3

Archaeal Digoxin and Bone Pathophysiology

Introduction

Global warming induces a genomic change in humans. Global warming induces endosymbiotic archaeal and RNA viroidal growth. The porphyrins form a template for the formation of RNA viroids, DNA viroids, prions, isoprenoids and polysaccharides. They can symbiose together to form primitive archaea. The archaea can further induce HIF alpha, aldose reductose and fructolysis resulting in further porphyrinogenesis and archaeal self replication. The primitive archaeal DNA is integrated along with RNA viroids which are converted to their corresponding DNA by the action of redox stress induced HERV reverse transcriptase into the human genome by the redox stress induced HERV integrase. The archaeal DNA sequences that are integrated into the human genome forms endogenous archaeal human genomic sequences akin to HERV sequences and can function as jumping genes regulating genomic DNA flexibility. The integrated endogenous genomic archaeal sequences can get expressed in the presence of redox stress forming endosymbiotic archaeal particles which can function as a new organelle called the archaeaons. The archaeaon can express the fructolytic pathway constituting an organelle called the fructosome, cholesterol catabolic pathway and digoxin synthetic forming an organelle called the steroidelle, the shikimic acid pathway forming an organelle called the neurotransminoid, antioxidant vitamin E and vitamin C synthetic organelle called the vitaminocyte as well as the glycosaminoglycan synthetic organelle called glycosaminoglycoid. The archaeaon secreting RNA viroids is called the viroidelle.

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 bone and joint disease.

The increase in endogenous EDLF, a potent inhibitor of membrane Na^+-K^+ ATPase, can decrease this enzyme activity. The results showed increased endogenous EDLF synthesis as evidenced by increased HMG CoA reductase activity, which functions as the rate limiting step of the isoprenoid pathway. Studies in our laboratory have demonstrated that EDLF is synthesized by the isoprenoid pathway. The endosymbiotic archaeal sequences in the human genome get expressed by redox stress and osmotic stress of global warming. This results in induction of HIF alpha which will upregulate fructolysis and glycolysis. In the setting of redox stress all glucose gets converted to fructose by the induction of enzymes aldose reductase and sorbitol dehydrogenase. Aldose reductase converts glucose to sorbitol and sorbitol dehydrogenase converts sorbitol to fructose. Since fructose is preferentially phosphorylated by ketohexokinases the cell is depleted of ATP and glucose phosphorylation comes

to a halt. Fructose becomes the dominant sugar that is metabolized by fructolysis in expressed archaeal particles in the cell functioning as organelle called fructosoids. The fructose is phosphorylated to fructose 1-phosphate which is acted upon by aldolase B which converts it into glyceraldehyde 3-phosphate and dihydroxy acetone phosphate. Glyceraldehyde 3-phosphate is converted to D 1, 3-biphosphoglycerate which is then converted to 3-phosphoglycerate. The 3-phosphoglycerate is converted to 2-phosphoglycerate. 2-phosphoglycerate is converted to phosphoenol pyruvate by the enzyme enolase. Phosphoenol pyruvate is converted to pyruvate by the enzyme pyruvic kinase. The archaeaon induces HIF alpha which upregulates fructolysis and glycolysis but inhibits pyruvate dehydrogenase. The forward metabolism of pyruvate is stopped. The dephosphorylation of phosphoenol pyruvate is inhibited in the setting of pyruvic kinase inhibition. Phosphoenol pyruvate enters the shikimic acid pathway where it is converted to chorismate. The shikimic acid is synthesized by a pathway starting from glyceraldehyde 3-phosphate. Glyceraldehyde 3-phosphate combines with the pentose phosphate pathway metabolite sedoheptulose 7-phosphate which is converted to erythrose 4-phosphate. The pentose phosphate pathway is upregulated in the presence of the suppression of glycolytic pathway. Erythrose 4-phosphate combines with phosphoenol pyruvate to generate shikimic acid. Shikimic acid combines with another molecule of phosphoenol pyruvate to generate chorismate. The chorismate is converted to prephenic acid and then to parahydroxy phenyl pyruvic acid. Parahydroxy phenyl pyruvic acid is converted to tyrosine and tryptophan as well as neuroactive alkaloids. The shikimic acid pathway is structured in expressed archaeaon organelle called the neurotransminoid. The fructolytic intermediates glyceraldehydes 3-phosphate and pyruvate are the starting points of the DXP pathway of cholesterol synthesis. Glyceraldehyde 3-phosphate combines with pyruvate to form 1-deoxy D-xylulose phosphate

(DOXP) which is then converted to 2-C methyl erythritol phosphate. 2-C methyl erythritol phosphate can be synthesized from erythrose 4-phosphate a metabolite of the shikimic acid pathway. DXP combines with MEP to form isopentenyl pyrophosphate which is converted to cholesterol. Cholesterol is catabolised by archaeal cholesterol oxidases to generate digoxin. The digoxin sugars digitoxose and rhamnose are synthesized by the upregulated pentose phosphate pathway. Glycolytic suppression leads to upregulation of the pentose phosphate pathway. The expressed archaeaon organelle concerned with cholesterol catabolism and digoxin synthesis is called the steroidelle. The suppresson of glycolysis and stimulation of fructolysis results in upregulation of the hexosamine pathway. Fructose is converted to fructose 6-phosphate by ketohexokinases. The fructose 6-phosphate is converted to glucosamine 6-phosphate by the action of glutamine fructose 6-phosphate amidotransferase (GFAT). Glucosamine 6- phosphate is converted to UDP N-acetyl glucosamine which is then converted to N-acetyl glucosamine and various amino sugars. UDP glucose is converted to UDP D-glucuronic acid. UDP D-glucuronic acid is converted to glucuronic acid. This forms the uronic acid synthetic pathway. Uronic acids and hexosamines form repeating units of glycosaminoglycans. In the setting of glycolytic suppression and fructolytic metabolism fructolysis leads to increase synthesis of hexosamines and GAG synthesis. The GAG synthesizing archaeaon particles are called the glycosaminoglycoids. The expressed archaeaon particles are capable of synthesizing antioxidant vitamin C and E. The UDP D-glucose is converted to UDP D-glucuronic acid. UDP D-glucuronic acid is converted to D-glucuronic acid. D-glucuronic acid is converted to L-gulonate by enzyme aldoketoreductases. L-gulonate is converted to L-gulonolactone by lactonase. L-gulonolactone is converted to ascorbic acid by the action of archaeal L-gulo oxidase. The vitamin E is synthesized from shikimate which is converted to tyrosine and then to parahydroxy phenyl

pyruvic acid. Parahydroxy phenyl pyruvic acid is converted to homogentisate. Homogentisate is converted to 2-methyl 6-phytyl benzoquinone which is converted to alpha tocopherol. 2-methyl 6-phytyl benzoquinone is converted to 2, 3-methyl 6-phytyl benzoquinone and gamma tocopherol. Vitamin E can also be synthesized by the DXP pathway. Glyceraldehyde 3-phosphate and pyruvate combined to form 1-deoxy D-xylulose 5-phosphate which is converted to 3-isopentenyl pyrophosphate. 3-isopentenyl pyrophosphate and dimethyl allyl pyrophosphate combined to form 2-methyl 6-phytyl benzoquinone which is converted to tocopherols. The ubiquinone another important membrane antioxidant and part of the mitochondrial electron transport chain is synthesized by the shikimic acid pathway and DXP pathway. The isoprenoid moiety of ubiquinone is contributed from the DXP pathway and the rest of it by tyrosine catabolism. The tyrosine is generated by the shikimic acid pathway. The archaeaon particles concerned with the synthesis of vitamin C, vitamin E and ubiquinone which are all antioxidants are called the vitaminocyte.

The human hypothalamus also produces an endogenous membrane Na^+-K^+ ATPase inhibitor called digoxin. Digoxin increases the intracellular calcium load and also leads on to intracellular magnesium depletion. An increase in the intracellular calcium load consequent to digoxin can affect the bone histopathology. The intracellular magnesium depletion consequent to digoxin can affect glycoconjugate metabolism. Hypomagnesemia has been reported to upregulate glycosaminoglycan metabolism. Digoxin can also function as an immunomodulator. Lithium, an exogenous membrane Na^+-K^+ ATPase inhibitor that can produce immune activation. Immunological mechanisms as well as alterations in the bone connective tissue matrix have been postulated to play an important role in the pathogenesis of degenerative osteoarthritis.

Articular cartilage is composed of two major macromolecular species: proteoglycans (PG) and collagen. The cartilage contains matrix

metalloproteinases (MMPs) which can degrade the proteoglycans. Lysosomal cathepsin present in the chondrocyte also plays a role in proteoglycan catabolism. The turnover of normal cartilage is effected through a degradative cascade for which the driving force is interleukin - 1 (IL-1) produced by mononuclear cells (including synovial lining cells) and synthesized by chondrocytes. IL 1 stimulates the synthesis and secretion of the latent MMPs and of the tissue plasminogen activator. Plasminogen can activate MMPs. IL-l suppresses PG synthesis. Lysosomal enzymes and MMPs account for much of the loss of the cartilage matrix in OA.

It was therefore considered pertinent to study digoxin synthesis and related alterations in glycoconjugate and free radical metabolism in the following degenerative bone and joint disease (cervical and lumbar spondylosis, degenerative osteoarthritis and postmenopausal osteoporosis). The patterns were compared to those obtained in right hemispheric and left hemispheric dominance to find out whether hemispheric dominance has any correlation with these disease states.[1-8]

Results

(1) The results showed that HMG CoA reductase activity, serum digoxin and dolichol were increased and serum ubiquinone, RBC membrane Na^+-K^+ ATPase activity and serum magnesium were reduced in degenerative bone disease (osteoarthritis and spondylosis). The results also showed that HMG CoA reductase activity, serum digoxin and dolichol were decreased and serum ubiquinone, RBC membrane Na^+-K^+ ATPase and serum magnesium increased in senile osteoporosis.

(2) 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 degenerative bone disease (spondylosis and osteoarthritis).

(3) The activity of enzymes involved in free radical scavenging like superoxide dismutase, catala.se, glutathione peroxidase, glutathione reductase and catalase is decreased in degenerative bone disease (spondylosis and osteoarthritis). There was a decrease in lipid peroxidation as evidenced by the decrease in the concentration of MDA, conjugated dienes, hydroperoxides and NO with increased antioxidant protection as indicated by an increase in ubiquinone and reduced glutathione in senile osteoporosis. The activity of enzymes involved in free radical scavenging like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and catalase is increased in postmenopausal senile osteoporosis.

(4) The results show an increase in the concentration of serum total GAG and carbohydrate components of glycoproteins (hexose, fucose and sialic acid) in degenerative bone disease (spondylosis and osteoarthritis). The increase in the carbohydrate components (total hexose, fucose and sialic acid) in the degenerative bone disease was not to the same extent in all cases suggesting qualitative change in glycoprotein structure. The pattern of change in individual GAG in the serum was different, though heparan sulphates (HS) and chondroitin sulphates (ChS) increased in degenerative bone disease (osteoarthritis and spondylosis). The activity of GAG degrading enzymes (beta glucuronidase, beta N-acetyl hexosaminidase, hyaluronidase and cathepsin-D) and that of glycohydrolases (beta galactosidase, beta fucosidase and beta glucosidase) showed significant increase in the serum in degenerative bone disease (osteoarthritis and spondylosis). The results show a decrease in the concentration of the serum total GAG and carbohydrate components of glycoproteins (hexose, fucose and sialic acid) in postmenopausal senile osteoporosis. The

decrease in the carbohydrate components (total hexose, fucose and sialic acid) in the disorders studied was not to the same extent in senile osteoporosis also suggesting qualitative change in glycoprotein structure. The pattern of change in the individual GAG in the serum was different, however heparan sulphate (HS) chondroitin sulphates (ChS) decreased in postmenopausal senile osteoporosis. The activity of GAG degrading enzymes (beta glucuronidase, beta N-acetyl hexosaminidase, hyaluronidase and cathepsin-D) and that of glycohydrolases (beta galactosidase, beta fucosidase and beta glucosidase) showed a significant decrease in the serum in postmenopausal senile osteoporosis.

(5) The cholesterol: phospholipid ratio of the RBC membrane is decreased in osteoporosis and increased in spondylosis and osteoarthritis. The concentration of total GAG, hexose and fucose content of glycoproteins increased in the serum and decreased in the RBC membrane in spondylosis and osteoarthritis. The concentration of total GAG, hexose and fucose content of glycoproteins decreased in the serum and increased in the RBC membrane in osteoporosis.

(6) 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.

Discussion

Archaeal Digoxin and Membrane Na^+-K^+ ATPase Inhibition in Relation to Bone and Joint Disease

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 decrease in the activity of HMG CoA reductase in postmenopausal senile osteoporosis suggests a downregulation of the isoprenoid pathway. There is a marked decrease in plasma digoxin and dolichol and this decrease may be a consequence of a decreased channeling of intermediates of the isoprenoid pathway for their biosynthesis. In this connection, incorporation of ^{14}C-acetate into digoxin in rat brain was shown, indicating that acetyl CoA is the precursor for digoxin biosynthesis in mammals also. The decrease in endogenous digoxin, a potent inhibitor of membrane Na^+-K^+ ATPase, can increase this enzyme activity. In postmenopausal senile osteoporosis there was significant stimulation of the RBC membrane Na^+-K^+ ATPase. The stimulation of Na^+-K^+ ATPase by digoxin is known to cause a decrease in intracellular calcium by decreased sodium - calcium exchange, decreased entry of calcium via the voltage gated calcium channel and decreased release of calcium from intracellular endoplasmic reticulum calcium stores. This decrease in intracellular calcium can cause an increase in the functional availability of intracellular magnesium because both these ions have an adversarial relationship. This increase in the availability of magnesium can cause increased mitochondrial ATP synthesis and further stimulation 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 the cell and ATP dependent sequestration of calcium within the endoplasmic reticulum. The increased intracellular magnesium related upregulated mitochondrial ATP

synthesis results in increased calcium extrusion from the cell. There is thus, a progressive stimulation of Na^{+}-K^{+} ATPase activity by digoxin. High intracellular magnesium and low intracellular calcium consequent to Na^{+}-K^{+} ATPase stimulation appear to be crucial to the pathophysiology of postmenopausal senile osteoporosis. The intracellular negative calcium signal and positive magnesium signal can regulate diverse cellular processes. Serum magnesium was assessed in postmenopausal osteoporosis and was found to be increased. Decrease in bone calcium load can lead to osteoporosis.

The increase in endogenous digoxin, a potent inhibitor or membrane Na^{+}-K^{+} ATPase, can decrease this enzyme activity in degenerative bone disease. In degenerative bone and joint disease (osteoarthritis and spondylosis), there was significant inhibition of the RBC membrane Na^{+}-K^{+} ATPase. 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. 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. The intracellular magnesium depletion 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 pathophysiology of degenerative bone disease. The intracellular positive calcium signal and negative magnesium signal can regulate diverse cellular processes. Serum magnesium was assessed in degenerative bone and joint disease (osteoarthritis and spondylosis) and was found to be reduced. Increased

intracellular calcium can lead to calcification that is noted in articular and ligamentous structures in cervical and lumbar spondylosis.[1-8]

Archaeal Digoxin and Regulation of Golgi Body / Lysosomal Function in Relation to Bone and Joint Disease

The archaeaon glycosaminoglycoid and fructosoid contributes to glycoconjugate synthesis and catabolism by the process of fructolysis. The elevation in the level of dolichol in degenerative bone disease (spondylosis and osteoarthritis) may suggest its increased availability of the N-glycosylation of proteins. In magnesium deficiency found in osteoarthritis and spondylosis; the glycolysis, citric acid cycle and oxidative phosphorylation are blocked and more glucose 6-phosphate is channelled for the synthesis of glycosaminoglycans (GAG). 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 a 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 glycohydrolases suggests a qualitative change in their structure. Proteoglycan complexes formed in the presence of altered calcium: magnesium ratios intracellularly, may be structurally abnormal and contribute to altered bone structure in osteoarthritis. Lysosomal stability is reduced and there is increased release of lysosomal enzymes, especially cathepsin-D contributing to bone and joint destruction. The protein processing defect can result in defective glycosylation of endogenous bone glycoprotein antigens with a consequent defective formation of MHC antigen complex. The MHC linked peptide transporter; a P-glycoprotein which transports MHC-antigen complex to the antigen presenting cell surface; has an ATP binding site. The peptide

transporter is dysfunctional in the presence of magnesium deficiency. This results in defective transport of MHC class-1 bone glycoprotein antigen complex to the antigen presenting cell surface for recognition by CD_4 or CD_8 cells. Defective presentation of endogenous bone glycoprotein antigen can explain the immune dysregulation contributing to osteoarthritis. A number of fucose and sialic acids containing natural ligands are involved in the trafficking of leukocytes, and similar breaches in the blood brain barrier and adhesion of the lymphocyte producing leukocyte trafficking and extravasation into the perivascular space have been described in osteoarthritis.

In postmenopausal senile osteoporosis, the hypodigoxinemia and resultant membrane Na^+-K^+ ATPase stimulation-related increased intracellular magnesium levels can affect the metabolism of glycosaminoglycans and glycoproteins. The decrease in the level of dolichol may suggest its decreased availability for the N-glycosylation of proteins. In intracellular magnesium excesses noted in osteoporosis, the glycolysis, citric acid cycle and oxidative phosphorylation are activated and less glucose 6-phosphate is channelled for the synthesis of glycosaminoglycans (GAG). The results show a decrease in the concentration of serum total GAG, and carbohydrate components of glycoproteins (hexose, fucose and sialic acid) in postmenopausal senile osteoporosis. The individual's GAG fractions in the serum-heparan sulphate (HS), chondroitin sulphates (ChS), heparin (H), hyaluronic acid (HA) and dermatan sulphate (DS) decreased in postmenopausal senile osteoporosis. The activity of GAG degrading enzymes (beta glucuronidase, beta N-acetyl hexosaminidase, hyaluronidase and cathepsin-D) and that of glycohydrolases (beta galactosidase, beta fucosidase and beta glucosidase) showed a significant decrease in serum in postmenopausal senile osteoporosis. Intracellular magnesium excess also results in increased ubiquitin-dependent proteolytic processing of glycoconjugates as it requires magnesium for its function. The

decrease in the activity of glycohydrolases and GAG degrading enzymes could be due to increased lysosomal stability. Increased lysosomal stability and defective degradation of glycoprotein - GAG complexes could lead to postmenopausal senile osteoporosis as it leads to defective bone remodelling. Reduction in the level of bone glycosaminoglycans can also affect the structural integrity of bone, leading to osteoporosis. The sulphated glycosaminoglycans have a negative charge and can be complex with calcium ions. This helps to retain the calcium ions in the bone matrix and contributes to the bone's structural integrity. The reduction in the level of sulphated glycosaminoglycans can thus, contribute to osteoporosis.[1-8]

Archaeal Digoxin and Alteration in Membrane Structure and Membrane Formation in Relation to Bone and Joint Disease

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 in osteoarthritis and spondylosis can lead to increased cholesterol synthesis and magnesium deficiency can inhibit phospholipid synthesis. Phospholipid degradation is increased owing to an increase in the intracellular calcium activating phospholipases A_2 and D. The cholesterol: phospholipid ratio of the RBC membrane was increased in spondylosis and osteoarthritis. 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. The glycoproteins, GAG and glycolipids of cellular membrane are formed in the endoplasmic reticulum, which is then budded of 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 the cholesterol: phospholipid ratio can produce changes in the conformation 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. Defective peroxisomal membranes lead to catalase dysfunction, which has been documented in these disorders.

The downregulation of the isoprenoid pathway in senile osteoporosis can lead to decreased cholesterol synthesis and magnesium excess can stimulate phospholipid synthesis. Phospholipid degradation is decreased owing to a decrease in intracellular calcium inhibiting phospholipase A_2 and D. The cholesterol: phospholipid ratio of the RC membrane was decreased in hypodigoxinemia. The concentration of total GAG, hexose and fucose of glycoprotein increased in the RBC membrane and decreased in the serum suggesting their increased incorporation into the membrane and defective membrane formation. The membrane trafficking depends upon GTPases and lipid kinases, which are crucially dependent on magnesium and are activated in magnesium excess. The change n membrane structure produced by an alteration in glycoconjugates and the cholesterol: phospholipid ratio can produce changes in the conformation of Na^+-K^+ ATPase resulting in further membrane Na^+-K^+ ATPase stimulation. The same changes can affect the structure of the organelle membrane. This results in increased lysosomal stability. Altered peroxisomal membranes could lead to a catalase hyperactivity noticed in hypodigoxinemic states.[1-8]

Archaeal Digoxin and Mitochondrial Dysfunction in Relation to Bone and Joint Disease

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 osteoarthritis and spondylosis which may be the result of low tyrosine levels, reported in degenerative bone and joint disease (spondylosis and osteoarthritis) consequent to digoxin's effect in preferentially promoting tryptophan transport over tyrosine. The aromatic ring portion of ubiquinone is derived from tyrosine. Ubiquinone, which is an important component of the mitochondrial electron transport chain, is a membrane antioxidant and contributes to free radical scavenging. The increase in intracellular calcium can open the mitochondrial PT pore, causing a collapse of the hydrogen gradient across the inner membrane and an 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 a superoxide ion, which produces lipid peroxidation. Ubiquinone deficiency also leads to reduced free radical scavenging. The increase in intracellular calcium may lead to an increased generation of NO by inducing the enzyme nitric oxide synthase, which combines with a superoxide radical to form peroxynitrite. Increased calcium can also activate phospholipase A_2, resulting in an 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 the glutathione synthase and

glutathione reductase functions. The mitochondrial superoxide dismutase leaks out and becomes dysfunctional with a calcium related opening of the mitochondrial PT pore and outer membrane rupture. The peroxisomal membrane is defective owing to a membrane Na^+-K^+ ATPase inhibition-related defect in membrane formation and leads to reduced catalase activity. Mitochondrial dysfunction-related free radical generation has been implicated in the pathogenesis of immune-mediated dysfunctions described in degenerative bone and joint disease (osteoarthritis).

The increased intracellular calcium-related opening of the mitochondrial PT pore also leads to a 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 an apoptosis-inducing factor and cytochrome C into the cytoplasm. This results in activation of caspase-9 and caspase-3. Caspase-9 can produce apoptosis of the cell. Apoptosis has been implicated in cell death. Increased apoptosis of osteoblast can contribute to degenerative bone and joint disease (osteoarthritis and cervical spondylosis).

The concentration of ubiquinone increased significantly in postmenopausal senile osteoporosis, which may be the result of increased tyrosine levels, consequent to digoxin deficiency promoting tyrosine transport over tryptophan. The decrease in intracellular calcium can stabilise the mitochondrial PT pore and improve mitochondrial function. Intracellular magnesium excess can lead to an increase in the activity of ATP synthase. All this leads to improved efficiency in mitochondrial oxidative phosphorylation and reduced free radical generation. Ubiquinone excess also leads to increased free radical scavenging. The decrease in intracellular calcium may lead to decreased generation of NO by inhibiting the enzyme nitric oxide synthase and reduced peroxynitrite formation. Decreased calcium can also inhibit phospholipase A_2 resulting in decreased generation of arachidonic acid and free radical formation. Decreased

generation of free radicals like the superoxide ion and hydroxyl radical can stabilise the cell membrane and stimulate membrane Na^+-K^+ ATPase. There was a decrease in lipid peroxidation as evidenced from the decrease in the concentration of MDA, conjugated dienes and hydroperoxides and the increase in free radical scavengers like ubiquinone and reduced glutathione in senile osteoporosis. The activity of enzymes involved in free radical scavenging like superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase is increased in postmenopausal senile osteoporosis suggesting increased free radical scavenging. The peroxisomal membrane is stabilised owing to membrane Na^+-K^+ ATPase stimulation-related alteration in membrane formation and leads to increased catalase activity. Glutathione is synthesized by the enzyme glutathione synthetase, which needs magnesium and ATP. The high intracellular magnesium consequent to Na^+-K^+ ATPase stimulation and the resulting increased ATP synthesis can result in increased synthesis of glutathione. Glutathione peroxidase, a selenium containing enzyme, oxidises reduced glutathione (GSH) to oxidised glutathione (GSSG) which is then rapidly reduced to GSH 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 excess due to membrane Na^+-K^+ ATPase stimulation leads to increased formation of glucose 6-phosphate and upregulation of the pentose phosphate pathway with consequent increased generation of NADPH. The glutathione system of free radical scavenging is activated in the presence of membrane Na^+-K^+ ATPase stimulation. Superoxide dismutase exists in a mitochondrial and cytoplasmic form. The stabilisation of the mitochondrial PT pore consequent to reduced intracellular calcium produces increased superoxide dismutase activity. Free radicals are required for osteoclastic activity and bone remodelling. The reduced generation of free

radicals owing to an increased efficiency of mitochondria leads to defective osteoclast related bone remodelling and postmenopausal senile osteoporosis. The decreased intracellular calcium related stabilisation of the mitochondrial PT pore also leads to downregulation of the apoptotic program and reduced apoptosis. The stabilisation of the mitochondrial PT pore leads to reduced release of the apoptosis inducing factor and cytochrome C into the cytoplasm. This results in inactivation of caspase-9 which produces cellular apoptosis. Apoptosis is an important component of bone remodelling. Defective apoptosis leads to defective bone remodelling and senile postmenopausal osteoporosis.[1-8]

Archaeal Digoxin and Immunoregulation in Relation to Bone and Joint Disease

The archaeaon fructosoid contributes to fructolysis and immune activation. Fructose can contribute to induction of NFKB and immune activation. The archaeaon steroidelle synthesized digoxin induces NFKB producing immune activation. In hyperdigoxinemia related degenerative bone disease (spondylosis and osteoarthritis) increased intracellular calcium activates the calcium dependent calcineurin signal transduction pathway which can produce T-cell activation and secretion of interleukin-l. This can also explain the immune activation leading to joint destruction in degenerative bone disease (osteoarthritis). 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.[1-8]

Archaeal Digoxin, Oncogene Activation and Bone and Joint Disease

The archaeaon secreting RNA viroids is called the viroidelle. The primitive archaeal DNA is integrated along with RNA viroids which are converted to their corresponding DNA by the action of redox stress induced HERV reverse

transcriptase into the human genome by the redox stress induced HERV integrase. The archaeal DNA sequences that are integrated into the human genome forms endogenous archaeal human genomic sequences akin to HERV sequences and can function as jumping genes regulating genomic DNA flexibility. The integrated endogenous genomic archaeal sequences can get expressed in the presence of redox stress forming endosymbiotic archaeal particles which can function as a new organelle called the archaeaons. In hyperdigoxinemic degenerative bone and joint disease, 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 osteoblastic proliferation. The decreased intracellular magnesium can produce dysfunction of the 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, Upregulation of the isoprenoid pathway can result in increased production of farnesyl phosphate, which can farnesylate the ras oncogene, producing its activation. Thus in the hyperdigoxinemic state-related degenerative bone and joint disease (spondylosis and osteoarthritis), there is increased growth potential of the osteoblast which leads to an increase in bone density and spondylosis. In the hypodigoxinemic postmenopausal senile osteoporosis, high intracellular magnesium and low intracellular calcium consequent to Na^+-K^+ ATPase stimulation appears to reduce the growth potential of the osteoblast. Decreased intracellular calcium inactivates phospholipase C beta which results in decreased production of diacyglycerol (DAG) with resultant inactivation of protein kinase C The protein kinase C (PKC) activation of the MAP kinase cascade is inhibited, resulting in a blockade of cellular proliferation. The increased intracellular magnesium can produce an increase in the GTPase activity of the alpha-subunit of G protein.

This results in ras oncogene inactivation, as more of the ras is bound to GDP rather than GTP and a reduction in osteoblastic growth potential. The downregulation of the isoprenoid pathway can result in decreased production of farnesyl phosphate which is required for ras oncogene activation. Therefore the ras oncogene is inactivated. In the hypodigoxinemic postmenopausal senile osteoporosis there is a reduction in the growth potential of the osteoblast. This contributes to the pathogenesis of senile postmenopausal osteoporosis.[1-8]

Archaeal Induced Hyperdigoxinemic State and Hemispheric Dominance in Relation to Bone and Joint Disease

The archaeaon related organelle - steroidelle, neurotransminoid and vitaminocyte contribute to hemispheric dominance. Thus, the degenerative bone and joint disease (spondylosis and osteoarthritis) represents the hyperdigoxinemic state. This leads to (1) increased free radical production and reduced scavenging leading to increased osteoclastic activity, (2) increased glycoconjugate synthesis and defective lysosomal stability, (3) immune activation. (4) membrane Na^+-K^+ ATPase inhibition related increased calcium load in the bone, (5) digoxin related increased apoptosis and (6) hyperdigoxinemia related increased osteoblastic proliferation potential. The hyperdigoxinemic state occurs in right hemispheric dominant individuals.

The postmenopausal senile osteoporosis is a hypodigoxinemic state. This leads to (1) reduced free radical production, (2) reduced glycoconjugate synthesis, (3) stable lysosomes which can contribute to defective osteoclastic activity and bone remodeling, (4) membrane Na^+-K^+ ATPase inhibition-related reduced intracellular calcium load, (5) hypodigoxinemia related immune inactivation, (6) hypodigoxinemia related reduced apoptosis and bone remodeling and (7) hypodigoxinemia related blockade of osteoblastic

proliferation. The pattern in postmenopausal senile osteoporosis is similar to the left hemispheric dominant state.

The hyperdigoxinemia related patterns seen in spondylosis and osteoarthritis correlates with right hemispheric dominance. Similarly the hypodigoxinemia related pattern in senile osteoporosis correlates with left hemispheric dominance. Hemispheric dominance and archaeal digoxin decide the predisposition to these disease states.[1-8]

References

[1] Kurup RK, Kurup PA. Hypothalamic digoxin and hemispheric chemical dominance relation to the pathogenesis of senile osteoporosis, degenerative osteoarthritis and spondylosis. *In. I. Neurosci*. 2003 Mar; 113(3): 341-59.

[2] Kurup RK, Kurup PA. Hypothalamic digoxin, cerebral dominance, and lipid metabolism. *Int. J Neurosci*. 2003 Jan; 113 (1): 107-15.

[3] Kurup RK, Kurup PA. Hypothalamic digoxin, hemispheric chemical dominance, and endocrine / metabolic / cellular regulation. *Int. J. Neurosci*. 2002 Dec; 112(12): 1421-38.

[4] Kurup RK, Kurup PA. Hypothalamic digoxin, cerebral dominance, and mitochondrial function / free radical metabolism. *Int. J Neurosci*. 2002 Dec; 112 (12): 1409-20.

[5] Kurup RK, Kurup PA. Hypothalamic digoxin, cerebral dominance, and Golgi body / lysosomal function. *Int. J Neurosci*. 2002 Dec; 112 (12): 1449-59.

[6] Kurup RK, Kurup PA. Hypothalamic digoxin cerebral dominance and membrane biochemistry. *Int. J Neurosci*. 2002 Dec: 112(12): 1439-47.

[7] Kurup RK, Kurup PA. Hypothalamic digoxin, cerebral chemical dominance, and calcium/magnesium metabolism. *Int. J. Neurosci*. 2003 Jul; 113(7): 999-1004.

[8] Kurup RK, Kurup PA. Endogenous hypodigoxinemia - related immune deficiency syndrome. *Int. J Neurosci*. 2003 Sep; 113(9): 1287-303.