

Chapter 8

**A Cholesterol and Actinide Dependent Shadow
Biosphere of Archaea and Viroids in Cancer**

Introduction

Endomyocardial Fibrosis along with the root wilt disease of coconut is endemic to Kerala with its radioactive actinide beach sands. Actinides like rutile, endogenous digoxin as well as organisms like phytoplasmas and viroids have been implicated in the etiology of cancer.^{1,2,3,4} Endogenous digoxin has been related to the pathogenesis of malignancy.⁴ The possibility of endogenous digoxin synthesis by actinide based primitive organism like archaea with a mevalonate pathway and cholesterol catabolism was considered.⁵⁻⁸ An actinide dependent shadow biosphere of archaea and viroids in cancer is described.^{7,9} Metal actinides in beach sands have been postulated to play a role in abiogenesis.⁷ A hypothesis of cholesterol as the primal prebiotic molecule synthesised on actinide surfaces with all other biomolecules arising from it and a self replicating cholesterol lipid organism as the initial life form is presented.

Materials and Methods

The following groups were included in the study: - Non-Hodgkin's Lymphoma and glioma. 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 Cholesterol substrate was prepared as described by Richmond.¹⁰ Aliquots were withdrawn at zero time immediately after mixing and after incubation at 37°C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.¹¹⁻¹³ 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 rutila increased their levels. The addition of antibiotics to the patient's plasma caused a decrease in all the parameters while addition of rutila increased their levels but the extent of change was more in patient's sera as compared to controls. The results are expressed in tables 1-7 as percentage change in the parameters after 1 hour incubation as compared to the values at zero time.

Table 1. *Effect of rutile and antibiotics on cytochrome F420 and PAH.*

| Group | CYT F420 % (Increase with Rutile) | | CYT F420 % (Decrease with Doxy+Cipro) | | PAH % change (Increase with Rutile) | | PAH % change (Decrease with Doxy+Cipro) | |
|--------|--------------------------------------|------|--|------|--|------|--|------|
| | Mean | ± SD | Mean | ± SD | Mean | ± SD | Mean | ± SD |
| Normal | 4.48 | 0.15 | 18.24 | 0.66 | 4.45 | 0.14 | 18.25 | 0.72 |
| NHL | 22.79 | 2.13 | 55.90 | 7.29 | 22.84 | 1.42 | 66.07 | 3.78 |
| Glioma | 22.70 | 1.87 | 60.46 | 8.06 | 23.73 | 1.38 | 65.20 | 6.20 |
| | F value 306.749 | | F value 130.054 | | F value 391.318 | | F value 257.996 | |
| | P value < 0.001 | | P value < 0.001 | | P value < 0.001 | | P value < 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 |
| NHL | 22.42 | 1.99 | 61.14 | 3.47 | 23.78 | 1.20 | 66.90 | 4.10 |
| Glioma | 22.29 | 2.05 | 58.70 | 7.34 | 22.29 | 2.05 | 67.03 | 5.97 |
| | F value 337.577 | | F value 356.621 | | F value 427.828 | | F value 654.453 | |
| | P value < 0.001 | | P value < 0.001 | | P value < 0.001 | | P value < 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 |
| NHL | 22.28 | 1.76 | 61.88 | 6.21 | 24.01 | 1.17 | 66.66 | 3.84 |
| Glioma | 22.92 | 1.48 | 61.91 | 7.56 | 23.37 | 1.31 | 63.97 | 3.62 |
| | F value 319.332 | | F value 199.553 | | F value 449.503 | | F value 673.081 | |
| | P value < 0.001 | | P value < 0.001 | | P value < 0.001 | | P value < 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 |
| NHL | 0.54 | 0.04 | 0.210 | 0.042 | 22.98 | 2.19 | 64.96 | 5.64 |
| Glioma | 0.51 | 0.05 | 0.213 | 0.033 | 23.41 | 1.41 | 58.70 | 7.34 |
| | F value 135.116 P value < 0.001 | | F value 71.706 P value < 0.001 | | F value 290.441 P value < 0.001 | | F value 203.651 P value < 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 |
| NHL | 21.19 | 1.61 | 58.57 | 7.47 | 22.53 | 2.41 | 64.29 | 5.44 |
| Glioma | 22.29 | 2.05 | 62.37 | 5.05 | 21.66 | 1.94 | 67.03 | 5.97 |
| | F value 321.255 P value < 0.001 | | F value 115.242 P value < 0.001 | | F value 292.065 P value < 0.001 | | F value 317.966 P value < 0.001 | |

Table 6. *Effect of rutile and antibiotics on hydrogen peroxide and delta amino levulinic acid.*

| Group | H ₂ O ₂ % (Increase with Rutile) | | H ₂ O ₂ % (Decrease with Doxy+Cipro) | | ALA % (Increase with Rutile) | | ALA % (Decrease with Doxy+Cipro) | |
|--------|---|------|---|------|------------------------------------|------|-------------------------------------|------|
| | Mean | ± SD | Mean | ± SD | Mean | ± SD | Mean | ± SD |
| Normal | 4.43 | 0.19 | 18.13 | 0.63 | 4.40 | 0.10 | 18.48 | 0.39 |
| NHL | 23.35 | 1.76 | 59.17 | 3.33 | 23.34 | 1.75 | 66.80 | 3.43 |
| Glioma | 23.29 | 1.67 | 60.52 | 5.38 | 22.29 | 2.05 | 61.91 | 7.56 |
| | F value 380.721 P value < 0.001 | | F value 171.228 P value < 0.001 | | F value 372.716 P value < 0.001 | | F value 556.411 P value < 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 |
| NHL | 23.81 | 1.90 | 66.95 | 3.67 | 23.12 | 1.71 | 65.12 | 5.58 |
| Glioma | 22.28 | 1.52 | 64.05 | 2.79 | 22.82 | 1.56 | 64.61 | 4.95 |
| | F value 403.394 P value < 0.001 | | F value 680.284 P value < 0.001 | | F value 348.867 P value < 0.001 | | F value 364.999 P value < 0.001 | |

Discussion

There was increase in cytochrome F420 indicating archaeal growth. The archaea can synthesise and use cholesterol as a carbon and energy source.^{6,14} The archaeal origin of the enzyme activities was indicated by antibiotic induced suppression. The study indicates the presence of actinide based archaea with an alternate actinide based enzymes or metalloenzymes in the system as indicated by rutile induced increase in enzyme activities.¹⁵ There was also an increase in archaeal HMG CoA reductase activity indicating increased cholesterol synthesis by the archaeal mevalonate pathway. The archaeal beta hydroxyl steroid dehydrogenase activity indicating digoxin synthesis and archaeal cholesterol hydroxylase activity indicating bile acid synthesis were increased.⁸ The archaeal cholesterol oxidase activity was increased resulting in generation of pyruvate and hydrogen peroxide.¹⁴ The pyruvate gets converted to glutamate and ammonia by the GABA shunt pathway. The archaeal aromatization of cholesterol generating PAH, serotonin and dopamine was also detected.¹⁶ The archaeal glycolytic hexokinase activity and archaeal extracellular ATP synthase activity were increased. The archaea can undergo magnetite and calcium carbonate mineralization and can exist as calcified nanoforms.¹⁷ There was an

increase in free RNA indicating self replicating RNA viroids and free DNA indicating generation of viroid complementary DNA strands by archaeal reverse transcriptase activity. The actinides modulate RNA folding and catalyse its ribozymal action. Digoxin can cut and paste the viroidal strands by modulating RNA splicing generating RNA viroidal diversity. The viroids are evolutionarily escaped archaeal group I introns which have retrotransposition and self splicing qualities.¹⁸ Archaeal pyruvate can produce histone deacetylase inhibition resulting in endogenous retroviral (HERV) reverse transcriptase and integrase expression. This can integrate the RNA viroidal complementary DNA into the noncoding region of eukaryotic non coding DNA using HERV integrase as has been described for borna and ebola viruses.¹⁹ The noncoding DNA is lengthened by integrating RNA viroidal complementary DNA with the integration going on as a continuing event. The archaea genome can also get integrated into human genome using integrase as has been described for trypanosomes.²⁰ The integrated viroids and archaea can undergo vertical transmission and can exist as genomic parasites.^{19,20} This increases the length and alters the grammar of the noncoding region producing memes or memory of acquired characters as well as eukaryotic speciation and individuality.²¹ The viroidal complementary DNA can function as jumping genes producing a dynamic genome important in storage of synaptic information, HLA gene expression and developmental gene expression. The RNA viroids can regulate mRNA function by RNA interference.¹⁸ The phenomena of RNA interference can modulate T cell and B cell function, insulin signaling lipid metabolism, cell growth and differentiation, apoptosis, neuronal transmission and euchromatin/heterochromatin expression. This can lead to oncogenesis.

The archaea and viroids can regulate the nervous system including the NMDA/GABA thalamocorticothalamic pathway mediating conscious perception.^{4,22} NMDA/GABA receptors can be modulated by digoxin induced

calcium oscillations resulting NMDA/GAD activity induction, PAH increasing NMDA activity and inducing GAD as well as viroid induced RNA interference.⁴ The cholesterol ring oxidase generated pyruvate can be converted by the GABA shunt pathway to glutamate and GABA. The dipolar PAH and archaeal magnetite in the setting of digoxin induced sodium potassium ATPase inhibition can produce a pumped phonon system mediated frohlich model superconducting state²² inducing quantal perception with nanoarchaeal sensed gravity producing the orchestrated reduction of the quantal possibilities to the macroscopic world.^{4,22} The archaea can regulate limbic lobe transmission with archaeal cholesterol aromatase/ring oxidase generated norepinephrine, dopamine, serotonin and acetyl choline.¹⁶ The higher degree of integration of the archaea into the genome produces increased digoxin synthesis producing right hemispheric dominance and lesser degree producing left hemispheric dominance.⁴ Right hemispheric dominance can lead to oncogenesis. The increased integration of archaea into the neuronal genome can produce increased cholesterol oxidase and aromatase mediated monoamine and NMDA transmission producing oncogenesis. Archaea and RNA viroid can bind the TLR receptor induce NF κ 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 κ B producing chronic immune activation.^{4,23} The archaea and viroid induced chronic immune activation and generation of superantigens can lead on to lymphoma. Archaea, viroids and digoxin can induce the host AKT PI3K, AMPK, HIF alpha and NF κ B producing the Warburg metabolic phenotype.²⁴ The increased glycolytic hexokinase activity, decrease in blood ATP, leakage of cytochrome C, increase in serum pyruvate and decrease in acetyl CoA indicates the generation of the Warburg phenotype. There is induction of glycolysis, inhibition of PDH activity and mitochondrial dysfunction resulting in inefficient energetics and cancer. The archaea and viroid generated cytokines can lead to

TNF alpha induced insulin resistance and cancer. The accumulated pyruvate enters the GABA shunt pathway and is converted to citrate which is acted upon by citrate lyase and converted to acetyl CoA, used for cholesterol synthesis.²⁴ The pyruvate can be converted to glutamate and ammonia which is oxidised by archaea for energy needs. The increased cholesterol substrate leads to increased archaeal growth and digoxin synthesis leading to metabolic channeling to the mevalonate pathway. The archaeal bile acids are steroidal hormones which can bind GPCR and modulate D2 regulating the conversion of T4 to T3 which activates uncoupling proteins, can activate NRF ½ inducing NQO1, GST, HOI reducing redox stress, can bind FXR regulating insulin receptor sensitivity and bind PXR inducing the bile acid shunt pathway of cholesterol detoxification.²⁵ The archaea and viroid induced monocyte activation and Warburg phenotype induced increased cholesterol synthesis leads to oncogenesis. The Warburg phenotype induced increased mitochondrial PT pore hexokinase, archaeal PAH and viroid induced RNA interference can lead on to malignant transformation. The digoxin and PAH induced increased intracellular calcium can lead to PT pore dysfunction and oncogenesis.⁴ The archaeal cholesterol catabolism can deplete the cell membranes of cholesterol resulting in organelle dysfunction and degeneration. 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. Endogenous retroviral sequences and prion protein like tumour suppressor protein can lead to oncogenesis.⁴

The metal actinides provide radiolytic energy, catalysis for oligomer formation and provide a coordinating ion for metalloenzymes all important in abiogenesis.⁷ The metal actinide surfaces would by surface metabolism generate acetate which could get converted to acetyl CoA and then to cholesterol which functions as the primal prebiotic molecule self organizing into self replicating

supramolecular systems, the lipid organism.^{9,26,27} Cholesterol by radiolysis by actinides would have formed PAH generating PAH aromatic organism.⁹ Cholesterol radiolysis would generate pyruvate which would get converted to amino acids, sugars, nucleotides, porphyrins, fatty acids and TCA acids. Anastase and rutile surfaces can produce polymerization of amino acids, isoprenyl residues, PAH and nucleotides to generate the initial lipid organism, PAH organism, prions and RNA viroids which would have symbiosed to generate the archaeal protocell. The archaea evolved into gram negative and gram positive bacteria with a mevalonate pathway which had a evolutionary advantage and the symbiosis of archaea with gram negative organism generated the eukaryotic cell.²⁸ The data supports the persistence of an actinide and cholesterol based shadow biosphere which throws light on the actinide based origin of life and cholesterol as the premier prebiotic molecule.

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