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Archaeal Digoxin, Hemispheric Chemical Dominance and Oncogenesis - Evidence from Multiple Myeloma

Introduction

Changes involving the isoprenoid pathway have been described in neoplasms. The isoprenoid pathway produces four key metabolites important in cellular function - digoxin (an endogenous Na^+-K^+ ATPase inhibitor), dolichol (important in N-glycosylation of proteins), ubiquinone (a component of the mitochondrial electron transport chain and membrane antioxidant) and cholesterol.

Alteration in membrane Na^+-K^+ ATPase has been described in oncogenesis suggesting a possible role for endogenous digoxin. An important feature of malignant transformation is loss of the cholesterol feedback inhibition mechanism that regulates cholesterol synthesis. Cancer cells seem to require an increased concentration of cholesterol and cholesterol precursors. Prevention of tumour-cell growth can be achieved by restricting either cholesterol availability or cholesterol synthesis. In vivo-and-cell-culture experiments have shown that lowering the plasma cholesterol concentration or intervening in the mevalonate pathway with HMG CoA reductase inhibitors decreases tumour growth. Another key protein in the internal signalling pathway that triggers cell growth is ras. ras is activated by hooking a 15 carbon farnesyl chain to ras by the enzyme farnesyl transferase. Farnesyl transferase inhibitors are used to block K-ras-driven tumours.

Digoxin, by its inhibition of Na^+-K^+ ATPase, can alter intracellular calcium / magnesium ratios in the cell leading to free radical generation. Alteration in ubiquinone which is a component of the mitochondrial electron transport chain and a membrane antioxidant can also lead to mitochondrial dysfunction and free radical generation. Defects in structure and function of mitochondria have been described in neoplasms. Free radical mechanisms have been implicated in tumourogenesis. Free radicals are required for the action of the oncogene coded growth factors. Digoxin induced membrane Na^+-K^+ ATPase inhibition can

produce magnesium depletion leading to altered glycoconjugate metabolism. Altered glycoproteins and dolichol have been described in neoplasms. The dolichol pathway is important in N-glycosylation of protein. Altered glycosylation of serum transferrin has been reported in neoplastic lesions. Abnormal glycoconjugates have been described in neoplastic disorders. A number of fucose and sialic acids containing carbohydrate ligands are important in malignant cell transformation. Glycosylation inhibitors are used to treat neoplasms. In multiple myeloma, interaction of tumour and host cells with adhesion and extracellular matrix molecules like heparan sulphate proteoglycan and syndecan are important.

Digoxin has been reported to regulate the transport of amino acids, especially the neutral amino acids. Tryptophan metabolism has also been implicated in neoplastic disorders and immune activation. lnterferons act by inducing the enzyme indoleamine 2,3-dioxygenase which catalyses the catabolism of tryptophan along the kynurenine pathway. This leads to tryptophan depletion and increase in the level of its metabolites kynurenine and quinolinic acid. Cachexia related to cancer has also been related to indoleamine 2,3-digoxygenase induction and depletion of tryptophan by enhancing its catabolism. The kynurenine pathway can also contribute to oncogenesis. Neurotransmitters could contribute to the regulation of the immune response. Elevated serotonin and reduced dopamine levels have been related immune activation and immunoproliferation. Tryptophan and tyrosine catabolism could be important in this respect with regard to immunoproliferative neoplasms like multiple myeloma.

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 cancer.

This study was undertaken to assess the following parameters in freshly diagnosed cases of multiple myeloma: (1) The isoprenoid pathway, (2) The tryptophan/tyrosine catabolic patterns, (3) Glycoconjugate metabolism, and (4) RBC membrane changes as a reflection of neoplastic cell membrane change (the isoprenoid pathway produces four metabolites which can regulate membrane function and structure - dolichol, digoxin. cholesterol and ubiquinone. A hypothesis implicating membrane Na^+-K^+ ATPase inhibition as pivotal to all these changes in multiple myeloma is also presented. Since digoxin can regulate multiple neurotransmitter systems it could possibly play a role in the genesis of cerebral dominance. The isoprenoid pathway and digoxin status was studied in individuals of differing hemispheric dominance in order to elucidate the role of cerebral dominance in the pathogenesis of multiple myeloma and neoplasms.

Materials and Methods

Fifteen freshly diagnosed cases of multiple myeloma were chosen randomly for the study from the orthopaedics and hematology wards of Medical College, Trivandrum and the Regional Cancer Centre, Trivandrum over a three year period. The age of the patients ranged from 50-60 years. All fifteen patients with multiple myeloma were right handed / left hemispheric dominant by the dichotic listening test. Informed consent was obtained from all the patients. The permission of the Ethics committee of the institute was also obtained. None of the subjects studied was under medication at the time of removal of blood. Samples were drawn before treatment was initiated. All the patients included in the study were non-smokers (active and passive). They were free of systemic diseases like hypertension and diabetes. Each patient had an age and sex matched healthy normal control.

Fifteen normal male healthy individuals (50-60 years of age) each of left handed / right hemispheric dominant, right handed / left hemispheric dominant and amphidextrous / bihemispheric dominant individuals diagnosed by the dichotic listening test were chosen for the study. This group was chosen at random from the general population of Trivandrum city. These individuals were not on any drugs like digoxin and were free from any systemic disease. All individuals in this group also were non-smokers (passive or active).

Fasting blood was removed from each of the patients for various estimations. RBCs were separated within 1 hour of collection of blood for the estimation of membrane Na^+-K^+ ATPase. Serum was used for the estimation of HMG CoA reductase activity. Plasma/serum was used for the estimation of the other parameters. All biochemicals used in this study were obtained from M/s Sigma Chemicals, USA. Activity of HMG CoA reductase of the plasma was determined using the method of Rao and Ramakrishnan by determining the ratio of HMG CoA to mevalonate. For the determination of Na^+-K^+ ATPase activity

of the erythrocyte membrane, the procedure described by Wallach and Kamat was used. Digoxin in the plasma was determined by the procedure described by Arun, Ravikumar, Leelamma and Kurup. For estimation of ubiquinone and dolichol in the plasma, the procedure described by Palmer, Maureen and Robert was used. Magnesium in the plasma 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 Innoye. Serotonin was estimated by the method of Curzon and Green and catecholamines by the method of Well-Malherbe. Quinolinic acid content of plasma was estimated by HPLC (C_{18} column micro Bondapak^{TM} 4.6 x 150 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). Nicotine, morphine and strychnine 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 (beta glucuronidase, beta N-acetyl hexosaminidase, hyaluronidase and cathepsin-D), activity of glycohydrolases (beta galactosidase, beta fucosidase and beta glucosidase) have been described earlier. Serum glycolipids (gangliosides, glycosyl diglycerides, cerebrosides and sulphatides) were estimated as described in methods in enzymology. Cholesterol was estimated by using 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 Wills and conjugated dienes and hydroperoxides by the procedure of Brien. Reduced glutathione was estimated the method of Beutler, Duran and Kelley. Extraction of erythrocytes for vitamin E was out according to the procedure described by Cohn, Rammel, Cunlifee and

Keiboom and Vitamin E estimated in the extract by HPLC (Waters HPLC, Nova Pak C_8 column (4.6 x 150 mm), solvent -acetonitrite: methanol: water (63:33:4), flow rate - 2 ml/min, detection (UV 280 nm). For vitamin E, the retention time was 3.5 mm under these conditions. Nitric oxide was estimated in the plasma by the method of Gabor and Allon. Iron binding capacity in plasma was estimated by the method of Wootton and ceruloplasmin by the method of Henry, Chiamori, Jacobs and Segalov. Serum albumin was estimated by the method of Spencer and Price. Free fatty acid was estimated by the method of Falholt, Lund and Fatholt. Statistical analysis was done by 'ANOVA'.

Results

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

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

(3) Nicotine aid strychnine could be detected n the plasma of patients with multiple myeloma but was not detectable in control serum. Morphine was not detected in the plasma of these patients.

(4) The concentration of total glycosaminoglycans (GAG) increased in the serum of multiple myeloma patients. The concentration of heparan sulphate (HS) heparin (H), chondroitin sulphates (ChS), hyaluronic acid and dermatan sulphate was increased in multiple myeloma. The concentration total hexose, fucose and sialic acid were increased in the glycoproteins of the serum in these patients. The concentration of

gangliosides, glycosyl-diglycerides, cerebroside and sulphatide showed significant increase in the serum of these patients.

(5) The activity of glycosaminoglycan (GAG) degrading enzymes - beta glucuronidase, beta N-acetyl hexoseaminidase, hyaluronidase and cathepsin-D - was increased in multiple myeloma when compared to the controls. The activity of beta galactosidase, beta fucosidase and beta glucosidase increased in multiple myeloma patients.

(6) The concentration of total GAG and hexose and fucose residues of glycoproteins in the RBC membrane decreased significantly in multiple myeloma. The concentration of RBC membrane cholesterol was unaltered in multiple myeloma while that of phospholipid decreased. The ratio of RBC membrane cholesterol phospholipids increased in multiple myeloma.

(7) The activity of superoxide dismutase (SOD), catalase, glutathione reductase and glutathione peroxidase in the erythrocytes decreased significantly in multiple myeloma. The concentration of MDA, hydroperoxides, conjugated dienes and NO increases significantly. The concentration of glutathione was decreased and of alpha tocopherol was unaltered in multiple myeloma. Iron binding capacity and ceruloplasmin decreased significantly in multiple myeloma while albumin was reduced.

(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 Multiple Myeloma

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 multiple myeloma there was significant inhibition of the RBC membrane Na^+-K^+ ATPase. The inhibition of Na^+-K^+ ATPase 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. The 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. 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 pathophysiology of multiple myeloma. Serum magnesium was assessed in multiple myeloma and was found to be reduced. 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. Intracellular magnesium depletion can produce defective phosphorylation of microtubule associated proteins (MAP) resulting in microtubule related spindle fibre dysfunction and chromosoma1 non-dysjunction. This produces the characteristic neoplastic cellular polyploidy and aneuploidy. Upregulation of the isoprenoid pathway can result in increased produclion of farnesyl phosphate which can farnesylate the ras oncogene producing its activation.

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

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. Two of the amino acids in this respect are important - tryptophan, a precursor for nicotine and strychnine and tyrosine a precursor for morphine. We have already shown the presence of endogenous nicotine and strychnine in the brain of rats loaded with tryptophan, and morphine in the brain of rats loaded with tyrosine. The results now obtained showed that the concentration of tryptophan, quinolinic acid and serotonin was higher in the plasma of patients with multiple myeloma

while that of tyrosine, dopamine and noradrenaline was lower. Serum of patients with multiple myeloma showed the presence of high amounts of nicotine in their serum. Morphine and strychnine were absent in the serum of these patients. Thus there is increase in tryptophan and its catabolites and a reduction in tyrosine and its catabolites in the patient's serum. This could be due to the fact that digoxin can regulate the neutral amino acid transport system with preferential promotion of tryptophan transport over tyrosine. The decrease in membrane Na^+-K^+ ATPase activity in all the disorders studied could be due to the fact that the hyperpolarising neurotransmitters (dopamine and noradrenaline) are reduced and the depolarising neuroactive compounds (serotonin, nicotine and quinolinic acid) are increased

The neurotransmitter pattern of reduced dopamine and noradrenaline, and increased serotonin can contribute to cancer related psychosis. This neurotransmitter pattern is common to multiple myeloma and schizophrenia. A schizoid state of mind can predispose the patients to the development of neoplasms. Alteration in natural killer cell activity has been reported in psychiatric disorders. Serotonin and acetyl choline promote cell proliferation and dedifferentiation by inhibiting adenyl cyclase or by activating phospholipase-C (PLC). Nicotine by binding to the nicotinic receptor promotes cholinergic transmission. Dopamine and noradrenaline elevate cyclic AMP levels and inhibit cell proliferation and differentiation. Increased quinolinic acid can lead to cancer related cachexia. Serotonin, dopamine and noradrenaline receptors have been demonstrated in the lymphocytes. It has been reported that during immune activation serotonin is increased with the corresponding reduction dopamine and noradrenaline in the brainstem monoaminergic nuclei. Thus elevated serotonin and reduced noradrenaline and dopamine can contribute to the immune activation ad immunoproliferation in multiple myeloma. Decreased morphine levels can

lead to increased metastatic property of tumours as morphine has a suppressing effect on tumour metastasis and tumour growth.

In the presence of hypomagnesemia, the magnesium block on the NMDA receptor is removed leading to NMDA excitotoxicity. The increased presynaptic neuronal calcium can produce cyclic AMP dependent phosphorylation of synapsins resulting in increased neurotransmitter release into the synaptic junction and vesicular recycling. Increased intracellular calcium in the post synaptic neuron can also activate the calcium dependent NMDA signal transduction. The plasma membrane neurotransmitter transporter (on the surface of the glial cell and presynaptic neuron) is coupled to a sodium gradient which is disrupted by the inhibition of Na^+-K^+ ATPase, resulting in decreased clearance of glutamate by presynaptic and glial uptake at the end of synaptic transmission. By these mechanisms, inhibition of Na^+-K^+ ATPase can promote glutamatergic transmission. The elevated levels of quinolinic acid and serotonin can also contribute to NMDA excitotoxicity. Quinolinic acid and serotonin are positive modulators of the NMDA receptor. Glutamate excitotoxicity has been implicated in the pathogenesis of neuronal degeneration. This could explain the increased incidence of paraneoplastic motor neuron disease in multiple myeloma. Increased glutamatergic transmission resulting in excitotoxicity has been implicated in cellular proliferation. Excitatory amino acids like glutamate can act as trophic factors and promote cellular proliferations.

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

The archaeaon glycosaminoglycoid and fructosoid contributes to glycoconjugate synthesis and catabolism by the process of fructolysis. The membrane Na^+-K^+ ATPase related magnesium depletion can affect the metabolism of glycosaminoglycans, glycoproteins and glycolipids. The

elevation in the level of dolichol may suggest its increased availability for N-glycosylation of proteins. Magnesium deficiency can lead to defective metabolism of sphinganine 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 glucose 6-phosphate is channelled for the synthesis of glycosaminoglycans (GAG). The results now obtained show an increase in the concentration of serum total GAG, individual GAG fractions, glycolipids and carbohydrate components of glycoproteins in multiple myeloma. The increase in the carbohydrate components - total hexose, fucose and sialic acid in multiple myeloma was not to the same extent suggesting qualitative change in glycoprotein structure. The activity of GAG degrading enzymes and that of glycohydrolases showed significant increase in the serum in multiple myeloma. 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 / magnesiurn ratios intracellularly may be structurally abnormal and resistant to lysosomal enzymes and may accumulate. In multiple myeloma, interaction of tumour and host cells with adhesion and extracellular matrix molecules like heparan sulphate, proteoglycan and syndecan are important. Elevated levels of heparan sulphate reported here may favour an upregulated interaction between tumour and host cells with adhesion and extracellular matrix molecules. This interaction

is important in the pathogenesis of myeloma. Accumulation of structurally abnormal glycoproteins leading to amyloid deposition has been described in myeloma. The abnormal glycoconjugate metabolism and lysosomal instability reported here may be important in amyloid deposition. Abnormal glycoconjugates accumulation can lead on to neuronal degeneration like motor neuron disease described in myeloma.

The protein processing defect can result in defective glycosylation of endogenous tumour 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. There is dysfunction of this 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 CD_4/CD_8 cell/NK cell. Defective presentation of exogenous viral antigens can produce immune evasion by the virus leading on to herpes viral persistence and oncogenesis in multiple myeloma. Kaposi's sarcoma associated herpes virus (KSHV) was found in the bone marrow dendritic cells of multiple myeloma patients but not in malignant plasma cells or bone marrow dendritic cells from normal individuals or patients with other malignancies. In addition the virus was detected in bone marrow dendritic cells from two out of eight patients with MGUS. Viral interleukin-6, the human homolog of which is a growth factor for myeloma was found to be transcribed in the myeloma bone marrow dendritic cells. KSHV may be required for transformation from MGUS to myeloma and perpetuate the growth of malignant plasma cells. Defective presentation of endogenous tumour antigens can lead to loss of NK cell (natural killer cell) immunosurveillance and oncogenesis. Altered cell surface glycoproteins, glycolipids and GAG can lead to defective contact inhibition and oncogenesis. A number of fucose and sialic acids

containing natural ligands have been implicated in neoplastic transformation and metastasis as also immune activation and lymphocytic proliferation.

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

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 RBC membrane cholesterol was unchanged while the phospholipids were reduced resulting in increased cholesterol: phospholipid ratio. The concentration of total GAG, hexose and fucose residues of glycoprotein 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. Similar 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. Lysosomal instability can contribute to abnormal glycoconjugate metabolism important in paraneoplastic neuronal degeneration and amylodogenesis. Defective peroxisomal membranes lead to catalase dysfunction which has been documented in multiple myeloma.

Archaeal Digoxin and Mitochondrial Dysfunction in Relation to Multiple Myeloma

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 most of the cases which may be the result of low tyrosine levels, reported in multiple myeloma 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 ca1ciun 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 the 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 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.

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. The free radicals and scavenging enzymes were estimated in all these disorders. There was 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 decrease in ubiquinone and reduced glutathione in multiple myeloma. The activity of enzymes involved in free radical scavenging like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and catalase is decreased in multiple myeloma suggesting reduced free radical scavenging. (alpha-tocopherol) values were unchanged in both neoplasms. In our study the iron binding capacity and serum ceruloplasmin are reduced suggesting increased amounts of free iron and copper, promoting free radical generation. Ceruloplasmin is a 132 KD monomeric copper oxidase which has been implicated in iron metabolism because of its catalytic oxidation of Fe^{2+} to Fe^{3+} (feroxidase activity). In the presence of iron in Fe^{2+} form, the conversion of H_2O_2 to hydroxyl radical is greatly increased. Low ceruloplasmin results in more of the iron to be in Fe^{2+} form. It has been shown that ceruloplasmin increases iron uptake by cells increasing the apparent affinity for the substrate by three times. Low ceruloplasmin levels can result in decreased iron uptake and this results in an increased amount of free iron. The intracellular magnesium deficiency can produce ribosomal dysfunction and inhibition of protein synthesis. The low iron binding capacity and low serum ceruloplasmin levels may be a consequence of reduced ferritin and ceruloplasmin synthesis. The peroxisomal membrane is defective owing to a membrane Na^{+}-K^{+} ATPase inhibition related defect in membrane formation and leads to reduced catalase activity. Glutathione is synthesized by the enzyme glutathione synthetase which

needs magnesium and ATP. The low intracellular magnesium consequent to Na^+-K^+ ATPase inhibition and the resulting low ATP can result in decreased synthesis of glutathione. Glutathione peroxidase, a selenium containing enzyme oxidises reduced glutathione (GSH) to oxidised glutathione (GSSG) which is then rapidly reduced to OSH by glutathione reductase. There is also a concomitant conversion of H_2O_2 to H_2O. The activity of glutathione reductase needs NADPH for the regeneration of GSH. This NADPH comes mostly from the pentose phosphate pathway. Intracellular magnesium deficiency due to membrane Na^+-K^+ ATPase inhibition leads to decreased formation of glucose-6-phosphate and down regulation of the pentose phosphate pathway with consequent decreased generation of NADPH. 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 dismutase and 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 the oncogenesis. Free radicals are required for the action of growth factors and promote cellular proliferation. Mitochondrial dysfunction and free radical generation can also contribute to neuronal neuronal degeneration like motor neuron disease described in multiple myeloma.

The increase intracellular calcium and ceramide related opening of the mitochondrial PT 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 cytochrome C into the cytoplasm. This results in activation of caspase-3. Caspase-3 can cleave and

inactivate P_{21} involved in linking DNA duplication to cell division resulting in a polyploidal cell and oncogenesis.

Archaeal Digoxin and Regulation of Cell Division, cell Proliferation and Neoplastic Transformation in Relation to Multiple Myeloma - 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 the calcium dependent calcineurin signal transduction pathway which can produce T-cell activation and secretion of interleukin-6 and TNF alpha. Interleukin-6 can stimulate the growth of myeloma cells by functioning as an autocrine growth factor. IL-6 was found to induce in vitro of myeloma cells. Myeloma cells spontaneously produced IL-6 and expressed IL-6 receptor. This can explain the immune activation in multiple myeloma and related paraneoplastic syndromes like motor neuron disease. Membrane Na^+-K^+ ATPase inhibition can produce immune activation and is reported to increase CD_4/CD_8 ratios as exemplified by action of lithium. Defective presentation of glycoprotein neuronal antigens to the CD_4 and CD_8 cell can explain the immune dysregulation and autoimmunity describe in paraneoplastic syndromes.

Archaeal Digoxin and Hemispheric Dominance in Relation to Multiple Myeloma

The archaeaon related organelle - steroidelle, neurotransminoid and vitaminocyte contribute to hemispheric dominance. Thus the defective isoprenoid pathway can promote oncogenesis and multiple myeloma by several mechanisms. (i). Altered calcium / magnesium ratios promoting ras oncogene activation and defective function of the P_{53} tumour suppressor gene, (ii) Protein

processing defect and defective presentation of tumour antigens and defective immunosurveillance. This can also lead to defective contact inhibition. Defective processing of viral glycoprotein antigens and their presentation can lead to viral persistance and oncogenesis, (iii) Mitochondrial defect and free radical generation. This also leads to caspase-3 activation and cleaving of P_{21} protein which couples cell division to DNA duplication, (iv) Intracellular magnesium depletion can produce defective phosphorylation of MAP (microtubule associated proteins) resulting in microtubule related spindle fibre dysfunction and cellular polyploidy and aneuploidy, (v) Digoxin related tryptophan / tyrosine transport defect leading to increase in neurotransmitters that promote cell proliferation (nicotine and serotonin) and decrease in neurotransmitters that inhibits cell proliferation (dopamine and noradrenaline). This also leads to quinolinic acid related cachexia and immunoproliferation, (vi) Increased production of farnesyl phosphate leading to farnesylation of ras oncogene and its activation.

The biochemical patterns obtained in right hemispheric chemically dominant individuals correlated with those obtained in multiple myeloma. Left handed / right hemispheric chemically dominant individuals 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 and its catabolites - serotonin, quinolinic acid, strychnine and nicotine while the levels of tyrosine and its catabolites - dopamine, noradrenaline and morphine were lower. The elevated digoxin levels produce increased tryptophan levels over tyrosine by its effect on neutral amino acid transport. The increased levels of depolarising tryptophan catabolites produced membrane Na^+-K^+ ATPase inhibition. The reverse patterns were obtained in right handed: left hemispheric chemically dominant individuals.

They had down regulated HMG CoA reductase activity with decreased di8oxin and dolichol levels and increased ubiquinone levels. The RBC membrane Na^+-K^+ ATPase activity was increased and serum magnesium levels elevated. The right handed / left hemispheric chemically dominant individuals had decreased levels of tryptophan and its catabolites - serotonin, quinolinic acid, strychnine and nicotine while the levels of tyrosine and its catabolites - dopamine, noradrenaline and morphine were increased. The low digoxin levels produce elevated tyrosine levels over tryptophan. The increased levels of hyperpolarising tyrosine catabolites produced membrane Na^+-K^+ ATPase stimulation. Thus chemical right hemispheric chemical dominance may predispose to oncogenesis by the hypothalamic archaeal digoxin hypersecretion occurring in that state. Chemical left hemispheric dominance and the related digoxin hyposecretion may protect against neoplasms and has an inhibitory effect on oncogenesis.

The biochemical patterns obtained in multiple myeloma are similar to those obtained in left handed / right hemispheric chemically dominant individuals diagnosed by the dichotic listening test. But all the patients with multiple myeloma were right handed/left hemispheric dominance by the dichotic listening test. Hemispheric chemical dominance has no correlation with handedness or the dichotic listening test. Multiple myeloma occurs in right hemispheric chemically dominant individuals and is a reflection of altered brain function.

References

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