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Combination therapy of dexamethasone with epigallocatechin enhances tibiotarsal bone articulation and modulates oxidative status correlates with cartilage cytokines expression in the early phase of experimental arthritis

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ABSTRACT

The inclusion of antioxidant for the treatment of arthritis, especially under the therapy with immunosuppressant, is motivated because antioxidant plays an essential role in disease progression and moreover, immunosuppressive treatment suffers redox homeostasis balance of the organism. The aim of the present study was to evaluate the enhancement of anti-arthritic effect of dexamethasone in combination with epigallocatechin on the progression of adjuvant-induced arthritis in rats. Adjuvant arthritic rats were treated with dexamethasone (0.2 mg/kg), epigallocatechin (100 mg/kg) and combination of dexamethasone (0.1 mg/kg) with epigallocatechin (100 mg/kg) daily for a period of 28 days. Paw swelling changes, estimation of serum albumin level, alteration of bone mineral density, histopathological, and radiographical analysis were assessed to evaluate the anti-arthritic effect. Lipid peroxidation and antioxidant enzyme activities in joint tissue homogenate were performed along with the expression of different pro-inflammatory cartilage cytokines like TNF-α and IL-6 . Dexamethasone and epigallocatechin combination potentiated both the antiarthritic (decrease of hind paw volume) and the antioxidant effect (lipid peroxidation, superoxide dismutase, glutathione reductase and catalase). In combination with dexamethasone, epigallocatechin markedly potentiated the beneficial effect of dexamethasone which resulted in more significant increment of serum albumin and bone mineral density. Improvement of anti-arthritic effect of combination therapy was supported by histopathological, radiographical alterations, and attenuation of over-expression of cartilage cytokines. Epigallocatechin act as potent antioxidant and combined administration of dexamethasone with epigallocatechin increased the anti-arthritic efficacy of basal dexamethasone therapy and suppressed the development phase of arthritic progression in rats.

1. Introduction

Rheumatoid arthritis is a chronic inflammatory autoimmune-mediated disorder characterized by cellular infiltration and proliferation of the synovial membrane, pannus formation, cartilage and bone erosion, leading to the progressive destruction of the joints through the interaction between infiltrating cells and mediators like cytokines, prostanoids and proteolytic enzymes (Firestein, 2003). In India, the prevalence of rheumatoid arthritis is found to be around 0.75% of the adult population (Mathew et al., 2009). The pathogenesis of rheumatoid arthritis is associated predominantly with the formation of free radicals and proinflammatory cytokines at the site of inflammation with low anti-oxidant status (Hassan et al., 2001).

A growing body of evidence indicates that O_2^- perpetuates the chronic inflammatory state associated with rheumatoid arthritis. Osteoclast (Key et al., 1990), chondrocytes (Henrotin et al., 1993), synovial cells (Tawara et al., 1991), neutrophils or macrophages (Bomalaski et al., 1989) and fragmented particles of degraded extracellular matrix which activates synovial cells and neutrophils to release reactive oxygen species are prime source of O_2^-. The involvement of the O_2^- in rheumatoid arthritis was suggested from studies performed in animal model (Hwang et al., 2009; Afonso et al., 2007) as well as in patients with active rheumatoid arthritis (Kundu et al., 2011). Thus, an approach to treating rheumatoid arthritis is to combat those reactive oxygen species with a compound having potent antioxidant activity along with traditional anti-arthritic agent.

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Dysregulated expression of pro-inflammatory cytokines including tumor necrosis factor (TNF-α), interleukin (IL)-1β, IL-6, interferon (IFN-γ), and anti-inflammatory cytokines including IL-10 (Feldmann et al., 1996) has been reported. TNF-α is expressed at many sites within the synovial membrane, including the cartilage or pannus junction (Chu et al., 1991). TNF-α, IL-1 and IL-6 are clearly involved in arthritis process, all three cytokines are present in synovial fluid and can be detected immunohistochemically in the inflamed rheumatoid synovium (Farahat et al., 1993). Recent progression in cytokine biology has provided a considerable amount of evidence for the pathological role of IL-6 in rheumatoid arthritis (Nishimoto, 2006). IL-6 is a pleiotropic cytokine with wide range of biological activities, including immuno-regulation, mediation of acute phase response, and effects on bone metabolism (Akira et al., 1993). Despite its important physiological roles, dysregulated over production of IL-6 is responsible for many systemic inflammatory manifestations observed in patients with rheumatoid arthritis (Kishimoto, 2005).

Several disease-modifying antirheumatic drugs have been used to control the progression of rheumatoid arthritis. While the majority of these disease-modifying antirheumatic drugs act as immunomodulatory drugs in rheumatoid arthritis (Hashimoto and Lipsky, 1993), some also act by inhibiting cytokines and endothelial cell proliferation (Koda et al., 1996). Glucocorticoids can be considered as disease-modifying antirheumatic drugs and having potent immunosuppressive effects and are widely used in the management of rheumatoid arthritis (Bijlsma et al., 2006). Glucocorticoids can down-regulate the expression of several inflammatory cytokines such as IL-1, TNF-α, and IL-6 (Barnes, 1998). Important evidence shows that glucocorticoids may have a disease modifying effect in addition to their well-documented anti-inflammatory actions, demonstrating the ability to substantially reduce the rate of radiologically detected disease progression (Krause et al., 2011), although there is an important question about the cost of this in terms of other, unwanted effects such as osteoporosis (O'Dell, 2004). In fact, therapeutic management of long-term pathologic conditions with glucocorticoids is often linked to a series of unwanted side effects (Da Silva et al., 2006; Dixon et al., 2011).

Side effects of glucocorticoids are dose dependent and since glucocorticoids are potent anti-inflammatory agents, therapeutic use would benefit greatly from the reduced burden of side effects, particularly those that affect the bone compartment (Yoon et al., 2012). Glucocorticoids induce rapid bone loss; greatly increase the risk of fractures (Weinstein, 2011) by the generation of reactive oxygen species mediated proapoptotic effects on osteoblastic cells (Almeida et al., 2011). Moreover, the generation of reactive oxygen species has been implicated in the mechanism by which TNF-α increases osteoblast apoptosis. This, along with the evidence that administration of antioxidants abrogates the age related increase in osteoblast apoptosis (Jilka et al., 2010) strongly support the notion that oxidative stress causally related to the osteoporosis. Therefore, combination of a glucocorticoid and a natural substance either it will be food products or dietary supplements with antioxidant activity may be a fruitful approach towards therapy of rheumatoid arthritis. In this regard, the applicability of the food product to develop a novel therapy for rheumatoid arthritis is really a challenging job. Frequently encountered issues in rheumatoid arthritis product development include (a) Lack of selecting an appropriate in vitro (animal or human systems) or an in vivo animal models for screening potentially active agents from food products, (b) Be short of designing and performing appropriate preclinical safety studies to support the use of a new molecular entity in human volunteers or patients, (c) Be lack of identifying the potential risk associated with combination therapy, particularly those with shared target organ toxicity or potential for pharmacokinetic interactions, (d) Deficit of balancing the potential need for therapeutic interactions early in the disease course with the need to avoid exposing patients with mild disease to agents that have toxicity or little record of safety, (e) Lack of adequate designing and potential long term safety monitoring, (f) Limited designing trials that dignifiedly show clinical efficacy. Moreover, lack of consistent manufacturing practice and quality standards, fear of adulteration and perceived deficiencies in scientific validation of efficacy and safety impede world wide acceptance of food products or dietary supplements. Despite these usual obstacles (-)-epigallocatechin-3-gallate which is a polyphenol from green tea extracts serves as a functional beverage and a component of dietary supplements risen to wide acceptance on the basis of his remarkable performance, high antioxidant status, and low toxicity. Epigallocatechin have attracted considerable attention for the prevention of oxidative stress-related diseases including cancer, cardiovascular diseases, and degenerative diseases (Trevisanato and Kim, 2000). Epigallocatechin was found to possesses antioxidant (Katiyar and Elmets, 2001), anti-inflammatory (Lin and Lin, 1997), anti-proliferative (Sachinidis et al., 2002), and anti-cancer activities (Mukhtar and Ahmad, 2000).

Prompted by the evidence, we tested the hypothesis that the potent suppressive effect of dexamethasone on bone formation may be caused by increasing oxidative level and antagonising the deleterious effect of dexamethasone can be modulated by the co-administration of epigallocatechin on the progression of adjuvant induced arthritis by examining the hind paw volume, estimation of serum albumin, assessment of bone mineral density, radiographic and histopathological analysis correlates with the antioxidant status and expression of cartilage cytokines.

2. Materials and methods

2.1. Materials

Dexamethasone, freund's complete adjuvant, biotinylated horseradish peroxidase, 3,3′-diaminobenzidine were purchased from Sigma Chemical Co (St. Louis, MO, USA). Rabbit anti-rat TNF-α and IL-6 polyclonal antibodies and biotinylated goat anti-rat IgG were purchased from ANASPEC Inc (San Jose, CA). Target retrieval solution was obtained from Dako Cytomation, (Carpinteria, CA). Avidin–biotin blocking kit was purchased from Vector Laboratories, (Burlingam, CA). Epigallocatechin was obtained from Chengdu Biopurify Phytochemicals Limited (China). All other chemicals and reagents used were of analytical grade and purchased in purest form available from local firms.

2.2. Animals and diet

Inbred male Sprague Dawley rats (150–180 g) were purchased from Indian Institute of Chemical Biology, Kolkata, India and were housed separately in animal cages (Tarsons) in a room and acclimated for 1 week under standard laboratory conditions at a temperature of (25 ± 1 °C), humidity (50–60%). They were exposed to 12:12 h light and dark cycle and allowed free access to both demineralized drinking water and commercial standard rat chow diet throughout the experimental period. All the experimental procedures involving animals were performed according to recommendations of "Institutional Animal Ethical Committee [Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA Regn. No. 1458/po/a/11/CPCSEA) India] for the care and use of laboratory animals were strictly followed throughout the experiment.