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Archaeal Digoxin Mediated Model for Multiple Sclerosis

Alteration in the isoprenoid pathway has been described in multiple sclerosis. Decreased ubiquinone levels have been reported in the serum of patients with multiple sclerosis. Increased urinary excretion of some organic acids which are degradation products from isoprenoid compounds have been reported in multiple sclerosis and it has been suggested that there is a defect in the isoprenoid pathway in this disorder. Increased serum endogenous digoxin like factor (EDLF) activity has been reported in some autoimmune syndromes.

Digoxin has been reported to regulate the transport of amino acids especially by neutral amino acids. Tryptophan and kynurenic metabolism has also been implicated immune mediated disorders. Alterations in quinolinic acid metabolism have been implicated in the pathological lesion of AIDS dementia and neurolupus. Saito et al. reported increased activities of kynurenine pathway enzymes in various tissues following systemic immune stimulation, in conjunction with macrophage infiltration of the affected tissue.

Digoxin can alter Ca^{++}/Mg^{++} in the cell leading on to free radical generation. Defective ubiquinone synthesis can also lead to mitochondrial dysfunction and free radical generation. Microglial activation has been reported in multiple sclerosis and free radical generation is important in this context.

Digoxin induced membrane Na^{+}-K^{+} ATPase inhibition can produce Mg^{++} depletion leading on to altered glycoconjugate metabolism. The dolichol pathway can regulate N-glycosylation of glycoproteins. A number of fucose and sialic acid containing natural ligands are common to the inflammatory acute phase response. A large body of research supports a role for small carbohydrate ligands in trafficking of leukocytes. Similar blood brain barrier changes and altered adhesion and trafficking of the lymphocyte have been described in MS. Galactosyl ceramide can be converted into sulfogalactosyl ceramide which is present in high amounts in myelin and is required for myelin integrity.[1-13]

This study was undertaken to assess, (1) the isoprenoid pathway (2) the tryptophan tyrosine catabolic patterns (3) Glycoconjugate metabolism in multiple sclerosis (4) RBC membrane changes as a reflection of neuronal and glial membrane change. A hypothesis implicating neuronal and glial membrane Na^{+}-K^{+} ATPase inhibition as pivotal to all these changes is also 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 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. 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 neurological disease.

Results

(1) The activity of HMG CoA reductase and the concentration of digoxin and dolichol were increased in MS. The concentration of serum ubiquinone, the activity of erythrocyte membrane Na^+-K^+ ATPase and serum magnesium were decreased in MS.

(2) The concentration of serum tryptophan, quinolinic acid and serotonin were increased in the plasma while that of tyrosine, dopamine and noradrenaline were decreased in MS.

(3) Morphine and strychnine were detected in the plasma of patients with MS and were undetectable in the control serum. Nicotine was not detected in the plasma of MS patients.

(4) The concentration of total GAG increased in the serum of MS patients. The concentration of hyaluronic acid (HA), heparan sulphate (HS) and heparin (H) was increased, while that of dermatan sulphate (DS) and chondroitin sulphates (ChS) was decreased in MS. The concentration total hexose, fucose and sialic acid were increased in the glycoproteins of the serum in MS. The concentration of gangliosides, glycosyl diglycerides, cerebroside and sulphatide showed significant increase in the serum in MS when compared to controls.

(5) The activity of GAG degrading enzymes beta glucuronidase, beta N-acetyl hexosaminidase, hyaluronidase, cathepsin-D, was increased in MS when compared to the controls. The activity of beta galactosidase and beta fucosidase increased in MS while that of beta glucosidase showed a decrease.

(6) The concentration of total GAG, hexose and fucose in the RBC membrane decreased significantly in MS. The concentration cholesterol and phospholipids were not significantly altered in the RBC membrane in MS

but the cholesterol: phospholipid ratio in the RBC membrane decreased significantly in MS.

(7) Concentration of total serum cholesterol in LDL cholesterol increased significantly while HDL cholesterol was reduced in the plasma in MS. Serum triglycerides and free fatty acids were unaltered in MS.

Discussion

Archaeal Digoxin and Lipid Metabolism - Membrane Na^+-K^+ ATPase Inhibition in Relation to Multiple Sclerosis

The archaeaon steroidelle contributes to lipid synthesis and metabolism. The archaeaon steroidelle DXP pathway and the upregulated pentose phosphate pathway contribute to digoxin synthesis. The results showed that HMG CoA reductase activity, serum digoxin and dolichol were increased in MS while serum ubiquinone was reduced. Previous studied in this laboratory has demonstrated incorporation of ^{14}C-acetate into digoxin in the rat brain indicating that acetyl CoA is the precursor for digoxin biosynthesis in mammals also. The elevated HMG CoA reductase activity correlates well with elevated digoxin levels and reduced RBC membrane Na^+-K^+ ATPase activity. The increase in endogenous digoxin, a potent inhibitor of membrane Na^+-K^+ ATPase, can decrease this enzyme activity. The inhibition of Na^+-K^+ ATPase by digoxin is known to cause an increase in intracellular calcium resulting from increased Na^+-Ca^{++} exchange, increased entry of calcium via the voltage gated calcium channel and increased release of calcium from intracellular endoplasmic reticulum calcium stores. This increase in intracellular calcium by displacing magnesium from its binding sites 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 inhibition of Na^+-K^+ ATPase, since the ATP-magnesium complex

is the actual substrate for this reaction. Cytosolic free calcium is normally buffered by two mechanisms, ATP dependent calcium extrusion from cell and ATP dependent sequestration of calcium within the endoplasmic reticulum. The magnesium related mitochondrial dysfunction results in defective calcium extrusion from the cell. There is thus a progressive inhibition of Na^+-K^+ ATPase activity first triggered by digoxin. Low intracellular magnesium and high intracellular calcium consequent to Na^+-K^+ ATPase inhibition appear to be crucial to the pathogenesis of MS. Serum magnesium was found to be reduced in MS.[1-13]

Archaeal Digoxin and Immunoregulation in Relation to Multiple Sclerosis - The Fructosoid, Steroidelle and Viroidelle

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. The archaeaon viroidelle secreted RNA viroids can produce immune activation by blocking mRNA function. Increased intracellular calcium 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 can also explain the immune activation in MS. TNF alpha can also bring about apoptosis of the cell. It binds to its receptor and activates caspase-9 an ICE protease which converts IL-1 beta precursor to IL-1 beta. IL-1 beta produces apoptosis of the oligodendrocyte, the myelin forming cell in MS. Membrane Na^+-K^+ ATPase inhibition can produce immune activation and is reported to increase CD_4/CD_8 ratios as exemplified by the action of lithium.[1-13]

Archaeal Digoxin and Regulation of Neurotransmitter Synthesis and Function in Relation to Multiple Sclerosis

The archaeaon neurotransminoid shikimic acid pathway contributes to tryptophan and tyrosine synthesis and catabolism generating neurotransmitters

and neuroactive alkaloids. Digoxin, apart from affecting cation transport is also reported to influence the transport of various metabolites across cellular membranes, including amino acids and various neurotransmitters. The results showed that the concentration of tryptophan, quinolinic acid and serotonin were found to be higher in the plasma of patients with MS while that of tyrosine, dopamine and norepinephrine were lower. Thus there is increase in tryptophan and its catabolites and a reduction in tyrosine and its catabolites in the serum of MS patients. This could be due to the fact that digoxin can regulate neutral amino acid transport system with preferential promotion of tryptophan transport over tyrosine. The decrease in membrane Na^+-K^+ ATPase activity in MS could be due to the fact that the hyperpolarising neurotransmitters (dopamine and noradrenaline) are reduced and the depolarising neuroactive compounds (serotonin and quinolinic acid) are increased. The increased levels of quinolinic acid acts as a NMDA agonist producing glutamate excitotoxicity and increasing the intraneuronal calcium load. Quinolinic acid has been implicated in immune activation in other autoimmune diseases like SLE and could contribute to the same in MS. 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 in the brainstem monoaminergic nuclei. Thus elevated serotonin and reduced noradrenaline and dopamine can contribute to the immune activation in MS. The neurotransmitter pattern of reduced dopamine and noradrenaline, and increased serotonin can contribute to the psychosis described in multiple sclerosis.

We had already shown the presence of endogenous morphine in the brain of rats loaded with tyrosine and endogenous strychnine and nicotine in the brain of rats loaded with tryptophan. Serum of patients with multiple sclerosis showed the presence of morphine and strychnine but nicotine was absent. The detection

of increased levels of morphine in multiple sclerosis despite low tyrosine levels may indicate its synthesis from other sources. Morphine has got an immunoregulatory function in the brain. It produces alteration in T-cell deficit in heroin addicts which was shown to consist of their inability to form rosettes on sheep erythrocytes. It has been found to inhibit the expression of antigenic markers for both T-helper and T-suppressor cells. In multiple sclerosis a CD_8 MHC class-1 restricted immune dysregulatory effect has been described. Morphine may contribute to this CD_8 MHC class-1 restricted T-cell defect in multiple sclerosis. Strychnine by blocking glycinergic transmission can contribute to the decreased inhibitory transmission in MS. Serum of patients with MS showed strychnine which can produce increase in intraneuronal calcium load leading to oligodendrocyte apoptosis and immune activation. No nicotine could be detected in MS.[1-13]

Archaeal Digoxin and Regulation of Golgi Body / Lysosomal Function in Relation to Multiple Sclerosis - The Glycosaminoglycoid

The archaeaon glycosaminoglycoid and fructosoid contributes to glycoconjugate synthesis and catabolism by the process of fructolysis. The low magnesium levels consequent to membrane Na^+-K^+ ATPase inhibition can affect the metabolism of glycosaminoglycans, glycoproteins and glycolipids. The elevation in the level of dolichol consequent to its increased synthesis may suggest its increased availability of N-glycosylation of proteins. Magnesium deficiency can lead on to defective metabolism of sponganine producing its accumulation which may lead to increased cerebroside and ganglioside synthesis. In magnesium deficiency the glycolysis, citric acid cycle and oxidative phosphorylation are blocked and more glycose 6-phosphate is channelled for the synthesis of glycosaminoglycans (GAG). The results showed an increase in the concentration of serum total GAG, glycolipids (ganglioside,

glycosyl-diglyceride, cerebrosides and sulphatides) and carbohydrate components of glycoproteins (hexose, fucose and sialic acid) in MS. The increase in the carbohydrate components of serum glycoproteins - total hexose, fucose and sialic acid was not to the same extent in MS suggesting qualitative change in glycoprotein structure. In MS the percentage change in total hexose, fucose and sialic acid when compared to the control is 54.3%, 20% and 33% respectively. The pattern of change in individual GAG in the serum was different. The concentration of hyaluronic acid, heparan sulphate and heparin was increased while that of dermatan sulphate and chondroitin sulphates was reduced in the serum of MS patients. The activity of GAG degrading enzymes (beta glucuronidase, beta N-acetyl hexosaminidase, hyaluronidase and cathepsin-D) was increased in the serum of MS patients. The activity of glycohydrolases - beta galactosidase and beta fucosidase was increased while that of beta glucosidase was decreased in the serum of MS patients. 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 consequent to qualitative change in their structure. Proteoglycan complexes formed in the presence of altered calcium / magnesium ratios intracellularly may be structurally abnormal and resistant to lysosomal enzymes and may accumulate.

The protein processing defect can result in defective glycosylation of endogenous myelin glycoprotein antigens and exogenous viral 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 which is dysfunctional in the presence of magnesium deficiency. This results in defective transport of the MHC class-1 myelin glycoprotein antigen complex to the antigen presenting cell surface for recognition by the CD_4 or CD_8 cell. Defective presentation of the endogenous myelin glycoprotein antigen can explain the immune dysregulation in MS. A CD_8 MHC class-1 restricted immune dysregulatory defect has been described in MS. Defective presentation of the exogenous viral or bacterial glycoprotein antigens can produce immune evasion by the virus / bacteria and viral / bacterial persistence as in the case of retrovirus, herpes virus and chlamydia induced demyelination. A number of fucose and sialic acids containing natural ligands that are involved in trafficking of leukocytes and similar breaches in the blood brain barrier and adhesion of the lymphocyte producing leukocyte trafficking and extravasation in to the perivascular space have been described in MS. Alteration in ganglioside, glycosyl-diglycerides, cerebrosides and sulphatides can affect the structural integrity of myelin. Defectively N-glycosylated myelin glycoproteins and alteration in GAG / proteoglycans of myelin can also affect the structural integrity of myelin leading on to demyelination.[1-13]

Archaeal Digoxin and Alteration in Membrane Structure and Membrane Formation in Relation to Multiple Sclerosis

The archaeaon steroidelle, glycosaminoglycoid and fructosoid contribute to cell membrane formation synthesizing cholesterol by the DXP pathway and glycosaminoglycans by fructolysis. The alteration in the isoprenoid pathway specifically, cholesterol as well as changes in glycoproteins and GAG can affect cellular membranes. The upregulation of the isoprenoid pathway can lead to increased cholesterol synthesis and magnesium deficiency can inhibit

phospholipid synthesis. Phospholipid degradation is increased owing to increase in intracellular calcium activating phospholipase A_2 and D. The total cholesterol and LDL cholesterol were increased and HDL cholesterol was reduced in the serum of MS patients. HDL cholesterol is important in neuronal regeneration. The membrane composition was assessed by RBC membrane cholesterol: phospholipid ratio, carbohydrate residues of glycoproteins and total glycosaminoglycans. The cholesterol: phospholipid ratio of the RBC membrane was decreased in MS. The concentration of total GAG, hexose and fucose of glycoprotein and cholesterol decreased in the RBC membrane and increased in the serum suggesting their reduced incorporation into the membrane and defective membrane formation. The glycoproteins, GAG and glycolipids of the cellular membrane are formed in the endoplasmic reticulum, which is then budded off as a vesicle which fuses with the golgi complex. The glycoconjugates are then transported via the golgi channel and the golgi vesicle fuses with the cell membrane. This trafficking 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 organelle membrane. This results in defective lysosomal stability and leakage of glycohydrolases and GAG degrading enzymes into the serum. Oligodendrocyte, the myelin forming cell in the central nervous system ensheaths several axons during the process of myelination. Alteration in the structure of the oligodendrocyte membrane can affect myelination. Remyelination following demyelination also depends upon heat-shock protein. Digoxin can regulate the function of heat-shock protein which coordinates protein folding and maturation. The heat-shock protein has an ATP/ADP switch domain that regulates its

conformation. Intracellular magnesium deficiency can produce dysfunction of the ATP/ADP switch domain.[1-13]

Archaeal Digoxin and Mitochondrial Dysfunction in Relation to Multiple Sclerosis - The Vitaminocyte

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 MS which may be the result of low tyrosine levels, consequent to digoxin's effect in preferentially promoting tryptophan transport over tyrosine. The aromatic ring portion of ubiquinone is derived from the 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 and ceramide 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 defect in mitochondrial oxidative phosphorylation, incomplete reduction of oxygen and generation of the superoxide ion. 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 calcium also can activate phospholipase A_2 resulting in increased generation of arachidonic acid which can undergo increased lipid peroxidation. Lipid peroxidation of myelin lipids can contribute to demyelination. Microglial activation and free radical generation has been implicated in the pathogenesis of immune mediated disorders like MS.

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 oligodendrocyte, the myelin forming cell in MS leading on to demyelination.[1-13]

Archaeal Digoxin and Hemispheric Dominance in Relation to Multiple Sclerosis

The archaeaon related organelle-steroidelle, neurotransminoid and vitaminocyte contribute to hemispheric dominance. Thus the isoprenoid pathway dysfunction is important in the pathogenesis of MS. This can operate at several levels, (1) Membrane Na^+-K^+ ATPase inhibition and immune activation, (2) Digoxin related tryptophan/tyrosine catabolic patterns and neurotransmitters chanes, (3) Alter glycoconjugate metabolism and changes in myelin structure and myelin glycoprotein antigen presentation, (4) Altered membrane formation and oligodendrocyte mediated myelination, (5) Mitochondrial dysfunction leading on to free radical generation and microglial activation / oligodendrocyte apoptosis. It may reflect a defective neuro-immune integration of the brain due to an upregulated isoprenoid pathway and paroxysmal hypothalamic archaeal digoxin hypersecretion. All these chemical features are suggestive of right hemispheric chemical dominance which can lead to multiple sclerosis.[1-13]

References

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