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Archaeal Digoxin Mediated Model for Systemic Lupus Erythematosis - Relation to Hemispheric Chemical Dominance

Introduction

The archaea produces an endogenous membrane Na^+-K^+ ATPase inhibitor, digoxin which is a steroidal glycoside. Digoxin is synthesized by the isoprenoid pathway. Increased level of digoxin has been documented in immune diseases like Kawasaki's disease. A viral infective theory for Kawasaki's disease has been postulated by several groups of workers. Membrane Na^+-K^+ ATPase inhibition leads to immune stimulation and increased in CD_4/CD_8 ratios as exemplified by the action of lithium. Digoxin can also modulate amino acid and neurotransmitter transport. There have been reports of increased activities of the tryptophan catabolic kynurenine pathway in various tissues following systemic immune stimulation, in conjunction with macrophage infiltration of the affected tissues. These results suggest that kynurenine metabolites may have some connection with immune response. Previous reports have demonstrated induction of indoleamine 2,3-dioxygenase and increased production of quinolinic acid in systemic lupus mediated by interferons. The isoprenoid pathway produces two other metabolites - ubiquinone and dolichol important in cellular metabolism. Ubiquinone functions as a free radical scavenger and dolichol is important in N-glycosylation of proteins.

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 km 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 NFKB. 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 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 autoimmune disease.

It was therefore considered pertinent to study digoxin status and digoxin synthesis in SLE. The glycoconjugate metabolism, free radical metabolism and RBC membrane composition were also studied in these groups of diseases. These parameters were also studied in patients with right hemispheric and left hemispheric dominance in order to find the correlation between hemispheric dominance and immune mediated diseases. The results are presented in this paper.

Materials and Methods

The following groups were included in the study: (1) 10 cases of SLE (ARA criteria); all the 15 patients with SLE were right handed heft hemispheric dominant by the dichotic listening test, (2) 15 patients with right hemispheric dominance, left hemispheric dominance and bihemispheric dominance respectively detected by the dichotic listening test, (3) Each patient had an age and sex matched bihemispheric dominant healthy control. The permission of the Ethics committee of the institute as well as informed consent from the patients / relatives was obtained for the study.

None of the subjects studied was under medication at the time of removal of blood. Fasting blood was removed in citrate tubes from each of the number of patients mentioned above. RBCs were separated within one hour of collection of blood for the estimation of membrane Na^+-K^+ ATPase. Serum was used for

the analysis of various parameters. The methodology used in the study was as follows: All biochemicals used in this study were obtained from M/s Sigrna Chemicals, USA. Activity of HMG CoA reductase of the serum was determined by the method of Rao and Ramakrishnan by determining the ratio of HMG CoA to mevalonate. For the determination of the RBC Na^+-K^+ ATPase activity of the erythrocyte membrane, the procedure described by Wallach and Kamat was used. Digoxin in the serum was determined by the procedure described by Arun, Ravikumar, Leelamma and Kurup. For estimation of ubiquinone and dolichol in the serum, the procedure described by Palmer, Maureen and Robert was used. Magnesium in the serum was estimated by atomic absorption spectrophotometry. Tryptophan was estimated by the method of Bloxam and Warren and tyrosine by the method of Wong, O'Flynn and Inouye. Serotonin was estimated by the method of Curzon and Green and catecholamines by the method of Well-Malherbe. Quinohnic acid content of serum was estimated by HPLC (C_{18} column micro Bondapak™ 4.6 x 140 mm), solvent system 0.01 M acetate buffer (pH 3.0) and methanol (6:4), flow rate 1.0 ml/min and detection UV (250 nm). Morphine, strychnine and nicotine were estimated by the method described by Arun, Ravikumar, Leelamma and Kurup. Details of the procedures used for the estimation of total and individual GAG, carbohydrate components of glycoproteins, activity of enzymes involved in the degradation of GAG and activity of glycohydrolases are described before. Serum glycolipids were estimated as described by Lowenstein. Cholesterol was estimated by using commercial kits supplied by Sigma Chemicals, USA. SOD was assayed by the method of Kakkar, Das and Viswanathan. Catalase activity was estimated by the method of Maehly and Chance, glutathione peroxidase by the method of Paglia and Valentine and glutathione reductase by the method of Horn and Burns. MDA was estimated by the method of Will and conjugated dienes and hydroperoxides by the procedure of Brien. Reduced glutathione was estimated by the method of

Beutler, Duran and Kelley. Nitric oxide was estimated in the plasma by the method of Gabor and Allon. Statistical analysis was done by 'ANOVA'.

Results

(1) The results showed that serum HMG CoA reductase activity, serum digoxin and dolichol were increased in SLE indicating upregulation of the isoprenoid pathway but serum ubiquinone, magnesium and RBC membrane Na^+-K^+ ATPase activity was reduced.

(2) The results showed that the concentration of tryptophan, quinolinic acid, serotonin, strychnine and nicotine was found to be higher in the serum of patients with SUE while that of tyrosine, dopamine, norepinephrine and morphine was lower.

(3) There was an increase in lipid peroxidation as evidenced from the increase in the concentration of MDA, conjugated dienes, hydroperoxides and NO with decreased antioxidant protection as indicated by a decrease in ubiquinone and reduced glutathione in SLE. The activity of enzymes involved in free radical scavenging like superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase is decreased in SLE suggesting reduced free radical scavenging.

(4) The results show an increase in the concentration of serum total and individual GAG fractions, glycolipids and carbohydrate components of glycoproteins in SLE. The activity of GAG degrading enzymes and that of glycohydrolases showed a significant increase in the serum in SLE.

(5) The cholesterol: phospholipid ratio of the RBC membrane was increased in SLE, The concentration of total GAG, hexose and fucose content of glycoprotein decreased in the RBC membrane and increased in the serum in SLE.

(6) The results showed that serum HMG CoA reductase activity serum digoxin and dolichol levels were increased and serum ubiquinone, magnesium and RBC membrane Na^+-K^+ ATPase activity were reduced in left handed / right hemispheric dominant individuals. The results also showed that serum HMG CoA reductase activity, serum digoxin and dolichol levels were decreased and serum ubiquinone, magnesium and RBC membrane Na^+-K^+ ATPase activity were increased in right handed / left hemispheric dominant individuals. The results showed that the concentration of tryptophan, quinolinic acid serotonin, strychnine and nicotine was found to be higher in the serum of left handed / right hemispheric dominant individuals while that of tyrosine, dopamine, morphine and norepinephrine was lower. The results also showed that the concentration of tryptophan, quinolinic acid serotonin, strychnine and nicotine was found to be lower in the serum of right handed / left hemispheric dominant individuals while that of tyrosine, dopamine, morphine and norepinephrine was higher.

Discussion

Archaeal Digoxin and Membrane Na^+-K^+ ATPase Inhibition in Relation to SLE

The archaeaon steroidelle DXP pathway and the upregulated pentose phosphate pathway contribute to digoxin synthesis. The increase in endogenous digoxin, a potent inhibitor of membrane Na^+-K^+ ATPase, can decrease this enzyme activity in SLE. There was increased synthesis of digoxin as evidenced by increased HMG CoA reductase activity. The inhibition of Na^+-K^+ ATPase by digoxin is known to cause an increase in intracellular calcium resulting from increased Na^+-Ca^{++} exchange, which displaces magnesium from its binding site and causes a decrease in the functional availability of magnesium. This decrease in the availability of magnesium can cause decreased mitochondrial ATP

formation which along with low magnesium can cause further progressive inhibition of Na^+-K^+ ATPase, since the ATP-magnesium complex is the actual substrate for this reaction. Low intracellular magnesium and high intracellular calcium consequent to Na^+-K^+ ATPase inhibition appear to be crucial to the pathophysiology of SLE.

Archaeal Digoxin and Immune Activation in Relation to SLE

The archaeaon fructosoid contributes to fructolysis and immune activation. Fructose can contribute to induction of NFKB and immune activation. The archaeaon steroidelle synthesized digoxin induces NFKB producing immune activation. In SLE increased intracellular calcium consequent to membrane Na^+-K^+ ATPase inhibition activates the calcium dependent calcineurin signal transduction pathway, which can produce T-cell activation and secretion of interleukin-3, 4, 5, 6 and TNF alpha. This immune activation can contribute to the genesis of SLE.

Archaeal Digoxin and Regulation of Neurotransmitter Synthesis and Function in Relation to SLE

The archaeaon neurotransminoid shikimic acid pathway contributes to tryptophan and tyrosine synthesis and catabolism generating neurotransmitters and neuroactive alkaloids. There is an increase in tryptophan and its catabolites and reduction in tyrosine and its catabolites in the serum of patients with SLE. This could be due to the fact digoxin can regulate neutral amino acid transport system with preferential promotion of tryptophan transport over tyrosine. In the presence of hypomagnesmia, the magnesium block on the NMDA receptor is removed leading to NMDA excitotoxicity. The elevated levels of quinolinic acid, strychnine and serotonin can also contribute to NMDA excitotoxicity as they are positive modulators of the NMDA receptor. NMDA excitotoxic

mechanisms have been postulated to contribute to neuronal death. Quinolinic acid has been implicated in immune activation in autoimmune diseases like SLE. Serotonin, dopamine and noradrenaline receptors have been demonstrated in the lymphocytes. It has been reported that during immune activation serotonin is increased with a corresponding reduction in dopamine and noradrenaline and this can contribute to the immune activation in SLE. The schizoid neurotransmitter pattern of reduced dopamine, noradrenaline and morphine and increased serotonin, strychnine and nicotine is common to SLE and could predispose to its development. A schizoid type of personality could predispose to the development of SLE. The present study shows that schizoid psychosis in neurolupus could as well be due to a primary neurotransmitter change.

Archaeal Digoxin and Regulation of Golgi Body / Lysosomal Function in Relation to SLE

The archaeaon glycosaminoglycoid and fructosoid contributes to glycoconjugate synthesis and catabolism by the process of fructolysis. The elevation in the level of dolichol in SEE may suggest its increased availability for N-glycosylation of proteins. Magnesium deficiency can lead to increased glycolipid and glycosaminoglycan synthesis. Intracellular magnesium deficiency also results in defective ubiquitin dependent proteolytic processing of glycoconjugates as it requires magnesium for its function. The increase in the activity of glycohydrolases and GAG degrading enzymes could be due to reduced lysosomal stability and consequent leakage of lysosomal enzymes into the serum. The increase in the concentration of carbohydrate components of glycoproteins and GAG inspite of increased activity of many glycohydrolases may be due to their possible resistance to cleavage by glycohydrolases / GAG degrading enzymes consequent to qualitative change in their structure. The protein processing defect can result in defective glycosylation of endogenous

neuronal glycoprotein antigens and exogenous bacterial glycoprotein antigens with consequent defective formation of MHC-antigen complex. The MHC linked peptide transporter, a P-glycoprotein which transports MHC antigen complex to the antigen presenting cell surface, has an ATP binding site. The peptide transporter is dysfunctional in the presence of magnesium deficiency. This results in defective transport of MHC class-l glycoprotein antigen complex to the antigen presenting cell surface for recognition by CD_4 or CD_8 cell. Defective presentation of the endogenous neuronal / nuclear glycoprotein antigen can explain the immune dysregulation and autoimmunity in neurolupus. Defective presentation of exogenous viral can produce immune evasion by the virus as in SLE. Viral and bacterial persistence has been implicated in the development of SLE also. The antinuclear antibodies in SLE are developed against methylated bases. Methylated bases are seen commonly in bacteria and rarely in humans. A number of fucose and sialic acid containing natural ligands are involved in trafficking of leukocytes and similar breaches in the blood brain barrier and resultant adhesion and trafficking of the lymphocyte and extravasation in to the perivascular space have been described in the brain in neurolupus.

Archaeal Digoxin and Alteration in Membrane Structure and Membrane Formation in Relation to SLE

The archaeaon steroidelle, glycosaminoglycoid and fructosoid contribute to cell membrane formation synthesizing cholesterol by the DXP pathway and glycosaminoglycans by fructolysis. The upregulation of the isoprenoid pathway can lead to increased cholesterol synthesis and magnesium deficiency can inhibit phospholipid synthesis in SLE. Phospholipid degradation is increased owing to an increase in intracellular calcium activating phospholipase A_2 and D. The cholesterol: phospholipid ratio of the RBC membrane was increased in SLE. The concentration of total GAG, hexose and fucose of glycoprotein decreased in

the RBC membrane and increased in the serum suggesting their reduced incorporation into the membrane and defective membrane formation. This trafficking of the glycoconjugates and lipids which are synthesized in the endoplasmic reticulum - golgi complex to the cell membrane depends upon GTPases and lipid kinases which are crucially dependent on magnesium and are defective in magnesium deficiency. The change in membrane structure produced by alteration in glycoconjugates and cholesterol phospholipid ratio can produce changes in the conformation of Na^+-K^+ ATPase resulting in further membrane Na^+-K^+ ATPase inhibition. The same changes can affect the structure of the lysosomal membrane. The results in defective lysosomal stability and leakage of glycohydrolases and GAG degrading enzymes into the serum.

Archaeal Digoxin and Mitochondrial Dysfunction in Relation to SLE

The archaeaon vitaminocyte contributes to the synthesis of ubiquinone and mitochondrial electron transport chain function. The mitochondrial function related free radical generation is regulated by the archaeaon vitaminocyte synthesized tocopherol and ascorbic acid. The concentration of ubiquinone decreased significantly in SLE, which may be the result of low tyrosine levels, reported in most of the disorders, consequent to digoxin's effect in preferentially promoting tryptophan transport over tyrosine. The aromatic ring portion of ubiquinone is derived from tyrosine. Ubiquinone, which is an important component of the mitochondrial electron transport chain, is a membrane antioxidant and contributes to free radical scavenging. The increase in intracellular calcium can open the mitochondrial PT pore causing a collapse of the hydrogen gradient across the inner membrane and uncoupling of the respiratory chain. Intracellular magnesium deficiency can lead to a defect in the function of ATP synthase. All this leads to defects in mitochondrial oxidative phosphorylation, incomplete reduction of oxygen and generation of superoxide

which produces lipid peroxidation. Ubiquinone deficiency also leads to reduced free radical scavenging. The increase in intracellular calcium may lead to increased generation of NO by inducing the enzyme nitric oxide synthase which combines with superoxide radical to form peroxynitrite. Increased intracellular calcium also can activate phospholipase A_2 resulting in increased generation of arachidonic acid which can undergo increased lipid peroxidation. Increased generation of free radicals like the superoxide ion and hydroxyl radical can produce lipid peroxidation and cell membrane damage which can further inactivate Na^+-K^+ ATPase, triggering the cycle of free radical generation once again. Magnesium deficiency can affect glutathione synthetase and glutathione reductase function. The mitochondrial superoxide dismutase leaks out and becomes dysfunctional with calcium related opening of the mitochondrial PT pore and outer membrane rupture. The peroxisomal membrane is defective owing to membrane Na^+-K^+ ATPase inhibition related defect in membrane formation and leads to reduced catalase activity. Mitochondrial dysfunction related free radical generation has been implicated in the pathogenesis of immune mediated diseases like neurolupus. The increased intracellular calcium and ceramide related opening of the mitochondrial PT pore also leads to volume dysregulation of the mitochondria, causing hyperosmolality of the matrix and expansion of the matrix space. The Outer membrane of the mitochondria ruptures and releases apoptosis inducing factor and cytochrome C into the cytoplasm. This results in activation of caspase-9. Caspase-9 can produce apoptosis of the cell. Apoptosis has been implicated in the genesis of cell death in SLE.

Archaeal Digoxin and Hemispheric Dominance in Relation to SLE

The archaeaon related organelle - steroidelle, neurotransminoid and vitaminocyte contribute to hemispheric dominance. The biochemical patterns obtained in neurolupus is similar to those obtained in left handed / right

hemispheric chemically dominant individuals by the dichotic listening test. But all the patients with neurolupus were right handed / left hemispheric dominant by the dichotic listening test. Hemispheric chemical dominance has no correlation with handedness or the dichotic listening test. Neurolupus occurs in right hemispheric chemically dominant individuals and is a reflection of altered brain function. Thus the immune mechanisms and the response to an invading bacteria / virus differ in the hypo and hyperdigoxinemic state. The hypodigoxinemic state is associated with immunosuppression. But there is no viral persistence in the hypodigoxinernic state. The hyperdigoxinemic state is associated with immunoactivation and viral persistence. There is an increased tendency for autoimmune diseases like SLE in the hyperdigoxinemic state.

Hypodigoxinemia is related to left hemispheric chemical dominance and hyperdigoxinemia with right hemispheric chemical dominance. The immune response and immune mediated disease in right hemispheric and left hemispheric chemical dominance differ. SLE is probably associated with right hemispheric chemical dominance and hyperdigoxinemia. Immunosuppression is associated with left hemispheric chemical dominance and hypodigoxinemia. Geschwind has postulated a relationship between cerebral lateralization and immune function. They observed a high frequency of left handedness in patients with immune disorders. Bardos, Degenne, Lebranchu, Biziere and Renoux demonstrated that lesions of the left neocortex in mice depress T-cell immunity, whereas lesions of right neocortex enhance T-cell immunity. These earlier reports are in agreement with our studies. Archaeal digoxin and hemispheric dominance may regulate immune function.

References

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