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A Cholesterol and Actinide Dependent  
Shadow Biosphere of Archaea and  
Viroids in Metabolic Syndrome - Type  
2 Diabetes Mellitus with Coronary  
Artery Disease and Stroke

## Introduction

Actinides like rutile, endogenous digoxin as well as organisms like phytoplasmas and viroids have been implicated in the etiology of metabolic syndrome X - type 2 diabetes mellitus, CVA and CAD.<sup>1-4</sup> Endogenous digoxin has been related to the pathogenesis of metabolic syndrome - type 2 diabetes mellitus, CVA and CAD.<sup>4</sup> The possibility of endogenous digoxin synthesis by actinide based primitive organism like archaea with a mevalonate pathway and cholesterol catabolism was considered.<sup>5-8</sup> An actinide dependent shadow biosphere of archaea and viroids in metabolic syndrome X, CVA and CAD is described.<sup>7,9</sup> Metal actinides in beach sands have been postulated to play a role in abiogenesis.<sup>7</sup> 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  $K_m$  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 NF $\kappa$ B. Oxidative stress can open the mitochondrial PT pore producing release of cyto C and activation of the caspase cascade of cell death. 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 produce endothelial dysfunction and vascular disease. 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 metabolic syndrome X.

## Materials and Methods

The following groups were included in the study: - metabolic syndrome X - type 2 diabetes mellitus, CVA and CAD. 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>10</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, 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.<sup>11-13</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 rutilic acid increased their levels. The addition of antibiotics to the patient's plasma caused a decrease in all the parameters while addition of rutilic acid 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 rutilic acid and antibiotics on cytochrome P450 and PAH.

Group	CYT P450 % (Increase with Rutilic acid)		CYT P450 % (Decrease with Doxo+Cipro)		PAH % change (Increase with Rutilic acid)		PAH % change (Decrease with Doxo+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
DM	22.59	1.86	57.05	8.45	23.40	1.55	65.77	5.27
CVA	22.29	1.66	59.02	7.50	23.23	1.97	65.89	5.05
CAD	22.06	1.61	57.81	6.04	23.46	1.91	61.56	4.61
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
DM	23.01	1.67	65.35	3.56	23.33	1.86	66.46	3.65
CVA	22.56	2.46	62.70	4.53	23.32	1.74	65.67	4.16
CAD	23.30	1.42	65.07	4.95	23.11	1.52	66.68	3.97
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
DM	23.06	1.65	62.25	6.24	23.72	1.73	66.25	3.69
CVA	22.86	2.58	66.53	5.59	23.15	1.62	66.48	4.17
CAD	22.38	2.38	60.65	5.27	23.00	1.64	66.67	4.21
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
DM	0.47	0.04	0.202	0.025	22.87	2.58	64.51	5.93
CVA	0.56	0.05	0.220	0.052	22.29	1.47	64.35	5.58
CAD	0.53	0.06	0.212	0.045	23.30	1.88	62.49	7.26
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
DM	20.67	1.38	58.75	8.12	23.23	1.88	65.11	5.14
CVA	21.21	2.36	58.73	8.10	21.11	2.25	64.20	5.38
CAD	21.07	1.79	63.90	7.13	22.47	2.17	65.97	4.62
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 <sub>2</sub> O <sub>2</sub> % (Increase with Rutile)		H <sub>2</sub> O <sub>2</sub> % (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
DM	23.27	1.53	58.91	6.09	22.87	1.84	66.31	3.68
CVA	23.32	1.71	63.15	7.62	23.45	1.79	66.32	3.63
CAD	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	e < 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
DM	24.10	1.61	65.78	4.43	22.73	2.46	65.87	4.35
CVA	23.43	1.57	66.30	3.57	22.98	1.50	65.13	4.87
CAD	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

There was increase in cytochrome F420 indicating archaeal growth. The archaea can synthesize and use cholesterol as a carbon and energy source.<sup>6, 14</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>15</sup> 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.<sup>8</sup> The archaeal cholesterol oxidase activity was increased resulting in generation of pyruvate and hydrogen peroxide.<sup>14</sup> 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.<sup>16</sup> 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.<sup>17</sup> 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.<sup>18</sup> 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.<sup>19</sup> 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.<sup>20</sup> The integrated viroids and archaea can undergo vertical transmission and can exist as genomic parasites.<sup>19, 20</sup> 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.<sup>21</sup> 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.<sup>18</sup> 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 contributes to the pathogenesis of metabolic syndrome - type 2 diabetes mellitus, CVA and CAD.

Archaea and RNA viroid can bind the TLR receptor induce NF $\kappa$ B producing immune activation and cytokine TNF alpha secretion. The archaeal DXP and mevalonate pathway metabolites can bind  $\gamma\delta$  TCR and digoxin induced calcium signaling can activate NF $\kappa$ B producing chronic immune activation.<sup>4, 23</sup> The archaea and viroid induced chronic immune activation and generation of superantigens can lead on to autoimmunity in metabolic syndrome X, CVA and CAD. Archaea, viroids and digoxin can induce the host AKT PI3K, AMPK, HIF alpha and NF $\kappa$ B producing the Warburg metabolic phenotype.<sup>24</sup> 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 metabolic syndrome. The archaea and viroid generated cytokines can lead to TNF alpha induced insulin resistance and Metabolic Syndrome - type 2 diabetes mellitus with CAD and CVA. 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.<sup>24</sup> 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<sub>2</sub> regulating the conversion of T<sub>4</sub> to T<sub>3</sub> which activates uncoupling proteins, can activate NRF<sup>1/2</sup> 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.<sup>25</sup> The archaea and viroid induced monocyte activation and Warburg phenotype induced increased cholesterol synthesis leads to atherogenesis. 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. Prion proteins and HERV sequences are related to metabolic syndrome - type 2 diabetes mellitus, CVA and CAD.<sup>4</sup> Thus the archaea and the viroids are crucial to the etiopathogenesis of metabolic syndrome - type 2 diabetes mellitus, CVA and CAD.

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