European Journal of Pharmacology 761 (2015) 273–278

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Immunopharmacology and inflammation

Menaquinone-7 as a novel pharmacological therapy in the treatment of rheumatoid arthritis: A clinical study

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ARTICLE INFO

Article history:
Received 19 April 2015
Received in revised form
4 June 2015
Accepted 5 June 2015
Available online 11 June 2015

Keywords:
Rheumatoid arthritis
Menaquinone-7
DAS28-ESR
ucOC
Matrix metalloproteinase.

ABSTRACT

Menaquinones (MKs) have been reported to induce apoptosis in rheumatoid arthritis (RA) synovial cells. Recently, menaquinone-4 (MK-4) was proven as a new potential agent for the treatment of RA. However, menaquinone-7 (MK-7) has greater bioavailability and efficacy than MK-4 after oral administration. Yet, the therapeutic benefits of MK-7 in the management of patients with RA have never been addressed. This study was designed to clarify the therapeutic role of MK-7 added to normal therapeutic regimen of RA in patients with different stages of the disease with a clinical follow up through a randomized clinical trial. In a cross sectional study, 84 RA patients (24 male, 60 female) (average age=47.2 years) were enrolled in this study. The patients were divided into MK-7 treated group (n=42) and MK-7 naïve group (n=42). MK-7 capsules were administered in a dose of 100 μg/day for three months in the first group without changing in other medications. The clinical and biochemical markers on RA patients treated with MK-7 and naïve group were assessed. In MK-7 treated group, serum concentrations of MK-7 were monitored before and after three months of MK-7 administration. In the cross sectional study, a significant decrease in MK-7 treated group for the levels of undercarboxylated osteocalcin (ucOC), erythrocyte sedimentation rate (ESR), disease activity score assessing 28 joints with ESR (DAS28-ESR), C-reactive protein (CRP) and matrix metalloproteinase (MMP-3) was found. In MK-7 treated group, a marked decrease in RA clinical and biochemical markers for moderate and good response compared to non-responders was observed in ucOC, ESR and DAS28-ESR. A marked increase in the levels of MK-7 for the moderate and good responders compared to non-responders was observed. The results suggest that MK-7 improves disease activity in RA patients. Therefore, MK-7 represents a new promising agent for RA in combination therapy with other disease modifying antirheumatic drugs.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting multiple small joints of the hands and feet (Firestein, 2003). Involvement of autoimmune system with the release of various inflammatory cytokines as interleukins (IL-1, IL-6 and IL-17) and tumor necrosis factor alpha (TNF-α) has been documented in pathogenesis of RA (Choy, 2012). The progressive destruction of these joints is mainly dependent on the evolution of hyperplastic synovial tissue, which in turns dependent on dysregulated proliferation and apoptosis (Qu et al., 1994). Methotrexate (MTX) is accepted as a standard therapy for RA and the mechanism of action of MTX is supposed to be its inhibitory effects on hyperplasia of synovial tissue. Therefore, any drug which could inhibit hyperplasia of synovial cells has a potential for promising anti-RA strategy (Okamoto, 2008).

Vitamin K is a generic term of compounds having a common 2-methyl-1,4-naphthoquinone nucleus and a variable alkyl substituent attached at the position 3, Vitamin K_1 (phylloquinone, PK) has a phytyl side chain and it is from plant origin, whereas vitamin K2 has a bacterial origin and it is represented by a group of analogs known as menaquinones-n (MK-n), where n represents the number of isoprenoid residues in the side chain (Suttie, 1993). Vitamins K not only works in blood coagulation, but also has a biochemical role in other organs as bone and cartilage (Kaneki et al., 2001).

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http://dx.doi.org/10.1016/j.ejphar.2015.06.014
0014-2999/

Many reports supported the possible role of vitamin K in bone diseases. It has been reported that osteoporosis and bone fractures frequently occurred after the long-term use of warfarin attributed to the inhibition of the effect of vitamin K-dependent factors during coagulation process (Liu et al., 1996). Vitamin K_2 consists of a family of related products known as menaquinones (MKs). These compounds are a series of chemical compounds (MK-1–MK-10) based on side chain chemical structure. It has been established that vitamin K has antitumor effects through its proapoptotic effects. The effect of vitamin K_2 (MK-4) on the proliferation of rheumatoid synovial cells and on the development of arthritis in the experimental model of collagen type II-induced arthritis (CIA) was studied. Results showed that MK-4 reduces the viability of synovial cells and thus be a novel treatment for RA (Okamoto et al., 2007). In addition, vitamin K_2 has been established to be useful in osteoporosis (Akiyama et al., 1994). The safety of vitamin K_2 (MK-4) in osteoporotic patients has been also reported (Hirao et al., 2008).

Recently, it was found that MK-4 reduced RA disease activity associated with a marked decrease in clinical and biochemical markers (Ebina et al., 2013 and Suzuki et al., 2013). Hence, MK-4 was recommended as a new agent for the treatment of RA either alone or in the setting of combination therapy with other disease modifying antirheumatic drugs. However, menaquinone-7 (MK-7) has greater bioavailability than menaquinone-4 after oral administration (Sato et al., 2012), the therapeutic utility of MK-7 in RA has never been addressed so far. Disease activity score 28 (DAS-28) using C-reactive protein underestimates disease activity and overestimates response criteria compared with DAS-28 using erythrocyte sedimentation rate (Matsui et al., 2007). Therefore, the present study was designed to investigate in a randomized clinical trial the influence of oral administration of MK-7 added to conventional antirheumatic therapy in cross-sectional study on clinical and biochemical metabolic parameters for RA in a prospective study. In addition, serum concentration of MK-7 pre- and post-treatment was assessed in RA patients.

2. Materials and methods

Eighty four RA patients (24 male and 60 female) diagnosed according to the 1987 revised American College of Rheumatology (ACR) criteria were enrolled in this cross sectional prospective study (Arnett et al., 1988). Demographic data of the patients were as follows; the mean age of patients was 47.2 ± 12.2 years old, and the mean disease duration was 9.8 ± 5.5 years. Methotrexate (MTX) was administered to 79.8% of patients (67 patients) at a dose of 7.5 mg/week at least for 3 months. The concomitant use of NSAIDs, immunosuppressive drug (leflunomide) and prednisolone was permitted provided that dosing was stable for at least 4 weeks before study. Among these 84 patients, 42 patients were treated with oral MK-7 in a daily dose of 100 μg in a soft gel capsule dosage form (Swanson Ultra, ND, USA) and the other 42 patients were taken as a control group without MK-7; both the groups were given conventional anti-rheumatic drugs (Table 1). Cross sectional study was selected to investigate the influence of MK-7 on rheumatoid activity for the following reasons (Hennekens and Buring, 1987): relatively quick and easy to conduct (no long periods of follow-up), multiple outcomes and exposures can be studied, able to measure prevalence for all factors under investigation as well as good for descriptive analyses and for generating hypotheses. The safety of MK-7 was recently studied by Pucaj et al. (2011) and proved to be safe with LD_{50} above 2000 mg/kg. The dose used in this study was 100 μg/day. In the cross sectional study, MK-7 treated group was compared with non-treated group (MK-7-naïve group). The clinical and biochemical parameters for bone health were assessed pre-treatment and post-treatment (at week 12) such as rheumatoid factor (RF), intact osteocalcin (OC), undercarboxylated osteocalcin (ucOC), matrix metalloproteinase-3 (MMP-3), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP), and disease activity score assessing 28 joints was calculated with ESR (DAS28-ESR). ESR and routine hematological counts were performed each month. This study was carried out between January, 2014 and January, 2015 on Egyptian population in Assiut University Hospitals. Patients treated with warfarin (vitamin K antagonist) were excluded from this study. The clinical effects of MK-7 on RA disease activity were assessed by DAS28-ESR as previously described (Nielung et al., 2015). Based on this evaluation, patients with an improvement in DAS28-ESR of ≤ 0.6 or a final DAS28-ESR > 5.1 were considered as non-responders. However, patients with an improvement in DAS28-ESR of > 1.2 and a final DAS28-ESR ≤ 3.2 were considered to have a good response. Differences in variables between MK-7-naïve group and MK-7-treated group were matched after 3 months of menaquinone-7 administration. Hands and feet radiographs were evaluated and scored using the Larsen method (Larsen et al., 1977). Radiographs of the hands and feet were available for all patients, and were estimated separately without knowledge of the identity of the patients. A blind consensus result was given when necessary. Serum ucOC levels were measured according to previous report by electrochemiluminescence immunoassay (ECLIA) (Sanko Junyaku, Co., Ltd., Tokyo, Japan) (Sokoll et al., 1995). Serum intact osteocalcin (intact OC) was also measured by enzyme immunoassay (EIA) (Mitsubishi Yuka, Tokyo, Japan) according to reported methodology (Merle and Delmas, 1990). Serum MMP-3 was measured by an enzyme immunoassay (EIA) system using Stromelysin-1-Kit (Fuji chemical Industries, Ltd., Japan) (Obata et al., 1992). All the patients were followed up at Assiut University Hospitals, and a written consent was obtained from each patient. The protocol of this study was approved by the Ethical Committee

Table 1
Biochemical characteristics of RA in MK-7-naive and MK-7-treated groups after 3 months of treatment (cross-sectional study).

	MK-7-naive group ($n=42$)	MK-7-treated group ($n=42$)	P value[a]
Age (years)	50.2 ± 10.8	44.2 ± 12.2	NS
Duration of disease (years)	9.8 ± 5.5	9.5 ± 6.2	NS
Body mass index (kg/m^2)	29.6 ± 1.5	26.8 ± 1.2	NS
RF positivity (% patients)	92.9	90.5	NS
Intact OC (ng/ml)	7.2 ± 0.5	8.2 ± 0.6	NS
ucOC (ng/ml)	4.7 ± 0.4	2.1 ± 0.5	0.01
MMP-3 (ng/ml)	210.4 ± 25.3	155.3 ± 12.6	0.01
CRP (mg/dl)	2.2 ± 0.4	1.5 ± 0.3	0.01
ESR (mm/h)	45.9 ± 19.7	26.1 ± 11.5	0.001
DAS28-ESR	5.38 ± 0.78	3.18 ± 0.65	0.001
MTX dose (mg/week)	7.5	7.5	NS
MTX usage (% patients)	83.3	78.5	NS
PSL dose (mg/week)	15	15	NS
PSL usage (% patients)	9.5	7.1	NS
NSAIDs dose (mg/day)	50	50	NS
NSAIDs usage (% patients)	23.8	21.4	NS
Leflunamide (mg/day)	20	20	NS
Leflunamide (% patients)	14.3	11.9	NS
MK-7 dose (μg/day)	–	100	–
MK-7 usage (% patients)	–	100	–
MK-7 serum conc. (ng/ml)	0.75 ± 0.56	5.8 ± 1.42	0.001

Data are mean ± S.E.M.
NS: not significant, RF: rheumatoid factor, OC: Osteocalcin, ucOC: undercarboxylated osteocalcin, MMP-3: matrix metalloproteinase, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, DAS28-ESR: disease activity score assessing 28 joints with ESR, MTX: methotrexate, PSL: prednisolone, NSAIDs: nonsteroidal anti-inflammatory drugs (diclofenac sodium), MK-7: menaquinone-7 capsules.

[a] Determined by Mann–Whitney test by SPSS program.