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Archaeal Digoxin Mediated Model for Cirrhosis Liver

The cardinal pathologic features of cirrhosis reflect irreversible chronic injury of the hepatic parenchyma and include extensive fibrosis in association with the formation of regenerative nodules. These features result from hepatocyte necrosis, collapse of the supporting reticulin network with subsequent connective tissue deposition, distortion of the vascular bed, and nodular regeneration of remaining liver parenchyma. The causes of cirrhosis include alcoholic, post viral or post necrotic. The finding of increased concordance of alcoholic liver disease among monozygotic twins compared to dizygotic twins ingesting excessive amounts of alcohol suggests that genetic factors may contribute, to the genesis of cirrhosis. The alcoholic cirrhosis passes through three pathological states - alcoholic fatty liver, alcoholic hepatitis and alcoholic cirrhosis. The isoprenoid pathway is a key regulatory pathway in the cell. It produces two metabolites important in the genesis of cirrhosis - digoxin and cholesterol. Archaeal digoxin by producing membrane Na^+-K^+ ATPase inhibition can contribute to intracellular hypomagnesemia which can upregulate connective tissue synthesis. The cholesterol pathway and lipoprotein synthesis can contribute to the genesis of fatty liver. Archaeal digoxin can also modulate tryptophan and tyrosine transport. Tyrosine can contribute to the synthesis of endogenous morphines. Alcoholic addiction, an important etiological factor for cirrhosis has been related to an endogenous morphine deficiency syndrome. Tryptophan catabolism can lead to the generation of quinolinic acid important in the pathogenesis of hepatic coma. It was therefore considered pertinent to study the isoprenoid pathway related biochemical cascade in alcoholic cirrhosis of the liver to find out whether changes in the pathway can modulate the predisposition to cirrhosis liver. As hypothalamic archaeal digoxin can modulate synaptic transmission of multiple neurotransmitter systems, the pathway was also assessed in individuals with differing hemispheric dominance to find out the role of hemispheric dominance in the pathogenesis of cirrhosis.

Results

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

(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 the patient group.

(3) Nicotine and strychnine were detected in the plasma of the patient group and were undetectable in control serum. Morphine was not detected in the plasma of the patient group while it was present in the control group.

(4) The concentration of total GAG increased in the serum of the patient group. The concentration of hyaluronic acid (HA), heparan sulphate (HS), heparin (H), dermatan sulphate (DS) and chondroitin sulphates (ChS) were increased in the patient group. The concentration of total hexose, fucose and sialic acid were increased in the glycoproteins of the serum in the patient group.

(5) The activity of GAG degrading enzymes beta glucuronidase, beta N-acetyl hexosaminidase, hyaluronidase and cathepsin-D, were increased in the patient group when compared to the controls. The activity of beta galactosidase, beta fucosidase and beta glucosidase increased in the patient group.

(6) The concentration of total GAG, hexose and fucose in the RBC membrane decreased significantly in the patient group. The concentration cholesterol increased and phospholipids decreased in RBC membrane in the patient group and the RBC membrane the cholesterol: phospholipid ratio increased significantly.

(7) The activity of superoxide dismutase (SOD), catalase, glutathione reductase and glutathione peroxidase in the erythrocytes decreased significantly in the patient group. In the patient group the concentration of MDA, hydroperoxides, conjugated dienes and NO increased significantly. The concentration of reduced glutathione decreased in the patient group.

(8) The results showed that HMG CoA reductase activity, serum digoxin and dolichol were increased and ubiquinone reduced in left handed / right hemispheric dominant individuals. The results also showed that HMG CoA reductase activity, serum digoxin and dolichol were decreased and ubiquinone 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 plasma 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 plasma 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 Cirrhosis Liver

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 cirrhosis liver while serum ubiquinone was reduced. Previous studies in this laboratory have demonstrated incorporation of ‘4C-acetate in to digoxin in 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 RC 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 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 cirrhosis liver. Serum magnesium was found to be reduced in cirrhosis liver.

Decreased intracellular magnesium can produce dysfunction of lipoprotein lipase leading to defective catabolism of triglycerides rich lipoproteins and hypertriglyceremia. In hypomagnesemia Lecithin cholesterol acyl transferase (LCAT) is defective and there is reduced formation of cholesterol esters in HDL. Magnesium deficiency has been reported to increase LDL cholesterol levels also. Increased intracellular calcium consequent to membrane Na^+-K^+ ATPase inhibition can open up the mitochondrial PT pore leading on to a mitochondrial

dysfunction. This leads on to defective mitochondrial beta oxidation of fatty acids and triglyceride accumulation. All these changes in lipid metabolism produced by digoxin can contribute to alcoholic fatty liver.

Archaeal Digoxin and Regulation of Neurotransmitter Synthesis and Function in Relation to Cirrhosis Liver

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 cirrhosis liver while that of tyrosine, dopamine and norepinephrine were lower. Thus there is an increase in tryptophan and its catabolites and a reduction in tyrosine and its catabolites in the serum of cirrhosis liver 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 cirrhosis liver 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. These abnormalities can contribute to the neurological complications of liver failure in cirrhosis. Reduced tyrosine levels can lead on to reduced dopamine synthesis contributing to the extrapyramidal syndrome and altered sensorium in liver failure consequent to cirrhosis. Increased quinolinic acid and serotonin levels can contribute to NMDA excitotoxicity as both are NMDA agonists. Quinolinic acid mediated NMDA excitotoxicity is important in the pathogenesis of neuronal dysfunction in hepatic failure.

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 cirrhosis liver showed the presence of strychnine and nicotine but morphine was absent. The absence of morphine in patients with cirrhosis liver is also significant. Endogenous morphine deficiency has been related to alcoholic addiction. This could be a contributory factor for cirrhosis liver. The presence of strychnine in patients with cirrhosis liver is important. Strychnine displaces glycine from its binding site. The glycine is free to bind to the glycine sensitive site of the NMDA receptor producing NMDA excitotoxicity important in neuronal dysfunction in cirrhosis.

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

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. Decrease in intracellular magnesium can produce changes in collagen and elastin biosynthesis and produce replacement fibrosis. In magnesium deficiency the glycolysis, citric acid cycle and oxidative phosphorylation are blocked and more glucose 6-phosphate is channelled for the synthesis of glycosaminoglycans (GAG). The results showed an increase in the concentration of serum total GAG, and carbohydrate components of glycoproteins (hexose, fucose and sialic acid) in cirrhosis liver. The increase in the carbohydrate components of serum glycoproteins - total hexose, fucose and sialic acid was not to the same extent in cirrhosis liver suggesting a qualitative change in glycoprotein structure. In

cirrhosis liver the percentage change in total hexose, fucose and sialic acid when compared to 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, heparin, dermatan sulphate and chondroitin sulphates were increased in the serum of cirrhosis liver patients. The activity of GAG degrading enzymes (beta glucuronidase, beta N-acetyl hexosaminidase, hyaluronidase and cathepsin-D) were increased in the serum of cirrhosis liver patients. The activities of glycohydrolases - beta galactosidase, beta fucosidase and beta glucosidase were increased in the serum of cirrhosis liver patients. 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 Ca^{++}/Mg^{++} ratios intracellularly may be structurally abnormal and resistant to lysosomal enzymes and may accumulate. The upregulated connective tissue macromolecular synthesis consequent to hypomagnesemia can predispose to cirrhosis liver.

The protein processing defect can result in defective glycosylation of exogenous viral glycoprotein antigens with consequent defective formation of MHC-viral glycoprotein 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 which is dysfunctional in the presence of magnesium deficiency. This results in defective transport of MHC class-1 viral glycoprotein antigen complex to the antigen presenting cell surface for recognition by CD_4 or CD_8 cell. Defective presentation of exogenous viral or bacterial glycoprotein antigens can produce immune evasion by the virus /

bacteria and viral/bacterial persistence as in the case of hepatitis virus B persistence in post necrotic cirrhosis of the liver. This can also contribute to defective immunity and increased predisposition to bacterial infections especially due to pneumococcus and mycobacterium tuberculosis in cirrhosis liver. Increased intracellular calcium activates calcium dependent calcineurin signal transduction pathway which can produce T-cell activation and secretion of interleukin - 3, 4, 5, 6, 8 and TNF alpha (Tumour necrosis factor alpha). This can also explain the immune activation and infiltration of inflammatory cell noticed in the alcoholic hepatitis stage of cirrhosis liver. TNF alpha can also bring about apoptosis of the cell. It binds to its receptor and activates caspase-9. Caspase-9 activation can produce hepatocyte apoptosis. 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. A number of fucose and sialic acid containing natural ligands are involved in the trafficking of leukocytes and adhesion of the lymphocyte producing leukocyte trafficking and extravasation in to the perivascular space as has been described in cirrhosis liver. In the alcoholic hepatitis stage the damaged hepatocytes contained mallory bodies or alcoholic hyaline. These are clumps of perinuclear, deeply eosinophilic material believed to represent aggregated intermediate filaments. Digoxin by producing magnesium depletion intracellularly can interfere with the function of the cytoskeletal proteins leading on to the intermediate filament aggregation.

Archaeal Digoxin and Alteration in Membrane Structure and Membrane Formation in Relation to Cirrhosis Liver

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 isoprenoid pathway can lead to increased cholesterol synthesis and magnesium deficiency can inhibit phospholipid synthesis. Phospholipid degradation is increased owing to an increase in intracellular calcium activating phospholipase A_2 and U. 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 increased in cirrhosis liver patients. 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 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 organelle membrane. This results in defective lysosomal stability and leakage of glycohydrolases and GAG degrading enzymes into the serum. Increased released of lysosomal enzymes can contribute to tissue destruction and necrosis in cirrhosis of the liver. Alteration in RBC membrane can lead on to the acanthocytosis noticed in cirrhosis liver.

Archaeal Digoxin and Mitochondrial Dysfunction in Relation to CIrrhosis Liver

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 cirrhosis liver which may be the result of low tyrosine levels, reported in cirrhosis liver 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 also 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 a defect in mitochondrial oxidative phosphorylation, incomplete reduction of oxygen and generation of superoxide ion 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 radicals to form peroxynitrite. Increased generation of NO can lead onto vasodilatation and the hyperdynamic circulation noticed in hepatic failure consequent to cirrhosis. Many of the cutaneous abnormalities of hepatic failure in cirrhosis like spider naevi and palmar erytherna can be related to increased nitric oxide synthesis. Increased 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 again. 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 cirrhosis liver. The activity of enzymes involved in free radical scavenging like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and catalase is decreased in cirrhosis liver suggesting reduced free radical scavenging. The peroxisomal membrane is defective owing to membrane Na^{+}-K^{+} ATPase inhibition related defect in membrane formation and leads to reduced catalase activity. Intracellular magnesium deficiency consequent to membrane Na^{+}-K^{+} ATPase inhibition can lead to inhibition of glutathione synthetase and glutathione peroxidase function. Thus the glutathione system of free radical scavenging is defective in the presence of membrane Na^{+}-K^{+} ATPase inhibition. Superoxide dismutase exists in a mitochondrial and cytoplasmic form. Opening of the mitochondrial PT pore produces hyperosmolality and matrix expansion rupturing the outer membrane, producing loss of the mitochondrial disniutase leading to a decrease in its activity. The reduction in catalase, superoxide dismutase (SOD), glutathione peroxidase and glutathione reductase suggests reduced free radical protection. Mitochondrial dysfunction related free radical generation has been implicated in the pathogenesis of tissue damage in cirrhosis liver.

Cell death is also mediated by increased intracellular Ca^{++} and ceramide related opening of the mitochondrial PT pore causing a collapse of the H^{+} gradient across the inner membrane and uncoupling of the respiratory chain. This also leads to volume dysregulation of mitochondria causing hyperosinolality of matrix and expansion of matrix space. The outer membrane of the mitochondria ruptures and releases AIF (apoptosis inducing factor) and

cyto C (cytochrome C) into the cytoplasm. This results in procaspase-9 activation to caspase-9 which produces cell death. Caspase-9 activates CAD (caspase activated deoxyribonuclease) which cleaves the nuclear membrane lamins and several proteins involved in cytoskeletal regulation like gelsolin which cleaves actin. Hepatocyte apoptosis has been reported to occur in cirrhosis liver. We have been able to demonstrate neuronal degeneration and apoptosis in digoxin injected rat brain.

Archaeal Digoxin and Regulation of Cell Division, Cell Proliferation and Neoplastic Transformation in Relation to Cirrhosis Liver - Relation to Immune Activation

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. Increased intracellular calcium activates phospholipase C beta which results in increased production of diacyglycerol (DAG) with resultant activation of protein kinase C. The protein kinase C (PKC) activates the MAP kinase cascade resulting in cellular proliferation. The decreased intracellular magnesium can produce dysfunction of GTPase activity of the alpha - subunit of G-protein. This results in ras oncogene activation, as more of the ras is bound to GTP rather than GDP. Phosphorylation mechanisms are required for the activation of the tumour suppressor gene P_{53}. The activation of P_{53} is impaired owing to intracellular magnesium deficiency producing a phosphorylation defect. Upregulation of isoprenoid pathway can result in increased production of farnesyl phosphate which can farnesylate the ras oncogene producing its activation. The ubiquitin system of catabolic processing of processing of proteins is important in the DNA repair mechanism. In the presence of intracellular magnesium deficiency ubiquitin protein catabolic processing and DNA repair mechanisms are defective and this could contribute to oncogenesis.

Thus there is an increased tendency for neoplastic transformation in patients with cirrhosis liver. There is increased incidence of hepatomas in patients with cirrhosis liver.

Archaeal Digoxin and Hemispheric Dominance in Relation to Cirrhosis Liver

The archaeaon related organelle - steroidelle, neurotransminoid and vitaminocyte contribute to hemispheric dominance. In left handed / right hemispheric dominant individuals there was a derangement of the isoprenoid pathway. They had an upregulated HMG CoA reductase activity with increased digoxin and dolichol levels and reduced ubiquinone levels. The RBC membrane Na^+-K^+ ATPase activity was reduced and serum magnesium depleted. The left handed / right hemispheric dominant individuals had increased levels of tryptophan, serotonin, quinolinic acid, strychnine and nicotine while the levels of tyrosine, dopamine, noradrenaline and morphine were lower. Thus an upregulated isoprenoid pathway, increased level of tryptophan and its catabolites, decreased levels of tyrosine and its catabolites and hyperdigoxinemia is suggestive of right hemispheric dominance. In left handed *I* right hemispheric dominant individuals the patterns were reversed. Cirrhosis liver occurs in right hemisphere dominant individuals and is a reflection of altered brain function occurring in right hemispheric dominant individuals.

References

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