Bone & Joint Research

Supplementary Material

10.1302/2046-3758.104.BJR-2020-0085.R2

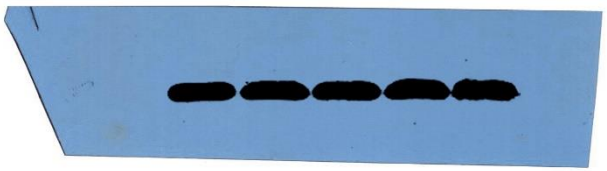

Fig. aa. Original Kodak film of total protein: p65.

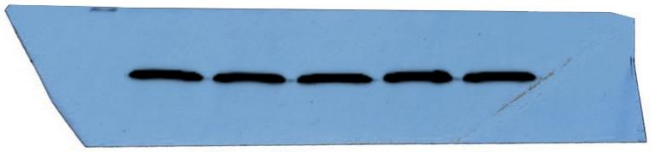

Fig. ab. Original Kodak film of total protein: glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

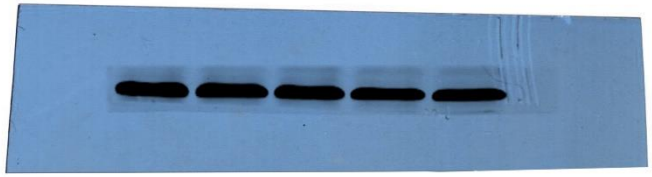

Fig. ac. Original Kodak film of total protein: p65.

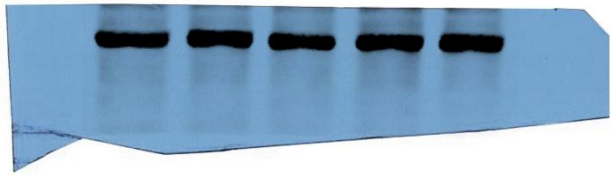

Fig. ad. Original Kodak film of total protein: GAPDH.

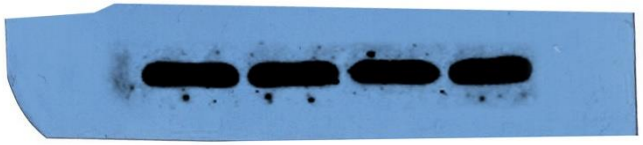

Fig. ae. Original Kodak film of total protein: p65.

Fig. af. Original Kodak film of total protein: GAPDH.

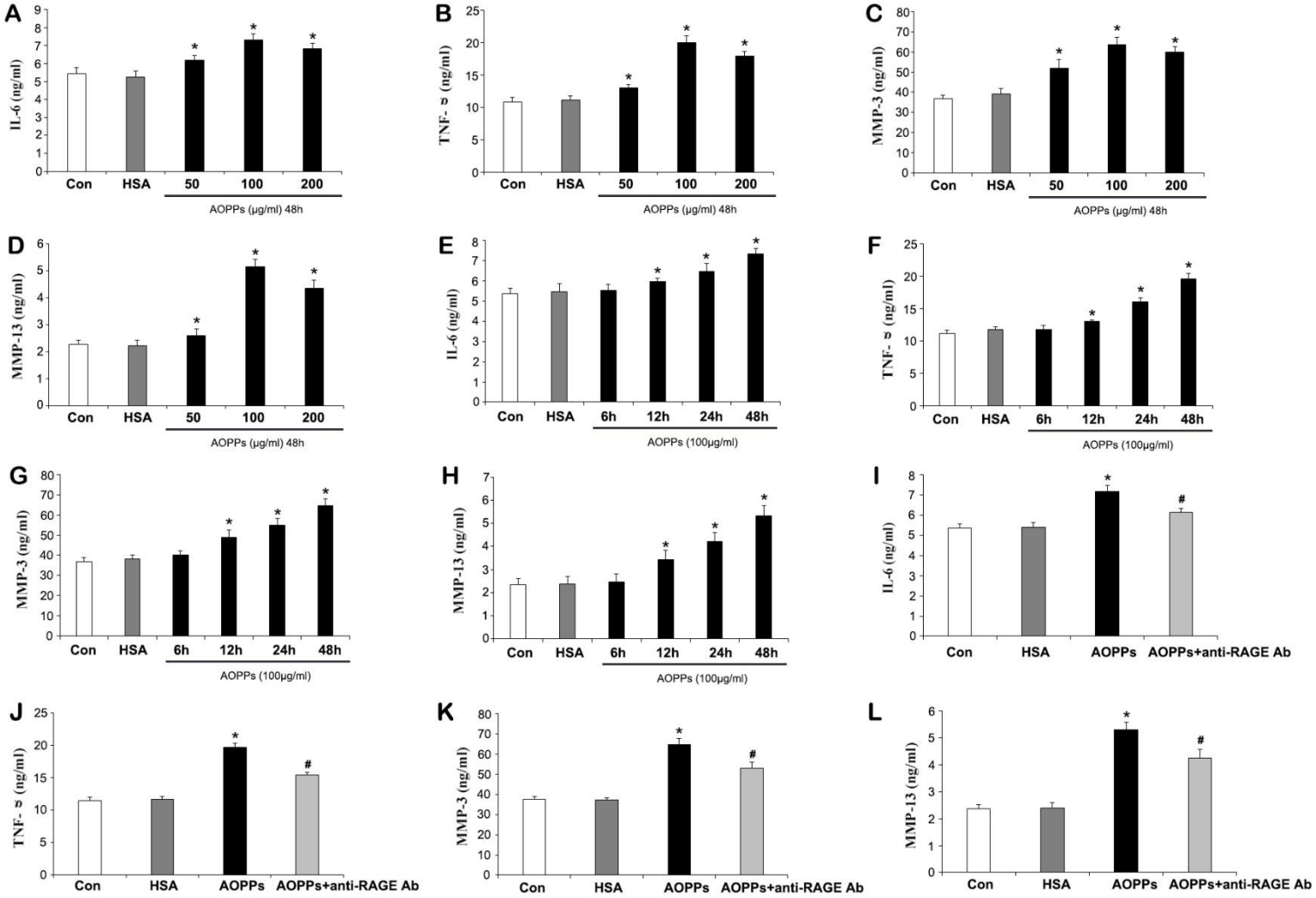
A
IL-6 (ng/ml)
Con
HSA
50
100
200
AOPPs (μg/ml) 48h
B
TNF-α (ng/ml)
Con
HSA
50
100
200
AOPPs (μg/ml) 48h
C
MMP-3 (ng/ml)
Con
HSA
50
100
200
AOPPs (μg/ml) 48h
D
MMP-13 (ng/ml)
Con
HSA
50
100
200
AOPPs (μg/ml) 48h
E
IL-6 (ng/ml)
Con
HSA
6h
12h
24h
48h
AOPPs (100μg/ml)
F
TNF-α (ng/ml)
Con
HSA
6h
12h
24h
48h
AOPPs (100μg/ml)
G
MMP-3 (ng/ml)
Con
HSA
6h
12h
24h
48h
AOPPs (100μg/ml)
H
MMP-13 (ng/ml)
Con
HSA
6h
12h
24h
48h
AOPPs (100μg/ml)
I
IL-6 (ng/ml)
Con
HSA
AOPPs
AOPPs+anti-RAGE Ab
J
TNF-α (ng/ml)
Con
HSA
AOPPs
AOPPs+anti-RAGE Ab
K
MMP-3 (ng/ml)
Con
HSA
AOPPs
AOPPs+anti-RAGE Ab
L
MMP-13 (ng/ml)
Con
HSA
AOPPs
AOPPs+anti-RAGE Ab

Fig. b. Interleukin-6 (IL-6), tumour necrosis factor alpha (TNFα), and matrix metalloproteinase (MMP) production and secretion in response to advanced oxidation protein products (AOPPs) treatment were quantified with enzyme-linked immunoabsorbent assay (ELISA) kits. a) to d) Cultured rheumatoid arthritis fibroblast-like synoviocytes (RA-FLSs) were incubated with the indicated concentrations of AOPP-human serum albumin (HSA), medium alone (control), or native HSA for 48 hours. e) to h) Cultured RA-FLSs were incubated for the indicated times with AOPP-HSA (100 μg/ml), medium alone (control), or native HSA (100 μg/ml). i) to l) Cultured RA-FLSs were incubated with medium alone (control), native HSA, AOPP-HSA (100 μg/ml), or AOPP-HSA (100 μg/ml) with a neutralizing antibody against receptor for advanced glycation end products (RAGE) (antiRAGE Ab, 10 μg/ml) for 48 hours. AOPPs

treatment increased the expression levels of IL-6, TNFα, and MMP in dose- (a) to d)) and time-dependent (e) to h)) manners.

Pretreatment with the antibody against RAGE effectively suppressed IL-6, TNFα, and MMP production (i) to l)). Data are presented as

the mean and standard deviation of triplicates. *$p < 0.05$ versus medium alone (control). #$p < 0.05$ versus the AOPP group.

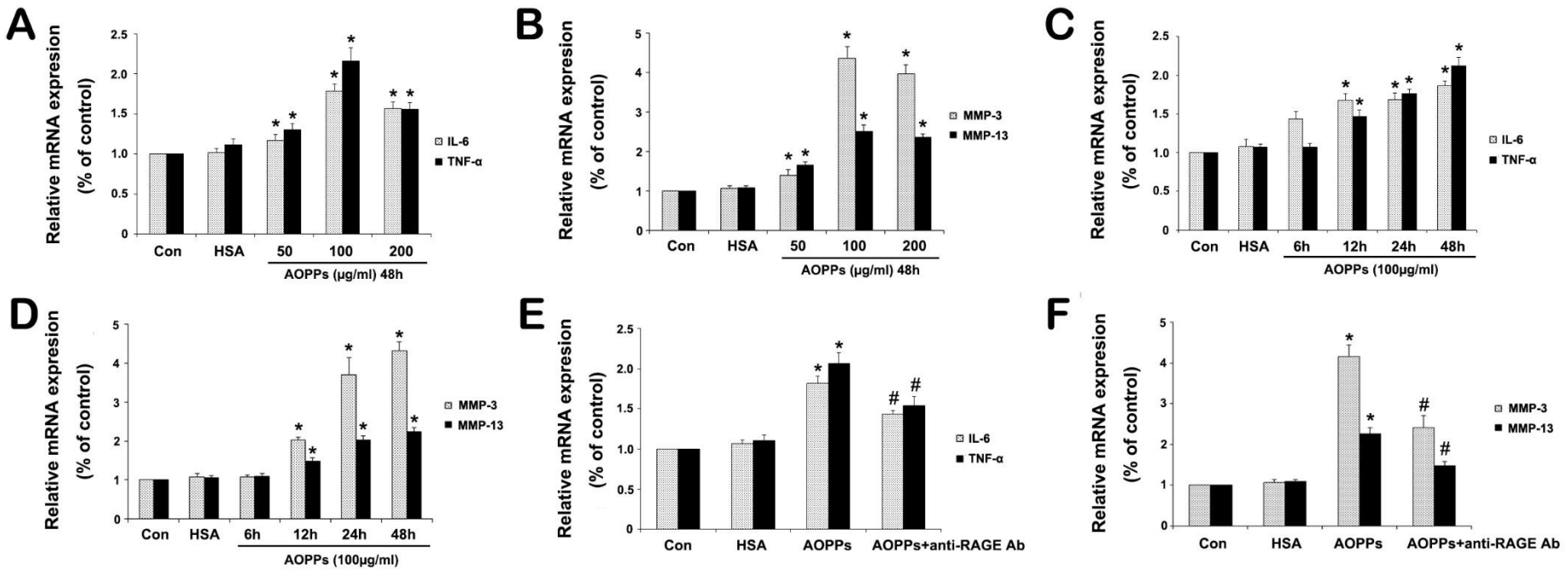

Fig. c. Advanced oxidation protein products (AOPPs) challenge increased the messenger RNA (mRNA) expression levels of interleukin-

6 (IL-6), tumour necrosis factor alpha (TNFα), and matrix metalloproteinases (MMPs) in rheumatoid arthritis fibroblast-like

synoviocytes (RA-FLSs). a) and b) Cultured RA-FLSs were incubated with the indicated concentrations of AOPP-human serum albumin (HSA), medium alone (control), or native HSA for 48 hours. c) and d) Cultured RA-FLSs were incubated for the indicated times with AOPP-HSA (100 μg/ml), medium alone (control), or native HSA (100 μg/ml). e) and f) Cultured RA-FLSs were incubated with medium alone (control), native HSA, AOPP-HSA (100 μg/ml), or AOPP-HSA (100 μg/ml) with a neutralizing antibody against receptor for advanced glycation end products (RAGE) (anti-RAGE Ab, 10 μg/ml) for 48 hours. The mRNA expression levels of IL-6, TNFα, and MMPs increased in dose-dependent (a) and b)) and time-dependent (c) and d)) manners. Pretreatment with the antibody against RAGE effectively suppressed the mRNA expression increases (e) and f)). Data are presented as the mean and standard deviation of

triplicates. *$p < 0.05$ versus medium alone (control). #$p < 0.05$ versus the AOPP group.