

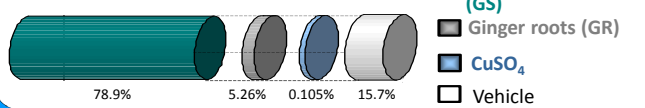
# DIFFERENTIAL IMPACT OF GLUCOSAMINE SULFATE AND CUIVRAMINE® ON THE IL-1 $\beta$ STIMULATED C-20/A4 CHONDROCYTE CELL LINE

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## INTRODUCTION:

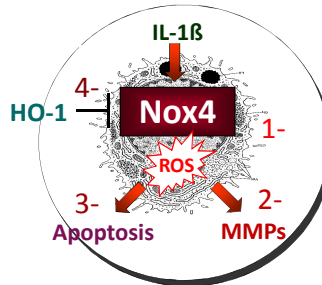
IL-1 $\beta$  activates reactive oxygen species (ROS) production, release of MMPs and chondrocyte apoptosis which leads to matrix breakdown and osteoarthritis. The ROS generating NADPH oxidase 4 (Nox4) could play a central role in this pathway (Grange et al, 2006). Nox4 activity is regulated by heme oxygenase-1 (HO-1) (Rousset et al, in preparation).

### Composition of Cuivramine (CA)



Glucosamine sulfate (GS) is approved as a symptomatic slow-acting drug for osteoarthritis. GS impact on structural features of OA is however modest. To go further, Cuivramine® (CA), a new dietary supplement has been developed. The aim of the study is to compare the effects of CA and GS on IL-1 $\beta$  stimulated C-20/A4 chondrocytes.

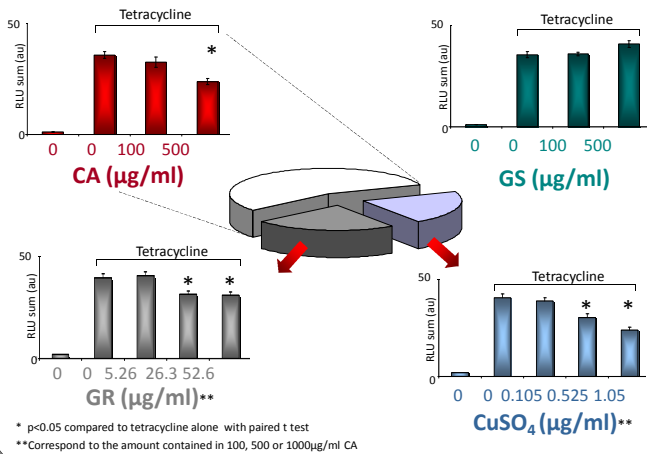
## Potential targets of CA on C-20/A4 chondrocytes



- 1- Nox4 activity
- 2- MMP/ADAMTS
- 3- Apoptosis
- 4- HO-1 expression

## 1- Nox4 Activity

Effects of CA and its components were assessed after 96h incubation on Nox4 activity with the tetracycline inducible HEK293 TREX\_No<sub>4</sub> cells (Serrander et al, 2007).

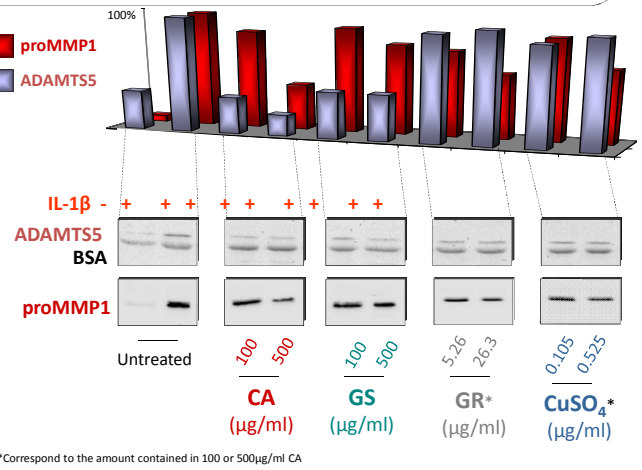


\* p<0.05 compared to tetracycline alone with paired t test  
 \*\* Correspond to the amount contained in 100, 500 or 1000μg/ml CA

**CA displays an indirect antioxidant effect dependant on Copper and Ginger**

## 2- MMP secretion

Impact of CA and its components on IL-1 $\beta$ -induced proMMP1 and ADAMT5 secretion by C-20/A4 chondrocytes. Western blot densitometry is normalized to BSA.

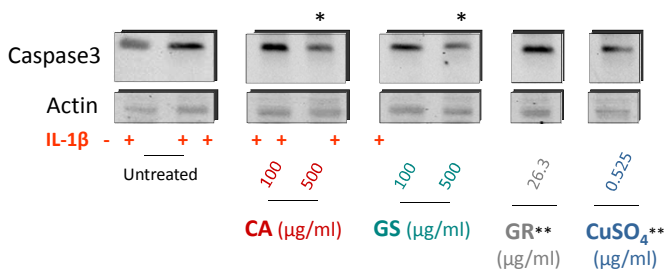


\*Correspond to the amount contained in 100 or 500μg/ml CA

**CA decreases ADAMT5 secretion via GS and MMP1 secretion via Copper and Ginger**

## 3- Apoptosis

Impact of 96h incubation with CA and its components on IL-1 $\beta$ -induced caspase 3 activation on the C-20/A4 chondrocytes cell line.

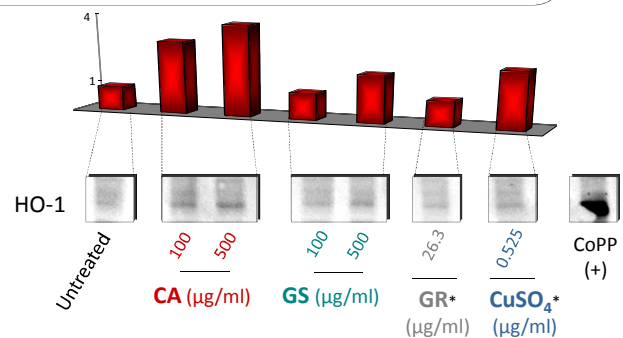


\* p<0.05 compared to IL-1 $\beta$  alone with paired t test  
 \*\* Correspond to the amount contained in 500μg/ml CA

**CA and GS reduce IL-1 $\beta$ -induced caspase 3 activation**

## 4- HO-1 expression

Impact of CA and its components on HO-1 expression by C-20/A4 chondrocytes after 96h incubation. CoPP is the positive control of HO-1 induction.



\*Correspond to the amount contained in 500μg/ml CA

**CA and GS increase HO-1 expression**

**CONCLUSION:** In this study we provided experimental evidences that glucosamine sulfate decreases ADAMT5 expression and apoptosis in the IL-1 $\beta$  stimulated C-20/A4 chondrocytes. In addition, ginger root and copper sulfate decrease the Nox4 regulated proMMP1 expression. Molecular mechanisms could imply a downregulation of Nox4 activity by the antioxidant protein HO-1. These findings emphasize *in vitro* the potential beneficial effects of Cuivramine in osteoarthritis.