**SUPPLEMENTAL MATERIAL**

**Therapeutic value of curcumin on the initiation and development of inflammation in Takayasu’s arteritis mediated by HSP65-induced CCL2 overexpression**

**Running Title:** Curcumin inhibits HSP65-induced CCL2 in TAK

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**Supplementary Figures**

**Supplementary Figure 1.** Co-expression of HSP65, IL-6, and IL-1β, in the aortic adventitia of TAK patients (n=8) and healthy control (n=6). **A,** Double-labelled immunofluorescence images of HSP65 and IL-6. **B,** Double-labelled immunofluorescence images of HSP65 and IL-1β. **C,** Correlation analysis of the expression of HSP65 (n = 8), IL-6 (r = 0.1145; p = 0.5775), and IL-1β (r = 0.2983; p = 0.1611). Spearman and Pearson correlation analysis were performed respectively.

**Supplementary Figure 2. A-C,** The level of JAK1 and JAK3 phosphorylation at 0, 15, 30, 60, 90, and 120 min following HSP65 (1 μg/mL) stimulation. Error bar represents SD. All experiments have been performed in triplicate.

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**Supplementary Figure 3. A,** The protein levels of pro-IL-1β and mature IL-1β in AAFs following stimulation with HSP65 (1 μg/mL), LPS (10 ng/mL), or HSP65 combined with Resatorvid (10 μM) for 12 h. **B,** The level of protein expression of IL-1β in the AAFs culture supernatants following stimulation with HSP65 alone or HSP65 combined with curcumin (CC), AG490, Tofacitinib, and MTX for 12 h. All experiments have been performed in triplicate

**Supplementary Tables**

**Supplementary Table I.** Clinical characteristics of the enrolled patients in IHC and IF experiments (n=8).

|  |  |
| --- | --- |
| Clinical characteristic | TAK patients (n=8) |
| Male | 7 (87.50%) |
| Age | 51.14±9.52 |
| ESR (mm/H) | 49.29±37.17 |
| CRP (mg/L) | 21.10±14.27 |
| Kerr score | 2-3 (median:2) |
| Imaging classification |  |
| IIa (n, %) | 8 (100.00%) |
| Reason for surgical treatment (n, %) |  |
| aortic aneurysm (n, %) | 2 (25.00%) |
| aortic regurgitation (n, %) | 3 (37.50%) |
| aortic dilatation and aortic regurgitation (n, %) | 2 (25.00%) |
| aortic aneurysm and aortic regurgitation (n, %) | 1 (12.50%) |

\* ESR, Erythrocyte sedimentation rate; †CRP, C-reactive protein.

**Supplementary Table II.** Clinical characteristics of the enrolled patients in curcumin treatment study (n=16).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameters | Curcumin treatment (7.5g bid, n=16) | |  |  |
| Baseline | 3months later | Baseline-3months later | P value |
| Demographics |  |  |  |  |
| Sex (female, %) | 15(93.8%) | | |  |
| Age (year) | 30.44±4.94 | | |  |
| Imaging classification |  | | |  |
| I (n, %) | 2 (12.5%) | | |  |
| IIa (n, %) | 1 (6.3%) | | |  |
| IIb (n, %) | 3 (18.8%) | | |  |
| III (n, %) | 0 (0%) | | |  |
| IV (n, %) | 2 (12.5%) | | |  |
| V (n, %) | 8 (50%) | | |  |
| Clinical manifestation |  |  |  |  |
| Absent/diminished pulse (n, %) | 2 (12.5%) | 2 (12.5%) |  |  |
| Hypertension (n, %) | 6 (37.5%) | 6 (37.5%) |  |  |
| Bruits (n, %) | 3 (18.8%) | 3 (18.8%) |  |  |
| Asymmetric hypertension (n, %) | 5 (31.3%) | 4 (31.3%) |  |  |
| Laboratory tests |  |  |  |  |
| Hb (g/L) | 123.563±19.43 | 120.438±17.74 | 3.13±13.41 | 0.18 |
| WBC (×109/L) | 8.89±2.21 | 9.46±1.97 | -0.58±1.96 | 0.13 |
| PLT (×109/L) | 262.31±72.13 | 254.44±82.06 | 7.88±64.14 | 0.32 |
| ESR (mm/H) | 17.88±17.22 | 20.13±18.14 | -2.25±17.53 | 0.49 |
| CRP (mg/L) | 6.90±7.67 | 14.45±22.77 | -7.56±22.29 | 0.14 |
| Treatment |  |  |  |  |
| GC dosage (mg) | 13.38±7.00 | 13.72±5.68 | -0.35±4.23 | 0.50 |
| Other IS agents | Tacrolimus (1); LEF (8); AZA (2); MMF (2); Rapamycin (2); HCQ (2) | Tacrolimus (1); LEF (5); AZA (1); MMF (2); Rapamycin (2); HCQ (2) |  |  |
| Disease activity index |  |  |  |  |
| Kerr score≥1 (n, %) | 11(68.6%) | 5 (31.2%) |  | **0.02** |
| Kerr score≥2 (n, %) | 6 (37.5%) | 3 (18.8%) |  | 0.19 |
| Serum detection |  |  |  |  |
| CCL2 (pg/ml) \* | 52.42±34.41 | 49.66±16.58 | 12.75±27.89 | **0.04** |
| IL-6 (pg/ml) | 38.01±11.61 | 35.27±8.64 | 2.74±11.52 | 0.18 |
| IL-1β (pg/ml) | 15.28±3.26 | 16.80±13.89 | -1.52±13.71 | 0.13 |

\* Hb, Hemoglobin; WBC, White blood cell; PLT, Platelets; ESR, Erythrocyte sedimentation rate; CRP, C-reactive protein. † LEF, Leflunomide; AZA, Azathioprine; MMF, Morphine mycophenolate; HCQ, Hydroxychloroquine.

‡ Paired t test (For data that conform to normal distribution) or Wilcoxon test (For data that did not conform to normal distribution) were used to analyze the differences of indicators of patients before and after curcumin treatment. Error bar represents SD