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ARTEMETHER SUPPRESSES MIGRATION AND INVASION OF FIBROBLAST-LIKE SYNOVIOCYTES THROUGH INHIBITION OF THE RHO SIGNALING PATHWAY IN RHEUMATOID ARTHRITIS

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Career situation of first and presenting author Student for a master or a PhD.

Introduction Experiments on collagen-induced arthritis rats showed that artemether, a new antimalarial drug derived from artemisinin, can reduce inflammatory cell infiltration, tissue edema, and bone erosion in the paws, which implied its potential efficacy in rheumatoid arthritis (RA). Fibroblast-like synoviocytes (FLS) in RA manifest tumor-like properties including increased adherence and invasiveness of adjacent cartilage and bone, resulting in joint destruction. However, effects of artemether on the aggressive properties of RA-FLS have not yet been elucidated.

Objectives The aim of this study was to investigate the role and underlying mechanism of artemether on the migration and invasion of RA-FLS.

Methods Synovial tissues were obtained by closed needle biopsy from 6 active RA patients and FLS were isolated and cultured *in vitro*. RA-FLS were treated with artemether at various concentrations, while methotrexate (MTX) and hydroxychloroquine (HCQ) were employed as controls. Cell viability, proliferation, cell-cycle, apoptosis, migration, invasion, and pseudopodium formation of RA-FLS were assessed by CCK-8 assays, EdU staining, Annexin V-FITC/PI staining, transwell assays, or F-actin staining respectively. The protein expression of RhoA, Rac1, and CDC42 were measured by Western blot.

Results

1. The IC₅₀ value of artemether, MTX and HCQ on RA-FLS were 988 μM, 181 nM and 5433 μM, respectively. Compared with untreated group, IC₅₀-20μM of artemether, IC₅₀-10nM of MTX and IC₅₀-20μM of HCQ showed no effect on the proliferation, cell-cycle or apoptosis of RA-FLS.
2. The transwell assays of migration and invasion showed that artemether (20 μM) significantly inhibited migration and invasion of RA-FLS and reduced lamellipodia and filopodia formations. Under the same IC₅₀ concentration, artemether (20 μM) showed similar inhibitory effects as MTX (10 nM), while HCQ (20 μM) showed no effect on migration and invasion of RA-FLS.
3. Rho family proteins play important roles in modulating cell migration and invasion. Western blot analysis showed that artemether (20 μM) and MTX (10 nM) significantly reduced the expression of RhoA, Rac1, and CDC42 in RA-FLS, which indicated that artemether could inhibit the Rho signaling pathway.

Conclusions Artemether can suppress the migration and invasion capability of RA-FLS through inhibiting the Rho signaling pathway.

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Disclosure of Interest None declared.

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CHEMOKINE CCL18 ENRICHED IN SYNOVIAL FLUID IS INVOLVED IN JOINT DESTRUCTION THROUGH PROMOTING MIGRATION OF FIBROBLAST-LIKE SYNOVIOCYTES IN RHEUMATOID ARTHRITIS

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Career situation of first and presenting author Young investigator.

Introduction CC chemokine ligand 18 (CCL18) which is either constitutively expressed or induced in monocytes/macrophages and dendritic cells has been reported to be highly expressed in peripheral blood and synovial fluid of rheumatoid arthritis (RA) patients compared with healthy controls, indicating the possible role of CCL18 in the development and pathogenesis of RA.

Objectives To explore the association of serum and synovial fluid CCL18 with clinical and radiographic outcome in RA and its potential effect on RA fibroblast-like synoviocytes (FLS).

Methods Consecutive patients with active RA (DAS28-CRP>2.6) were recruited. Synovial fluid was collected from inflamed joints if available. Demographic and clinical data were collected according to the 2017 EULAR recommendation. Serum and synovial fluid CCL18 was detected by ELISA. RA-FLS was cultured *in vitro* with RA synovial fluid and neutralizing antibody to CCL18. Migration/invasion ability was analyzed by Transwell assay.

Results

1. Among 83 RA patients, age (median and IQR, similarly hereinafter) was 50 (41~58) years old and 65 patients (78%) were female, with median disease duration 36 (12~102) months, median DAS28-CRP 5.0 (4.6~6.1).
2. Serum CCL18 was 107 (80~126) ng/mL, which was significantly higher than healthy controls [n=25, 51 (29~70) ng/mL, p<0.001]. Serum CCL18 correlated slightly but significantly with CRP (r=0.385, p<0.001), ESR (r=0.239, p=0.03), PrGA (r=0.249, p=0.03), DAS28-CRP (r=0.368, p=0.001), DAS28-ESR (r=0.336, p=0.003), SDAI (r=0.360, p=0.001), CDAI (r=0.328, p=0.004) and HAQ (r=0.325, p=0.004).
3. Synovial fluid CCL18 of 31 patients was 719 (415~1271) ng/mL, which was significantly higher than corresponding serum level (Paired test, p<0.001). Among them, 13 patients who were treated according to treat to target strategy received X-ray assessment of hand/wrist both at baseline and month 12. Six patients who had one-year radiographic progression (a change of the Sharp/van der Heijde modified sharp score ≥0.5 units) showed higher synovial fluid CCL18 than other 7 patients without radiographic progression [1481 (1244~2034) ng/mL vs. 458 (405~681) ng/mL, p=0.004].
4. When incubated with RA synovial fluid, the migration ability of RA-FLS was significantly increased; but this effect was inhibited by neutralizing antibody to CCL18.

Conclusions CCL18 elevates especially in synovial fluid of RA patients, which may correlate with one-year radiographic progression through promoting migration of RA-FLS.

Disclosure of Interest None declared.