



Cholesterol synthesis is the trigger and isoprenoid dependent interleukin-6 mediated inflammation is the common causative factor and therapeutic target for atherosclerotic vascular disease and age-related disorders including osteoporosis and type 2 diabetes

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Summary This is a unifying theory that cholesterol metabolites (isoprenoids) are an integral component of the signaling pathway for interleukin-6 (IL-6) mediated inflammation. IL-6 inflammation is the common causative origin for atherosclerosis, peripheral vascular disease, coronary artery disease, and age-related disorders including osteoporosis, dementia, Alzheimer's disease and type 2 diabetes. Therapeutic effects of bisphosphonates and statins are mediated by isoprenoid depletion.

Statins and bisphosphonates act in the cholesterol pathway to deplete isoprenoids. Anti-inflammatory properties of statins and bisphosphonates are due to isoprenoid depletion with subsequent inhibition of IL-6 mediated inflammation. Therapeutic targets for the prevention and control of all the above diseases should focus on cholesterol metabolites and IL-6 mediated inflammation. Prevention of atherosclerotic vascular disease and age-related disorders will be by utilization of cholesterol lowering agents or techniques and/or treatment with statins and/or bisphosphonates to inhibit IL-6 inflammation through regulation of cholesterol metabolism.

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Cholesterol synthesis, inflammation and aging theory

Cholesterol synthesis is the trigger and isoprenoid dependent interleukin 6 (IL-6) mediated inflammation is the common causative factor and therapeutic

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Cardiovascular disease (CVD) is the leading cause of death and disability in developed nations and is increasing rapidly in the developing world. By the year 2020, it is estimated that CVD will surpass infectious diseases as the world's leading cause of death and disability. Atherosclerotic vascular disease (ASVD), which encompasses coronary heart disease, cerebrovascular disease, and peripheral arterial disease, is responsible for the majority of cases of CVD in both developing and developed countries [1]. Atherosclerosis, a progressive disease characterized by the accumulation of lipids and fibrous elements in the arteries, constitutes the single most important contributor to this growing burden of cardiovascular disease. The link between lipid metabolism and atherosclerosis dominated the thinking until the 1980s. Over the last 15 years, however, a prominent role for inflammation in the pathogenesis of atherosclerosis has been established [2]. Now atherosclerosis is considered as an inflammation-mediated disease driven by complex interactions between leukocytes, platelets and cells of the vessel wall.

Endothelial injury is the first and crucial step in the pathogenesis of atherosclerosis. A plethora of genetically determined and epigenetic factors, such as oxidized low-density lipoprotein (LDL), free radicals (e.g., due to cigarette smoking), hypertension, diabetes mellitus, elevated plasma homocysteine, infectious microorganisms, autoimmune reactions, and combinations thereof, have been identified as etiological principles. Endothelial injury triggers inflammation with increased adhesiveness and activation of leukocytes (mainly monocytes) and platelets, which is accompanied by the production of cytokines, chemokines, vasoactive molecules and growth factors.

The hallmark of the early atherosclerotic lesion is the cholesterol ester-laden (CE-laden) macrophage foam cell [3]. Progressive "free" cholesterol (FC) loading of lesional macrophages leads to a series of phospholipid-related adaptive responses. These adaptive responses eventually fail, leading to macrophage death. Macrophage death by either necrosis or apoptosis leads to lesional necrosis, release of cellular proteases, inflammatory cytokines, and prothrombotic molecules, which could contribute to plaque instability, plaque rupture, and acute thrombotic vascular occlusion [4]. Indeed, necrotic areas of advanced atherosclerotic lesions are known to be associated

with death of macrophages, and ruptured plaques from human lesions have been shown to be enriched in apoptotic macrophages.

The presence of apoptotic and necrotic macrophages in atherosclerotic lesions has been well documented in many human and animal studies [5].

What is missing in the current theories and thinking is the direct molecular link (exclusive of macrophage death), between cholesterol and inflammation, and between inflammation and atherosclerosis, peripheral vascular disease, coronary artery disease, and age-related disorders including osteoporosis, arthritis, type 2 diabetes, dementia and Alzheimer's disease.

The acute phase response occurs prior to antibody-mediated immunological defense. It occurs in response to an inflammatory response brought on by injury and trauma, neoplasm, or disordered immunological activity. A local reaction at the site of injury or infection leads to an activation of cytokines (specifically, IL-6, IL-1, TNF- α , and interferons) that triggers a systemic response consisting of leukocytosis; increases in glucocorticoid production; increases in erythrocyte sedimentation rates, fever, activation of complement and clotting cascades; decreases in serum zinc and iron; and an increase in plasma levels of acute phase proteins, C-reactive protein (CRP), serum amyloid A, fibrinogen, and other proteins [6].

Levels of cytokines involved in the acute phase response – TNF- α , IL-1, IL-6, and fibrinogen – have been shown to be elevated in cases of unstable angina related to aneurysm and have been positively correlated with the risk of primary and recurrent myocardial infarction and death. The risk associated with these elevated levels remains constant even when the data is adjusted for other major risk factors: blood pressure, total and HDL cholesterol, body mass index, diabetes, alcohol use, family history, and exercise frequency [7]. Elevated levels of highly sensitive C-reactive protein (hs-CRP) have been related to increased risk of cardiovascular disease, myocardial infarction, and coronary artery disease (CAD) deaths among individuals with angina pectoris [8]. Assayed levels of hs-CRP can increase 100 times over normal levels within 24–48 h after an acute inflammatory stimulus. However, in long term prospective studies inter-individual variations in hs-CRP levels may occur over long periods of time, in the absence of trauma or acute infection. Elevated levels of hs-CRP have shown a doubling of risk both for ischemic stroke in hypertensive men and women and for peripheral artery disease [9].

Recent studies are now demonstrating that IL-6 and TNF- α are stronger predictors of cardiovascular

disease than C-reactive protein. In the health, aging and body composition study [10], people with the highest IL-6 levels were two to five times more likely to have a heart attack, stroke or other cardiovascular episode than those with the lowest levels. High blood levels of TNF- α increased the risk of heart disease by 79% and of heart failure by 121%. High levels of C-reactive protein increased the risk of heart failure by 160% compared to those with low levels, but they did not significantly raise the risk of a first stroke or heart attack.

As expected, the incidence of cardiovascular disease was high for people with the conventional risk factors – smoking, high blood pressure, high cholesterol and the like. But for participants free of those risk factors, the inflammation-related molecules were better predictors of heart disease. In the health, aging and body composition study [11], IL-6 levels were associated with the highest risks for subclinical cardiovascular disease as well as for clinical cardiovascular disease.

Cholesterol metabolism

Normal healthy adults synthesize cholesterol at a rate of approximately 1 g/day and consume approximately 0.3 g/day. A relatively constant level of cholesterol in the body (150–200 mg/dL) is maintained primarily by controlling the level of de novo synthesis. The level of cholesterol synthesis is regulated in part by the dietary intake of cholesterol. Cholesterol from both diet and synthesis is utilized in the formation of membranes and in the synthesis of the steroid hormones and bile acids. The greatest proportion of cholesterol is used in bile acid synthesis [12]. Cholesterol synthesis occurs in the cytoplasm and microsomes from the two-carbon acetate group of acetyl-CoA. The process has 10 major steps:

1. Synthesis begins when acetyl-CoA is derived from an oxidation reaction in the mitochondria and is transported to the cytoplasm.
2. Two moles of acetyl-CoA are condensed, forming acetoacetyl-CoA. Acetoacetyl-CoA and a third mole of acetyl-CoA are converted to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) by the action of HMG-CoA synthase.
3. HMG-CoA is converted to mevalonate, in a rate limiting step catalyzed by the enzyme HMG-CoA reductase, (HMGR).
4. Mevalonate is then activated by three successive phosphorylations, yielding 5-pyrophosphomevalonate.

5. After phosphorylation, an ATP-dependent decarboxylation yields isopentenyl pyrophosphate, (IPP), an activated isoprenoid molecule. Isopentenyl pyrophosphate is in equilibrium with its isomer, dimethylallyl pyrophosphate, DMAPP.
6. One molecule of IPP condenses with one molecule of DMAPP to generate geranyl pyrophosphate, (GPP). This step is catalyzed by GPP synthase.
7. GPP further condenses with another IPP molecule to yield farnesyl pyrophosphate, (FPP). This step is catalyzed by FPP synthase.
8. FPP condenses with another IPP molecule to yield geranylgeranyl pyrophosphate (GGPP). This step is catalyzed by GGPP synthase.
9. The head-to-tail condensation of two molecules of FPP yielding Squalene, is catalyzed by squalene synthase.
10. Squalene undergoes a two-step cyclization to yield lanosterol.
11. Lanosterol is converted to cholesterol, through a series of 19 additional reactions.

There is a complex regulatory system to co-ordinate the biosynthesis of cholesterol with the availability of dietary cholesterol.

Activation of IL-6 inflammation by cholesterol metabolites

Isoprenoid precursors are necessary for post-translational lipid modification (prenylation) and, hence, the function of Ras and other small guanine triphosphatases (GTPases) [14].

These GTPase proteins such as Ras, Rho, Rac, and Rab (particularly Rho) are intracellular signaling proteins that, when activated, are involved in receptor-coupled transduction of signals from extracellular stimuli to cytoplasm and the nucleus. The Rho proteins belong to the Ras superfamily. The Ras proteins alternate between an inactivated GDP-bound form and activated GTP-bound form, allowing them to act as molecular switches for growth and differentiation signals. Prenylation is a process involving the binding of hydrophobic isoprenoid groups consisting of farnesyl or geranylgeranyl residues to the C-terminal region of Ras protein superfamily.

Farnesyl pyrophosphate and geranylgeranyl pyrophosphate are metabolic products of mevalonate that are able to supply prenyl groups. The prenylation is conducted by prenyl transferases. The hydrophobic prenyl groups are necessary to anchor the Ras superfamily proteins to intracellular

membranes so that they can be translocated to the plasma membrane [15]. The final cell-membrane fixation is necessary for Ras proteins to participate in their specific interactions [16]. The activity of the small GTPase, Rac1, plays a role in various cellular processes including cytoskeletal rearrangement, gene transcription, and malignant transformation. The IL-6 receptor system consists of an IL-6 specific binding molecule, IL-6R and a signal transducer, gp130. Following gp130 dimerization, IL-6 activates multiple signaling pathways (Ras dependent MAP Kinase cascade, STAT1–STAT3 heterodimer pathway, and STAT3 homodimer pathway). Several other cytokines belonging to the IL-6 family including oncostatin M, IL-11, leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF) and cardiotropin-1 (CT-1) use gp130 as a common signal transducing molecule and therefore have similar biological activities [17]. Constitutively active Rac1 (Rac V12) stimulates the activation of STAT3, a member of the family of signal transducers and activators of transcription (STATs) [18]. The activity of Rac1 leads to STAT3 translocation to the nucleus coincident with STAT3-dependent gene expression. The expression of Vav (Delta1-187), a constitutively active guanine nucleotide exchange factor for the Rho GTPases, or activated forms of Ras or Rho family members, leads to STAT3-specific activation. Rac1 induces STAT3 activation through an indirect mechanism that involves the autocrine production and action of IL-6, a known mediator of STAT3 response. Rac V12 expression results in the induction of the IL-6 and IL-6 receptor genes and neutralizing antibodies directed against the IL-6 receptor block Rac1-induced STAT3 activation. Inhibition of the nuclear factor- κ B activation or disruption of IL-6-mediated signaling through the expression of I κ B α S32AS36A and suppressor of cytokine signaling 3, respectively, blocks Rac1-induced STAT3 activation. The induction of an autocrine IL-6 activation loop through which Rac1 mediates STAT3 activation is a link between oncogenic GTPase activity and Janus kinase/STAT signaling. The ability of leukemic cells to express IL-6 has been shown to correlate with clonal activation of N-RAS [19].

Activation of IL-6 inflammation by activated monocytes in the inflammatory response to infection and trauma

HMG-CoA reductase generates mevalonate, the precursor of a complex series of isoprenoids mole-

cules that are necessary for post-translational lipid modification (prenylation) and, hence, the function of intracellular signaling proteins that, when activated, are involved in expression of IL-6 mediated inflammation. Tissue injury, subsequent to a physical, chemical or biological insult, results in an inflammatory response associated with invasion of the area by immune cells, which include monocytes, T helper cells, lymphocytes, neutrophils, eosinophils, and other cells such as fibroblasts and endothelial cells. Isoprenoids are required for NADPH oxidase activity. Reduced nicotinamide adenine dinucleotide phosphate in granulocytes via low-molecular-weight (LMW) GTP-binding protein isoprenylation. Isoprenoid generation through the mevalonate pathway is a requirement for IL-8 and IL-6 induction by activated monocytic cells in vitro. In human monocytic THP-1 cells, pretreatment with isoprenoid synthesis inhibitors (HMG-CoA reductase inhibitor lovastatin or compactin at 10 mM) attenuates production of IL-6 and IL-8 by 50–90% in response to lipopolysaccharide, granulocyte-macrophage colony-stimulating factor, and phorbol myristate acetate. Coincubation of reductase inhibitor-treated cells with mevalonate prevents the attenuation of IL-8 production by reductase inhibitors [20]. Fluvastatin has decreased (and mevalonate rescued) signaling molecules within membrane rafts in monocytes in parallel with effects on tyrosine phosphorylation events. In addition, fluvastatin blocks Fc γ receptor mediated immune complex trafficking, blocks activation of MAP kinases (ERK and p38), and inhibits downstream inflammatory mediator release (MMP-1 and IL-6) [21]. Lovastatin and sodium phenylacetate (NaPA) inhibit lipopolysaccharide (LPS) and cytokine-mediated production of NO and expression of iNOS in rat primary astrocytes. This inhibition is not due to depletion of end products of mevalonate pathway (e.g., cholesterol and ubiquinone). The inhibition of LPS-mediated induction of iNOS by FPT inhibitor II, an inhibitor of Ras farnesyl protein transferase, suggests that farnesylation of p21(ras) or other proteins regulates the induction of iNOS [22].

Bacterial infection as typified by periodontal disease is associated with inflammation and the inflammatory response, with generation of isoprenoids by activated monocytes. Bacteria also directly synthesize isoprenoid molecules by a mevalonate – independent (non-MVA) pathway (see Fig. 1). The synthesis of IPP and DMAPP via the non-MVA pathway starts with the formation of 1-deoxy-Dxylulose-5-phosphate (DOXP) by two glycolytic intermediates, pyruvate and glyceraldehyde-3-phosphate [23]. These isoprenoids may be

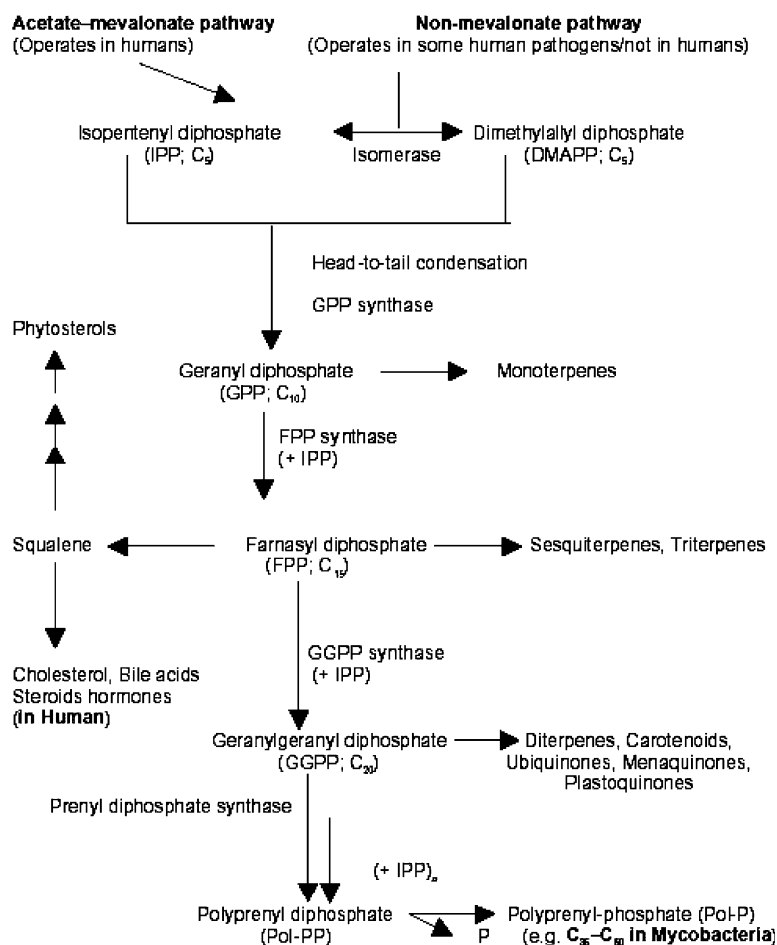


Figure 1 Isoprenoid synthesis [13].

involved in the cell-wall biosynthesis and may also play a role in direct activation of biologically active mediators [24]. Periodontal disease is characterized by adherence and colonization of the tooth enamel and root surface by saccharolytic, aerobic *Streptococcus* species, and other bacteria. This sets the stage for *Fusobacterium nucleatum* to coaggregate with these early colonizers and to permit late colonizers, including dental pathogens, to eventually form a biofilm. These complex interactions result in the release of factors that lead to tooth decay [25]. Chronic bacterial infections are risk factors for coronary heart disease. Dental health is significantly worse in patients with acute myocardial infarction than in controls. The association remained valid after adjustment for age, social class, smoking, serum lipid concentrations, and the presence of diabetes [26]. People with periodontal disease have a factor of 2 higher risk of dying from cardiovascular disease. By comparison smokers only have a 60% increased risk [27]. C-reactive proteins and pro-inflammatory cytokines are released during periodontal flare-ups and capa-

ble of eliciting effects associated with atherosclerosis and coronary heart disease [28]. The presence of oral infections is also associated with cerebrovascular disease, stroke, preterm births, osteoporosis [29] and type 2 diabetes. In patients with both diabetes and periodontal disease, treatment of periodontal infections markedly improves the management of their diabetes [30].

Inhibition of cholesterol pathway by statins

The main effect of statins is the decrease of serum level of low-density lipoprotein (LDL) cholesterol, due to the inhibition of intracellular cholesterol biosynthesis. A minor effect is the decrease of serum triglycerides. Statins inhibit HMG-CoA reductase and decrease the production of mevalonate, geranyl pyrophosphate, and farnesyl pyrophosphate, and subsequent products on the way to construction of the cholesterol molecule. Thus, statins

could inhibit inflammation, by inhibition of the cholesterol pathway and intracellularly interfering with Ras superfamily protein function [31]. Statins decrease matrix metalloproteinase-1 expression through inhibition of Rho [32]. Statin therapy has been demonstrated to provide significant reductions in non-high-density lipoprotein cholesterol, and to decrease cardiovascular morbidity and mortality.

Inhibition of cholesterol pathway by bisphosphonates

Recent findings suggest that alendronate and other N-containing bisphosphonates inhibit the isoprenoid biosynthesis pathway and interfere with protein prenylation, as a result of reduced geranylgeranyl diphosphate levels. Farnesyl diphosphate (FPP) synthase has been identified as the mevalonate pathway enzyme inhibited by bisphosphonates [33]. A wide range of bisphosphonates have a significant correlation between potency for inhibition of recombinant human FPP synthase in vitro and anti-resorptive potency in vivo, suggesting that this enzyme is the major pharmacologic target of these drugs. Inhibition of FPP synthase prevents the formation of FPP and its derivative GGPP. These isoprenoid lipids are necessary for the post-translational lipid modification (prenylation) of small GTPase proteins such as Ras, Rho, Rac, and Rab. The effects of nitrogen-containing bisphosphonates on osteoclasts can be overcome by addition of components of the mevalonate pathway, which bypass the inhibition of FPP synthase and restore protein prenylation. In particular, geranylgeraniol (a cell-permeable form of GGPP) prevents inhibition of resorption by nitrogen-containing bisphosphonates in vitro [34]. In patients with Paget's bone disease treated with intravenous pamidronate, decrease of bone ALP and total ALP is associated with a significant increase in HDL-C and a significant decrease in LDL-C [35].

Statins and interleukin-6

The ability of HMG-CoA reductase inhibitors to lower C-reactive protein levels brought into question the mechanisms of action of the statin drugs. Because these medications lower incidences of acute cardiovascular events as well as decreasing morbidity and mortality well before the effects of

lowered LDL cholesterol can be expected to occur, questions have been asked about whether they may work independently of LDL-lowering mechanisms. In patients with familial hypercholesterolemia (FH) and non-familial hypercholesterolemia (NFH), treatment with atorvastatin has significantly reduced IL-6 levels with a significant and progressive increase in brachial artery endothelial-dependent flow mediated dilatation (FMD) [36].

Bisphosphonates and interleukin-6

Because of various modes of action observed in studies, bisphosphonates have been classified into two groups. Bisphosphonates (such as clodronate and etidronate) that closely resemble pyrophosphate – a normal byproduct of human metabolism – are incorporated into adenosine triphosphate (ATP) analogues, which create compounds that are believed to build up and lead to osteoclast death. The newest generation of bisphosphonates, which contain nitrogen (such as pamidronate, alendronate, risedronate, and ibandronate), are believed to inhibit protein prenylation (post-translational modification) within the mevalonate pathway. The mevalonate pathway is responsible for the biosynthesis of cholesterol, other sterols, and isoprenoid lipids. Isoprenoid lipids are key in the prenylation of intracellular signaling proteins (GTPases) that, when activated, regulate a number of processes, including osteoclast activity. It is believed that by impeding the function of these regulatory proteins, bisphosphonates block osteoclast functioning and cause apoptosis [37].

In patients with Paget's disease of bone, bisphosphonate therapy is associated with a significant reduction of IL-6 soluble receptor (sIL-6R) serum levels [38]. Bisphosphonates inhibit the production of pro-inflammatory cytokine IL-6 in tumoral cell lines of human osteoblastic phenotype (MG63 and SaOs cells), and in peripheral blood mononuclear cells (PBMC) [39]. Bisphosphonates also inhibit IL-1 and TNF- α stimulated IL-6 release in cultures of human osteoblastic osteosarcoma cells [40]. Osteoblasts exposed to small amounts of bisphosphonate elaborate a soluble inhibitor, which interferes with osteoclast formation and development [41]. Pamidronate and other bisphosphonates inhibit osteoclastic resorption and decrease serum levels of the inflammatory cytokine IL-6 in patients with multiple myeloma [42].

Atherosclerosis and statins

Measurement of carotid arterial intima-media thickness (IMT) using B-mode ultrasonography is a non-invasive and powerful tool to evaluate early atherosclerotic lesions. There has been increasing use of this imaging technique in observational and interventional studies of lipid-lowering agents over the last decade. These observational studies clearly demonstrate an association between carotid IMT and atherosclerotic disease. Use of atorvastatin 80 mg daily for aggressive lowering of plasma low-density lipoprotein cholesterol (LDL-C) concentrations to below current target levels is associated with significant IMT regression compared with results obtained with less aggressive plasma LDL-C lowering [43].

Atherosclerosis and bisphosphonates

In subjects with type 2 diabetes associated with osteopenia, one year of therapy with cyclical etidronate (200 mg/day for 2 weeks every 3 months) produced a significant decrease in carotid arterial intima-media thickness (mean \pm SE, -0.038 ± 0.011 mm), when compared with control subjects (0.023 ± 0.015 mm; $P < 0.005$) [44]. Administration of ethane-1-hydroxy-1,1-diphosphonate (EHDP) to swine with pre-established atherosclerosis results in lower lesion calcium concentration, smaller lesions and a decrease in the area of lesions involved in necrosis [45].

Type 2 diabetes and interleukin-6

Circulating levels of IL-6 are raised in insulin resistant states such as obesity, impaired glucose tolerance (IGT), and type 2 diabetes mellitus (DM). Growing evidence suggests that IL-6 is not only produced by fat cells but is also capable of inducing insulin resistance in these cells. The expected result of this *in vivo*, would be to increase adipose mass and subsequently body mass index (BMI). The IL-6 -174G > C common functional gene variant has consistently been associated with increased plasma IL-6, insulin resistance, and increased cardiovascular risk [46]. In The Women's Health Study (an ongoing US primary prevention, randomized clinical trial initiated in 1992), elevated levels of the inflammatory markers IL-6 and C-reactive pro-

tein (CRP) have been associated with development of type 2 DM in healthy middle-aged women [47].

Type 2 diabetes and bisphosphonates

Advanced glycation end products (AGE) form at an accelerated rate in diabetes and induce angiogenesis through overgeneration of autocrine vascular endothelial growth factor (VEGF). The nitrogen-containing bisphosphonate, incadronate disodium completely inhibits AGE-induced increase in DNA synthesis as well as tube formation of human microvascular endothelial cells (EC). Furthermore, incadronate disodium significantly prevents transcriptional activation of nuclear factor- κ B and activator protein-1 and the subsequent up-regulation of VEGF mRNA levels in AGE-exposed EC. Incadronate disodium blocks the AGE-signaling pathway in microvascular EC through inhibition of protein farnesylation and may be a promising remedy for treatment of patients with proliferative diabetic retinopathy [48]. Charcot neuroarthropathy has been recognized for over 130 years and yet it remains a major cause of morbidity for patients with diabetes mellitus and a continuing challenge for physicians. The underlying cause is thought to be trauma in a neuropathic foot that leads to a complex series of pathological processes culminating in bone and joint destruction and subsequent deformity. The bisphosphonate pamidronate is used in the management of acute diabetic Charcot neuroarthropathy. Infusion of 90 mg of pamidronate is associated with an improvement in symptoms including reduction in bone turnover, urinary deoxypyridinoline and bone-specific alkaline phosphatase [49].

Type 2 diabetes and statins

Type 2 diabetes is associated with a substantially increased risk of cardiovascular disease, but the role of lipid-lowering therapy with statins for the primary prevention of cardiovascular disease in diabetes is inadequately defined. Atorvastatin 10 mg daily has been administered for primary prevention of major cardiovascular events in patients with type 2 diabetes without high concentrations of LDL-cholesterol. Acute coronary heart disease events were reduced by 36%, coronary re-vascularisations by 31%, and rate of stroke by 48%. Atorvastatin reduced the death rate by 27%. No excess of adverse events was noted with this treatment [50].

Osteoporosis and interleukin-6

Osteoporosis is a condition that is common with aging and especially in post-menopausal women. The etiology has often been ascribed to abnormalities in calcium metabolism. However many patients with osteopenia/osteoporosis have in common pain and inflammation and many inflammatory pain syndromes have osteopenia/osteoporosis as an accompanying feature [51]. A notable example is the osteoporosis that is often present in Complex Regional Pain Syndrome/Reflex sympathetic dystrophy (CRPS-I/RSD) [52]. IL-6 mediated inflammation has been shown to contribute to the process of bone remodeling. This it does by stimulating osteoclastogenesis and osteoclast activity [53]. Elevated levels of IL-6 have been observed in conditions of rapid skeletal turnover and hypercalcemia as in Paget's disease and multiple myeloma. In multiple myeloma, radiologic examinations reveals osteolytic lesion and the most common finding is diffuse osteopenia [54]. Adhesion of multiple myeloma cells to stromal cells triggers IL-6 secretion by the stromal cells [55]. This results in increased osteoclastic activity that in turn results in osteoporosis, painful osteolytic lesions and hypercalcemia characteristic of multiple myeloma [56]. In their youth, women are protected from osteoporosis because of the presence of sufficient levels of estrogen. Estrogen blocks the osteoblast's synthesis of IL-6. Estrogen may also antagonize the IL-6 receptors. Decline in estrogen production is often associated with osteopenia/osteoporosis in post-menopausal women. Estrogen's ability to repress IL-6 expression was first recognized in human endometrial stromal cells [57]. Additional clues came from the observations that menopause or ovariectomy resulted in increased IL-6 serum levels [58], increased IL-6 mRNA levels in bone cells and increased IL-6 secretion by mononuclear cells [59]. Further evidence for estrogen's ability to repress IL-6 expression is derived from studies, which demonstrated that estradiol inhibits bone marrow stromal cell and osteoblastic cell IL-6 protein and mRNA production in vitro [60] and that estradiol was as effective as neutralizing antibody to IL-6 in suppressing osteoclast development in murine bone cell cultures or in ovariectomized mice [61].

Osteoporosis and bisphosphonates

Bisphosphonates are inorganic chemical compounds that bind to hydroxyapatite in bone and prevent osteoclastic absorption of bone. Nitrogen-containing bisphosphonates (N-BPs) are potent inhibitors of bone resorption widely used in the treatment of osteoporosis and other bone degrading disorders including Paget's disease of bone, hypercalcemia associated with malignancy, metastatic bone diseases, such as breast cancer, multiple myeloma, and arthritis [62]. At the tissue level, N-BPs reduce bone turnover and increase bone mass and mineralization. This is measured clinically as an increase in bone mineral density and bone strength and a decrease in fracture risk. N-BPs localize preferentially at sites of bone resorption, where mineral is exposed, are taken up by osteoclasts and inhibit osteoclastic activity. At the molecular level, N-BPs inhibit an enzyme in the cholesterol synthesis pathway, farnesyl diphosphate synthase. As a result, there is a reduction in the lipid geranylgeranyl diphosphate, which prenylates GTPases required for cytoskeletal organization and vesicular traffic in the osteoclast, leading to osteoclast inactivation [63].

Osteoporosis and statins

3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) have been shown to stimulate bone formation in laboratory studies, both in vitro and in vivo. Statin use in most, but not all observational studies is associated with a reduced risk of fracture, particularly hip fracture, even after adjustment for the confounding effects of age, weight and other medication use. This beneficial effect has not been observed in clinical trials designed to assess cardiovascular endpoints [64]. Men using statin drugs are more likely to have a greater BMD of the spine ($p < 0.005$), and men who receive statin drugs for more than 2 year are approximately half as likely to develop osteoporosis. A similar effect is observed in women taking statins for any length of time [65]. Statin use in women is associated with a 3% greater adjusted BMD at the femoral neck, and BMD tends to be greater at the spine and whole body [66]. Nitrogen-containing bisphosphonate drugs inhibit the mevalonate pathway, preventing the production of isoprenoids, which consequently results in the inhibition of osteoclast formation and osteoclast function. Statins decrease the hepatic biosynthesis of cholesterol by blocking the mevalonate pathway, and can affect bone metabolism in vivo through effects on osteoclastic bone resorption. The ability of statin

compounds to inhibit bone resorption is directly related to HMG-CoA reductase activity [67].

Conclusion

Cholesterol synthesis is the trigger and IL-6 mediated inflammation is the common causative factor and therapeutic target for atherosclerosis, peripheral vascular disease, coronary artery disease, and age-related disorders including osteoporosis, and, type 2 diabetes.

Isoprenoids, which are intermediates, generated in the cholesterol biosynthesis pathway, play a more significant role than the end product cholesterol, in activation of IL-6 mediated inflammation. Isoprenoids are generated by endogenous cellular cholesterol synthesis in the body as well as by cholesterol synthesis in activated monocytes during the inflammatory response. Isoprenoids are an integral component of the signaling pathway for IL-6 mediated inflammation.

Control of dietary cholesterol is necessary but regulation of endogenous cholesterol synthesis is more important in the prevention and treatment of atherosclerosis, peripheral vascular disease, coronary artery disease, and age-related disorders including osteoporosis, type 2 diabetes and Alzheimer's disease.

Prompt treatment of infection such as in periodontal disease will prevent or reduce the generation of isoprenoids and induction of IL-6 mediated inflammation by activated monocytes.

Statins and bisphosphonates have similar mechanisms of action and act on similar diseases in the following ways:

1. Statins and bisphosphonates inhibit the mevalonate to cholesterol conversion pathway and cause isoprenoid depletion; statins inhibit the enzyme HMG-CoA reductase and bisphosphonates inhibit the enzyme FPP synthase.
2. Statins and bisphosphonates inhibit the signaling pathway for IL-6 mediated inflammation by isoprenoid depletion.
3. Statins and bisphosphonates inhibit bone resorption.
4. Combination treatment with statins, bisphosphonates and a cholesterol lowering agent or technique will be the most effective method of prevention and treatment of atherosclerosis, peripheral vascular disease, coronary artery disease, and age-related disorders including osteoporosis, type 2 diabetes and Alzheimer's disease.

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