

Chapter 5

Endosymbiotic Archaeal Digoxin Mediated
Model for Creutzfeldt Jakob's Disease

Introduction

The endosymbiotic archaea produces an endogenous membrane $\text{Na}^+\text{-K}^+$ 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 $\text{Na}^+\text{-K}^+$ 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. Saito has reported 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 immune mediated diseases by the action of 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.

It was therefore considered pertinent to study digoxin status and digoxin synthesis in CJD. 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) 7 cases of CJD (CSF prion positive / characteristic EEG), (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 were 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 $\text{Na}^+ - \text{K}^+$ 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 Sigma 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 $\text{Na}^+ - \text{K}^+$ 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 et al. For estimation of ubiquinone and dolichol in the serum, the procedure described by Palmer et al. 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 et al. Serotonin was estimated by the method of Curzon and Green and catecholamines by the method of Well-Malherbe. Quinolinic acid content of serum was estimated by HPLC (C_{18} column micro BondapakTM 4.6 x 140 mm), solvent system 0.01 M acetate buffer (pH 3.0) and methanol (6:4), flow rate 1.0 ml/minute and detection UV 250 nm). Morphine, strychnine and nicotine were estimated by

the method described by Arun et al. 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 in methods in enzymology. Cholesterol was estimated by using commercial kits supplied by Sigma Chemicals, USA. SOD was assayed by the method of Nishikimi et al. as modified by Kakkar et al. Catalase activity was estimated by the method of Maehly and Chance, glutathione peroxidase by the method of Paglia and Valentine as modified by Lawrence and Burk and glutathione reductase by the method of Horn and Burns. MDA was estimated by the method of Wills and conjugated dienes and hydroperoxides by the procedure of Brien. Reduced glutathione was estimated by the method of Beutler et al. 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 CJD indicating upregulation of the isoprenoid pathway but serum ubiquinone, magnesium and RBC membrane $\text{Na}^+ - \text{K}^+$ 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 CJD 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 CJD. The activity of enzymes involved

in free radical scavenging like superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase is decreased in CJD suggesting reduced free radical scavenging. There was a decrease in lipid peroxidation with increased antioxidant protection as indicated by an increase in ubiquinone and reduced glutathione in recurrent respiratory infection. The activity of enzymes involved in free radical scavenging is increased in recurrent respiratory infection suggesting increased free radical scavenging.

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

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

(6) The results showed that serum HMG CoA reductase activity serum digoxin and dolichol levels were increased and serum ubiquinone, magnesium and RBC membrane $\text{Na}^+\text{-K}^+$ 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 $\text{Na}^+\text{-K}^+$ 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 $\text{Na}^+\text{-K}^+$ ATPase Inhibition in Relation to CJD

The archaeon steroidelle DXP pathway and the upregulated pentose phosphate pathway contribute to digoxin synthesis. The increase in endogenous digoxin, a potent inhibitor of membrane $\text{Na}^+\text{-K}^+$ ATPase, can decrease this enzyme activity in CJD. There was increased synthesis of digoxin as evidenced by increased HMG CoA reductase activity. The inhibition of $\text{Na}^+\text{-K}^+$ ATPase by digoxin is known to cause an increase in intracellular calcium resulting from increased $\text{Na}^+\text{-Ca}^{++}$ 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 $\text{Na}^+\text{-K}^+$ ATPase, since ATP-magnesium complex is the actual substrate for this reaction. Low intracellular magnesium and high intracellular calcium consequent to $\text{Na}^+\text{-K}^+$ ATPase inhibition appear to be crucial to the pathophysiology of CJD.

Intracellular magnesium deficiency can result in disruption of ribosomal function. The protein synthetic machinery and DNA transcription is halted as both require magnesium for their function. When the normal protein transcription machinery is halted a primitive form of self replication of proteins especially metalloproteins comes in to play. Prions are copper containing metalloproteins. Dielectric proteins molecules can store information in the quantum state. The perceived element in quantal or subliminal perception could be matter dependent electric and magnetic fields. The brain functions as a quantum computer with the quantum computer memory elements constituted of superconducting quantum interference device - the SQUIDS which can exist as superpositions of macroscopic states. Bose condensation, the basis of

superconductivity is achievable at room temperature in the Frohlich model in biological systems. The dielectric protein molecules of the neuronal membrane are excellent electric dipole oscillators which exist under a steep neuronal membrane voltage gradient. The individual oscillators are energised with a constant source of pumping energy from outside, by digoxin binding to membrane of $\text{Na}^+ \text{-K}^+$ ATPase and producing a paroxysmal depolarisation shift in the neuronal membrane. This prevents the dipole oscillators from ever settling into thermal equilibrium with the cytoplasm and interstitial fluid which is always kept at constant temperature. There are connections between the hypothalamus and cerebral cortex and digoxin may serve as a neurotransmitter for these synapses. Bose condensed states produced by digoxin mediated dielectric protein molecular pumped phonon system could be used to store information which might be encoded - all within the lowest collective frequency mode - by appropriately adjusting the amplitudes of and phase relations between the dipole oscillators. Such dielectric protein molecules especially metalloproteins like prions could be able to act as a template for the synthesis of other prion molecules or be able to organise their self replications in the neuronal quantal state. Thus prion self replication can happen in the hyperdigoxinemic quantal state.

In CJD increased intracellular calcium consequent to membrane $\text{Na}^+ \text{-K}^+$ 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 (Tumour necrosis factor alpha). This immune activation can contribute to the genesis of CJD.

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

The archaeon 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 CJD. This could be due to the fact that digoxin can regulate neutral amino acid transport system with preferential promotion of tryptophan transport over tyrosine. In the presence of hypomagnesemia, 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 in CJD. Quinolinic acid has been implicated in immune activation in immune mediated diseases and could contribute to the same in CJD. 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 CJD. The schizoid neurotransmitter pattern of reduced dopamine, noradrenaline and morphine and increased serotonin, strychnine and nicotine is common to CJD and could predispose to its development. A schizoid type of personality could predispose to the development of CJD. Early onset form of CJD can also have in neuropsychiatric presentation.

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

The archaeon glycosaminoglycoid and fructosoid contributes to glycoconjugate synthesis and catabolism by the process of fructolysis. The

elevation in the level of dolichol in CJD 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 in spite 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. Previous reports of accumulation of abnormal glycoproteins include beta amyloid in the case of CJD.

The prion itself is a glycoprotein which is defectively processed owing to a glycosylation defect and resists catabolism by lysosomal enzymes and accumulates in the brain in CJD. Interaction between HS-proteoglycan and ChS-proteoglycan with proteins like beta amyloid and prions and reduced proteolytic digestion of these complexes leading on to their accumulation in the neurons has been reported in CJD. The protein processing defect can result in defective glycosylation of endogenous neuronal glycoprotein antigens and exogenous prion glycoprotein antigens with consequent defective formation of the MHC antigen complex. The MHC linked peptide transporter, a P-glycoprotein which transports the 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 the MHC class-1 glycoprotein antigen complex to the antigen presenting cell surface for recognition by the CD₄ or CD₈ cell. Defective presentation of exogenous prion antigens can produce immune evasion by the prion as in CJD.

A number of fucose and sialic acids containing natural ligands are involved in trafficking of leukocytes and similar breaches in blood brain barrier and resultant adhesion and trafficking of the lymphocyte and extravasation into the perivascular space have been described in the brain in CJD.

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

The archaeon 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 CJD. Phospholipid degradation is increased owing to an increase in intracellular calcium activating phospholipase A₂ and D. The cholesterol: phospholipid ratio of the RBC membrane was increased in CJD. 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. This results in defective lysosomal stability and leakage of glycohydrolases and GAG degrading enzymes into the serum. Prion is a membrane protein and change in neuronal membrane can affect prion replication.

Archaeal Digoxin and Mitochondrial Dysfunction in Relation to CJD

The archaeon vitaminocyte contributes to the synthesis of ubiquinone and mitochondrial electron transport chain function. The mitochondrial function related free radical generation is regulated by the archaeon vitaminocyte synthesized tocopherol and ascorbic acid. The concentration of ubiquinone decreased significantly in CJD, 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 a 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 the superoxide radical to form peroxynitrite. Increased intracellular calcium also can activate phospholipase A₂ 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 a membrane $\text{Na}^+ - \text{K}^+$ 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 CJD. 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 neuronal degeneration and probably in CJD.

Archaeal Digoxin and Hemispheric Dominance in Relation to CJD

The archaeon related organelle - steroidelle, neurotransminoid and vitaminocyte contribute to hemispheric dominance. 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 hypodigoxinemic state. The hyperdigoxinemic state is associated with immunoactivation. It could also lead to persistent infections like CJD and prion disease.

Hypodigoxinemia is related to left hemispheric dominance and hyperdigoxinemia with right hemispheric dominance. The immune response and immune mediated disease in right hemispheric and left hemispheric dominance differ. CJD is probably associated with right hemispheric dominance and hyperdigoxinemia. Immunosuppression is associated with left hemispheric 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 et al. 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. Hypothalamic archaeal digoxin and hemispheric dominance may regulate immune function.

References

- [1] Kurup RK, Kurup PA. *Hypothalamic Digoxin, Cerebral Dominance and Brain Function in Health and Diseases*. New York: Nova Medical Books, 2009.

