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Protective role of theophylline and their interaction with nitric oxide (NO) in adjuvant-induced rheumatoid arthritis in rats

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ABSTRACT

Theophylline (non-specific PDE inhibitor) and their interactions with nitric oxide modulators were evaluated in adjuvant-induced arthritic model of rats. Wistar rats (200–300 g), 8 animals per group were used in the study. The animals were injected with 0.1 mL of squalene and 0.2 mL of complete Freund's adjuvant on day (0) in sub-planter region of right hind paw controls received only saline. The treatment with theophylline and nitric oxide modulators were done from day 14 to day 28. Arthritis indexes, ankle diameter, paw volume, and body weight were determined to assess RA progression from day (0) to day 28. On day 28 animals were sacrificed and their blood collected for IL-10 and TNF-α cytokine levels and hind paw for pathological analysis. Synovial fluid from joint spaces of CFA inoculated rats was collected to estimate TNF-α level in synovial fluid. The data obtained was analyzed by two-way ANOVA followed by the Newman–Keuls post-hoc test. Theophylline (10 and 20 mg/kg) significantly decreased adjuvant induced increased arthritis-index, paw volume and ankle diameter ($p < 0.05$ in all parameters) compared to only adjuvant control group. It also reversed adjuvant induced slight decrease in body weight to normalcy. L-Arginine 100 mg/kg + theophylline 20 mg/kg suppressed TNF-α and elevates IL-10 level as well as reversed adjuvant-induced elevated arthritic parameters as compared to only adjuvant and prednisone group ($p < 0.001$). Synovial TNF-α level of adjuvant only group was several fold higher than its serum level. Treatment with theophylline 20 mg/kg significantly reduces synovial TNF-α level as compared to adjuvant only group. Theophylline 20 mg/kg + L-NAME 10 mg/kg significantly reversed these adjuvant-induced changes in immunological, histopathological and arthritis parameters ($p < 0.05$).

1. Introduction

Rheumatoid arthritis (RA) is an inflammatory autoimmune disorder; however its etiology is still largely unknown. Multiple joints are affected due to it and erosion of cartilages also occurs in rheumatoid arthritis. Rheumatoid arthritis (RA) is a lifelong progressive disease and significant morbidity and premature mortality are attributed to it. Its worldwide prevalence ranges from 0.3% and 1% and is more common in developed countries. Prevalence of RA in India is reported to be 0.75% in the adult population [1]. Although there drugs available for symptomatic treatment of RA, few affect the underlying pathological mechanism of RA.

Nitric oxide (NO) produced endogenously has a very important role in inflammatory pathways. It is also involved in many physiological processes. Biological effects of NO depend on environmental and pathophysiological conditions, it can produce totally opposite biological effects depending on environmental and pathophysiological conditions [2–4]. Researchers have evaluated role of free radicals in stress-induced immunological changes and have established that elevated oxidative markers may be responsible for suppressed immune cell functions [5]. Destruction of inflamed joints within the synovium occurs due to auto-immune disorder produced by oxidative stress [6]. Free radicals having oxidative activity generated by NO such as reactive nitrogen species and reactive oxygen species mediate the cartilage and joint destruction in RA [7]. NO also promotes synovial inflammation by mediating in signal transduction of various inflammatory mediators and has emerged as an important mediator of RA. Increased NO levels have been reported in serum and synovial fluid of RA patients [8]. Thus NO modulators may be of importance and may have role in pathogenesis of RA.

Phosphodiesterase (PDE) inhibitors are promisingly used in the treatment of asthma, as PDE inhibitors such as Theophylline suppress the immunopathology of asthma [9]. Intracellular levels of cAMP in leukocytes are elevated by PDE inhibitors, which then inhibits TNF-α production [10], due to which they were possibly used for treatment of RA. Various researches have reported that, non-selective as well as selective PDE-4 inhibitors are useful in amelioration of inflammation in different models of autoimmune encephalitis and collagen induced arthritis [11,12]. It has been also reported that anti-inflammatory

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