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**Endosymbiotic Actinidic Archaeal Cholesterol Catabolic  
Syndrome - Hypocholesterolemia and Human Diseases**

## Introduction

Actinidic archaea have been implicated in the pathogenesis of schizophrenia, malignancy, metabolic syndrome x, autoimmune disease and neuronal degeneration.<sup>1-9</sup> 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.<sup>10-17</sup>

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.<sup>10-17</sup>

## 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+ciprofloxacin and doxycycline each in a concentration of 1 mg/ml. Cholesterol substrate was prepared as described by Richmond.<sup>18</sup> Aliquots were withdrawn at zero time immediately after mixing and after incubation at 37 °C for 1 hour.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.<sup>19-21</sup> Cytochrome F420 was estimated fluorimetrically (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 rutil increased their levels. The addition of antibiotics to the patient's plasma caused a decrease in all the parameters while addition of rutil 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
AIDS	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
AIDS	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
AIDS	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 <sub>2</sub> O <sub>2</sub> % (Increase with Rutile)		H <sub>2</sub> O <sub>2</sub> % (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
AIDS	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.<sup>22-24</sup> 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.<sup>22-24</sup> The archaeal beta hydroxyl steroid dehydrogenase activity indicating digoxin synthesis and archaeal cholesterol hydroxylase activity indicating bile acid synthesis were increased.<sup>22-24</sup> The archaeal cholesterol oxidase activity was increased resulting in generation of pyruvate and hydrogen peroxide.<sup>22-24</sup> The pyruvate gets converted to glutamate and ammonia by the GABA shunt pathway. The archaeal aromatization of cholesterol generating PAH was also detected.<sup>22-24</sup> 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.<sup>25</sup> 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.<sup>10-17</sup>

The gut endotoxins and lipopolysaccharides are absorbed along with fat producing the syndrome of metabolic endotoxaemia. The endotoxins and lipopolysaccharides 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 lipopolysaccharides resulting in metabolic syndrome x, neuronal degenerations and autoimmune disease.<sup>10-17</sup>

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.<sup>10-17</sup> The endotoxins and lipopolysaccharides 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.<sup>10-17</sup>



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.<sup>10-17</sup>

## 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., Ambroggi, 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.

