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RNA Viroids- Role in Metabolic/Endocrine Regulation – The Pathogenesis of Metabolic Syndrome with Coronary Artery Disease and Stroke

Introduction

The human body synthesizes an endogenous sodium potassium ATPase inhibitor digoxin which plays a role in neuro-immuno-endocrine integration as well as in cardiovascular/metabolic disorders. Endomyocardial fibrosis (EMF) along with the root wilt disease of coconut is endemic to Kerala with its radioactive actinide beach sands. Actinides like rutile producing intracellular magnesium deficiency due to rutile-magnesium exchange sites in the cell membrane has been implicated in the etiology of EMF[1]. Endogenous digoxin, a steroidal glycoside which functions as a membrane sodium potassium ATPase inhibitor has also been related to its etiology due to the intracellular magnesium deficiency it produces[2]. Organisms like phytoplasmas and viroids have also been demonstrated to play a role in the etiology of these diseases[3, 4]. Endogenous digoxin has been related to the pathogenesis of type 2 diabetes mellitus, coronary artery disease and cerebrovascular disease[2]. The possibility of endogenous digoxin synthesis by actinide based primitive organism like archaea with a mevalonate pathway and cholesterol catabolism was considered[5-8]. An actinide dependent shadow biosphere of archaea and viroids in the above mentioned disease states is described[6]. The intracellular endosymbionts archaea and their intron derived viroids constitute the third element regulating the human body. A hypothesis regarding the role of endosymbiotic actinidic archaea and viroids in metabolic/endocrine regulation is put forward.

Materials and Methods

Informed consent of the subjects and the approval of the ethics committee were obtained for the study. The following groups were included in the study: – type 2

diabetes mellitus, coronary artery disease- acute coronary syndrome and acute cerebrovascular thrombotic stroke. The coronary artery disease and cerebrovascular disease patients chosen for the study did not have type 2 diabetes mellitus as a risk factor. 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[9]. Aliquots were withdrawn at zero time immediately after mixing and after incubation at 37 ℃ for 1 hour. The following estimations were carried out: – Cytochrome F420, free RNA, free DNA, polycyclic aromatic hydrocarbon, hydrogen peroxide, pyruvate, ammonia, glutamate, hexokinase, ATP synthase, HMG CoA reductase, digoxin and bile acids[10-13]. Cytochrome F420 was estimated flourimetrically (excitation wavelength 420 nm and emission wavelength 520 nm). Polycyclic aromatic hydrocarbon was estimated by measuring hydrogen peroxide liberated by using glucose reagent. The statistical analysis was done by ANOVA.

Results

The parameters checked as indicated above were: – cytochrome F420, free RNA, free DNA, muramic acid, polycyclic aromatic hydrocarbon, hydrogen peroxide, serotonin, pyruvate, ammonia, glutamate, cytochrome C, hexokinase, ATP synthase, HMG CoA reductase, digoxin and bile acids. 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 1-6 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)	
	Mean	**± SD**	**Mean**	**± SD**
Normal	4.48	0.15	18.24	0.66
DM	22.59	1.86	57.05	8.45
CAD	22.76	2.26	60.49	6.86
CVA	21.01	2.29	62.37	8.01
F value	306.749		130.054	
P value	< 0.001		< 0.001	

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

Group	DNA % change (Increase with Rutile)		DNA % change (Decrease with Doxy)		RNA % change (Increase with Rutile)		RNA % change (Decrease with Doxy)	
	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
CAD	23.12	1.71	65.12	5.58	24.01	1.17	66.66	3.84
CVA	22.51	1.85	63.56	5.29	22.95	1.90	66.39	3.83
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 PAH.*

Group	HMG CoA R % change (Increase with Rutile)		HMG CoA R % change (Decrease with Doxy)		PAH % change (Increase with Rutile)		PAH % change (Decrease with Doxy)	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Normal	4.30	0.20	18.35	0.35	4.45	0.14	18.25	0.72
DM	23.06	1.65	62.25	6.24	23.40	1.55	65.77	5.27
CAD	23.63	1.58	61.19	7.03	22.22	2.33	61.73	6.33
CVA	22.51	2.47	60.77	5.89	23.87	1.64	66.01	5.78
F value	319.332		199.553		391.318		257.996	
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)	
	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
CAD	0.49	0.07	0.202	0.021	22.22	2.44	63.47	6.98
CVA	0.51	0.07	0.195	0.023	22.33	2.18	62.20	6.33
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)		Hexokinase % change (Increase with Rutile)		Hexokinase % change (Decrease with Doxy)	
	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
CAD	20.16	1.07	57.08	9.83	21.88	2.11	65.02	4.40
CVA	20.60	1.81	58.97	7.03	21.98	2.12	65.78	6.08
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 ATP synthase and hydrogen peroxide.*

Group	ATP synthase % (Increase with Rutile)		ATP synthase % (Decrease with Doxy)		H_2O_2 % (Increase with Rutile)		H_2O_2 % (Decrease with Doxy)	
	Mean	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**
Normal	4.40	0.11	18.78	0.11	4.43	0.19	18.13	0.63
DM	23.72	1.73	66.25	3.69	23.27	1.53	58.91	6.09
CAD	23.78	1.20	66.90	4.10	23.24	1.85	57.08	7.42
CVA	23.47	1.60	66.27	3.88	23.32	1.60	61.45	7.01
F value	449.503		673.081		380.721		171.228	
P value	< 0.001		< 0.001		< 0.001		< 0.001	

Abbreviations

DM: Type 2 diabetes mellitus

CAD: Coronary artery disease

CVA: Cerebrovascular thrombosis

Discussion

There was increase in cytochrome F420 indicating archaeal growth in type 2 diabetes mellitus, coronary artery disease and cerebrovascular disease. The archaea can synthesize and use cholesterol as a carbon and energy source[14, 15]. 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[16]. 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[7]. The archaeal

cholesterol oxidase activity was increased resulting in generation of pyruvate and hydrogen peroxide[15]. 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[17]. 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[18].

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 probably escaped archaeal group I introns which have retrotransposition and self splicing qualities[19]. The decrease in free self replicating RNA and DNA with the addition of antibiotics indicates that the RNA viroids are derived from archaeal introns. 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[20-22]. The RNA viroids can regulate mRNA function by RNA interference[19]. The phenomena of RNA interference can modulate euchromatin/heterochromatin expression. RNA viroidal mRNA interference plays a role in the pathogenesis of type 2 diabetes mellitus, coronary artery disease and cerebrovascular disease. Viroidal RNA mediated mRNA interference can modulate lipid metabolism triggering of dyslipidemias important in atherogenesis. The viroidal RNA modulation of T cell and B cell function by mRNA interference can lead to immune activation. Monocytic infiltration of the vascular wall is important in atherogenesis. Insulin resistance due to TNF alpha

modulation of the insulin receptor can contribute to type 2 diabetes mellitus. The viroidal RNA mediated mRNA interference can also modulate insulin signalling and secretion leading onto type 2 diabetes mellitus[23-32].

Archaea and RNA viroid can bind the TLR receptor induce NFKB producing immune activation and cytokine TNF alpha secretion. The archaeal DXP and mevalonate pathway metabolites can bind γδ TCR and digoxin induced calcium signaling can activate NFKB producing chronic immune activation or systemic inflammatory reaction[2, 33]. The archaea and viroid induced chronic immune activation and generation of superantigens. Immune activation results in induction of NADPH oxidase which generates hydrogen peroxide. Cholesterol oxidase activity also generates hydrogen peroxide. Hydrogen peroxide can increase protein tyrosine kinase activity and suppress protein phosphatase activity increasing insulin receptor function. Immune activated NOX and bacterial cholesterol oxidase can thus regulate insulin receptor function. Immune activation can also produce insulin resistance. TNF alpha produced by chronic immune activation can modulate the insulin receptor producing insulin resistance[30]. Chronic immune activation and cholesterol oxidase generated hydrogen peroxide can induce neutral sphingomyelinase generating ceramide producing insulin resistance[34]. Immune activation and NFKB induction can suppress the nuclear receptors LXR, PXR and FXR. LXR suppression by NFKB stimulates HMG CoA reductase activity and suppresses cholesterol 7 alpha hydroxylase activity[35]. This stimulates cholesterol synthesis and inhibits its degradation via the bile acid pathway. PXR suppression by NFKB prevents cholesterol detoxification via the bile acid shunt pathway[36]. Thus LXR and PXR suppression by NFKB produces acute cholesterol toxicity. This NFKB induced suppression of LXR and PXR can contribute to increased lipid and cholesterol synthesis contributing to obesity. FXR suppression can also lead to insulin resistance, dyslipidemias and increased connective tissue MPS deposition in vessel wall and atherogenesis. The cholesterol toxicity can

lead to lipoprotein and cholesterol uptake by monocytes in the vessel wall producing atherogenesis. The archaea and viroid induced chronic immune activation can lead to monocyte infiltration of the vessel wall. This sets the stage for the atherogenetic process[29]. The increased cholesterol synthesis is important in stimulating archaeal growth which uses cholesterol as a carbon and energy source.

Archaea, viroids and digoxin can induce the host AKT PI3K, AMPK, HIF alpha and NFKB producing the Warburg metabolic phenotype[37]. 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. Inefficient energetics owing to the Warburg's phenotype can contribute to metabolic syndrome x. 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[37]. The pyruvate can be converted to glutamate and ammonia which is oxidised by archaea for energy needs. Ammonia can stimulate membrane sodium potassium ATPase increasing ATP utilisation, produce mitochondrial transmembrane potential changes and produce mitochondrial dysfunction important in type 2 diabetes mellitus. The increased cholesterol substrate also leads to increased archaeal growth and digoxin synthesis due to metabolic channeling to the mevalonate pathway.

Digoxin can produce sodium potassium ATPase inhibition and inward movement of plasma membrane cholesterol. This produces defective SREBP sensing, increased HMG CoA reductase activity and cholesterol synthesis[28]. The digoxin induced inward movement of plasma membrane cholesterol can alter membrane cholesterol/sphingomyelin ratio producing modified lipid microdomains[38]. The digoxin induced lipid microdomain modulation can regulate the GPCR couple

adrenaline, noradrenaline, glucagon and neuropeptide receptors as well as protein tyrosine kinase linked insulin receptor. The digoxin mediated inhibition of nuclear membrane sodium potassium ATPase can modulate nuclear membrane lipid microdomains and steroidal/thyroxine DNA receptor function. Thus endogenous digoxin can modulate all the endocrine receptors by regulating lipid microdomains. Hyperdigoxinemia is important in the pathogenesis of atherogenesis and metabolic syndrome X. Digoxin induced sodium potassium ATPase inhibition results in an ATP sparing effect[39]. Eighty percent of the ATP generated is used to run the sodium-potassium ATPase pump. The digoxin inhibition of the sodium-potassium ATPase spares this ATP which is then used for lipid synthesis. Thus endogenous digoxin and the shadow biosphere generated Warburg phenotype can produce increased lipid synthesis and obesity important in metabolic syndrome X. Fat fuels insulin resistance by binding to the toll receptor and producing immune activation and immune infiltration of the adipose tissue. Digoxin can also increase lymphocytic intracellular calcium which leads on to induction of NFKB and immune activation[2]. The archaeal cholesterol catabolism can deplete the lymphocytic cell membranes of cholesterol resulting in alteration of lymphocytic cell membrane microdomains related receptors producing immune activation.

The archaeal bile acids are steroidal hormones[40]. The archaeal bile acids can bind GPCR and modulate D2 regulating the conversion of T4 to T3. T3 activates uncoupling proteins reducing redox stress. Bile acids can also activate NRF ½ inducing NQO1, GST, HOI reducing redox stress. Bile acids can bind FXR regulating insulin receptor sensitivity and bind PXR inducing the bile acid shunt pathway of cholesterol detoxification. 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.

Thus the archaeal bile acids have a role opposite to digoxin and help to increase insulin sensitivity.

The cholesterol ring oxidase generated pyruvate can be converted by the GABA shunt pathway to glutamate. Glutamatergic transmission can lead to immune activation, atherogenesis and increased insulin signalling/release. The archaeal cholesterol aromatase can generate PAH[17]. The PAH can also lead to insulin resistance and atherogenesis. Particulate pollution has been related to metabolic syndrome X, type 2 diabetes mellitus and vascular thrombosis.

The higher degree of integration of the archaea and viroids into the genome produces increased digoxin synthesis producing right hemispheric dominance and lesser degree producing left hemispheric dominance[2]. Right hemispheric dominance can lead to type 2 diabetes mellitus and vascular thrombosis. Thus the actinide, viroid and mevalonate pathway bacteria induced metabolic, genetic, immune and neuronal transmission changes can lead onto metabolic syndrome X and atherogenesis.

An actinide dependent shadow biosphere of archaea and viroids is described in type 2 diabetes mellitus, coronary artery disease- acute coronary syndrome and acute cerebrovascular thrombosis contributing to their pathogenesis. The archaea secreted digoxin and archaeal viroids serves as a messenger regulating metabolic and endocrine systems.

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