AND

Carrageenan-induced thermal inflammatory processes, has been recognized as a predictor of cardiovascular risk. 

Results: Since gemcabene reduced hCRP in humans, we investigated the mechanism of hCRP reduction and efficacy of anti-inflammatory activity in animal models of arthritis and pain. In human hepatoma cell line, PLC/PY75, gemcabene showed dose-dependent inhibition of IL-6 and IL-1ß-induced hCRP production by -70% inhibition at 2 ìM. In TNF-α-stimulated primary human coronary artery endothelial cells, both IL-6 and IL-1ß productions were inhibited by gemcabene in a dose-dependent manner (70% at 2NM). Transfection studies with human CRP regulatory sequences in human embryonic kidney 2 cell showed a 25-fold increase in IL-6 and IL-1ß stimulated CRP transcription, which was reduced by gemcabene (50%) at 2 ìM, suggesting transcriptional down-regulation of CRP. Site-directed mutation of CEBP, NF-κB, and STAT sites of the human CRP promoter suggested that the overlapping downstream CEBP and NF-κB binding sites are important for gemcabene-mediated down-regulation of CRP promoter. STAT3 response element, while needed for IL-6-induced expression of CRP, is not required for gemcabene-mediated inhibition. Identification of the protein, in a gel-shift assay, that interacts with CEBP binding sites revealed it to be CEBPβ, Anti-inflammatory efficacy of gemcabene was evaluated in a rat model of monosodium iodoacetate (MIA)-induced osteoarthritis (OA) and carrageenan-induced thermal hyperalgesia (CTH). Gemcabene improved joint score (50% at 30 mg/kg/d for 2 wk) in MIA and attenuated paw withdrawal latency (60% at 30 mg/kg/d and 97% at 100 mg/kg/d, compared to untreated control) in the CTH model. These findings were further confirmed by an IL-6-64k lane migration study showing 63% and 71% reduction in hind paw withdrawal at 10 and 30 mg/kg/d, respectively. Gemcabene decreases CRP by CEBPβ and NF-κB mediated transcriptional mechanism, and attenuates inflammation-induced OA and hyperalgesia.

INTRODUCTION

A number of studies for the past 15 years suggest that atherosclerosis, the main cause of coronary artery disease (CAD), is an inflammatory disease in which inflammation plays a key role in setting the stage as well as causing the progression of atherosclerosis (1). C-reactive protein (CRP), an acute phase reactant released during inflammatory processes, has been recognized as a predictor of cardiovascular risk. 

Background

Atherosclerosis occurs via inflammatory and immune responses that occur in response to injury and remodelling of the arterial wall, with accumulation of lipids in the arterial intima. Patients with elevated CRP levels have a higher risk of cardiovascular disease (CVD) (2). The C-reaktive protein (CRP), an acute phase reactant released during inflammatory processes, has been recognized as a predictor of cardiovascular risk.

HYPOTHESES

1. Gemcabene lowers CRP in humans by a mechanism that involves direct effect on CRP gene regulation leading to reduced secretion.

2. The anti-inflammatory effect of gemcabene attenuates osteoarthritis and hyperalgesia in animal disease models.

EXPERIMENTAL DESIGN AND ANALYSES

Cell-based Studies: Cell-based studies in PLC/PY75, HepG2, and HCAEC cells were carried out to investigate gemcabene-mediated inhibition of CRP secretion. This was carried out both in the uninduced and proinflammatory cytokine-induced conditions. Cytokine-treated (100 ng/ml IL-6 and 10 ng/ml IL-1ß) HepG2 and HCAEC cells were exposed to varying concentrations of gemcabene. After 24 hours, the production of CRP was determined. The dose-dependent inhibition of CRP was determined.

Figure 4: Effect of Gemcabene on MIA-induced changes in hind paw withdrawal.

CONCLUSIONS & CLINICAL IMPLICATIONS

• Gemcabene inhibits cytokine-induced CRP secretion in hepatoma and endothelial cells, and IL-6 and IL-1ß-induced human CRP transcription in hepatoma cell line.

• Gemcabene inhibits IL-6-induced CRP promoter activity via the CRP protein binding site located from -182 to -150.

• The NF-κB site overlapping the downstream CEBP may be involved in the gemcabene- and human CRP promoter activity.

• In these studies, gemcabene had the ability to induce IL-6- and IL-1ß-induced CRP promoter activity, but failed to influence gemcabene-induced inhibition.

• The NF-κB site overlapping the downstream CEBP may be involved in the gemcabene- and human CRP promoter activity.

• The NF-κB site overlapping the downstream CEBP may be involved in the gemcabene- and human CRP promoter activity.

• Gemcabene attenuated MIA-induced, IL-6-64k, collagen, and carrageenan-injured osteoarthritis and hyperalgesia in animal models.

REFERENCES