

# Etiopathogenic mechanisms of tobacco constituents in rheumatoid arthritis

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## ABSTRACT

Active smoking is considered a risk factor for rheumatoid arthritis. Smokers show respiratory extra-articular manifestations and complications, such as interstitial lung disease and chronic obstructive pulmonary disease. Smokers may receive a more intensive drug therapy than non-smokers, but they have a poor prognosis. Smoker's resistance to therapy may be caused by the pharmacokinetic interactions between drugs and tobacco constituents. The present account of some of those thousands of components of the gas and tar phase of cigarette smoke (polynuclear aromatic hydrocarbons, quinones, cyanide, heavy metals, bacterial endotoxins, nicotine and carbon monoxide) may help explain the inconclusive incrimination of tobacco use in the development of rheumatoid arthritis and convince more clinicians to recommend smoking cessation.

**Keywords:** tobacco, cigarette smoke, rheumatoid arthritis, risk factors

## INTRODUCTION

Twin studies show that genetic (the rheumatoid epitope HLA-DRB1) and environmental factors are equally important in the susceptibility to rheumatoid arthritis (RA). Active smoking is a major risk factor for RA, especially in male patients and for the forms testing positive for the rheumatoid factor (RF) and/or anti-cyclic citrullinated peptide (CCP) antibodies, which account for approximately one third of RA cases. However, there is no general agreement whether tobacco use accelerates or delays the development of this disease, as some studies found no significant differences between smokers and non-smokers (1,2).

The disease activity was found to be influenced by active smoking, which leads to increased inflammation, with a higher number of swollen joints, an increased DAS28 score and higher levels of C-reactive protein (CRP), serum fibrinogen, acute phase reactants, circulating polymorphonuclear cells, pro-inflammatory cytokines (TNF-alpha, IL-6) and an increased activity of autoreactive B cells (3). This leads to a more intensive treatment in smoking patients with rheumatoid arthritis. Active smoking may also be responsible for the rapid radiological pro-

gression, mediated by the increased production of anti-citrullinated protein antibodies (ACPA) in RA cases with a duration of more than 10 years, even if the effect of biological therapies or systemic glucocorticoids used in these patients may reverse this situation. Extra-articular manifestations, such as subcutaneous nodules, interstitial lung disease and vasculitis, are often seen in smokers. The anti-pneumococcal vaccination recommended for infection control in RA patients is less efficient in active smokers. Smokers have an earlier onset of the disease and a two-fold risk of mortality from rheumatoid arthritis compared to non-smokers or former smokers. Smoking increases the risk for treatment cessation. Active smoking at the initiation of treatment leads to poor results after three or six months of anti-TNF-alpha therapy (e.g. infliximab, etanercept and adalimumab). Smokers receive a more intensive therapy with DMARDs than non-smokers. Tobacco use leads to a poor response to methotrexate administered in monotherapy. Smoker's resistance to therapy may be caused by the pharmacokinetic interactions between drugs and tobacco constituents that stimulate the metabolic rate (4, 5).

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Smoking cessation may have a positive effect on RA patients, but the risk in former smokers is minimized only after 10 or even 20 years. The prolonged duration of smoking (for more than 20 years) is a higher risk factor for RA than the number of cigarettes per day (more than 15 cigarettes daily) (3). Smokers suffering from RA have difficulties quitting due to the lack of awareness of the risks posed by smoking in this disease, the lack of alternatives for pain management or for the tobacco use itself (6). Passive smoking showed no risk for RA (7). However, a certain risk was found only in women and subsequent to childhood exposure (8). Maternal smoking during pregnancy may induce an increased risk of juvenile RA in girls (9).

### **EFFECTS OF CIGARETTE SMOKING ON THE RESPIRATORY TRACT**

A report from a British Hospital has shown that more than half of all male patients who died from RA in 2015 and 2016 had respiratory involvement (10). The smoking-induced inflammation of the respiratory tract mucosa, leading to an increased permeability of pulmonary capillaries, is responsible for an accelerated protein citrullination in the bronchial tract, by converting arginine into citrulline by peptidylarginine deiminase (PAD) enzymes (6). The positively charged protein arginine is turned into a neutral immunogenic citrulline. Tobacco use stimulates the population of antigen presenting cells in the lungs, triggering anti-citrulline autoimmunity and RA (3). The citrullination involves protein and peptide modifications that lead to a loss in immune tolerance. Bronchoalveolar lavage shows increased levels of macrophages, neutrophils, monocytes and eosinophils and increased concentrations of immunoglobulin M and immunoglobulin G. Bronchoalveolar lavage obtained from smokers suffering from RA contains cells with citrullinated proteins, suggesting the direct impact of tobacco on protein citrullination in lungs, an organ which is directly exposed to smoke. The lymphocytes in the bronchial tree have limited proliferative responses to mitogens. Smoke residues induce an abnormal surface of alveolar macrophages with abnormal cytokine reactions.

Besides citrullination, carbamylation and lipid peroxidation mediated by cyanate from cigarette smoke and by reactive oxygen species, respectively, lead to the formation of autoantigenic proteins, that

determine the formation of autoantibodies specific to autoimmune disorders, such as RA (11).

The oral cavity is another extra-articular site of smoking-induced production of anti-citrullinated protein antibodies, leading to the development of periodontitis in RA patients.

### **HARMFUL TOBACCO CONSTITUENTS**

Cigarette smoke has a gas and a tar phase (with at least 96 toxins, as estimated by the US Food and Drug Administration), but there are thousands of constituents in the leaves of tobacco, such as polynuclear aromatic hydrocarbons (benzo[a]pyrene), nitric oxide, aromatic amines, benzene, carbon monoxide, vinyl chloride, bacterial endotoxins and heavy metals (aluminium, arsenic, cadmium, cobalt, chromium, copper, mercury, nickel, lead, vanadium) (12,13,14).

#### **Reactive oxygen species**

Cigarette smoke releases numerous reactive oxygen species (ROS), such as hydroxyl, superoxide, hydrogen peroxide, singlet oxygen, which affect the epithelial cells of the upper airways and the cartilage through DNA damage and lipid peroxidation, by generating peroxy radicals from polyunsaturated fatty acids. Lipid peroxidation is responsible for the rapid development of atherosclerosis in RA patients. ROS activate signaling pathways, such as transcription factors, protein phosphatases and protein kinases. The upregulation of chemokines, adhesion molecules and glycation end-products, the activation of interleukin 8 and TNF $\alpha$ , the release of intracellular proteins from damaged cells, the exacerbation of autoreactive B-cell processes, impaired function of antigen-presenting cells and of T cells and the oxidative stress caused by ROS removal through antioxidant defence mechanisms are all involved in the etiopathogenesis of RA. Type II collagen oxidized by ROS is frequently seen in the synovial fluid and serum of RA patients (15,16).

#### **Benzo(a)pyrene**

Benzo(a)pyrene is a hydrocarbon that induces mutations in the tumor suppressor gene p53 of RA synoviocytes, which was associated with an unchecked synovial proliferation in rodent studies (17).

Benzo(a)pyrene significantly enhances the activation of NF- $\kappa$ B, which is overexpressed in the RA synovium, leading to an increased TNF $\alpha$ -induced proinflammatory cytokine production (e.g. interleukin 6, interleukin 1 $\beta$ ) and to the accumulation of ROS, as the result of formation of benzo(a)pyrene-quinone metabolites. NF- $\kappa$ B mediates the production of proinflammatory cytokines in mesenchymal cells, lymphocytes, macrophages, and fibroblasts. Due to its increased proinflammatory effects, benzo(a)pyrene significantly reduces the inhibitory effect of adalimumab and infliximab (18, 19).

Other polycyclic aromatic hydrocarbons than benzo(a)pyrene, such as 3-methylcholanthrene and dioxins, activate the aryl hydrocarbon receptor (AhR) which aggravates RA in smoking patients by differentiating and activating T helper 17 cells. A high AhR expression in the synovial membrane is correlated to smoking habits (20).

### Quinones

Hydroquinone is a derivative of benzene, a phenolic compound with the most prooxidative properties of all tobacco constituents. Hydroquinone has aggravated systemic and local RA symptoms in *in vivo* rodent models, due to the migration of aryl hydrocarbon receptors into synovia, higher serum levels of anti-citrullinated peptides and of proinflammatory cytokines. The large number of neutrophils in the synovial fluid activate aryl hydrocarbon receptors and the interleukin 17 pathway, which are involved in immune-mediated diseases (21, 22).

### Cyanide

Cigarette smoke contains hydrocyanic acid, which is absorbed into lipophilic phases in the lungs of smokers. Cyanide alters the distribution of gold nanoparticles during chrysotherapy for RA, indicating that it affects not only the lungs. Cyanide forms stable complexes with essential metals such as iron, zinc and copper, which diminishes the dietary absorption of these anti-inflammatory minerals. Cyanide is also achieved from cigarette smoke in inflammatory areas from the reaction of hydrogen peroxide with the myeloperoxidase from neutrophils or with eosinophil peroxidase. Cyanide detoxification involves the production of a less toxic thiocyanate, which nevertheless may induce chronic inflammation (23).

### Heavy metals (arsenic, cadmium, cobalt, chromium, mercury, nickel, lead)

The tobacco plant (*Nicotiana tabacum*) is a source of trace elements, obtained from a high absorption capacity from a growing environment full of fertilizers and even arsenical pesticides (24).

The mean values of cadmium, nickel and lead are significantly higher (up to 2- to 3-fold higher) in the blood and hair samples of smokers with rheumatoid arthritis patients than in non-smoking RA patients and healthy controls (25). Increased levels of heavy toxic metals facilitate the formation of reactive oxygen species. Cadmium is particularly harmful, because the absorption of cadmium is very similar with those of essential minerals, such as calcium, zinc and iron. High levels of cadmium induce oxidative stress and autoimmune diseases, such as RA. The reaction between oxygen and free metallic ions produces hydroxyl radicals. Imbalances of essential and toxic metals are found in RA patients (e.g. zinc deficits are correlated with an excess of cadmium) and the metabolism of essential metals is strongly affected by heavy metals. Higher concentrations of cobalt, cadmium, chromium and copper are seen in the blood of smokers with RA, whereas calcium, magnesium, iron and zinc have decreased values. Chromium and cobalt ions (Cr<sup>3+</sup> and Co<sup>2+</sup>) affect the macrophage-like cells. Lead damages osteoblasts and chondrocytes also due to the oxidative stress (26). The cadmium from cigarette smoke reacts with previously inhaled fine particulate matter generated by the environmental pollution, generating cadmium oxide nanoparticles, which stimulates protein citrullination in lung epithelial cells, causing RA (11). Toxic metals, such as mercury, nickel, lead and arsenic induce oxidative stress by depleting glutathione and protein-bound sulfhydryl groups (26).

### Bacterial endotoxins

Smoking involves also the inhalation of pro-inflammatory bacterial endotoxins, Gram-negative bacterial products, such as lipopolysaccharides, which stimulate the immune cells and are present in the joints of smoker affected by RA. Endotoxins form complexes with procollagen and generate nuclear factor kappa B (NF- $\kappa$ B), leading to the inflammation and destruction of cartilages. Bacterial endotoxins and the smoking-induced alterations of the microbiota in the airway tract induce inflammation and pro-

duction of ACPA, due to the fact that microorganisms also express citrullinated proteins (27).

### Other toxic compounds

Cigarette smoke releases 2,3,7,8-tetrachlorodibenzo-P-dioxin which is highly present in the synovial fluid and determines the expression of proinflammatory cytokines such as interleukin 1b, interleukin 6 and interleukin 8, by binding to the aryl hydrocarbon receptor, whose effect is transmitted through NF- $\kappa$ B and extracellular signal-regulated kinase signalling cascades. The carcinogen substance 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butane found in lung cancer cells increases the survival of fibroblast-like synoviocytes, which are involved in RA pathogenesis (14, 28, 29).

## PROTECTIVE TOBACCO CONSTITUENTS

### Nicotine

Nicotine is a tertiary cholinomimetic alkaloid, a cholinergic agonist that regulates the production of proinflammatory cytokines of macrophages. Nicotine suppresses the activation of TNF- $\alpha$ -induced nuclear factor NF- $\kappa$ B signaling and inhibits the production of interleukin 1, interleukin 6 and TNF- $\alpha$ , with reduced TNF- $\alpha$  expression in synovial tissue. Its protective properties against inflammation shown by *in vivo* and *in vitro* studies incriminate other constituents of tobacco in the onset mechanisms of RA. Nicotine may have damaging effects only in very large doses, which are toxic both in humans and animals, by decreasing blood flow and inhibiting tissue regeneration in RA affected joints. Nicotine administration in rodents prior to antigen immunization aggravated arthritis, while nicotine administration after the onset of arthritis led to the amelioration of symptoms (16).

A strong argument for a lack of involvement of nicotine in RA etiology is the observation that chewed tobacco shows no risk for RA, which indicates that most harmful chemicals are to be found in smoke. Low concentrations of nicotine may even ameliorate symptoms and inhibit bone destruction in RA murine models (30, 31).

### Carbon monoxide

Carbon monoxide (CO) may have been thought to enhance immune reactions, due to the fact that it is lethal in high concentrations, but recent studies have shown that CO has anti-inflammatory properties mediated by the mitogen-activated protein kinases, which help protect cells against reactive oxygen species and reactive nitrogen species.

*In vivo* rodent studies have shown therapeutic effects of low dose CO inhalation, with successful arthritis remission. Low levels of CO help to inhibit the activation of interleukin 6 and interleukin 8 in lung cells and macrophages and limit the release of TNF $\alpha$ . Similar effects are achieved in hypoxia experiments (32).

### Heavy metals (copper)

Low concentrations of copper in the scalp hair and blood samples of RA patients of both genders indicate that copper deficiency can be considered a risk factor for RA. In late 1940s, a copper complex, Cupralene, was successfully used for RA treatment. Copper salts were more effective than gold salts in the early stages of the disease.

Besides treatment with copper-containing agents, the use of the anti-inflammatory copper-dependent metalloenzyme superoxide dismutase was also found helpful for the control of RA symptoms (24).

## CONCLUSIONS

The harmful effects of tobacco constituents contradicted by a few instances of a protective role help explain the contradictory results of studies on RA characteristics in smoker and non-smoker patients. The thorough study of at least some of those thousands of components of the gas and tar phase of cigarette smoke may improve the results of existing RA therapies. The role played by some of these toxins in the etiology of rheumatoid arthritis could convince more practitioners to develop programs for smoking cessation among their RA patients.

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