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Original Research Article

Effects of Simvastatin and Ezetimibe on Rats' Parotid in Experimental Freund's Adjuvant Arthritis

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ABSTRACT

Statins and ezetimibe, except from their lipid lowering action, have been reported to exert anti-inflammatory effects. It is interesting to investigate if there is any clinical efficacy of these drugs on rheumatoid arthritis.

The aim of the present study was to investigate the effect of simvastatin and ezetimibe on parotid glands' morphological alterations and on serum indexes in arthritic rats.

Twenty-eight Wistar rats were divided into 4 groups. Group A, B and C were injected with 0.1ml Freund's complete adjuvant in the paw. After the arthritic symptoms' manifestation, Group B received simvastatin 0.1mg/100g per os and group C ezetimibe 3mg/kg for 14 days. Group D served as control.

Total proteins, rheumatoid factor, TNF-a, paw diameter, parotid and adrenal weight, as well as parotid histology were negatively affected under the influence of Freund's complete adjuvant. Simvastatin and ezetimibe administration significantly ameliorated all the above parameters.

An amelioration in adjuvant arthritic laboratory findings and in parotid histology was demonstrated under simvastatin and ezetimibe administration, implying that both drugs possess a potential antiinflammatory action.

Keywords: simvastatin, ezetimibe, parotid gland, rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis is characterized by an autoimmune induced inflammatory reaction to the peripheral synovial joints. Apart from its affection on synovial membranes, it possesses systemic manifestations such as haematological, pulmonary, neurological and cardiovascular dysfunctions. [(1)] It has been also demonstrated that the experimental arthritis, induced by Complete Freund's adjuvant, leads to morphological and histological changes of parotid, with increased gland weight and surface and chronic infiltration of parotid tissue, as well as atrophy and fibrosis of the acini. [(2)]

Statins, apart from their main use in the inhibition of cholesterol biosynthesis and their lipid lowering effect, may also justify additional indications including the management of diseases, such as inflammation and cancer. [(3)] Ezetimibe is an inhibitor of cholesterol absorption in the intestine. [(4)]

Mäki-Petäjä et al investigated the effect of simvastatin and ezetimibe on inflammation, endothelial dysfunction and arterial stiffness in rheumatoid arthritic patients, and demonstrated that both drugs

reduce disease activity and inflammatory parameters to a similar extent, suggesting that cholesterol lowering factors may have anti-inflammatory effect improving vascular function in rheumatoid arthritis. [(5)]

Freund's adjuvant induced arthritis in rats is an extensively studied experimental model of systemic polyarthritis, widely applied for the screening of anti-arthritic drugs. [(6-8)]

The aim of the present study is to investigate the possible effect of simvastatin and ezetimibe on the morphological parotid glands and serum arthritic indexes in rats, induced by complete Freund's adjuvant arthritis.

MATERIAL AND METHODS

Twenty-eight male Wistar rats (mean body weight 300 ± 15gr) were divided into 4 groups (n=7). The animals of groups A, B and C were injected in the right-hind paw with 0,1ml Freund's complete adjuvant [(FCA) - 0,5mg/ ml of *Mycobacterium tuberculosis* (H37Ra, ATCC 25177), heat killed and dried, 0.85 ml paraffin oil and 0.15 ml mannide monooleate, SIGMA]. Freud's adjuvant injection caused the swelling of the rat paw within 24 hours. On the second day, after the arthritic symptoms' manifestation, Group B received simvastatin 0,1mg/100g per os daily (Zocor, Vianex) and Group C ezetimibe 3mg/kg daily (Ezetrol, Vianex), *via* a gastroesophageal catheter, for 14 days. Group D served as control.

On the 15th day all animals were anaesthetized and sacrificed. Blood was collected and immediately stored at 4 ^{o} C. Rheumatoid factor serum levels were estimated through nephelometry using specific rat kit (Dade-Behring, Germany). [(2)]

Serum total proteins and albumin concentration were measured by colorimetric method with the bromcresol green assay while C-reactive protein (CRP) levels were calculated on a routine clinical chemistry analyzer, Synchron LX 20, by immunoturbidimetry, (Beckman Coulter, Inc.). [(9)]

Adrenals and parotid glands were isolated, removed and weighed. Parotids were preserved in a 10% buffered formaldehyde fixative solution (Merck). Paraffin-embedded tissue sections (5 μm) were stained with haematoxylin-eosin for further histological examination. [(9,10)]

Statistical analysis was performed *via* t-test between experimental arthritis group (A) -control (D), group (A) - simvastatin group (B) and group (A) - ezetimibe group (C).

The animals were housed under 12/12h light/darkness with *ad libitum* water and food access. They were treated according to the Guide for the Care and Use of Laboratory Animals (2011) 8th Edition National Research Council (US).

The animal experiments were carried out in accordance with the guidelines for the care and use of experimental animals that are in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC. All efforts were made to minimize animals suffering and the number of them used.

Experimental permission of the Greek Ethical EL.25BIO 009 was obtained.

RESULTS

Adjuvant arthritis model was assessed through the enhanced rat right paw diameter. Simvastatin and ezetimibe reduced the degree of paw swelling ($p<0,01$).

Rheumatoid factor, parotid and adrenal weight were increased and decreased under simvastatin and ezetimibe treatment ($p<0.05$).

Total serum proteins and albumin levels were decreased under the influence of Freund's complete adjuvant ($p<0.01$), while simvastatin and ezetimibe seem to ameliorate the first ($p<0.05$) but not the albumin.

Changes in C- reactive protein (CRP) were not statistically significant.IL1-β increased ($p<0.01$) with slight amelioration and TNF-α increased ($p<0.01$)

with significant amelioration, under simvastatin and ezetimibe.

The values of the examined parameters (laboratory findings and morphological observations) in the four groups are shown in Table 1.

Table 1: Values of the examined parameters in the four experimental groups

	Group A FCA	Group B FCA Simvastatin	Group C FCA Ezetimibe	Group D Control
Total proteins mg/dl	5,2±0,2**	6,2±0,3*	6.1±0,02*	7±0,05
Albumin levels mg/dl	3.6±0,02**	3,7±0,1	3.8±0,01	4,6±0,1
CRP mg/dl	0,15±0,01	0,12±0,01	0,13±0,01	0,11±0,02
Rheumatoid Factor mg/dl	17,5±2,5**	12,2±1*	14,5±2,5*	11,9±2
TNF-α pg/ml	827.5±17.8**	635±16.5*	685.4±18.2*	520.9±3.5
IL1-β pg/ml	243.5±15.7**	205±11,2 *	217.8±9	147.7±6.1
Paw diameter mm	1,84±0,51	1,52±0,18**	1,65±02**	0,62±0,14
Adrenal weight mg	0,068±0,008	0,059±0,012	0,0587±0,1	0,052±0,011*
Parotid weight mg	42,18±0,07*	38,57±5,5*	37,84±3,2*	32,5±0,8

* p<0,05 **p<0,01 FCA : Freund's complete adjuvant

Parotid glands' histological investigation showed an altered structure under the influence of the Freud's adjuvant. Histological and macromorphological changes in parotid glands are shown in figures 1-5.

Macromorphological changes of parotid gland and normal parotid gland (group D): (1) secretory duct, (2) acini, (haematoxylin-eosinX250), are presented in Fig 1 and 2

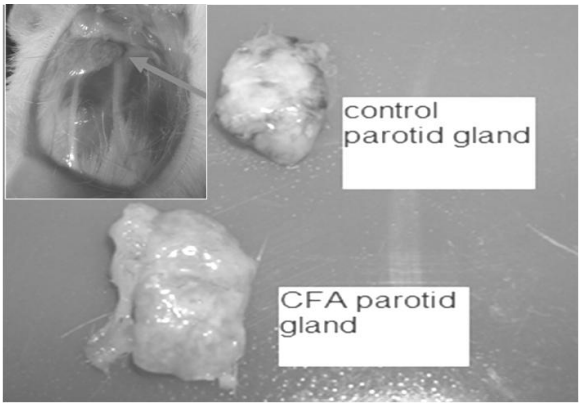

FIG.1 Macromorphological changes of parotid gland

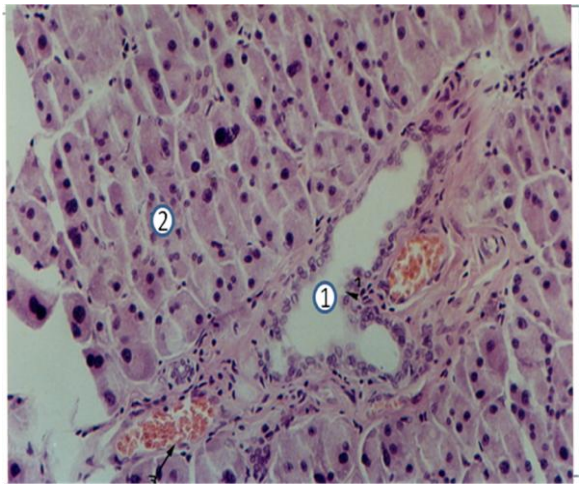

FIG2. Normal parotid gland (group D): (1) secretory duct, (2) acini, (haematoxylin-eosinX250)

Parotid gland histology shows focal chronic inflammation (1), ductal dilatation (2), severe acinar atrophy and fibrosis with variable acinic cell nuclear polymorphism and sialostasis (haematoxylin-eosin). (Fig 3)

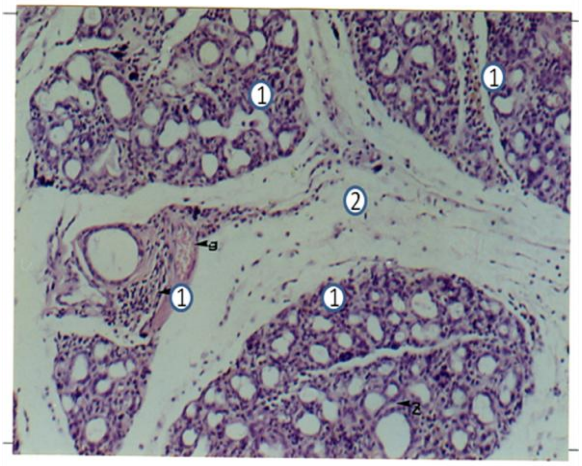

FIG3. Parotid gland following experimental arthritis group A with (1) chronic inflammatory foci, mild sclerosis and oedema and ductal dilatation (2) severe acinar atrophy (haematoxylin-eosin X100).

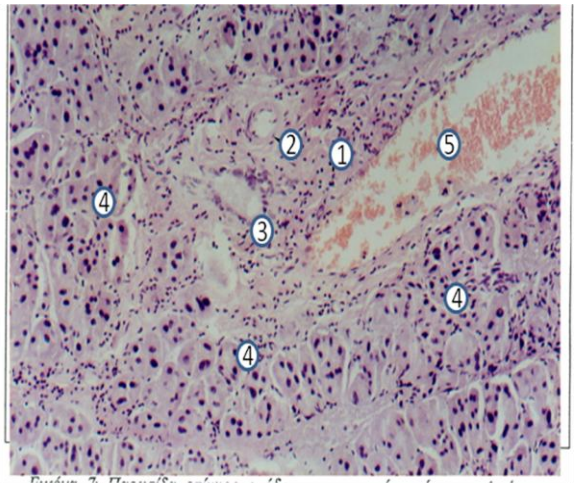

FIG4. Parotid gland FCA + simvastatin (group B): (1) focal mild chronic inflammation, (2) arteriol (3) normal duct, (4)normal acini, (5) congested venule (haematoxylin-eosinX 100)

Under simvastatin treatment (group B), parotid gland morphology shows milder inflammation without any further amelioration. (Fig 4)

Ezetimibe treatment (group C) on parotid gland histology presents fewer foci of chronic inflammation, as well as milder ductal dilatation, sialectasis and acinar atrophy. (Fig 5)

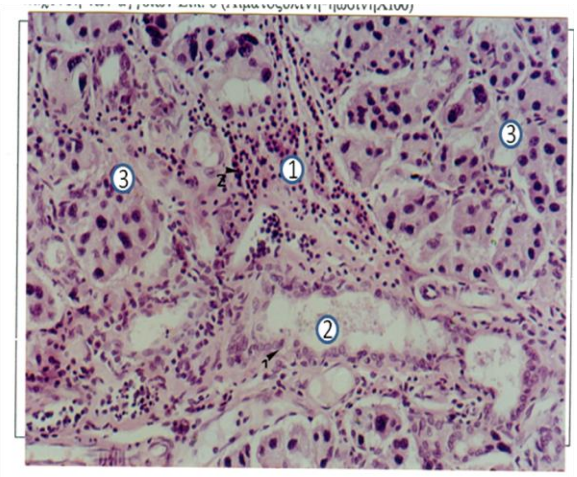

FIG5. Parotid gland group C experimental arthritis and ezetimibe treatment (1) chronic focal inflammation, (2) mild ductal dilatation and sialostasis, (3) mild acinar atrophy. (haematoxylin-eosinX 250).

DISCUSSION

Freund's adjuvant-induced arthritis in the rat is an experimental model reproducing some immunological aspects of rheumatoid arthritis.

A lot of extra-articular organs involvements e.g. vision abnormalities, salivary gland function impairment, cardiovascular disorder, coronary heart disease, have been recognized as major determinants of morbidity and mortality in patients with rheumatoid arthritis. [(11,12)]

The present study examined if parameters as total protein and albumin levels, CRP, TNF-a, IL1-β, rheumatoid factor, paw diameter and adrenal weight, were altered in the adjuvant arthritic group compared to the control group. Moreover, if parotid gland morphology is affected under the influence of experimental adjuvant arthritis. [(2)]

Some of the adjuvant arthritic parameters demonstrated statistically significant amelioration under simvastatin and ezetimibe administration. These were total serum proteins, rheumatoid factor, TNF-α, paw diameter, adrenal and parotid weight.

Numerous studies refer to the ability of statins to reduce serum levels of C-reactive protein (CRP). a plasma protein among the most expressed ones in acute phase inflammation cases being a known biomarker for inflammatory states. [(13-21)] In our study changes in C- reactive protein (CRP) were not statistically significant.

Patients with rheumatoid arthritis or other degenerative diseases like hyperlipidaemia have been shown to have reduced salivary flow. [(22-25)] The involvement of salivary glands in rheumatoid arthritis appears to be a complication of connective tissue disease. This gland dysfunction may be reflected in the histological findings of the arthritic subjects in the present study.

In parotid histology, the excess of the inflammatory infiltration was reduced under both lipid lowering drugs and more by ezetimibe.

On the other hand statins' anti-inflammatory effects may contribute to their beneficial role in cardiovascular disease prevention, since it is suggested that active rheumatoid arthritis patients have increased prevalence of specific cardiovascular risk factors. Nevertheless it is recommended that

in patients with rheumatoid arthritis, statin therapy should be considered only when cholesterol levels are elevated despite appropriate dietary treatment. [(26)]

Present results demonstrate a potential improvement of the Freund's complete adjuvant's noxious process, under simvastatin and ezetimibe treatment. Similar findings by Bracht L et al 2012 demonstrated analogous effectiveness in reducing the inflammatory response of this arthritis, under simvastatin plus ezetimibe administration but proved the presence of liver toxic effects. [(27)]

Finally it may be concluded that lipid lowering drugs seem to act favorably in the inflammation treatment. Further studies are needed to elucidate this action and if their benefits outweigh their probable hepatotoxicity, which could limit their use as an antiinflammatory agent.

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