



Oxidative stress and nutritional prevention in autoimmune rheumatic diseases

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Abstract

The hypothesis that oxidative stress favours flogistic and immune processes inducing autoimmune rheumatic diseases (ARDs) and their complications is still under discussion. In this review we take into consideration both the aetiopathological role of the diet in such diseases and the possible efficacy of dietary supports as adjuvants for the usual specific therapies. Moreover, we shall examine the hypothetical pathophysiological role of oxidative stress on ARDs and their complications, the methods for its evaluation and the possibility of intervening on oxidative pathways by means of nutritional modulation. It is possible that in the future we will be able to control connective pathology by associating an immuno-modulating therapy ('re-educating') with natural products having an anti-oxidant activity to current immunosuppressive treatment (which has potentially toxic effects).

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1. Role of the diet and dietary supports in autoimmune rheumatic disease

In 1880, Sir Jonathan Hutchinson suggested some healthy-life rules in the treatment of systemic lupus erythematosus (SLE). They included selected aliments and dietary support with cod-liver oil [1].

More than 100 years later, the role of the diet and the dietary supports is still under discussion. The complexity of the pathology and the number

of concurrent factors often frustrate the efforts made in attributing symptomatic remission to a specific therapeutic support. We are dealing, in fact, with pathologies marked by time-variant clinical patterns, showing periods of spontaneous remission and influenced by many environmental factors able to sustain the illness' activity or favour its flares.

In a recent review—'Diets, dietary supplements and nutritional therapies in rheumatic disease'—Henderson and Panush [2] critically examined approximately 200 publications on the subject and they did not find conclusive data about the role of diet in autoimmune rheumatic diseases (ARDs). In that review the authors highlight some consid-

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erations about the possible protective role of ω -3 fatty acids in rheumatoid arthritis and that antioxidant vitamin A, E and C concentrations are decreased in RA and SLE [2–4].

2. What role does oxidative stress have on autoimmune rheumatic disease?

The reactive oxygen species (ROS) (superoxide, hydrogen peroxide, hydroxyl radicals and nitric oxide) are physiologic activators of transcription factors for pro-inflammatory cytokines as:

(1) Activator protein-1 (AP-1) that is a transcription factor of the gene for collagenases, TNF- α , IL8, IL9, IL3 and IFN γ , adhesion molecules related to the formation of atherosclerotic plaques and genes for the cell-division cycle;

(2) Nuclear factor- κ B (NF- κ B) that activates the promoters of the genes for IL1, IL6, IL8 and TNF- α cytokines together with the genes for the inducible form of nitric oxide (i-NO), e-selectin and VCAM-1 adhesion molecules, class I and II HLA antigens, IL2-receptors and several acute-phase proteins [5].

From this background we may suggest some ideas about the role of oxidative stress in ARD, and therefore some lines for clinical investigation.

(a) The majority of the previously mentioned cytokines increase during the flares of ARD, as well as the i-NO; similarly, the endothelium, macrophages and antigen presenting cells show an increased expression of e-selectin and VCAM-1 during disease activity, or the class I and II HLA antigens are highly expressed on the activated lymphocytes. Therefore, we must suppose that the oxidative stress may trigger inflammatory activity and therefore it may induce a flare of the disease.

(b) The activation of monocytes, endothelial and poly-morpho-nucleated cells, as it appears during infections, surgical interventions and traumas, induces an exalted release of ROS. As we discussed, the biologic effect is the exalted production of pro-inflammatory cytokines, the expression of adhesion molecules and the exalted production of i-NO, which sustains the immune reactions leading to inflammation and organ damage. From these points we may suggest how

traumatic or flogistic events trigger and/or maintain the flares of ARD.

(c) We suppose that the exalted production of ROS has a central role in the pathogenesis of two major complications: early atherosclerosis, with consequent cardiovascular pathology, and osteoporosis.

3. Survival curves for systemic lupus erythematosus

In SLE, biological and clinical advances give us a better understanding of the disease, leading to a more effective clinical control. Therefore, it has been possible to observe a bimodal survival curve where deaths directly related to disease only account for approximately 30%, while cardiovascular accidents for early atherosclerosis and infections are the major cause of mortality [6].

4. Oxidative stress and autoimmune rheumatic disease are concurrent causes of early atherosclerosis

Oxidative stress favours a sequence of proathrogenetic events: the production of pro-inflammatory cytokines, macrophagic activation, cellular proliferation of the cellularis mucosae, endothelial activation, the synthesis of ox-LDL and the production of the i-NO [7], and, finally, the ossifying activity of the cells of the muscularis mucosae. The same events are found in ARDs, particularly in the SLE. This disease is characterized by vascular inflammation, endothelial and macrophagic activation, ox-LDL-synthesis, production anti-ox-LDL antibodies, as well as dyslipohaemia, hypertension, lymphocyte hyperactivation and organ damage. It is possible that SLE rapidly leads to the formation of atherogenetic lesions and, consequently, to the cardiovascular diseases partially by means of elevated oxidative stress observed in this disease.

5. Early atherosclerosis and ensuing vascular damage are serious complications of SLE

A recent study [8] examined the 'risk factors for cardiovascular disease in systemic lupus ery-

thematosus'. Eight relevant risk factors are reported, six of them are related to vascular damage following early atherosclerosis, while lupus anticoagulants and homocysteine levels represent independent vascular damage risk factors. Higher cumulative cortisone is not reported in this study as an independent vascular risk factor, but it is a risk factor for early atherosclerosis because it is related to a marked aggressiveness of the illness. Experiments on animal models confirm this interpretation, as they showed that steroid's anti-atherosclerotic effect could be in turn related to its anti-inflammatory [9] and—given the autoimmune pathogenic hypothesis about atherosclerosis—immunosuppressive activity.

6. Association between atherosclerosis and osteoporosis, the role of oxidative stress

In women affected by SLE with associated cardiovascular damages, a greater incidence of osteoporosis has been observed [8]. The association between atherosclerosis and osteoporosis is not confined to this disease, as it has also been found in post-menopausal women [10]. The pro-inflammatory cytokines, which have been reported to play a pivotal role in atherogenesis, likewise favour osteoporosis, as shown by a study on post-menopausal women [11].

In addition, oxidative stress plays a primary role in the pathogenesis of both diseases. Indeed, *in vitro* tests with mice cell lines showed that the ROS enhance the activity of osteoclasts [12] and calcifying vascular cells, while inhibiting osteoblastic cells [13].

7. Which clinical and therapeutic measures are capable of reducing oxidative stress' effect of the disease?

From the above-mentioned data it could be suggested that the oxidative stress favours flogistic and immune processes sustaining ARD and their complications (like early atherosclerosis and osteoporosis). On the other hand, ARD and its complications start, increase and maintain oxidative processes, giving thereby rise to a vicious circle (oxidative stress—disease activity and expression

of some complications—oxidative stress). These elements loop into a steady and self-sustained worsening of the clinical symptoms.

The persistent production of large amounts of ROS may induce changes in signal transduction and gene expression determining a chronic oxidative stress condition [14].

Therefore, the specific treatment of the disease (corticosteroids, anti-inflammatory, immunomodulatory and immunosuppressive drugs) can indirectly reduce oxidative stress and, partially, directly inhibit some oxidative processes. Treating oxidative stress by means of nutritional modulation could represent an optional complementary tool that could be developed in the near future in order to reduce treatment with standard drugs.

8. How to evaluate oxidative stress in the 'at risk' population?

As far as oxidative stress plays an important role in favouring inflammation and vice versa, it is mandatory to evaluate oxidative stress in the population at risk even if the practical role of this evaluation is still a debatable matter. Anyway, as reported by Halliwell, some apparently healthy human beings show higher rates of lipid peroxidation than others, therefore as we do not administer antihypertensive drugs to patients in clinical trials without checking blood pressure, so why should we give anti-oxidants without checking that they have decreased oxidant status? [15].

But which measurements can we perform? According to Ref. [16], we can evaluate the level of oxidative stress by means of checking some categories of by-products of the oxidization process or by the evaluation of dietary intake.

Catabolites of lipidic peroxidation (i.e. thiobarbituric acid reactant substances) [17].

Catabolites of protein oxidation (i.e. oxidized LDLs) [18].

Catabolites of DNA oxidation (thymidine glycol and 8-HO-2'-desoxyguanosine) [19].

Detection of the derivatives of reactive oxygen metabolites (D-Rom test) [20].

Evaluation of anti-oxidant dietary intake (i.e. Mediterranean Adequacy Index) [21].

9. Will dietary derived substances capable of reducing oxidative stress play a therapeutic role in autoimmune rheumatic diseases?

If we suggest a direct pathogenetic role for oxidative stress in ARD, we may suppose that therapeutic and dietary choices capable of maintaining oxidative stress could be of clinical benefit.

As previously mentioned, laboratory and clinical data sustaining this idea are the following:

1. patients with ARD showed a reduced anti-oxidant condition (reduction of vitamins A, C and E), which was supposed to play a pathogenic role for the disease [22,23];
2. serum values of vitamins with anti-oxidant activity (alpha-tocopherol, carotene and retinol), measured in samples stored in a serum bank from blood donors who developed after 2–15 years RA or SLE, were lower than for the controls [22];
3. high vitamin-E doses administered to RA-patients were effective in reducing pain symptomatology [24,25].

Furthermore, a hypothetical field of intervention takes into account foods rich in anti-oxidants: in fact, in in vitro studies they have shown anti-inflammatory effects linked to the down-regulation of NF- κ B.

Green tea has shown significant anti-inflammatory actions in cell culture systems in vivo experimental inflammatory processes [26]. These biological effects of green tea are determined by tea polyphenols, known also as tea catechins, which may inhibit NF- κ B activation. Epigallocatechin 3-gallate (EGCG), the main polyphenol of green tea, may inhibit TNF- α -induced degradation of I κ B and activation of NF- κ B in cancer and normal cells [27]. The impairment of NF- κ B activity observed by treatment with EGCG seems to be a consequent to the decrease of IKK activity. Moreover, EGCG specifically inhibits IKK activity in cytosolic extracts of TNF- α -stimulated cells [28].

Sesquiterpene lactones derived by herbal preparations from *Asteraceae* plants commonly used in alternative medicine for rheumatoid arthritis [29] specifically strong inhibit NF- κ B pathway in in

vitro models [30,31]. Sesquiterpene lactone parthenolide selectively inhibits activation of the NF- κ B pathway by targeting IKK [30] and/or preventing the degradation of I κ B- α and I κ B- β , without interfering with ROS formation, in in vitro experiments [31].

Capsiate and its dihydroderivatives (capsaicin (CPS) and nordihydrocapsiate (CPT)) that are the major capsaicinoids of sweet pepper inhibit NF- κ B activation in response to different agents including TNF- α .

CPS itself does not affect the DNA-binding ability of NF- κ B but it prevents I κ B kinase activation and I κ B- α degradation in a dose-dependent manner [32].

Curcumin, the most important component of turmeric—a spice component of curry—commonly adopted as an anti-inflammatory compound in eastern medicinal practices, shows inhibition of NF- κ B activation in a concentration-dependent manner in endothelial cells [33].

Resveratrol, an edible polyphenolic stilbene found in the skin of red grapes, which has anti-oxidant properties similar to vitamin E, has been reported as being capable of suppressing IL-1 β -induced activation of NF- κ B in acute myeloid leukemia cells [34].

A fermented wheat germ extract with standardized benzoquinone content (Avemar[®]) has shown an improvement of clinical and laboratory parameters on mice subject to SLE [35]. Authors postulate that results may be related to a rebalancing of the lymphocytes subclasses Th1/Th2 (inhibition of IL4 and IL10 production). This product contains large amounts of chinolonic and flavonoids having an intense anti-oxidant activity. It is therefore likely that the immunomodulatory therapeutic effect can be ascribed to them [35].

Nevertheless, there are no experimental studies in vivo which demonstrate the therapeutic potential as an anti-inflammatory of all the previous substances in ARD. Clinical trials are in progress at our unit to verify the activity of Avemar[®] in SLE patients.

In conclusion, during ARDs a lack of anti-oxidant vitaminic factors has been reported. These vitamins, like diet derived substances of vegetal origin, could represent a complementary therapeutic

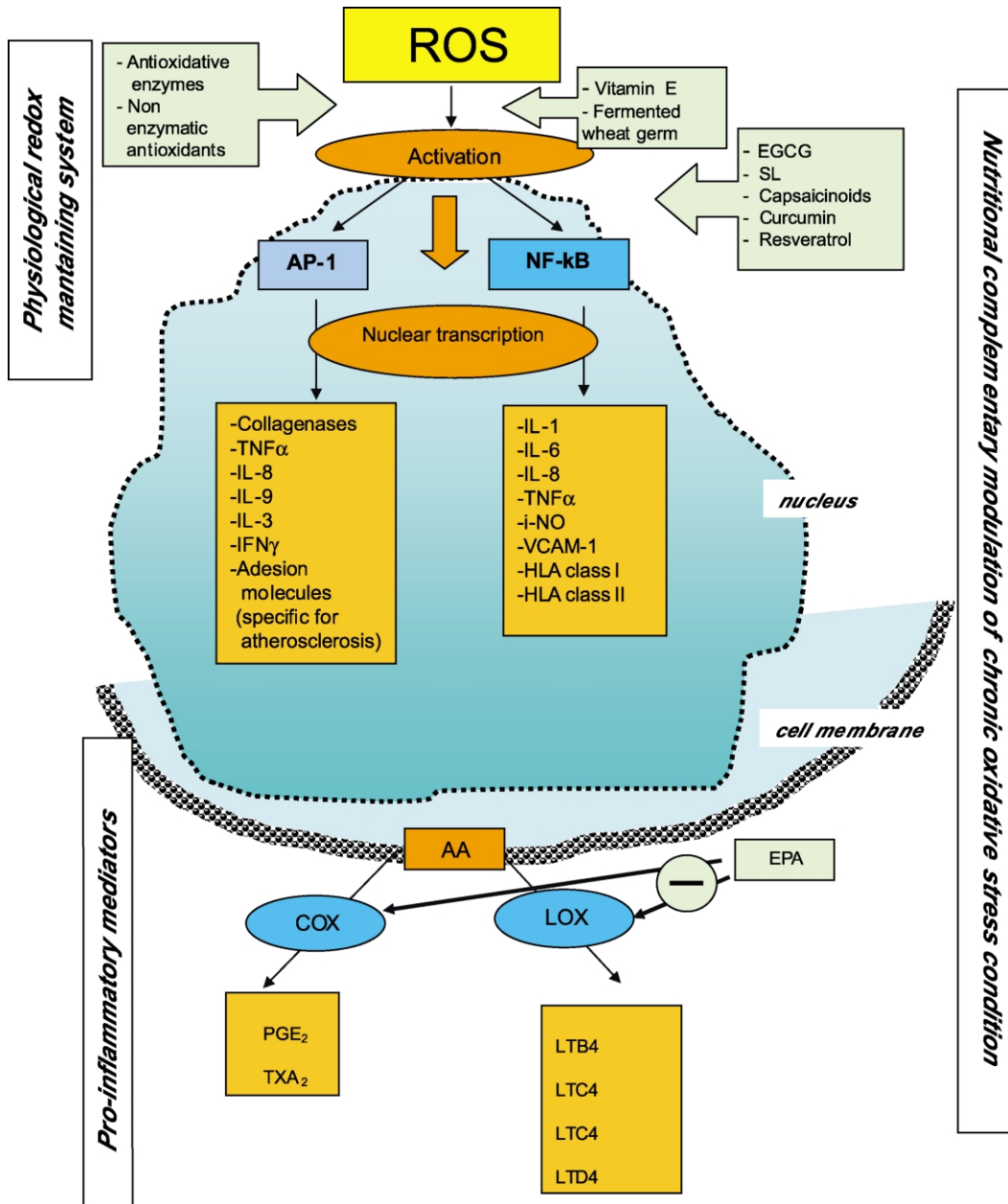


Fig. 1. Possible role of nutritional intervention as complementary therapy in chronic oxidative stress: signal transduction and gene expression may be down regulated by anti-oxidant natural systems and their precursor (cysteine, glutamate, anti-oxidant vitamins), by diet derived anti-oxidants substances, by diet derived inhibitors of NF-κB or by n-3 fatty acids. ROS, reactive oxygen species; EGCG, epigallocatechin 3-gallate; SL, sesquiterpene lactones; AP-1, activator protein-1; NF-κB, nuclear factor-κB; AA, arachidonic acid; EPA, eicosapentaenoic acid; COX, cyclooxygenase; LOX, lipoxygenase; PG, prostaglandines; TX, tromboxans; LT, leucotriens.

tic option by reducing the dysregulation of ROS steady state, throughout various mechanisms, of which the inhibition of the NF- κ B activation could be the most important. As far as clinical efficacy is concerned, the results of vitamin E treatment in RA and rheumatic polyarthritis are encouraging, while controlled clinical studies on vegetal anti-oxidant compounds are still lacking, as well as information about their pharmacokinetics and toxicity.

Therefore, further trials are mandatory to verify, possibly in large multicentric studies, the efficacy of vitamins and efficacy and safety of natural anti-inflammatory substances as well as better understanding the mechanisms of their action and their role in the nutritional prevention of ARD.

10. Summary

Oxidative stress plays a substantial role not only in the pathogenesis of ARD and their complications, but also on specific disease activity. Thus, an imbalance in the pro- and anti-inflammatory molecules due to the dysregulation of redox homeostasis oxidants/anti-oxidants could play a role in the pathophysiology of ARD. Furthermore, a defect in the apoptosis of pro-inflammatory T cells could maintain inflammatory activity in ARD.

Controlled clinical tests are mandatory in order to verify, *in vivo*, the potential therapeutic effect of products with anti-oxidant activity Fig. 1.

It is possible that in the future we will be able to control ARD by associating to immunosuppressive treatment (having potentially toxic effects) an immuno-modulating therapy ('re-educating') with natural products expressing anti-oxidant activity. The causative events of the ARD and of their clinical behaviour are still largely unknown and we cannot look forward to establishing sound results in the short time with natural re-educating substances. Clinical experience tells us that a flare does not take long to manifest its aggressiveness, while we need a lot of time and therapy to get a remission.

Anti-oxidants should play a modulating role without abolishing the oxidising processes but, as a matter of fact, we should consider and remember that

1. ROS play a physiological task in cellular functions and are essential for the protection from infectious agents;
2. NF- κ B and AP-1 are transcription factors necessary to the activation of genes synthesising proteins indispensable to the normal activities of the cells.

Take-home messages

- Anti-oxidant vitamin A, E and C concentrations are decreased in RA and SLE.
- Vegetarian diet and eicosa-pentanoic acid or derivatives of fish oil can provide clinical benefits on RA whereas in SLE this role is still debatable.
- The clinical use of vitamin E in the treatment of RA and rheumatic polyarthritis is encouraging.
- The ROS are physiologic activators of transcription factors for pro-inflammatory cytokines activating protein-1 and nuclear factor- κ B (NF- κ B).
- Food derived compounds activation show in *in vitro* studies to have anti-inflammatory effects linked to the down-regulation of NF- κ B.
- Controlled clinical studies on natural compounds are still lacking, as well as information about their pharmacokinetics and toxicity.
- The evaluation of oxidative stress and of nutritional intake is mandatory in the clinical approach of patients affected by autoimmune rheumatic diseases.

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The World of Autoimmunity; Literature Synopsis

Autoantibodies associated with reproductive failure

Autoimmune factors are associated with some of the cases of reproductive failure. Sherer et al. (*J. Assist Reprod Gen* 2003;20:53) reviewed the various autoantibodies associated with reproductive failure. These antibodies were found mainly in patients having systemic lupus erythematosus or the antiphospholipid syndrome. These include the classical antiphospholipid antibodies: anti-cardiolipin, anti-phosphatidylserine, anti-phosphatidylethanolamine, and anti-beta-2-glycoprotein-I. However, other autoantibodies which are also part of the spectrum of antiphospholipid antibodies have been described in the setting of reproductive failure. These include: antibodies directed to prothombin, thromboplastin, and mitochondrial M5 type antibodies. In addition, based on mainly animal studies and some observational studies other autoantibodies are also implicated in the pathogenesis of reproductive failure including those directed to laminin, thyroglobulin, corpus luteum, prolactin, poly(ADP-ribose) and lymphocytotoxic antibodies. The manifestations of reproductive failure are various with respect to their association with autoantibodies. Some are associated with infertility, whereas others with recurrent early or late pregnancy loss. The strength of evidence of this association differs between antibodies, but it is the rationale for further research of the role and association of these and other autoantibodies in cases of reproductive failure. Such studies would be able to shed light on the pathogenesis of some cases which are currently termed as 'idiopathic', and might also contribute to better management of these patients.

Graves' disease and allergic rhinitis

Takeoka et al. (*Int Arch Allergy Immunol* 2003;132:268) followed 10 patients having Graves' disease for 18 months. Five of them had seasonal allergic rhinitis. All had attacks of allergic rhinitis caused by cedar pollen in early March. In addition, the levels of peripheral eosinophil counts, pollen-specific IgE activity, anti-thyroglobulin and anti-thyroid peroxidase autoantibodies significantly increased. Moreover, in 3 of these patients, serum levels of anti-TSH-receptor antibodies also increased. On the other hand, the 5 remaining patients without allergic rhinitis had no change in the above-mentioned parameters. These results support an association between allergy and autoimmunity, as allergic rhinitis was associated with autoantibody production in a Th2-dependent thyroid disease.

Anti-endothelial cell antibodies in scleroderma-associated pulmonary fibrosis

The role of anti-endothelial cell antibodies in scleroderma is emphasized in a study by Wusirika et al. (*Am J Clin Pathol* 2003;120:596). Serum samples from 49 patients having scleroderma were incubated with rodent lung tissue sections and visualized with fluorescinated human anti-IgG. Among scleroderma patients, there was a significant positive correlation between intensity of indirect immunofluorescence staining and pulmonary fibrosis and hypertension. Western blot analysis has also been performed with endothelial cells extracts using serum samples from scleroderma patients and control subjects. The controls had negative results, whereas the western blot analysis revealed antibody binding to proteins in extracts of human endothelial cells in all patients having pulmonary disease. These results support a role of anti-endothelial cell antibodies in pulmonary disease in scleroderma patients.