University of Nevada, Reno

Coronary Artery Disease: A Disease Review, an Examination of a Mechanism Involving the Risk Factor Homocysteine-Thiolactone, and a Case Study.

A thesis submitted in partial fulfillment
of the requirements for the degree of

BACHELOR OF SCIENCE, BIOCHEMISTRY AND MOLECULAR BIOLOGY

by

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May, 2015

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Abstract

Coronary artery disease (CAD) presents itself with significant frequency in the human population across the globe. The acute condition affects in some form, almost half of the middle aged male population in the US. The chronic condition develops over time to finally evolve into the acute state which presents itself as chest pain and results in a myocardial infarction. The field of biochemistry has discovered a risk factor, homocystein thiolactone (Hcy-thiolactone), that plays a rule in exacerbating the chronic and acute processes of CAD. Mechanistic theory and experimentation reveals that Paraoxinase I is capable of negating Hcy-thiolactone's pathologic effects. Manipulation of PON1 shows promise in reducing the risk of the acute phase of CAD development. A patient case study is provided that illustrations a common presentation of the acute condition. An understanding of the identification and treatment process with regard to the clinical appearance of CAD can greatly reduce morbidity.

Acknowledgements

I have much gratitude to Dr. Josh Baker for supervising my research endeavor and providing useful insight on researching my mechanistic aspect of the disease. I would also like to show my appreciation for Tenille Smith MSIV, whose instruction guided the successful formation of my case study. Finally I want to thank Gina Sella for her unfaltering instruction and support that allowed me to create this thesis.

Table of Contents

List of Tables

List of Figures

Chapter1: Disease Review

Introduction

An overview of coronary artery disease has been formed including the clinical presentation and treatment of the condition as well as the pathophysiology. The condition may present itself acutely or chronically, both of which are explored in great detail which regard to signs and symptoms as well as treatment plans. The pathophysiology of the chronic state and how it leads to the acute state is outlined. A molecular mechanism is introduced that has implications in CAD.

Coronary artery disease is a condition that may be benign at first and can prove fatal over time. It is responsible for the largest number of deaths around the world and as such is a condition being vigorously researched and treatments being developed. Examination of the inflammatory roots of the disease's pathophysiology will give insight into the current treatment options available.

Clinical Features and Symptoms

The hallmark symptom of CAD is angina pectoris, or simply chest pain, and is sometimes visualized with the patient clutching their chest in severe or acute situations. Varying levels of angina pectoris exist with the least concerning being low risk or stable angina and the most concerning being high risk or unstable angina. Stable angina is specifically defined as having no significant change in frequency, duration, precipitating causes, or ease of relief. Usually this can be managed for at least 60 days without any complication but unstable angina arises when chest pain that was previously chronic has an increase in frequency and/or duration. New triggers to the chest pain as well as the inability to relieve the pain with past successful therapies elicit the consideration of

unstable angina. Patient's having chest pain for the first time or presenting with evidence of recent myocardial damage must be considered in the unstable angina category until further evaluation. Normally patients with stable angina can be treated under the observation of their primary care physician. However, unstable angina is of greater severity and is usually referred to a cardiologist by the primary care physician.

Instances in which the chest pain is acute, and most of the time severe, falls into the hands of emergency medicine and is categorized as acute coronary syndrome. Chest pain in these cases is often accompanied by diaphoresis (excess sweating). Other accompanying symptoms include upper abdominal pain, back pain, throat/jaw pain, arm pain, nausea, shortness of breath, and/or dizziness. Expeditious treatment in the emergency department with cardiology consult is critical to patient survival with evidence of myocardial damage or dysfunction with diagnostic testing.

Significant CAD can also be present in individuals are asymptomatic. This is known as silent myocardial ischemia and is discovered by electrocardiogram findings pointing at previous myocardial damage. It has been noted that this condition has higher prevalence among individuals with diabetes mellitus, older age, prior heart attack history, or past revascularization procedures.

CAD also presents itself in patients for the first time, although rarely, as sudden cardiac death. This is highly acute, difficult to treat, and is best prepared for by recognizing people at risk for CAD before allowing this nature of presentation to occur.

Studies have shown that the occurrences of CAD are much higher in males than females. In the US, signs and symptoms appear in approximately half of middle-aged men and one-third of middle aged women. As such, women are generally less likely to be

considered at risk for CAD even when presenting with similar symptoms as men. Women additionally tend to show the less typical symptoms, such as shortness of breath, vomiting, or jaw pain, more often than men (Hanson, Fareed, Argenio, Agunwamba, & Hanson, 2013).

Risk Factors

Being of the male gender or having a past family history of CAD are factors that currently can't be changed that increase CAD risk. Factors that can be personally influenced include tobacco use, hyperlipidema, hypertension, diabetes mellitus, obesity, and a sedentary lifestyle. Individuals can reduce their risk for CAD with regard to the pervious factors with smoking cessation, diet changes, regular exercise, and medication use. Recently evidence has shown the evaluation of lipoprotein A, highly sensitive C-reactive protein, fibrinogen, and homocystein can aid in earlier treatment and prevention of CAD (Hanson et al., 2013).

Pathophysiology

Several years ago, an overhaul in the causative effects of CAD has shown that the atherosclerotic aspect is less indicative of a cholesterol storage problem and more line with a chronic inflammatory disorder. Treatment to prevent the chronic lesion formation representative of CAD will stop the thrombotic acute phase of the disease. The inflammatory component of the disease involves a complex interaction between the cells of the artery and the white blood cells in the lumen via various signaling molecules. The condition is initiated by bacterial products or risk factors including dyslipidemia, vasoconstrictor hormones, the products of glycoxidation associated with hyperglycemia, or proinflammatory cytokines derived from excess adipose tissue. These initiators

increase the production of signaling molecules on the inner surface of the arterial wall which results in an increased leukocyte presence, mainly composed of mononuclear phagocytes and T lymphocytes, outside the arterial intima. Chemoattractant cytokines induce the transmigration of the leukocytes into the intima as seen in figure 1.

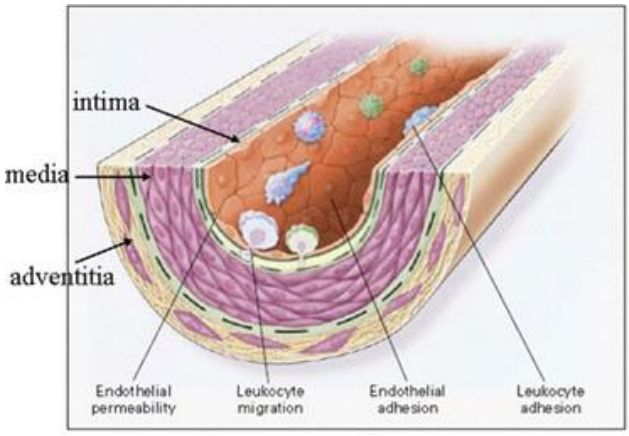

Figure 1. Cross section of an artery displaying leukocyte migration.
Source: *Pathophysiology of Coronary Artery Disease*

Signals associated with risk factors for atherosclerosis regulate these chemoattractant cytokines. Inflammatory processes are underway once the leukocytes and endogenous cells of the arterial wall communicate. Prostanoids and other derivatives of arachidonic acid as well as autacoids such as histamine mediate the inflammatory response. The prostanoids play a role in the regulation of communication between cells while histamine increases the permeability of the blood vessel. The large increase in activity in the intima prompts the entry of smooth muscle cells from the arterial media and the resulting acceleration of extracellular matrix development. Matrix metalloproteinases are then secreted by smooth muscle cells, endothelial cells, and monocytes which have a role in matrix reconstruction of arteries and myocardium. This enlargement of the matrix causes

a subsequent increase in lipoprotein binding by proteoglycans. Lipoproteins become more vulnerable to oxidative modification and glycation when bound to proteoglycans, the products of which exacerbate the inflammatory response. In the midst of Cell proliferation and cell death all occur within the lesion. Leukocytes overwhelmed with lipid particles die and release tissue factor which elevates thrombotic risk. Sometimes calcification similar to process involved in the construction of bone tissue occurs. The plethora of lipids accumulating amidst the cell deaths aggregates to structure the infamous necrotic plaque.

Development of the chronic condition over time ultimately leads to the presentation of acute coronary syndrome, or the emergent aspect of coronary artery disease. Thrombosis is the formation of a blood clot within the blood vessel that increases the likelihood of vessel blockage. The thrombotic environment arising from a plaque is initiated when the fibrous cap protecting the tissue within the plaque is disrupted. Disruption leads to coagulation, thrombosis of the clot, and finally restriction of the blood flow. Thrombosis can also be caused by superficial erosion, intraplaque hemorrhage, or the erosion of a calcified nodule although they are not too common. The clotting process is composed of two elements, the “fluid phase” determinants which increases the disposition for clotting and the “solid state” determinants mainly responsible for the acute clotting. The “solid state” exists after disruption of the atherosclerotic plaque surface leads to contact with the fibrinogen in the extracellular matrix as seen in figure 2.

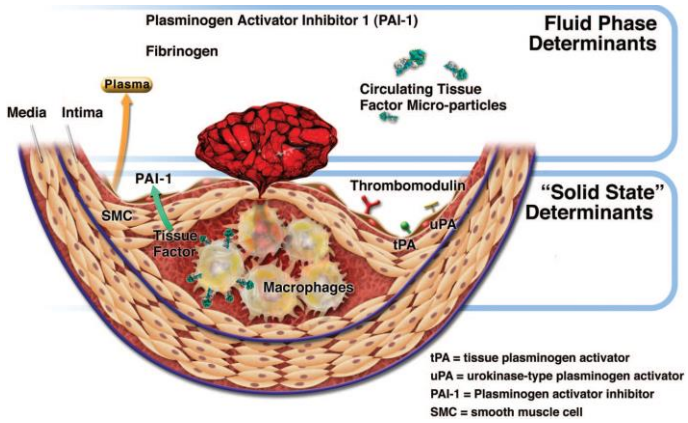

Figure 2. Plaque rupture with imminent thrombosis.
Source: *Pathophysiology of Coronary Artery Disease*

This contact causes platelet activation and subsequent clotting. Simultaneously, macrophages and smooth muscle cells secrete tissue factor that starts the coagulation cascade. Development of the thrombus exacerbates the process via positive feedback by increasing platelet activation as well as inducing the clotting effects of other cells in the plaque. Fibrinogen is soluble in the blood and can be transformed into fibrin which binds to each other as insoluble fibers that aid in the clotting process. This along with secretion of von Willebrand, a glycoprotein involved with coagulation, create a dense network of platelets connected to each other and suspended in a fibrin mesh (Libby & Theroux, 2005). The high platelet density nature of this thrombus gives it the characteristic "white" arterial thrombus appearance contrary to the high blood cell and fibrin composition of the "red" venous thrombus. (Jerjes-Sanchez, 2005). Blood levels of fibrinogen, tissue factor micro-particles, and Plasminogen Activor Inhibitor 1 (PAI-1) are all categorized under the "fluid phase" determinants influencing thrombosis. PAI-1 specifically plays an interesting role by suppressing natural processes of the body that disrupt thrombolytic presence. Mechanisms that inhibit urokinase-like and tissue-type plasminogen activators,

therefore reducing excessive coagulation, are limited to some extent by PAI-1. This leads to an increased tendency for clotting to occur and develop and as such displays high levels of PAI-1 as a disposition toward coronary artery disease. Diabetes and obesity are examples of conditions in which PAI-1 tends to have higher than normal levels in the blood stream. (Libby & Theroux, 2005).

Diagnostic Testing and Diagnosis

The gold standard for diagnosis of CAD is the use of the coronary angiogram. However due to its invasiveness and generally high cost, it is normally not the first form of testing completed. Lab results, electrocardiogram findings, stress tests, and imaging findings are all useful in determining the presence of CAD whether chronic or acute. Acute coronary syndrome, aortic dissection, pulmonary embolism, pericarditis, gastroesophageal reflux disease, peptic ulcer disease, biliary problems, and musculoskeletal causes are all differential diagnoses for acute onset chest pain.

Lab results give some insight into whether a patient is experiencing an acute exacerbation of CAD. Elevated levels of Cardiac biomarkers (myoglobin and CK isoforms), troponin, and creatinine kinase MB (CK-MB) may all indicate some extent of myocardial infarction (MI) as seen in figure 3.

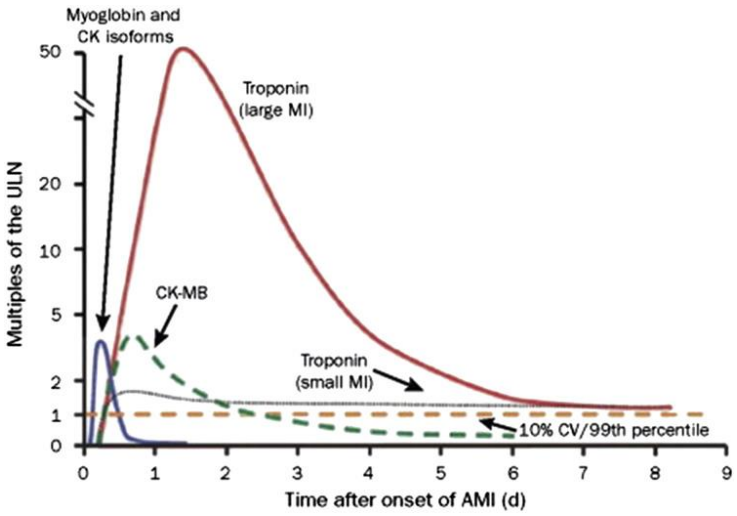

Figure 3. Biomarkers of acute myocardial infarction.
Source: *Coronary Artery Disease*

Unfortunately these results aren't completely decisive. Myoglobin and CK isoforms can be elevated without any occurrence of MI. Increased CK-MD levels can also be associated with skeletal myopathy/trauma, cardiac trauma, myocarditis, severe hypothyroidism, seizures, cardioversion/defibrillation, and renal failure. Increased troponin levels can also be associated with several of the previous conditions as well as heart failure, pulmonary embolism, demand ischemia, coronary angioplasty, or myocardial infiltration.

The electrocardiogram is one of the most commonly utilized cardiac tests and is critical for the diagnosis of acute coronary syndrome. Specifically the focus is centralized on the status of ST segments as seen in Figure 4.

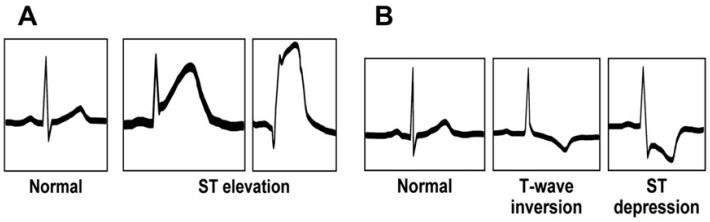

Figure 4. ST segment elevation, depression, and T wave inversion on an ECG
Source: *Coronary Artery Disease*

Elevated ST segments indicate cardiac ischemia although a lack of elevation may not necessarily it out. Some depression in ST segments along with T-wave inversion points at unstable angina. The presence of Q waves indicates the patient may have had a past myocardial infarction.

Stress tests allow the heart to be evaluated while under the conditions of exertion and utilize the ECG as well as blood pressure observation throughout the test. Patients asymptomatic of CAD may develop those symptoms while under stress. Exertional hypotension will occur if there is severe heart failure or if CAD as affecting multiple vessels of the heart. Fixed cardiac output, volume depletion, and the effects of vasodilators can also result in exertional hypotension. The opposing condition of exertional hypertension is not as informative of a finding as exertional hypotension but does indicate a higher chance for developing hypertension. In cases where patients are unable to complete a stress test due to orthopedic or medical issues, their heart can be stimulated to imitate conditions of exertions with Dobutamine being a commonly used stimulant.

The level of calcium detected in the coronary arteries of the heart, scored according to the Agatson score, gives an idea of the risk a patient has to develop CAD. This is due to the common presence of calcium in atherosclerotic plaques characteristic

of CAD. The technique is completed by having a CT scan of the heart done followed by determination of the Agatson score. A score of 0 means there is no identifiable disease, 1-99 shows mild disease, 100-399 shows moderate disease, and 400+ shows severe disease.

Cardiac catheterization and angiography is the most informative diagnostic method in that it displays the heart's blood flow status in real time. The procedure is completed by catheter insertion via blood vessels in the groin, arm, or neck and movement to the vessels of the heart. From there a dye is injected and imaging of the blood flow is taken to show the efficiency of blood flow through the vessels and heart and whether there is any sort of obstruction. The angiogram is merely for imaging but catheter placement is utilized for angioplasty, stenting or valvuloplasty (Hanson et al., 2013).

Treatment

A patient presenting with the acute version of CAD should be given oxygen, aspirin, β – blockers, nitrates, and morphine given that they do not have any contraindications against any of those treatments. Continuous observation is also a must with the use of constant ECG, blood pressure, and oxygen saturation monitoring. This allows for immediate detection of abnormal heart rhythms, rates, and heart failure.

Aspirin acts as a blood thinner and so reduced the coagulation ability of the blood and consequently alleviating a thrombotic environment. The recommended dosage for acute CAD is orally chewed 162 to 325 mg. Clopidogrel must be taken simultaneously if the patient has an allergy to aspirin. β – blockers decrease heart rate and strength of contractions which reduces stress on the left ventricular wall and cardiac oxygen demand. It is usually given intravenously for acute cases. Contraindications for β – blockers

include cardiogenic shock, moderate to severe LV failure, second- or third-degree atrioventricular heart block, hypotension with systolic blood pressure less than 100 mm Hg, sinus bradycardia with heart rate less than 69, or severe reactive airway disease. Nitrates, specifically nitroglycerin, is administered sublingually for the first dosage and intravenously for following dosages. Its function is the relaxation of smooth muscle which reduces left ventricular preload stress, dilates coronary vessels, and reverses vasospasms. Morphine is a pain medication administered intravenously during episodes of acute coronary syndrome. Supplemental oxygen provides a plethora of oxygen for the body to utilize so that ischemia due to a lack of oxygen in the environment is not an issue.

Concrete studies have shown that many acute coronary syndrome cases with ST segment elevation are caused by thrombosis to the coronary vessels, blockage, and resulting ischemia. As such, the use of blood thinners, or medications that decrease clotting efficiency, have been essential to reducing the effects of thrombosis related myocardial infarction. The physical widening of blocked vessels, known as angioplasty, is another technique used to treat acute coronary syndrome. Stents are wire meshes that hold the structure of vessels and keep them open.

The chronic CAD condition does not require immediate intervention but can be treated over a period of time. The use of aspirin, β – blockers, nitrates, calcium channel blockers, and statins allow for chronic alleviation of CAD and reduction of risk factors. Procedural treatments include angioplasty, stent placement, coronary artery bypass grafting and minimally invasive coronary artery bypass (Hanson et al., 2013).

Review of Disease

In the past, the treatment and prevention of coronary artery disease was focused on cholesterol metabolism and storage but has now moved to a more inflammatory perspective. Extensive research has begun with the thrombosis aspect of the disease including how people with increased risk for thrombosis are at increased risk for CAD. Recent studies centering around homocysteine (Hcy) and its erred modification to homocysteine thiolactone have revealed the connection of these proteins to CAD risk. Homocysteine thiolactone, created via and error in an editing reaction of homocysteine, causes homocysteinylation (N-Hcy-"protein") of various proteins. The homocysteinylation of fibrinogen (N-Hcy-fibrinogen) in particular transforms normal fibrinogen into version more resistant to lysis. The result is a circulatory system more prone to coagulation and consequential ischemia (Jakubowski, 2008).

Conclusion

The machinations of CAD are becoming more and more translucent with much more treatment capability today than decades ago. The critically acute onset now can still be treated for and with a lower mortality rate. However, **i**ncreased awareness of the manifestation of CAD will better equip the population to prevent or diagnose and treat the disease. Research is still ongoing to further elucidate the causes of CAD and ultimately render the acute state a mostly preventable condition.

Chapter 2: The Cardioprotective Role of Paraoxinase 1 in HDL via Hydrolysis of Homocysteine Thiolactone, A Risk Factor for Cardiovascular Disease.

Introduction

The pathological consequences of homocysteine thiolactone (Hcy-thiolactone) in CAD have focused research on the enzyme responsible, PON1, for reducing Hcy-thiolactone's negative effects. Several correlative studies, both in laboratory and clinical settings, have been completed that display PON1's biochemical relationship with Hcy-thiolactone. Directed evolution has been used to create variants of PON1 that have been used to discover the structure of PON1 and a likely mechanism of hydrolysis of Hcy-thiolactonase. Ironically, a study has shown how PON1 possibly undergoes N-homocysteinylation itself. Research focusing on increasing the expression of PON1 by hyperactivation of the enzyme or overexpression of the gene in humans shows promise into reducing the risk of CAD development or exacerbation.

Now that research has expanded from the cholesterol storage aspects of coronary artery disease and into the inflammatory aspects, much attention is being brought to methods of alleviating the inflammation state. Thrombosis occurs with disruption of the inflammation site, a plaque in an arterial wall, where contents within the plaque spew into the bloodstream causing coagulation and the formation of a thrombus. Regulation of a person's thrombotic tendencies shows promise into regulating, to some extent, coronary artery disease itself. This is where homocysteine thiolactone (Hcy-thiolactone) presents itself as a causal agent of coronary artery disease with respect to the coagulation component. Simultaneously, Paraoxinase 1 (PON 1) presents itself as an enzyme known to have a detoxification role in the human body with the proposed hydrolysis of Hcy-

thiolactone. Discovering the natural mechanism of degrading Hcy-thiolactone opens the way to inducing Paraoxinase 1 to specifically increase hydrolysis of Hcy-thiolactone or discovering artificial methods of reducing Hcy-thiolactone levels.

Homocysteine is an intermediate molecule in the metabolic pathways of methionine and cysteine as seen in figure 5.

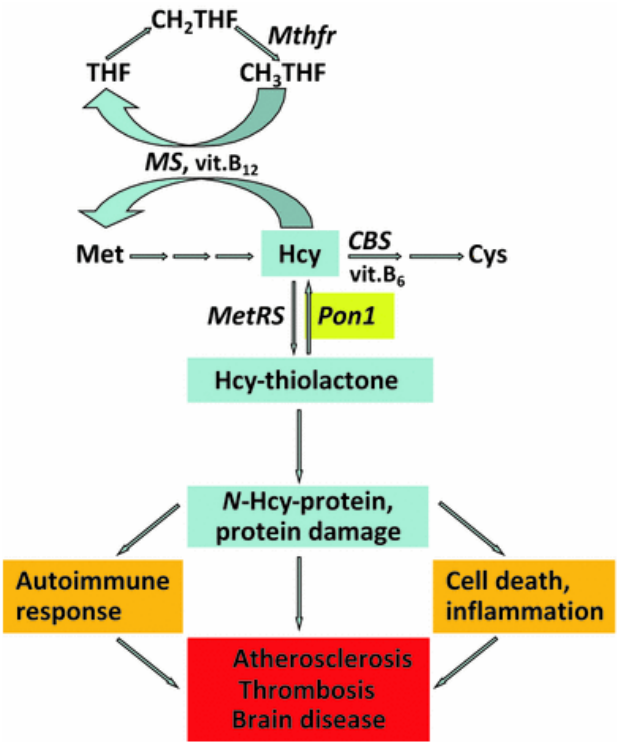

Figure 5. Pathophysiological hypothesis of homocysteine thiolactone
Source: *The pathophysiological hypothesis of homocysteine thiolactone-mediated vascular disease*

It is formed from methylation reactions from methionine with first the activation of methionine by ATP to form S-adenosylmethionine (AdoMet). Transfer of a methyl group from AdoMet forms S-adenosylhomocysteine (AdoHcy) and a following hydrolysis converts AdoHcy into Hcy. At this point, Hcy can be remethylated to methionine

catalyzed by Met synthase with vitamin B_{12} as a cofactor and 5,10-mthyl-tetrahydrofolate as a reactant. Hcy can also undergo a transsulferation to cysteine starting with the help of cystathionine β-synthase (CBS) with vitamin B_6 as a cofactor. The pathophysiological relevance of homocysteine to CAD starts when homocysteine does not undergo the normal remethylation or transsulfuration reactions and instead is accidentally selected by methionyl-tRNA synthetase (MetRS) in an error-editing reaction in protein biosynthesis as seen in figure 6.

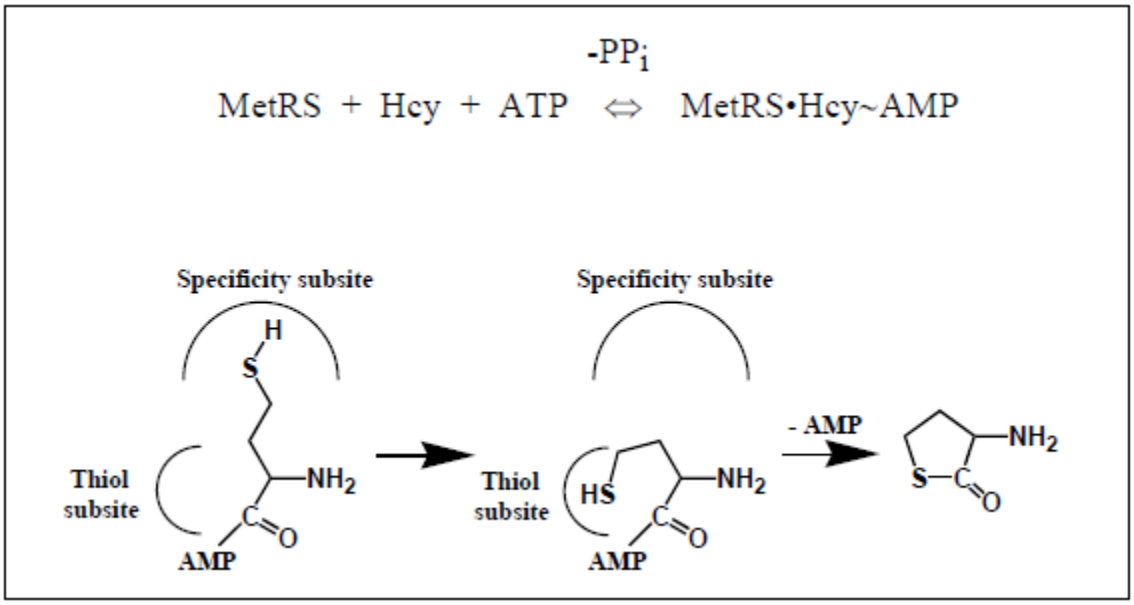

Figure 6. Error-editing reaction where methionyl-tRNA mistakenly selects homocysteine and converts it to Hcy-thiolactone
Source: *The pathophysiological hypothesis of homocysteine thiolactone-mediated vascular disease*

The accidental selection of homocsyteine by MetRS results in the metabolism of the thioester, homocysteine thiolactone. The reaction involves MetRS interacting with homocysteine at its active site and using ATP to catalyze the formation of the thioester. Genetic mutations that cause enzymes involved in the remethylation or transsulfuration

of homocysteine to become altered increases the flux through the Hcy-thiolactone pathway. The condition in which homocysteine is produced in excessive levels in the blood plasma is called hyperhomocysteinemia and it was the examination of this condition that led researchers to discover the causal relationship between homocysteine and subsequently homocysteine thiolactone and vascular disease. Increased levels of homocysteine and the consequent Hcy- thiolactone have been found to be strongly correlated with vascular problems, including atherothrombosis (Jakubowski, 2008). One study found that baboons given diets enriched with Hcy-thiolactone or direct Hcy-thiolactone infusions have had a higher rate of atherosclerosis development when compared to baboons with normal diets (Harker et al, 1947). Another study's results displayed a positive correlation between the presence of Hcy-thiolactone and the initiation of apoptosis in human vascular endothelial cells (Kerkeni et al, 2006). First developed in 1997, the Hcy-thiolactone hypothesis explains that the pathological issues caused by an increase in homocysteine are due to the conversion to Hcy-thiolactone and its resulting behavior. It is common to find heightened levels of Hcy-thiolactone in patients predisposed to atherothrombosis and so further validates the theory. Several mechanisms of Hcy-thiolactone that create an environment favoring atherothrombosis include modification of proteins by homocysteinylation, oxidative stress, inflammation, endothelial dysfunction, and thrombosis. Hcy-thiolactone's ability to induce a form of protein modification known as *N*-homocysteinylation is due to its nature as a reactive metabolite. The modification itself occurs with the formation of amide bonds with lysine residues within the protein's amino acid sequence as seen in figure 7.

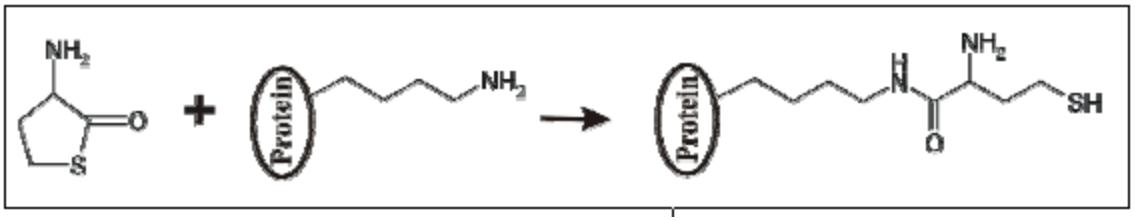

Figure 7. General mechanism of *N*-homocysteinylation of proteins by Hcy-thiolactone by reacting with the lysine residue of a protein
Source: *The pathophysiological hypothesis of homocysteine thiolactone-mediated vascular disease*

N-homocysteinylation causes a protein to lose its functionality or leads to an alteration of its functionality. One protein in particular that undergoes this transformation by Hcy-thiolactone is fibrinogen and *N*-Hcy-fibrinogen it was first discovered in vitro in human fibroblasts and endothelial cells (Jakubowski, 2000). *N*-Hcy-fibrinogen along with other affected proteins have been found in increased quantities in aortic lesions of mice with hyperhomocysteinemia with the use of anti-*N*-Hcy-protein antibodies (Perła-Kaján, 2008**)**. Vascular lesions are the foundation upon which the inflammatory aspect of coronary artery disease takes hold. This relation between vascular lesions, *N*-Hcy-proteins, and a large presence of homocysteine solidifies the Hcy-thiolactone pathway as a causal agent of CAD. This relation is cemented further more from the positive correlations between *N*-homocysteinylation of proteins and the triggering of the auto-immune response and increasing vascular inflammation. *N*-Hcy-fibrinogen presents a role more critical than that of other proteins in increasing thrombotic tendencies in human vasculature. This was determined when it was discovered that blood clots, composed of fibrinogen, degraded more slowly when the fibrinogen was exposed to Hcy-thiolactone and allowed to undergo *N*-homocysteinylation. It is proposed that the altered protein is able to sustain the clot formation for a longer period of time because the *N*-

homocysteinylation affects lysine resides that are near the sites for activating and binding plasminogen. Consequently, plasminogen has a hard time being converted to plasmin and gaining the ability to break apart blood clots. This mechanistic hypothesis for fibrinogen alteration can be supported by a correlation between the negative effects of high plasma Hcy-thiolactone levels and lessened dissolution capability of blood clots. With *N*-Hcy-fibrinogen a building block to a sturdier form of vascular clotting, it is evident that Hcy-thiolactone's mechanism of *N*-homocysteinylation is a major culprit in the thrombotic elements of CAD. *N*-Hcy-proteins in general serve as an exacerbating agent of autoimmunogenicity in humans by instigating the formation of IgG auto-antibodies that target *N*-Hcy-Lys epitopes. CAD and stroke patient's especially display a strong correlation between elevated levels of anti-*N*-Hcy proteins and anti-*N*-Hcy-protein IgG auto-antibodies. Another strong correlation is revealed between plasma homocysteine levels and Anti-N-Hcy-protein auto-antibodies, showing that the increased presence of homocysteine leads to Hcy-thiolactone formation and subsequent transformation of proteins. Any protein that has undergone homocysteinylation is at risk of being bound by the IgG auto-antibodies and then being formed into antigen-antibody complexes. The auto-immune response elicited by N-Hcy-proteins has pathological implications in CAD when these complexes arise on the surfaces of blood vessels. Macrophages phagocytize endothelial cells covered in the complexes with the antibodies exposed. This results in vascular damage and is followed by lesions when the surface is repeatedly repaired. With lesions being the foundation of CAD, it can be seen how detrimental the presence of these auto-antibodies can be to CAD patients (Jakubowski, 2008).

Fortunately a molecule exists, Paraoxinase 1 (PON1), which is naturally able to

hydrolyze Hcy-thiolactone in the human body. It was initially discovered and named for its ability to hydrolyze the oganosphosphate paraoxon. PON1's relation to atherosclerosis arose when it came to light that its presence affected the outcome of atherosclerosis in mice. Mice deficient in PON1 would develop atherosclerosis more frequently than wild type mice when on high fat diets. Contrarily, transgenic mice that have three copies of the human PON1 gene would be less likely to develop atherosclerosis (Jakub, 2008). Several correlative studies, both in laboratory and clinical settings, have been completed that display PON1's biochemical relationship with Hcy-thiolactone. Directed evolution has been used to create variants of PON1 that have been used to discover the structure of PON1 and a likely mechanism of hydrolysis of Hcy-thiolactonase. Ironically, a study has shown how PON1 possibly undergoes N-homocysteinylation itself.

Methods & Results

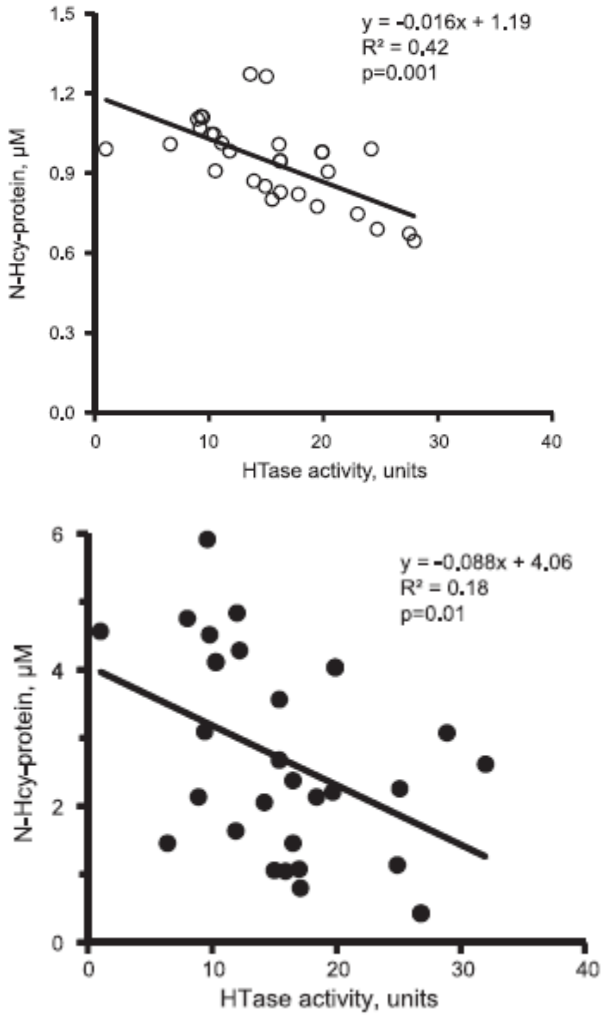

Figure 8. N-Hcy-protein levels with respect to HTase activity in vitro and human blood plasma. In vitro *N*-Hcy-protein levels with respect to HTase activity (Left). Human blood plasma *N*-Hcy-protein levels with respect to human serum HTase activity (Right). The in vivo data was obtained by measuring plasma N-Hcy-protein levels and HTase activity in cystathionin β-synthase-deficient patients. The in vivo data was obtained by radioactively labeling Hcy-thiolactone.

Source: *Paraoxonase 1 Protects against Protein N-homocysteinylation in Humans*

Additions	HTase activity
	%
None	41 ± 1
EDTA (2 mM)	8 ± 0.4
Ca	
0.007 mM	58 ± 1
0.03 mM	75 ± 1
0.12 –2 mM	100 ± 1
Mg	8 ± 0.4
Mn	4 ± 0.2
Zn	8 ± 0.4
Cd	12 ± 0.5
Co	24 ± 1
Fe	30 ± 0.6
Ni	
0.12 mM	12 ± 1
0.5 mM	29 ± 1
2 mM	67 ± 1
Cu	25 ± 1
Pb	20 ± 1

Table 1. HTase reactivation with respect to the addition of various metals.
Source: *Calcium-dependent Human Serum Homocysteine Thiolactone Hydrolase: A Protective Mechanism Against Protein N-Homocysteinylation*

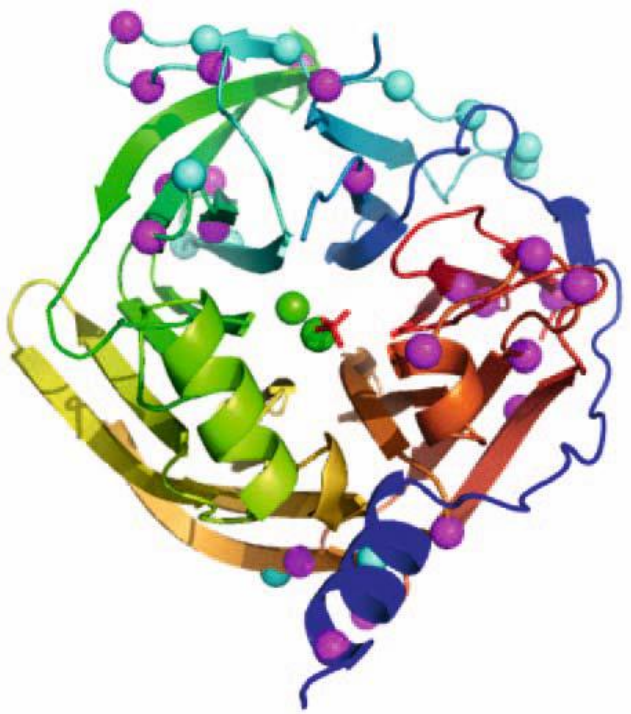

Figure 9. Recombinant PON1 variant G6E2. Directed evolution was used to create the recombinant PON1 variant G6E2 for experimentation to simulate human PON1. The structure was determined using xray crystallography. Two calcium ions are displayed as green spheres in the center of the molecule along with the phosphate ion as a red stick model.
Source: *3-D Structure of Serum Paraoxonase 1 Sheds Light on Its Activity, Stability, Solubility and Crystallizability*

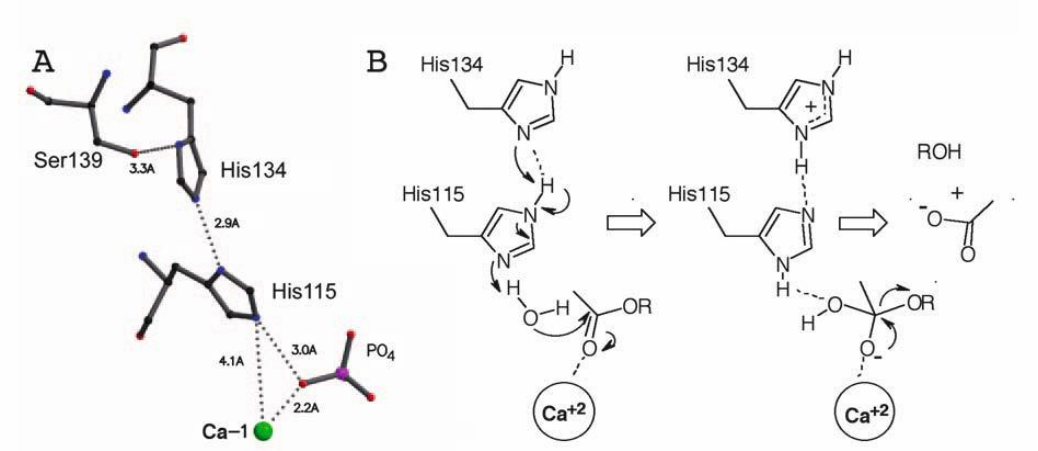

Figure 10. Proposed active site and catalytic mechanism of PON1. Determined from analysis of the structure in figure 6.
Source: *3-D Structure of Serum Paraoxonase 1 Sheds Light on Its Activity, Stability, Solubility and Crystallizability*

	Aliphatic lactones	**Phenyl acetate**	**Paraoxon**	**Dihydrocumarin (lactone)**
PON1's conformation	Closed	Closed	Open	Closed
Bond cleaved	C–O	C–O	P–O	C–O
Putative reaction intermediate	Tetrahedral	Tetrahedral	Pentavalent	Tetrahedral
Intermediate reacts with	H115 E53	H115 E53	D269 E53	D269 E53

Table 2. How different substrates are accepted by the different conformations of PON1
Source: *Paraoxonase 1 and Homocysteine Metabolism*

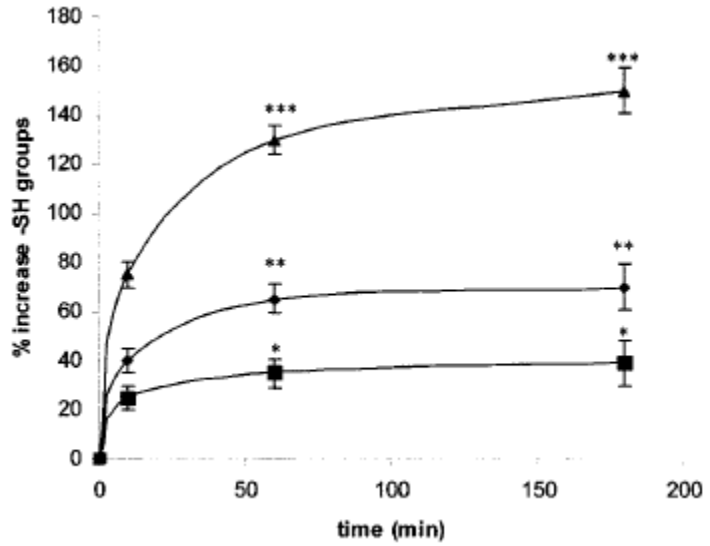

Figure 11. HDL-SH formation with respect to Hcy-thioloactone concentration. The increase over time in –SH groups in HDL incubated when exposed to three concentrations of Hcy-thiolactone [10 µmol/L (square), 100 µmol/L (diamond); and 1 mmol/L (triangle) Hcy-thiolactone]
Source: *Effect of Homocysteinylation on Human High-density Lipoproteins: A Correlation with Paraoxonase Activity*

Discussion

PON1 has been found to be associated with apolipoprotein A1 on an HDL particle (Jakubowski 2012). This allows for some experimentation with the PON1 enzyme by using HDL particles since PON1 is unstable and usually aggregates when not in the presence of detergents (Harel et al, 2007). Therefore the activity level of PON1 can still be observed without removal from the HDL complex and displays an interesting correlation with respect to *N*-Hcy-protein levels as can be seen in Figure 4. There is a strong negative correlation between the activity level of an HTase protein, PON1, and *N*-Hcy-protein levels in vivo in the human blood serum as well as when the same conditions are replicated in vitro. This cements the foundation of PON1's role in reducing *N*-Hcy-protein levels via a mechanism somewhere in the homocysteinylation pathway (Perla-Kaján, 2010). Analysis of the enzyme with respect to reactivation by metals (Figure 5) shows that calcium plays a major functional role in the PON1's ability to reduce *N*-Hcy-protein levels. This inference can be made from the fact that the enzyme reactivates with a significantly high frequency in the presence of calcium ions when compared to other metals (Jakubowski, 2000). Determining the reason for PON1's dependence on calcium requires a visualization of the enzyme which can be seen in Figure 6. The two calcium ions lie in the center of the enzyme along with a phosphate ion and compose the catalytic aspect of the enzyme. Figure 7 shows a catalytic mechanism of the active site, which includes an upper calcium ion, a phosphate ion, and a histidine dyad, for substrates such as phenyl and 2-naphthylacetate (Harel et al, 2007). This gives insight into the active site's multi-substrate functionality and further experimentation reveals that PON1 is found in a closed formation when hydrolyzing aliphatic lactones, specifically Hcy-

thiolactone. One suggestion is that the Try71 of PON1 interacts with the lactone ring while Ile74 interacts with the alkyl side chain of Hcy-thiolactone to hold it in place for hydrolysis (Perla-Kaján, 2012). Interestingly, it has been discovered that PON1 can undergo homocysteinylation, and subsequent inactivation, by Hcy-thiolactone. Figure 9 shows how the percentage of sulfhydryl groups present in an incubating HDL solution increase with the increased concentration of Hcy-thiolactone. Since the formation of sulfhydryl groups is an indicator of Hcy-thiolactone activity, it shows how PON1 itself can be victim to homocysteinylation. Research focusing on increasing the expression of PON1 by hyperactivation of the enzyme or overexpression of the gene in humans shows promise into reducing the risk of CAD development or exacerbation.

Chapter 3: A Case Study

The case study presented here follows the treatment and diagnosis of a 56 year old Caucasian male presenting with acute chest pain. Acute coronary syndrome, which commonly presents itself as chest pain, affects in some form almost half of the middle aged male population in the US. The frequency with which this condition afflicts people highlights the importance of being able to identify and treat the condition in various levels of the medical field. This case study serves the purpose of illustrating a general manner in which this acute condition can be addressed and resolved.

Leo Cichlid

Chief Complaint

Leo Cichlid presents to the emergency department with a chief complaint of chest pain.

History of Present Illness

Leo Cichlid is a 56 year old Caucasian male with a history of hypertension complaining of constant sharp central chest pain onset about an hour ago. He reports he has been having intermittent chest pains over the past month with a severity of 6/10 that lasts about thirty seconds. He states these episodes of chest pain appear to be provoked by exertion and generally resolve after 30 seconds with rest. Leo states today he was mowing his lawn when he started to feel the chest pain again but this time with a severity of 8/10. He reports his chest pain has been constant and was radiating to his left arm at onset but not currently. He states the pain was intense for the first 30 min and is still present now but slightly less severe. Leo reports his current chest pain is also exacerbated

with exertion. He states he had associated diaphoresis and mild shortness of breath. He denies he has had any palpitations, leg swelling, nausea, vomiting, hematemesis, abdominal pain, melena, headache, fever, cough, sputum production, or hemoptysis. Leo reports he has a history of hypertension but denies he has any history of heart attacks, pulmonary embolism, deep vein thrombosis, COPD, or diabetes. He states he normally takes a 75 mg aspirin every day but hasn't been taking them over the past week because he ran out. He denies having any past surgeries. Leo denies any history of family members dying at a young age (before age 60) from heart attacks. He reports he has no known allergies. He states he has been smoking about a quarter pack of cigarettes a day for the past 20 years. Leo states he drinks alcohol occasionally but denies any drug use, particularly cocaine or methamphetamine.

Review of Systems

Constitutional: Positive for diaphoresis. Negative for fever.

Cardiovascular: Positive for sharp central chest pain. Negative for palpitations.

Pulmonary: Positive for mild shortness of breath. Negative for cough, sputum production, or hemoptysis.

Gastrointestinal: Negative for nausea, vomiting, hematemesis, abdominal pain, or melena.

Musculoskeletal: Positive for left arm pain. Negative for leg swelling.

Neurologic: Negative for headache.

Past Medical History

Hypertension.

Denies any history of heart attacks, pulmonary embolism, deep vein thrombosis, COPD,

or diabetes.

Surgical History

None.

Family History

Denies any history of family members dying at a young age (before age 60) from heart attacks.

Social History

Smokes about a quarter pack of cigarettes a day with a 5 pack-year history.

Drinks alcohol occasionally.

Denies any drug use, particularly cocaine or methamphetamine.

Current Medications

Daily aspirin (75 mg).

Allergies

No known allergies.

Physical Exam

Vital signs:

Oxygen saturation: 96% Heart rate: 74 Blood pressure: 137/84

Respiratory rate: 12 Temperature: 98.3^{o}F Height: 5’11’’

Weight: 180 pounds

Exam findings:

Constitution: Well developed, Well nourished, Not obese, Alert, Mild distress.

HENT: No signs of trauma, Bilateral external ears normal, Bilateral TM’s normal, Oropharynx moist and without exudates, Nose normal.

Eyes: Pupils are equal and reactive, Conjunctiva normal, Non-icteric.

Neck: Supple, Trachea midline, Normal range of motion, No tenderness.

Lymphatic: No lymphadenopathy noted.

Cardiovascular: Regular rate and rhythm, No murmurs. Point of maximal impact is not laterally displaced.

Thorax & Lungs: Normal breath sounds, Mild respiratory distress, No wheezing, No chest tenderness to palpation.

Abdomen: Bowel sounds normal, Soft, No tenderness, No rebound, No guarding, No masses, No pulsatile masses.

Skin: Warm, Dry, No erythema, No rash, Koi fish tattoo on the upper back.

Back: No bony tenderness, No CVA tenderness.

Extremities: Intact distal pulses, No edema, No tenderness, No cyanosis, Negative Homans' sign.

Musculoskeletal: Good range of motion in all major joints. No tenderness to palpation or major deformities noted.

Neurologic: Awake and alert x3, Normal motor function, Normal sensory function, No focal deficits noted.

Psychiatric: Affect normal, Judgment normal, Mood normal.

Diagnostic Studies

Laboratory results:

	Patient values	Normal range
Complete Blood Count:		
RBC	4.7 x 10^{12} cells/L	4.2-5.9 x 10^{12} cells/L
Hemoglobin	16 g/dL	14-17 g/dL (Male)
Hematocrit	48%	41-51 % (Male)
MCV	92 fL	80-100 fL
MCH	29.7 pg	28-32 pg
MCHC	33.3 g/dL	32-36 g/dL
White Cell Count	4.3 x 10^{9}	4.0-10.0 x 10^{9} /L
Neutrophil Count (Absolute)	2.4 x 10^{9}	2.0-6.2 x 10^{9} /L
Neutrophil Count (Relative)	56.2 %	50.0-62.0 %
Band neutrophils (Absolute)	1.9 x 10^{8}	1.2-6.0 x 10^{8} /L
Band neutrophils (Relative)	4.4 %	3.0-6.0 %
Lymphocyte (Absolute)	1.4 x 10^{9}	1.0-4.0 x 10^{9} /L
Lymphocyte (Relative)	33.5 %	25.0-40.0 %
Monocyte Count (Absolute)	1.5 x 10^{8}	1.2-7.0 x 10^{8} /L
Monocyte Count (Relative)	3.5 %	3.0-7.0 %
Eosinophil Count (Absolute)	9.0 x 10^{7}	0.0-3.0 x 10^{8} /L
Eosinophil Count (Relative)	2.1 %	0.0-3.0 %
Basophil Count (Absolute)	1.3 x 10^{7}	0.0-1.0 x 10^{8} /L
Basophil Count (Relative)	0.3 %	0.0-1.0 %

	Patient values	Normal range
Platelet Count	260 x 10^9 /L	150-350 x 10^9 /L
MPV	9.4 fL	7.5-11.5 fL
Comprehensive Metabolic Panel:		
Albumin	4.5 g/dL	3.9-5.0 g/dL
Alkaline phosphatase	84 IU/L	44-147 IUL
ALT	22 IU/L	8-37 IUL
AST	17 IU/L	10-34 IU/L
BUN	10 mg/dL	7-20 mg/dL
Calcium	9.1 mg/dL	8.5-10.9 mg/dL
Chloride	98 mmol/L	96-106 mmol/L
	Patient values	Normal range
CO_2	25 mmol/L	20-29 mmol/L
Creatinine	1.2 mg/dL	0.8-1.4 mg/dL
Glucose	83 mg/dL	70-100 mg/dL
Potassium	4.0 mEq/L	3.7-5.2 mEq/L
Sodium	137 mEq/L	136-144 mEq/L
Total bilirubin	0.4 mg/dL	0.2-1.9 mg/dL
Total protein	7.8 g/dL	6.3-7.9 g/dL
Prothrombin Time:	13.0 seconds	11-13.5 seconds
Partial Thromboplastin Time:	33 seconds	25-35 seconds
Troponin T:	0.80 ng/mL	0-0.10 ng/mL
Brain Natriutetic Peptide Test:	280 pg/mL	0-100 pg/mL

	Patient values	Normal range
C-Reactive Protein:	4.2 mg/L	0.0-1.0 mg/L

EKG:

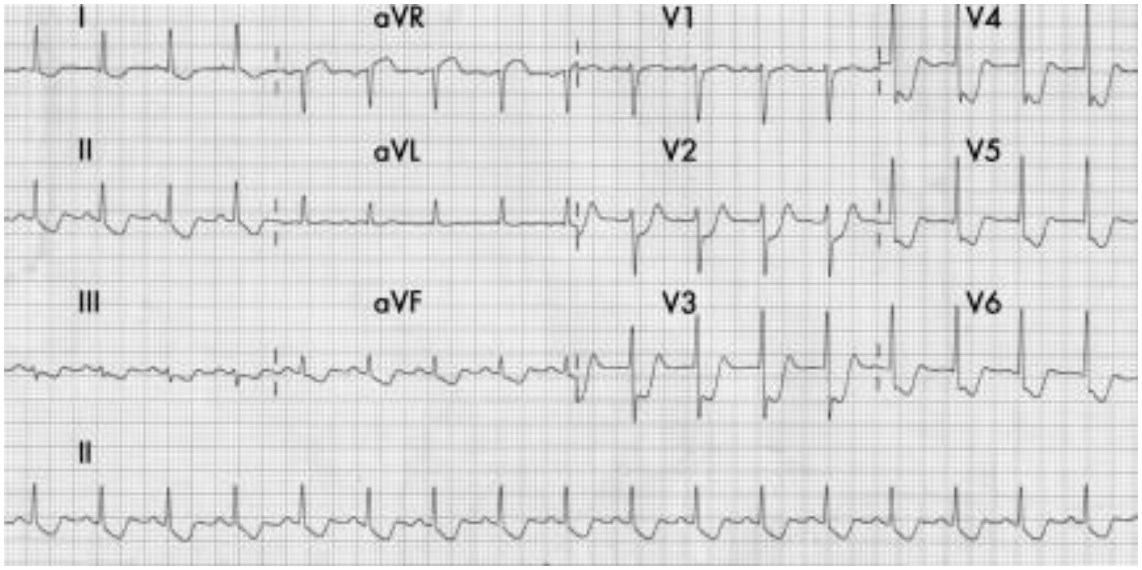

Figure 12. 12 lead EKG. A 2 mm ST segment elevation is present in lead aVR and 1 mm ST segment elevation is present in lead V1. Diffuse ST segment depressions are present in leads V2-V6, I aVI, II, and aVF.
Source: *ECG Showing Features of Total Left Main Coronary Artery Occlusion*

Chest X-ray:

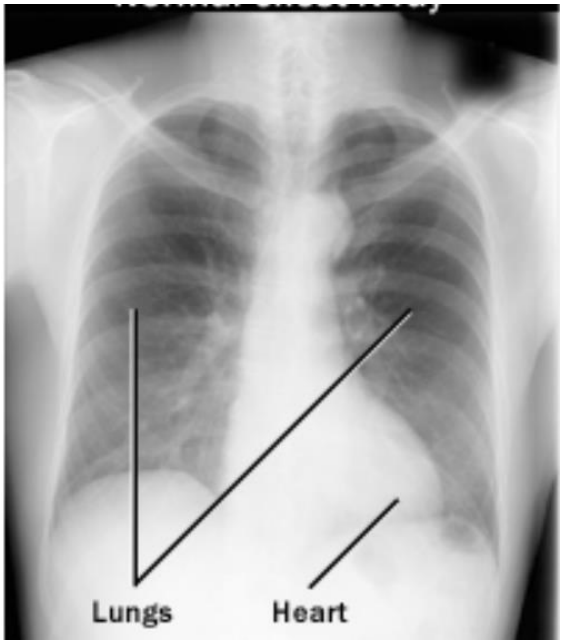

Figure 13. Chest xray. The radiologist interpreted the chest xray as normal with no acute findings.
Source: *Pneumonia: MedlinePlus Medical Encyclopedia*

Coronary Angiogram:

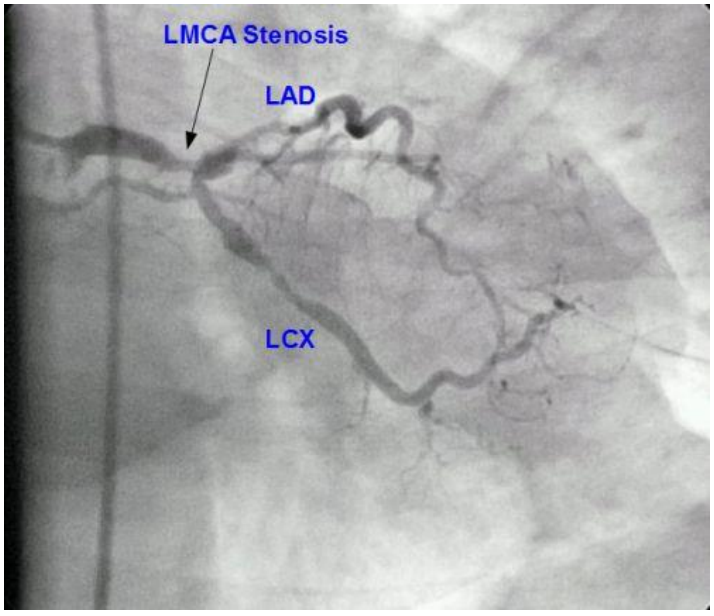

Figure 14. Coronary angiogram. The left main coronary artery was found to be in stenosis with a 40 % occlusion.
Source: *Cardiophile for Medical Professionals*

Medical Decision Making

Differential Diagnoses:

When a chief complaint of chest pain presents itself clinically it is usually first suspected of being cardiac in nature. However, pulmonary and gastrointestinal sources must also be taken into account. Several differential diagnosis and the reasoning behind their consideration follow:

Acute coronary syndrome (myocardial infarction) is of high probability considering the acute nature of the patient's chest pain as well as his history of hypertension and smoking cigarettes. If ACS is the case, the patient may currently be having a heart attack or had one since onset of the chest pain.

Unstable angina is another possibility as seen by the increase in intensity of the chest pain that he has been feeling over the past month. This presentation may signify risk for myocardial infarction (Unstable Angina: MedlinePlus Medical Encyclopedia, 2012).

Aortic dissection usually occurs when the inner lining of the aorta separates from the other layers, causing bleeding into the open space. It often presents with acute severe chest pain although usually as a tearing or ripping quality. The patient's history of hypertension is a risk factor (Aortic Dissection: MedlinePlus Medical Encyclopedia, 2012).

Pulmonary embolism is the sudden blockage of an artery in the lungs and may cause chest pain similar to a heart attack. The patient's history of smoking gives him some predisposition to blood clot formation although he states he has had no history of blood clots (DVT or PE). He also appears to have no leg swelling or pain which is

indicative of DVT and normally precedes PE (Pulmonary Embolism: MedlinePlus Medical Encyclopedia, 2014).

Pericarditis occurs when the pericardium becomes inflamed and usually presents with chest pain. It is often caused by infection and the initial indication appears weak since the patient has no associated fever (Pericarditis: MedlinePlus Medical Encyclopedia, 2012).

Pneumonia can culminate into chest pain but is commonly associated with coughing, sputum production, fever, shortness of breath and/or chills. It can lead to sepsis and so has reasonable concern but the patient doesn't appear to illustrate this (Pneumonia: MedlinePlus Medical Encyclopedia, 2014).

Boerhaave syndrome (esophageal rupture) is the perforation of the esophagus, causing chest pain and difficulty swallowing. The patient has a weak indication of this as he has no history of GI procedures since most ruptures are caused by injury during such procedures. He also has had no violent vomiting or chest trauma (Esophageal perforation: MedlinePlus Medical Encyclopedia, 2012).

Costocondritis is a common cause of chest pain and results from the inflammation of the cartilages connecting the ribs to the sternum. This is a very weak differential as the patient had no tenderness with palpation of the chest wall (Costochondritis: MedlinePlus Medical Encyclopedia, 2012).

Diagnosis and Treatment:

The patient's symptoms raised high concern for a cardiac pathophysiology and he was immediately put on supplemental oxygen via a nonrebreather (Hanson et. al, 2013). He was also immediately treated with PO chewable aspirin, IV metoprolol, PO sublingual

nitroglycerin, and IV morphine since he had no contraindications. Aspirin was used to inhibit blood clotting. Metoprolol was used to reduce the patient's blood pressure, treat his chest pain, and to increase outpatient survival in case he was having a myocardial infarction. Nitroglycerin reduces the chance of ischemia by relaxing the heart muscles. Morphine was given for pain control and to reduce anxiety. The EKG completed soon after patient arrival displayed ST segment elevations indicative of possibly a left main coronary artery occlusion or at least some form of myocardial infarction. A prompt bedside chest X-ray showed the patient had no signs of esophageal rupture or pneumonia such as airspace opacity. With initial medications administered and the cardiologist consulting, the patient was taken off to the catheter lab to have an angiogram. Critical lab results resulted showing the patient had increased Troponin T, B-Type Natriutetic Peptide, and C-reactive Protein levels which further solidified the idea the patient was having a heart attack. The lab results showed no signs of infection from the presence of his various blood cells. His electrolytes were at normal levels as well indicating normal kidney and liver function. With the angiogram, the patient's was found to have a 40 % occlusion of the left main coronary artery and a diagnosis of coronary artery disease and myocardial infarction. He was immediately taken to emergency coronary bypass surgery. Complications from a coronary artery bypass graft include heart muscle damage, arrhythmia development, bleeding, renal failure, and/or wound infection. The patient did not have any acute complications during his procedure. He was admitted to the hospital for observation and rehabilitation. The patient was discharged five days later with prescriptions for an anticoagulant, a beta blocker, an ACE inhibitor, a nitrate, and a statin medication. He followed up with his new cardiologist and was put on a preventative plan

to avoid subsequent heart attacks and for wound care to avoid infection. He was specifically put on medications to treat his hypertension and given a statin to reduce CAD risk. Leo followed his chronic treatment plan accordingly and finally heeded his primary care physician's advice to top smoking cigarettes.

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