in gut firmicutes and decrease in bacteroides have been related to metabolic syndrome x. Chlamydial infections have been related to

vascular disease. Low cholesterol leads to lack of lipoprotein binding to endotoxins.[10-17] The endotoxins and lipo-polysaccharides are not detoxified.

Viral diseases have been related to the pathogenesis of schizophrenia, malignancy, metabolic syndrome x, autoimmune disease and neuronal degeneration. The virus binds to lipid microdomains in the cell membrane. Cholesterol depletion leads to alteration in lipid microdomains and increased entry of virus in the cell. Herpes virus infection and borna virus disease leads to schizophrenia. Enterovirus infection has been associated with motor neuron disease. Corona virus infection predisposes to Parkinson's disease. Herpes virus infection is implicated in Alzheimer's disease. Herpes virus infection and EBV infections predisposed to SLE. Retroviral infection - exogenous and endogenous have been related to schizophrenia, malignancy, metabolic syndrome x, autoimmune disease and neuronal degeneration. CMV infection and herpes infection has been related to atherogenesis. Prion disease has been related to alterations in cholesterol metabolism. Thus a cholesterol depleted state can lead to increased predilection to viral infection and systemic disease.[10-17]

The actinidic archaea uses cholesterol catabolism to generate energy. The cholesterol catabolizing enzymes of the archaea are dependent on actinides. The archaeal cholesterol catabolism leads to a cholesterol depleted state and systemic disease. Cholesterol depleted state have been related to high mortality. This can be described as the endosymbiotic actinidic archaeal cholesterol catabolic syndrome.[10-17]

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] Marini, A., Carulli, G., Azzarà, A., Grassi, B., Ambrogi, F. (1989). Serum cholesterol and triglycerides in hematological malignancies. *Acta Haematol*, 81(2), 75-9.

[11] Jacobs, D., Blackburn, H., Higgins, M. (1992). Report of the Conference on Low Blood Cholesterol: Mortality Associations. *Circulation*, 86(3), 1046-60.

[12] Suarez, E. C. (1999). Relations of trait depression and anxiety to low lipid and lipoprotein concentrations in healthy young adult women. *Psychosom Med*, 61(3), 273-9.

[13] Woo, D., Kissela, B. M., Khoury, J. C. (2004). Hypercholesterolemia, HMG-CoA reductase inhibitors, and risk of intracerebral hemorrhage: a case-control study. Stroke, 35(6), 1360-4.

[14] Schatz, I. J., Masaki, K., Yano, K., Chen, R., Rodriguez, B. L., Curb, J. D. (2001). Cholesterol and all-cause mortality in elderly people from the Honolulu Heart Program: a cohort study. *Lancet*, 358 (9279), 351-5.

[15] Onder, G., Landi, F., Volpato, S., (2003). Serum cholesterol levels and in-hospital mortality in the elderly. *Am J Med*, 115(4), 265-71.

[16] Gordon, B. R., Parker, T. S., Levine, D. M. (2001). Relationship of hypolipidemia to cytokine concentrations and outcomes in critically ill surgical patients. *Crit Care Med*, 29(8), 1563-8.

[17] Jacobs, Jr., D. R., Iribarren, C. (2000). Low Cholesterol and Nonatherosclerotic Disease Risk: A Persistently Perplexing Question. *American Journal of Epidemiology*, Vol. 151, No. 8.

[18] 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.

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

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

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

[22] 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.

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

[24] 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.

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

Chapter 4

Global Warming Induced Endosymbiotic Actinidic Archaeal Synthesis of Bile Acids from Cholesterol Regulates Cellular Function

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.

Endomyocardial fibrosis along with the root wilt disease of coconut is also endemic to Kerala with its radioactive actinide beach sands. Actinides like rutile as well as organisms like phytoplasmas and viroids have been implicated in the etiology of these diseases.[1-4] Bile acids are considered as steroidal hormones with endocrine, metabolic and neuroregulatory functions. Bile acids has been related to the pathogenesis of schizophrenia, malignancy, metabolic syndrome x, autoimmune disease and neuronal degeneration.[4] The possibility of endogenous bile acid 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]

The induction of the Warburg phenotype results in inhibition of pyruvate dehydrogenase and defective generation of acetyl CoA. Acetyl CoA is the substrate for cholesterol synthesis by the isoprenoid pathway. This results in defective cholesterol synthesis and cholesterol deficient state. Defective cholesterol synthesis and catabolism can lead to cholesterol depletion and low cholesterol levels. Low cholesterol level can result in bile acid deficiency and systemic disease.

Materials and Methods

The following groups were included in the study: - endomyocardial fibrosis, Alzheimer's disease, multiple sclerosis, non-Hodgkin's lymphoma, metabolic syndrome x with cerebrovascular thrombosis and coronary artery disease, schizophrenia, autism, seizure disorder, Creutzfeldt Jakob's disease and

acquired immunodeficiency syndrome. 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 and bile acids.[11-13] Cytochrome F420 was estimated flourimetrically (excitation wavelength 420 nm and emission wavelength 520 nm). 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 table 1 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 archaeal bile acids.*

Group	CYT F420 % (Increase with Rutile)		CYT F420 % (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	4.48	0.15	18.24	0.66	4.29	0.18	18.15	0.58
Schizo	23.24	2.01	58.72	7.08	23.20	1.87	57.04	4.27
Seizure	23.46	1.87	59.27	8.86	22.61	2.22	66.62	4.99
AD	23.12	2.00	56.90	6.94	22.12	2.19	62.86	6.28
MS	22.12	1.81	61.33	9.82	21.95	2.11	65.46	5.79
NHL	22.79	2.13	55.90	7.29	22.98	2.19	64.96	5.64
DM	22.59	1.86	57.05	8.45	22.87	2.58	64.51	5.93
AIDS	22.29	1.66	59.02	7.50	22.29	1.47	64.35	5.58
CJD	22.06	1.61	57.81	6.04	23.30	1.88	62.49	7.26
Autism	21.68	1.90	57.93	9.64	22.21	2.04	63.84	6.16
EMF	22.70	1.87	60.46	8.06	23.41	1.41	58.70	7.34
F value	306.749		130.054		290.441		203.651	
P value	< 0.001		< 0.001		< 0.001		< 0.001	

Discussion

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] The archaeal cholesterol hydroxylase activity indicating bile acid synthesis were increased.[8]

The archaeal bile acids are steroidal hormones which can bind GPCR and modulate D2 regulating the conversion of T4 to T3 which activates uncoupling proteins reducing redox stress. Bile acids can activate the transcription factor NRF ½ inducing NQO1, GST, HOI reducing redox stress. Bile acids are neuroprotective and help to prevent neurodegenerative process. Bile acids can bind FXR regulating insulin receptor sensitivity. Bile acid deficiency leads to insulin resistance and metabolic syndrome x. Bile acids can bind PXR inducing the bile acid shunt pathway of cholesterol detoxification.[25] Bile acid deficiency leads to cholesterol toxicity. Bile acid binding to FXR receptor inhibits tissue fibrosis and connective tissue deposition. Bile acid deficiency may play a role in MPS and collagen deposition in EMF, CCP, MNG and mucoid angiopathy. Bile acids can bind macrophage GPCR and VDR producing immunosuppression and inhibiting NFKB. This helps to modulate the archaea and viroid induced chronic immune activation. Bile acid deficiency can contribute to autoimmune disease. The archaeal bile acids are chemically diverse and structurally different from human bile acids. The archaeal bile acids can bind olfactory GPCR receptors and stimulate the limbic lobe producing a sense of social identity. The dominance of archaeal bile acids over human bile acids in stimulating the olfactory GPCR - limbic lobe pathway leads to loss of social identity and schizophrenia / autism. Bile acids tend to have an inhibitory effect on cell proliferation and prevent malignant transformation.[16]

Archaeal bile acids binding to VDR has an inhibitory effect on cell proliferation. Bile acids thus can modulate cell death and cell proliferation. It can regulate metabolism via modulating mitochondrial uncoupling proteins and via FXR insulin receptor sensitivity. Bile acids can modulate limbic lobe and

brain functions via GPCR receptor and olfactory pathways. Bile acids can regulate immune function via macrophage GPCR and VDR. Archaeal bile acids can produce neuro-immuno-endocrine integration. Archaeal bile acids deficiency can contribute to the pathogenesis of endomyocardial fibrosis, Alzheimer's disease, multiple sclerosis, non-Hodgkin's lymphoma, metabolic syndrome x with cerebrovascular thrombosis and coronary artery disease, schizophrenia, autism, seizure disorder, Creutzfeldt Jakob disease and acquired immunodeficiency syndrome.

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] 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.

Chapter 5

Endosymbiotic Actinidic Archaea and Viroidal Induced Warburg Phenotype Can Be Reversed by a Modified Vegetarian High Fiber, High Medium Chain Triglyceride Ketogenic Diet

Introduction

Actinidic archaea has been related to the pathogenesis of schizophrenia, malignancy, metabolic syndrome x, autoimmune disease and neuronal degeneration. An actinide dependent shadow biosphere of archaea and viroids in the above mentioned disease states is described. The actinidic archaeal and viroid induced Warburg phenotype contributes to the pathology of the disease states mentioned. The possibility of administration of high medium chain triglyceride, high fiber ketogenic diet on actinide based primitive organism like archaea with a mevalonate pathway and cholesterol catabolism was considered in these disease states.[1-10] The effect of a high medium chain triglyceride and a high fiber modified vegetarian ketogenic diet on the Warburg phenotype was also studied.

The ketogenic diet is a high-fat, adequate-protein, low-carbohydrate diet that in medicine is used primarily to treat difficult-to-control (refractory) epilepsy in children. The diet mimics aspects of starvation by forcing the body to burn fats rather than carbohydrates. However, if there is very little carbohydrate in the diet, the liver converts fat into fatty acids and ketone bodies. The ketone bodies pass into the brain and replace glucose as an energy source. An elevated level of ketone bodies in the blood, a state known as ketosis, leads to a reduction in the frequency of epileptic seizures. The ketogenic diet results in adaptive changes to brain energy metabolism that increases the energy reserves; ketone bodies are a more efficient fuel than glucose, and the number of mitochondria is increased. This may help the neurons to remain stable in the face of increased energy demand during a seizure, and may confer a neuroprotective effect.[10-15]

Dietary fiber and medium chain triglycerides have antiviral and antibacterial effects. A low carbohydrate diet generates lesser glucose for the body and inhibits glycolysis. Dietary fiber generates short chain fatty acids butyrate and propionate which are immunosuppressive. The decrease in cytokines has inhibitory effect on the generation of the Warburg phenotype. The results of the study on the effect of a high fiber, high MCT vegetarian ketogenic diet on the actinidic archaea and viroid induced Warburg phenotype are presented in this paper.[10-15]

Materials and Methods

The following groups were included in the study: - endomyocardial fibrosis, Alzheimer's disease, multiple sclerosis, non-Hodgkin's lymphoma, metabolic syndrome x with cerebrovascular thrombosis and coronary artery disease, schizophrenia, autism, seizure disorder, Creutzfeldt Jakob's disease and acquired immunodeficiency syndrome. 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 was drawn from the (1) in freshly diagnosed cases in the fasting state before treatment was initiated and (2) after a 15-days modified high fiber, high MCT vegetarian ketogenic diet of medium chain triglycerides (150 g of coconut oil), fiber (45 g of banana stem fiber) and vegetable proteins (black gram protein 100 g/day) with 50 g of carbohydrate (black gram polysaccharide). 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.[16]

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, hexokinase activity and archaeal cholesterol oxidase activity as measured by hydrogen peroxide liberation.[17-19] Cytochrome F420 was estimated flourimetrically (excitation wavelength 420 nm and emission wavelength 520 nm). 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 1-5 as percentage change in the parameters after 1 hour incubation as compared to the values at zero time. The patients on modified ketogenic diet showed a decrease in all the parameters. Vegetarian ketogenic diets based on high fiber and high medium chain triglycerides has a inhibitory effect on the growth of archaea and viroids as well as archaeal cholesterol oxidase activity. The vegetarian ketogenic diet with its high fiber and high MCT content reversed the Warburg phenotype has indicated by a reduction in hexokinase activity.

Table 1. *Effect of rutile, antibiotics and ketogenic diet on cytochrome F420.*

Group	CYT F420 % (Increase with Rutile)		CYT F420 % (Decrease with Doxy+Cipro)		CYT F420 % (Decrease with Ketogenic diet)	
	Mean	± SD	Mean	± SD	Mean	± SD
Normal	4.48	0.15	18.24	0.66	18.25	0.72
Schizo	23.24	2.01	58.72	7.08	59.49	4.30
Seizure	23.46	1.87	59.27	8.86	57.69	5.29
AD	23.12	2.00	56.90	6.94	60.91	7.59
MS	22.12	1.81	61.33	9.82	59.84	7.62
NHL	22.79	2.13	55.90	7.29	66.07	3.78
DM	22.59	1.86	57.05	8.45	65.77	5.27
AIDS	22.29	1.66	59.02	7.50	65.89	5.05
CJD	22.06	1.61	57.81	6.04	61.56	4.61
Autism	21.68	1.90	57.93	9.64	64.48	6.90
EMF	22.70	1.87	60.46	8.06	65.20	6.20
F value	306.749		130.054		257.996	
P value	< 0.001		< 0.001		< 0.001	

Table 2. *Effect of rutile, antibiotics and ketogenic diet on free RNA.*

Group	RNA % change (Increase with Rutile)		RNA % change (Decrease with Doxy+Cipro)		RNA % change (Decrease with Ketogenic diet)	
	Mean	± SD	Mean	± SD	Mean	± SD
Normal	4.37	0.13	18.38	0.48	18.15	0.58
Schizo	23.59	1.83	65.69	3.94	57.04	4.27
Seizure	23.08	1.87	65.09	3.48	66.62	4.99
AD	23.29	1.92	65.39	3.95	62.86	6.28
MS	23.29	1.98	67.46	3.96	65.46	5.79
NHL	23.78	1.20	66.90	4.10	64.96	5.64
DM	23.33	1.86	66.46	3.65	64.51	5.93
AIDS	23.32	1.74	65.67	4.16	64.35	5.58
CJD	23.11	1.52	66.68	3.97	62.49	7.26
Autism	23.33	1.35	66.83	3.27	63.84	6.16
EMF	22.29	2.05	67.03	5.97	58.70	7.34
F value	427.828		654.453		203.651	
P value	< 0.001		< 0.001		0.001	

Table 3. *Effect of rutile, antibiotics and ketogenic diet on DNA.*

Group	DNA % change (Increase with Rutile)		DNA % change (Decrease with Doxy+Cipro)		DNA % change (Decrease with Ketogenic diet)	
	Mean	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**
Normal	4.37	0.15	18.39	0.38	18.78	0.11
Schizo	23.28	1.70	61.41	3.36	67.39	3.13
Seizure	23.40	1.51	63.68	4.66	66.15	4.09
AD	23.52	1.65	64.15	4.60	66.21	3.69
MS	22.62	1.38	63.82	5.53	67.05	3.00
NHL	22.42	1.99	61.14	3.47	66.66	3.84
DM	23.01	1.67	65.35	3.56	66.25	3.69
AIDS	22.56	2.46	62.70	4.53	66.48	4.17
CJD	23.30	1.42	65.07	4.95	66.67	4.21
Autism	22.12	2.44	63.69	5.14	66.86	4.21
EMF	22.29	2.05	58.70	7.34	63.97	3.62
F value	337.577		356.621		673.081	
P value	< 0.001		< 0.001		< 0.001	

Table 4. *Effect of rutile, antibiotics and ketogenic diet on hexokinase activity.*

Group	Hexokinase % change (Increase with Rutile)		Hexokinase % change (Decrease with Doxy+Cipro)		Hexokinase % change (Decrease with Ketogenic diet)	
	Mean	± SD	Mean	± SD	Mean	± SD
Normal	4.21	0.16	18.56	0.76	18.43	0.82
Schizo	23.01	2.61	65.87	5.27	61.23	9.73
Seizure	23.33	1.79	62.50	5.56	62.76	8.52
AD	22.96	2.12	65.11	5.91	56.40	8.59
MS	22.81	1.91	63.47	5.81	60.28	9.22
NHL	22.53	2.41	64.29	5.44	58.57	7.47
DM	23.23	1.88	65.11	5.14	58.75	8.12
AIDS	21.11	2.25	64.20	5.38	58.73	8.10
CJD	22.47	2.17	65.97	4.62	63.90	7.13
Autism	22.88	1.87	65.45	5.08	58.45	6.66
EMF	21.66	1.94	67.03	5.97	62.37	5.05
F value	292.065		317.966		115.242	
P value	< 0.001		< 0.001		< 0.001	

Table 5. *Effect of rutile, antibiotics and ketogenic diet on cholesterol oxidase activity.*

Group	Cholesterol oxidase activity % (Increase with Rutile)		Cholesterol oxidase activity % (Decrease with Doxy+Cipro)		Cholesterol oxidase activity % (Decrease with Ketogenic diet)	
	Mean	± SD	Mean	± SD	Mean	± SD
Normal	4.43	0.19	18.13	0.63	18.48	0.39
Schizo	22.50	1.66	60.21	7.42	66.39	4.20
Seizure	23.81	1.19	61.08	7.38	67.23	3.45
AD	22.65	2.48	60.19	6.98	66.50	3.58
MS	21.14	1.20	60.53	4.70	67.10	3.82
NHL	23.35	1.76	59.17	3.33	66.80	3.43
DM	23.27	1.53	58.91	6.09	66.31	3.68
AIDS	23.32	1.71	63.15	7.62	66.32	3.63
CJD	22.86	1.91	63.66	6.88	68.53	2.65
Autism	23.52	1.49	63.24	7.36	66.65	4.26
EMF	23.29	1.67	60.52	5.38	61.91	7.56
F value	380.721		171.228		556.411	
P value	< 0.001		< 0.001		< 0.001	

Discussion

There was increase in cytochrome F420 indicating archaeal growth. The archaea can synthesize and use cholesterol as a carbon and energy source as indicated by cholesterol oxidase activity.[20-22] 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.[20-22] The archaeal cholesterol oxidase activity was increased

resulting in generation of hydrogen peroxide.[20-22] The archaeal glycolytic hexokinase activity were increased. The archaea can undergo magnetite and calcium carbonate mineralization and can exist as calcified nanoforms.[17] 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 high fiber and high MCT modified vegetarian ketogenic diet can block archaeal and viroidal multiplication. Fiber and MCT have a antiarchaeal and antiviroidal effect.[11-15]

Archaea can induce the host AKT PI3K, AMPK, HIF alpha and NFKB producing the Warburg metabolic phenotype.[10] The increased glycolytic hexokinase activity indicates the generation of the Warburg phenotype. A high fiber and high MCT modified vegetarian ketogenic diet can inhibit hexokinase activity and glycolysis and reverse the Warburg phenotype. The generation of the Warburg phenotype is due to activation of HIF alpha. This stimulates anaerobic glycolysis, inhibits pyruvate dehydrogenase, inhibits mitochondrial oxidative phosphorylation, stimulates heme oxygenase, stimulates VEGF and activates nitric oxide synthase. The low carbohydrate diet generates less of glucose and inhibits the glycolytic pathway. This reverses the Warburg phenotype. The high fiber intake generates short chain fatty acids butyrate and propionate. Short chain fatty acids bind to lymphocyte GPCR receptors and are immunosuppressive. The reduction in cytokine generation inhibits the Warburg phenotype. The antiarchaeal and antiviroidal action of MCT and dietary fiber also inhibits the generation of the Warburg phenotype.[11-15]

The Warburg phenotype generates malignant, autoimmune, neurodegenerative, metabolic syndrome x and schizophrenic pathologies. The Warburg phenotype can lead to increased cell proliferation and malignant transformation. The mitochondrial PT pore hexokinase is increased leading onto

cell proliferation. There is induction of glycolysis, inhibition of PDH activity and mitochondrial dysfunction resulting in inefficient energetics and metabolic syndrome. The archaea and viroid generated cytokines can lead to TNF alpha induced insulin resistance and metabolic syndrome x. The increase in glycolysis can activate glyceraldehyde 3 phosphate dehydrogenase which gets translocated to the nucleus after polyadenylation. The PARP enzyme is activated by glycolysis mediated redox stress. This can produce nuclear cell death and neuronal degeneration. The increase in the glycolytic enzyme fructose 1,6 diphosphatase increases the pentose phosphate pathway. This generates NADPH which activates NOX. NOX activation is related to NMDA activation and glutamate excitotoxicity. This leads onto neuronal degeneration.[10]

The increase in glycolysis activates the enzyme fructose 1,6 diphosphatase which activates the pentose phosphate pathway liberating NADPH. This increases NOX activity generating free radical stress and H_2O_2. Free radical stress is related to insulin resistance and metabolic syndrome x. Free radicals can activate NFKB producing immune activation and autoimmune disease. Free radicals can open the mitochondrial PT pore, produce release of cyto C and activate the caspase cascade. This produces cell death and neuronal degeneration. The free radicals can activate NMDA receptor and induce the enzyme GAD generating GABA. This activates the NMDA/GABA thalamo-cortico-thalamic pathway mediating conscious perception. Increased free radical generation can also intitiate schizophrenia. Free radicals can also produce oncogene activation and malignant transformation. Free radicals can produce HDAC inhibition and HERV generation. The encapsulation of HERV particles in phospholipids vesicles can mediate the generation of the acquired immunodeficiency syndrome. Free radicals can also promote atherogenesis.[10]

The lymphocytes depend on glycolysis for its energy needs. The increase in glycolysis owing to the induction of Warburg phenotype can lead to immune activation. Immune activation can lead to autoimmune disease. TNF alpha can activate the NMDA receptor leading to glutamate excitotoxicity and neuronal degeneration. TNF alpha activating NMDA receptor can contribute to schizophrenia. TNF alpha can induce expression of HERV particles contributing to generation of acquired immunodeficiency syndrome. Immune activation has also been related to malignant transformation mediated by NFKB. TNF alpha can also act upon the insulin receptor producing insulin resistance. NOX activation consequent to the generation of the Warburg phenotype also activates the insulin receptor. Thus there is a hyperinsulinemic state leading on to metabolic syndrome x.[10]

Thus the induction of the Warburg phenotype can lead to malignancy, autoimmune disease, metabolic syndrome x, neuropsychiatric disease and neuronal degeneration. The Warburg phenotype leads to inhibition of pyruvate dehydrogenase and accumulation of pyruvate. 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. 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 further induction of the Warburg phenotype.[10]

A ketogenic diet is normal diet of the primitive hunter-gatherer humans. It is based upon a low carbohydrate, high saturated fat and high protein diet. In this study, a modified ketogenic diet was used. It included high medium chain triglycerides from coconut oil, high fiber from banana stem, high black gram protein and low black gram polysaccharide as source of carbohydrate. It was a modified vegetarian ketogenic diet high in MCT and fiber. This diet has got an

antiviroidal and antiarchaeal activity and can reverse the Warburg phenotype, the basis of diverse malignant, autoimmune, neurodegenerative, metabolic syndrome x and schizophrenic pathologies.[11-15]

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

[11] Liu, Y. M. (2008). Medium-chain triglyceride (MCT) ketogenic therapy. *Epilepsia,;* 49 S(8), 33-6.

[12] Gasior, M., Rogawski, M. A., Hartman, A. L. (2006). Neuroprotective and disease-modifying effects of the ketogenic diet. *Behav Pharmacol*, 17(5-6), 431-9.

[13] Maalouf, M., Rho, J. M., Mattson, M. P. (2009). The neuroprotective properties of calorie restriction, the ketogenic diet, and ketone bodies. *Brain Res Rev*, 59(2), 293-315.

[14] Barbary, O. M., El-Sohaimy, S. A., El-Saadani, M. A., Zeitoun, A. M. A. (2010). Antioxidant, antimicrobial and anti-HCV activities of lignan extracted from flaxseed. *Research Journal of Agriculture and Biological Sciences*, 6(3), 247-256.

[15] Lieberman, S., Enig, M. G., Preuss, H. G. (2006). A review of monolaurin and lauric acid. Natural virucidal and Bactericidal agents. *Alternative and Complementary Therapies*. 1, 310-314.

[16] 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.

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

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

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

[20] 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.

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

[22] 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.

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