

The Incredible Costs of Chronic Diseases: Why they Occur and Possible Preventions and/or Treatments

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Abstract

The United States government spends 3 trillion dollars on disease treatment each and every year. Chronic diseases are responsible for 86% of these health care costs. Chronic diseases are linked to 70% of the deaths that occur each year. The costs worldwide are likely even greater. After the age of 50, at least 50% of the people from the US have at least one chronic disease. About 1/3 of the US population has some form of diabetes or pre-diabetes. Why does this continue and certainly it is a major factor in the debt of the United States which is approximately 20 trillion dollars. How can the government and our medical people including scientists allow this outrage to continue?

Is it because we do not understand the cause of chronic diseases? Certainly we have not developed effective medications and or treatments; so even if we knew what the root cause of chronic diseases could we prevent or reverse them?

Dr Peter Barnes, M.D., Ph.D. is the premier scientist in the world who studies acute and chronic lung diseases. He has found that acute diseases can be effectively treated with steroidal anti-inflammatory drugs e.g., asthma; but chronic diseases like chronic obstructive pulmonary disease are not effectively treated with those same steroidal anti-inflammatory drugs. The difference between the two diseases forms the blueprint of what causes chronic diseases other than those caused by genetic defects which are relatively rare. The major difference between acute (treatable diseases) and chronic (essentially untreatable or poorly treatable diseases) is the excessive generation of peroxide called peroxynitrite (OONO⁻). Chronic diseases produce excessive amounts of peroxynitrite and this can create massive biochemical damage to the cell particles (mitochondria) that allow life to continue and produce necessary energy and key enzymatic proteins are damaged as well as the DNA, and RNA-the master molecules of life. Excessive peroxynitrite is the linchpin of chronic diseases.

Therefore the key to controlling chronic diseases is to control excessive peroxynitrite. This prevents the damage to our bodies from nitration, nitrosylation and nitrosation all major damages caused by peroxynitrite. How do we control these diseases -we need to find suitable targets of peroxynitrite damage that are non-toxic and exist in a continuous state to fight this toxic chemical. There are peroxynitrite catalytic antagonists and some vitamins like vitamin C and different forms of vitamin E are targets which destroy peroxynitrite and these have been shown to be somewhat effective against chronic diseases. We must introduce the peroxynitrite antagonists early in the disease state before the diseases become irreversible.

The excessive peroxynitrite actually damages the epigenetic mechanism (histone deacetylase) by which steroids exert their anti-inflammatory action. But, if we can suppress peroxynitrite early in the chronic disease state -chronic diseases can be become acute and very treatable diseases.

Keywords: Acute; Chronic diseases; Peroxynitrite; Nitration; Nitrosylation; Nitrosation; Mitochondria

Introduction

Each and every year the United States Government spends several trillion dollars to combat or treat chronic diseases with little change in the foreseeable future. Chronic diseases are responsible for 86% of our health care costs [1]. The cost worldwide must be even greater. Chronic diseases are responsible for the majority of deaths- probably in the area of 70% of the deaths. Why does this continue? It is because the basis of chronic diseases is not well understood so the treatments for these diseases are palliative or superficial and never getting to the actual basis of these diseases. If one believed the media it could be surmised that oxidants cause damage possibly by free radicals which could be prevented by eating or swallowing multiple antioxidants (cause gain of electrons). But biochemically oxidation is crucial to metabolize sugars and other nutrients so we can produce the energy to power our body. Oxidation (loss of electrons) is important in control or stimulation of a variety of important biochemical pathways. Siess of Germany developed the idea of oxidative stress which is the ratio of oxidants/antioxidants >1 or in other words there are more oxidants than antioxidants to oppose them [2].

There are a few problems with this basic concept because a person could have oxidative stress locally but not generally and it probably would be a transitory imbalance. The other problem with this idea is a person could take considerable amounts of antioxidants and see little to no result in a given chronic disease state. If antioxidants were at the basis for disease control why are they not more effective in disease states?

As we shall see from this review it is almost certain that nitrosative stress which includes, nitration of unsaturated organic structures, nitrosylation of sulfhydryls and nitrosation of amines are likely the real

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culprits in chronic diseases. They likely stem from the generation of peroxynitrite (OONO-) or one of its derivatives that when produced in excess makes acute (treatable diseases) to become chronic and mostly untreatable diseases. See cartoon Figure 1 of the likely chemical culprit in chronic diseases.

There have been trillions of dollars spent to treat chronic diseases and most treatments have been effective to a trivial extent or ineffective at all. For example multiple millions of dollars have been spent of treatment for cancer and for many people the chemotherapy or treatment often produces a few months of extra life but often they are in agony for much of the treatment. Most cancer chemotherapy is fairly non-selective for the cancer versus the normal cells. Since the normal cells die at a substantial rate, the side effects from these mostly poisonous substances are generally very substantial.

How effective have our medical and research community been in treating, arthritis, cancer, neurodegenerative diseases, brain damage, diabetes, diseases of the kidney, liver, pancreas, eye, hearing, lung, heart diseases which are chronic and the many vascular diseases, stroke etc. Answer-not very effective since these chronic diseases are the main causes of death.

There must be a defect in our basic understanding of chronic diseases. The key questions are the following:

- What causes acute and chronic diseases?
- How can we treat or prevent these diseases?

The major driving force in almost all disease states is inflammation. There are two major types of inflammation:

1. Acute
2. Chronic

How does acute inflammation happen? Two different types of inflammatory cells play major roles in inflammation. They are short lived neutrophils and long lived macrophages and these are both white cells that appear in both blood and tissues. The DNA of these cells is covered with positively charged histone proteins. When acute inflammation occurs a DNA transcription factor called nuclear factor kappa b (nf-kappa b) is activated which stimulates an enzyme called histone acetyl transferase (HAT). The histones are acetylated (with a two carbon acetate group similar to acetic acid) which changes the charge between the DNA and histones causing the histones to peel away from the DNA and the inflammatory genes of the DNA are stimulated to produce the gene products of inflammation.

This turn on process for inflammation is an epigenetic (non-gene regulation via a control histone-acetylated DNA on- switch).

How is acute inflammation stopped? The gold standard of anti-inflammatory drugs are steroidal anti-inflammatories which are used to inhibit or stop acute inflammation e.g., dexamethasone or prednisone etc.

How do steroids stop acute inflammation? They stimulate an enzyme called histone deacetylase 2 or (HDAC 2) linked to the nf kappa b-DNA transcription factor. This enzyme cuts off the acetate groups from the inflammatory- activated histones causing the positive histone proteins once again to be charged and is attracted to and covers the negative DNA, therefore shutting down the inflammatory process.

Therefore, the inflammatory off- switch is the deacetylation of histones from the inflammatory cell DNA which creates opposite charges between histones and DNA which causes acute inflammation to cease.

During this acute inflammatory process, the macrophages are also stimulated to produce a sufficient amount of superoxide (which is oxygen with an extra electron) which combines with available nitric oxide (.NO) which also carries a free electron. These two gaseous substances dissolved in bio-fluids react at diffusional speed (10^{-9} sec) to produce a strongly oxidizing/nitrating substance called peroxynitrite (OONO-) first in relatively small amounts in acute inflammation and therefore OONO- does not have a major effect on the acute inflammatory process mechanism. See standard Figures 2 and 3 for inflammation diagram and inflammatory cellular effects linked to peroxynitrite or its metabolites.

We were studying silicosis which is caused by inhaling fine sand particles into the lung and in our studies on rats so affected, we found a 10 fold jump in peroxynitrite based luminol luminescence 24 hours after silica placement- Antonini [3]. This is due to the nf-kappa b induction of nitric oxide (NO) synthase 2 (a highly inducible enzyme) which can produce large amounts of nitric oxide from macrophages. When dexamethasone was given acutely and simultaneously with the silica the induction of nitric oxide and thus peroxynitrite was stopped completely [4,5]. Therefore, when steroids are given during acute inflammation, they are very effective anti-inflammatory drugs stopping the acute inflammatory process.

Steroids are ineffective for chronic inflammation. After the silica remained in rat lung for 6 weeks and rat lung macrophages were assayed for peroxynitrite, we observed a 1000 fold increase in peroxynitrite-luminol luminescence compared to the control without silica [4]. This is a superior model of both acute and chronic inflammation. Later, it was shown by us that the actual source of inflammation is actually calcium ions which use silica as a carrier. When the calcium is removed or chelated using the membrane permeable INDO 1-AM, the inflammatory killing capacity is thwarted completely. This is a portion of the proof that black lung disease or coal worker's pneumoconiosis is actually caused by silica contaminated with calcium in the coal dust which can be easily viewed by silica examination via X-ray microanalysis [6].

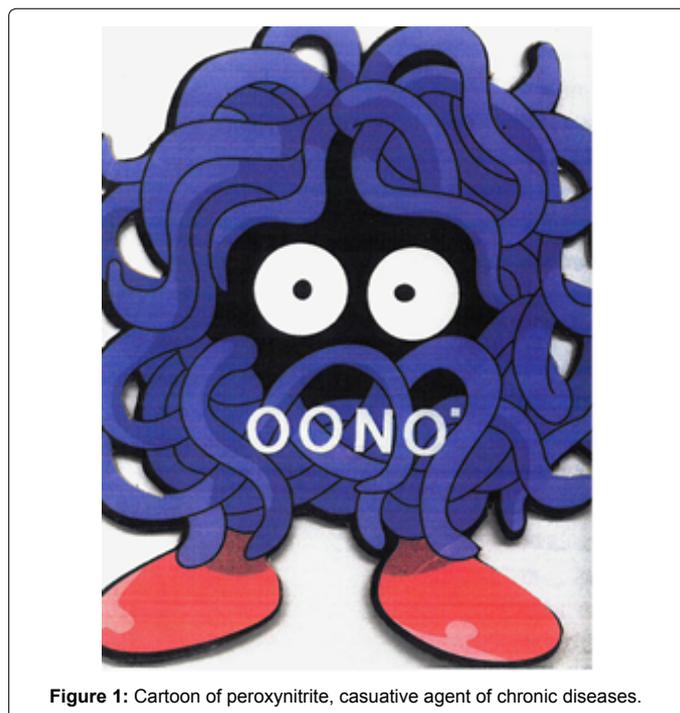
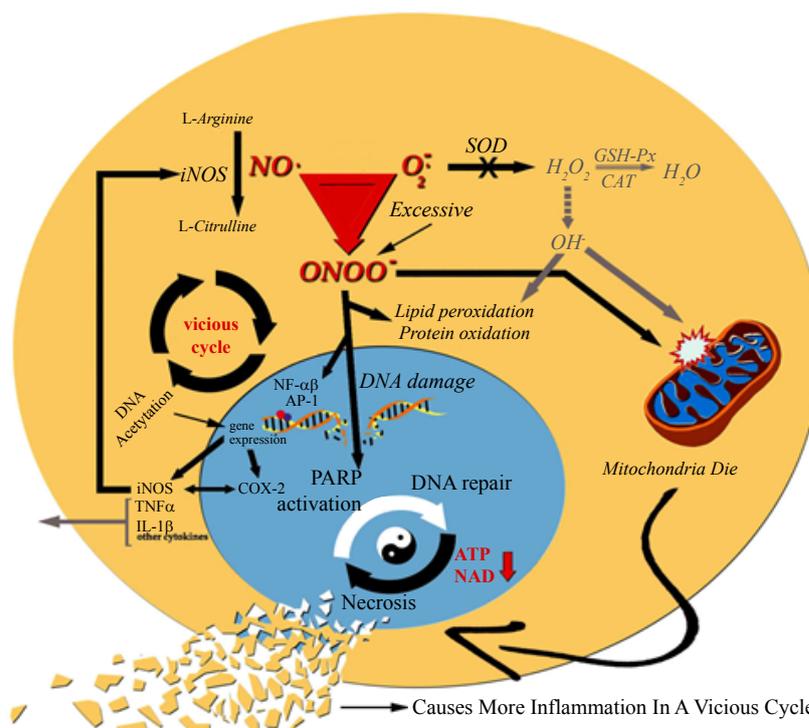


Figure 1: Cartoon of peroxynitrite, casuative agent of chronic diseases.

Toxic Mechanisms Caused By Peroxynitrite (ONOO⁻) or Derivatives Creates Nitrosative Stress



Oxidation stress is often an early and key event that activates numerous pathways involved in several cancer and development of chronic diseases. If the causative agent (e.g, hyperglycemia, cigarette smoking, UV lights and chemical toxicants) persists, eventually iNOS is activated and ONOO⁻ is formed. By then, cellular stress is transformed from oxidative only to nitro-oxidative. ONOO⁻ exerts its harmful effects directly and indirectly. It causes activation of transcriptional factors leading to pro-inflammatory gene expression. During this process, nitro oxidative stress also involves an inflammatory response. Interactions between transcriptional factors and pro-inflammatory products lead to a vicious cycle of damage. The cytokines spread the inflammatory signals through the circulation. Unless excess O₂⁻ and iNOS⁻ derived NO production are terminated, this mechanism continues to propagate damage within cell. Moreover ONOO⁻ directly damage all macromolecules including lipids, proteins and DNA. ONOO⁻ induced DNA damage is sensed by DNA repair enzymes, in particular poly (ADP ribose) polymerase (PARP). In presence of severe genomic damage, overactivation of PARP causes cellular NAD⁺ and ATP depletion by attempting a repair process. This drives cells into an energy crisis eventually leading to necrosis. This futile mechanism, so called suicide hypothesis of PARP activation, is reportedly involved in many diseases relative to nitro-oxidative stress. Since mitochondrion has its own DNA and PARP enzyme, this pathophysiologic process also takes place within the mitochondrion. It is well known that, both oxygen and nitrogen based radicals are prone to directly damage this organelle. Consumption of the majority of NAD⁺ by PARP also slows the rate of glycolysis and mitochondrial respiration, and eventually leads to cellular dysfunction and death.

Figure 2: Basic mechanism of peroxynitrite-induced toxicity and related pathways (As described by Korkmaz in the year 2008).

At this time, in chronic silicosis, anti-inflammatory steroids do not inhibit peroxynitrite-based luminol luminescence since this is chronic inflammation.

This occurs because the silicosis over a prolonged period of time caused the inflammation to be activated from acute to chronic inflammation [6]. Why is chronic inflammatory disease insensitive to steroidal anti-inflammatory drugs?

Peter Barnes et al. have explained this unusual phenomena with great clarity and they have written many articles after the major one cited here [7]. He or they demonstrated that excessive nitration by peroxynitrite attacks key tyrosines in the enzyme histone deacetylase 2 (HDAC-2). This destroys the HDAC-2 enzyme activity and prevents its anti-inflammatory action which is necessary for the actions of steroidal glucocorticoids (steroids). If histone deacetylase 2 has its active sites damaged via nitration, the enzyme cannot strip the acetates from the histones and steroid action becomes ineffective. The key to understanding chronic inflammation is to know that peroxynitrite in

high chronic doses destroys the epigenetic mechanism used by the cell to create steroidal anti-inflammatory action. If steroids are ineffective in treating chronic inflammation there are limited options to inhibit chronic inflammation; although tumor necrosis alpha inhibitors have proven somewhat effective; however, they can cause lymphoma and other cancers in some people and these drugs complicate treatment of tuberculosis and other diseases. Chronic inflammatory diseases are caused by excessive peroxynitrite generation, and HDAC-2 gene silencing then occurs from excessive peroxynitrite. Inactivation of the steroidal epigenetic gene mechanism renders steroids ineffective causing steroid resistance. Excessive peroxynitrite caused DNA damage/repair and cellular necrosis via PARP activation /DNA repair and necrosis. When cells die from necrosis they disintegrate into unusual particles that are basically un-recyclable. Furthermore necrosis causes a chronic cycle of chronic inflammation. Therefore, chronic diseases are creating a non-ending cycle of cell death that ends in the demise of the individual so affected.

CHRONIC INFLAMMATION (CHRONIC DISEASES)

*Excessive Nitration Of Histone Deacetylase 2 (HDAC)
Creates Steroid Resistance*

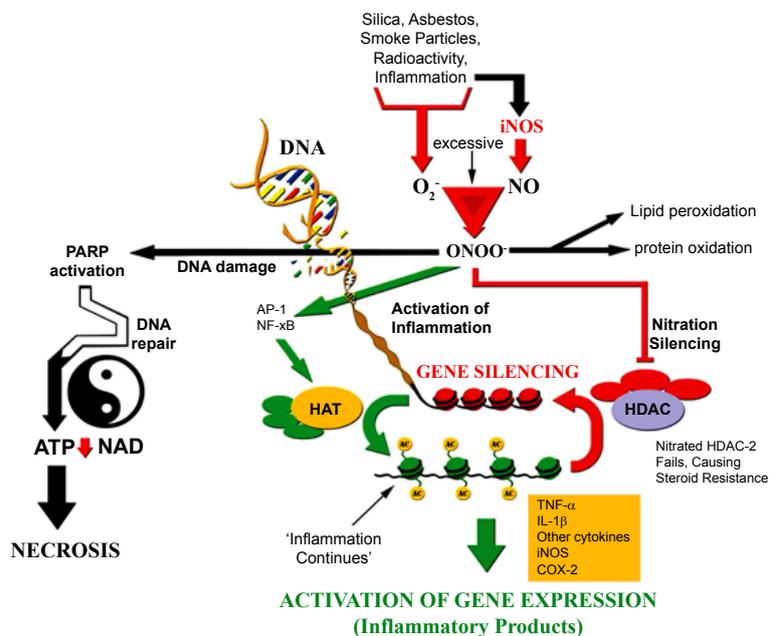


Figure 3: Lessons-learned from treatment of patients with COPD and proposed overall mechanism of ONOO⁻ induced cell toxicity. NF-κB and AP-1 switch on inflammatory genes by inducing several co-activators (e.g., p300/CBP) that have intrinsic HAT activity. Gene transcription only occurs when the chromatin structure is opened up, with unwinding and acetylation of Histones/DNA so that RNA polymerase II and basal transcription complexes can now bind to the naked DNA to initiate transcription. Glucocorticoids switch off multiple inflammatory genes that have been activated by NF-κB and AP-1 during the chronic inflammatory process. Both activation of HDAC and inhibition of HAT may be involved in glucocorticoid-dependent gene silencing. As found in patients with COPD, ONOO⁻ may block the HDAC activity, thereby cause glucocorticoid resistance. This mechanism may partly explain the controversy that antioxidants that only have the capability of scavenging superoxide, but not peroxynitrite may fail in a variety of chronic oxidative stress.

Are there alternative treatments for chronic diseases and brain damage diseases, chronic traumatic encephalopathy (cte) and/or trauma? Since excessive peroxynitrite or its derivatives are a major root cause of chronic diseases, it seems likely that controlling the excessive amount of peroxynitrite would be a logical step particularly if done early in the disease state before major chronicity occurred.

The key is to control the action of peroxynitrite to prevent or treat chronic diseases before they become untreatable with steroids.

There have been over 14,000 papers on various aspects of peroxynitrite generation, control etc., However, excessive peroxynitrite has been demonstrated to be very difficult to control- particularly in the brain- since 98% of drugs do not cross the blood-brain barrier. In addition, a drug that controls the peroxynitrite very effectively could cause immunosuppression, since it plays an important role in killing invaders e.g., bacteria, virus, fungus, parasites etc. A key problem is that peroxynitrite is made continuously and therefore any drug which destroys it must be there and working in a continuous manner. In addition, it must be in a useful concentration everywhere macrophages occur which essentially is in all major vascular and tissue portions of the body. Further, this drug or supplement must produce very little toxicity of its own or it would not be very effective.

Since peroxynitrite is made in macrophages which are found both in blood and tissues-The anti-nitration protein target must occur in a continuous manner and in an active state everywhere. Does such a compound exist without causing major toxicity?

This narrows the field down to very few substances. Some of the most effective substances which destroy OONO⁻ are known peroxynitrite-decomposition catalysts and several have been used and have been found to be effective in animal models of pain and brain damage. Iron porphyrinate (FeTPPS) has shown to be effective in an early model of brain damage associated with Huntington's disease model in rats [8]. Stavniichuck [9] has demonstrated that FeTMPS inhibited diabetic peripheral neuropathy [10,11] demonstrated that SR 110 and SR135 peroxynitrite decomposition catalysts inhibited diabetes and enhanced beta cell production of insulin in diabetic mice.

Salvemini [12] used peroxynitrite decomposition catalysts to lessen peroxynitrite and inhibit opiate antinociceptive tolerance. Van Dyke [13,14] prevented diabetes 1 and 2 using carboxy PTIO, and nitration targets to lower nitric oxide and peroxynitrite in a short term diabetes model using streptozotocin- treated rats.

Since peroxynitrite is composed of superoxide (O_2^-) and nitric oxide ($\cdot NO$) and when both gases with free electrons react together at tremendous speed by having the two free electrons pair-peroxynitrite (OONO⁻) forms. Theoretically one could limit the peroxynitrite production by delimiting production of nitric oxide and/or superoxide but since both molecules have important physiological roles that would likely be ineffective and counterproductive. However, it has been found that producing excessive nitric oxide relative to the amount of superoxide can inhibit formation of peroxynitrite- likely from a feedback like mechanism.

Could we treat chronic diseases early before the disease gets into an irreversible state causing death?

Is it possible to detect diseases early before a chronic state is reached so that a cure is possible? Diseases like diabetes type 1 and 2, Parkinson's disease, Alzheimer's disease, cancer, heart and other chronic inflammatory diseases like neurodegenerative diseases are certainly more treatable if they can be detected before they go into an irreversible state. All of these diseases are linked to excessive nitration-- and therefore, excessive nitration could be used to detect the disease early. Therefore a sensitive and selective protein nitration assay for a key inflammatory protein, which might be detected early in the disease state, and before the diseases greatly progress to produce massive nitration damage would likely be effective before major irreversible damage is done.

Further, if effective non-toxic nitration inhibitors could be found, the extent of nitration for a given protein would decrease, which would become a measurement of effectiveness of disease treatment. We are developing such an assay using the principles of luminescence which we have developed for a variety of diseases.

In addition, the vascular system is very dependent on the production of nitric oxide in the inner cellular walls formed from epithelial cells. In order to maintain healthy blood vessels it is necessary to produce sufficient nitric oxide which is a major vasodilating substance in the vascular walls themselves- in order to prevent hypertension or high blood pressure. This can be also helped by ingesting sustained release 6-8b grams/day in divided doses of L-arginine, L-citrulline or lesser doses of nitrates from foods like raw spinach, dark green lettuce or beets or beet juice. See Figure 4 which depicts peroxynitrite chronic disease chemistry occurring in blood vessels.

In addition, in the diseases of type 1 and 2 diabetes it has been shown by us and many other researchers [15-26] that the death and

illness of these diseases is linked to both excess sugar but also by excess nitration since even when blood sugar is well maintained diabetes of either type continues to persist. The pathological consequences of diabetes are depicted in Figure 5.

Why Hasn't Science Done a Better Job in Conquering These Chronic Diseases? Much of the money spent on disease states is done by scientists and physicians who generally have been taught to think with a group mentality. The National Institutes of Health and other Health Organizations get caught up in the complexities and the politics of science. Money is spent on projects that cannot make any major impact on prevention or understanding the mechanisms of how the disease toxicity is actually occurring. Chronic diseases are the result of overactive immunity greatly linked to macrophage stimulation. Chronic stimulation of macrophages causes chronic inflammation to occur. We can control this situation but it will take properly directed funding and courageous people that actually have an interest in preventing diseases rather than treating diseases after the die is cast. I believe that politics and ignorance has led us to support the NIH rather blindly without asking why haven't these chronic problems been mostly solved? Certainly the FDA and the major drug companies need to have an adjustment in attitude. What is needed is to focus on what is important to human health and not how do we make the most money with drugs that barely make a difference to the health of most humans. I predict that if we take the correct route to discovery, it is certainly possible for almost all humans to live a long, healthy, productive and happy lives.

Summary

Excessive efforts have been expended on the belief that the basis of chronic diseases is linked to gene defects or deficiency of antioxidants which disallow the control of toxic free radicals and the production of aging itself. Based on data presently available, these concepts are far

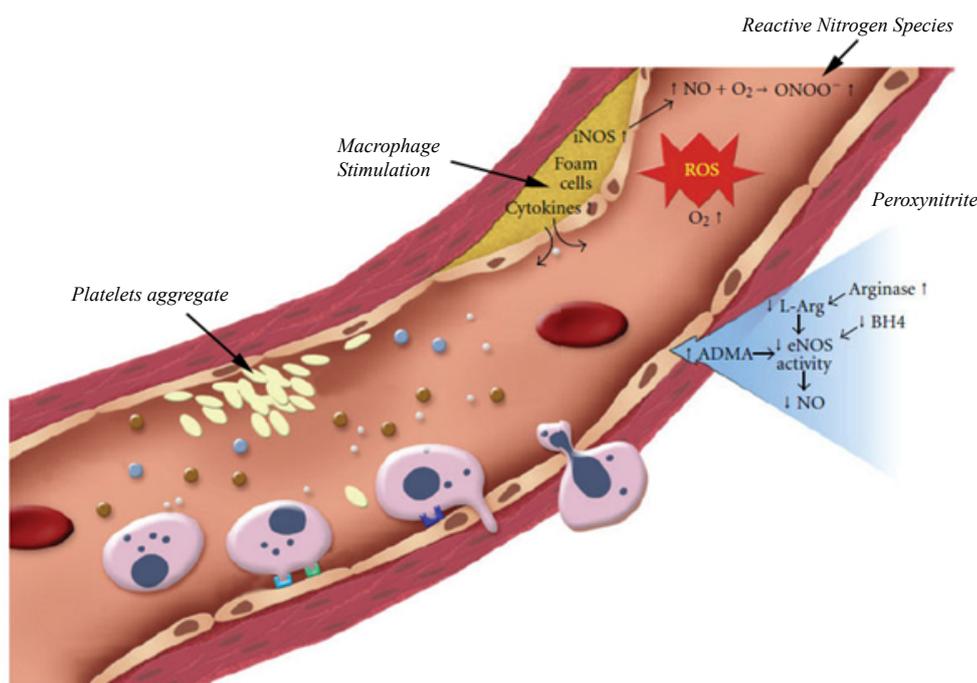


Figure 4: Vascular inflammation associated with chronic diseases.

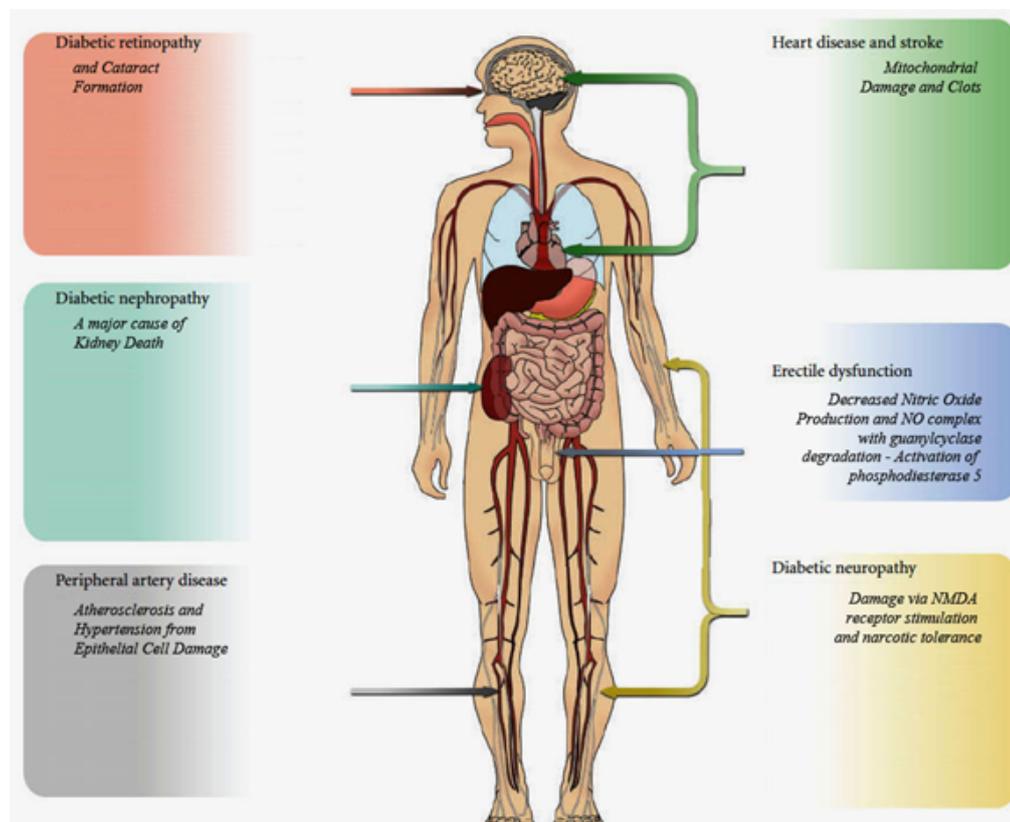


Figure 5: Pathology associated with diabetes.

from the important toxic mechanisms which actually control most untreatable chronic diseases.

Clearly, it is a chemical attack on epigenetic regulatory systems which renders our best anti-inflammatory drugs useless. Since chronic diseases are mostly caused by chronic inflammation, we must focus our attention on the chemistry causing damage to the epigenetic- control mechanism namely histone deacetylase 2 and prevention of nitration by peroxynitrite damage to this key enzyme as well as nitration damage to DNA, RNA, proteins containing tyrosines and tryptophans as well as key lipids and sulfhydryl containing molecules. It is excessive nitration that causes most of damage causing chronic diseases. We must control this excessive nitration to live happy, healthy and long productive lives.

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