primary synovial fluid cells were collected at the time of joint replacement and cultured with CS (200 μg/ml) and GLU (200 μg/ml), singly or in combination, with the addition of LPS and HA (n=2/group). After normalisation for cell viability, all results were expressed as fold change from the negative control (media only). One-way ANOVA with Dunnett’s post-hoc test was performed using GraphPad Prism.

**Results:** CS and GLU in combination (200 μg/ml of each) significantly reduced NF-κB activity by 70% compared to the control group (LPS/HA only). Although CS (200 μg/ml) alone did not reduce NF-κB activity, the addition of the lower concentration of CS (10–50 μg/ml) to GLU (200 μg/ml) significantly reduced NF-κB activity compared with GLU (200 μg/ml) alone. Addition of lower concentrations of GLU (10–50 μg/ml) to CS (200 μg/ml) modestly reduced NF-κB activity (Fig 1). Similar trends were observed in secreted pro-inflammatory cytokines (IL-1β, IL-6, IFN-γ, and TNF-α): namely, CS and GLU in combination significantly attenuated the LPS/HA mediated pro-inflammatory responses (p<0.05) (Fig 1). Although, a diverse range of inflammatory responses to the LPS/HA activation was observed, constitutive pro-inflammatory cytokine production by primary synovial fluid cells was reduced by the combination of CS and GLU.

**Conclusions:** Inflammatory reactions of THP-1 macrophages, induced by physiologically relevant concentrations of LPS and HA fragments, were suppressed synergistically by the combination of physiologically achievable concentrations of CS and GLU. A similar trend was observed in primary synovial fluid cells but further investigations are required. These data could explain, at least in part, the clinical efficacy of CS and GLU in combination observed in OA patients.

**REFERENCE:**


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**EVALUATION OF ANTI-INFLAMMATORY EFFECTS OF NAPROXEN SODIUM ON HUMAN OSTEARTHRITIS SYNOVIAL CELLS**

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**Background:** Inflammation is increasingly recognised as an essential factor in the pathogenesis and progression of osteoarthritis (OA). Etafiltralide imaging for activated macrophage quantification in knee joints confirms a high prevalence (~70%–80%) of joint inflammation in association with OA. Naproxen sodium is a non-steroidal anti-inflammatory drug that is widely available over-the-counter (OTC) and has been shown to be effective in different pain models including OA.

**Objectives:** Evaluate the ability of naproxen sodium to block the inflammatory responses and to reduce the activated inflammatory responses of a human monocyte cell line and primary human synovial fluid (SF) cells in vitro.

**Methods:** The immortalised human monocyctic cell line, THP-1, was grown and differentiated into mature macrophages using lipopolysaccharide (LPS) and hyaluronic acid (HA) fragments (n=8/group). After a further 24 hours, the cell culture supernatants were assessed for NF-κB activity, pro-inflammatory cytokines (IL-1β, IL-6, IFN-γ, and TNF-α), and prostaglandin E2 (PGE2). Cell viability was assessed using PrestoBlue reagent. Primary human SF cells were collected at the time of knee joint replacement for OA and treated 24 hours with naproxen sodium with and without the addition of LPS/HA (n=2/group). All results were expressed as fold change from the negative control (media only) after normalisation for cell viability. One-way ANOVA with Dunnett’s post-hoc tests were done using GraphPad Prism.

**Results:** Compared to the placebo group, NF-κB activity of THP-1 cells was significantly reduced by as little as 28.9 mg/L naproxen sodium (corresponding to a trough plasma concentration achieved by a daily oral dose of 55 mg naproxen sodium) when added before or after the activation by LPS/HA (84% and 78% NF-κB activity reduction, respectively) (Fig 1). When cells were treated before the activation with 33 mg/L naproxen sodium (corresponding to the concentration achieved by a 220 mg daily OTC dose), NF-κB activity was reduced 79% and IL-6 secretion was reduced by 77%. Cyclooxygenase enzyme activity, represented by PGE2 production, was reduced to basal levels by as little as 28.2 mg/L naproxen sodium (p<0.05) when cells were treated either before or after the activation. Primary human SF cells treated with LPS/HA showed a striking increase in cytokine secretion ranging from 50-fold (IL-8) to 600-fold (IL-6). Cytokine production was reduced by naproxen sodium but a rebound phenomenon was observed for the highest concentration of 55 mg/L, which may indicate the cell stress response.

**Conclusions:** Naproxen sodium at low dose can both prevent and reduce inflammatory responses of a human monocyctic cell line and primary human SF cells in vitro. These results highlight the potent activity of the OTC dose of naproxen sodium to dramatically reduce PGE2, NF-κB activity and cytokine production.

**REFERENCES:**

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