Investigation of curcuminoids as novel inhibitors of matrix metalloproteinase-13 for treatment of osteoarthritis

Mary Anand, Matthew A. Fisher

Dept of Chemistry, Saint Vincent College, Latrobe, PA, United States.

Abstract

Osteoarthritis is a degenerative joint disease affecting 32.5 million Americans that leads to progressive loss of mobility. One of the defining features of osteoarthritis is the degradation of articular cartilage, which is accelerated by overexpression of matrix metalloproteinase-13 (MMP-13), an enzyme which cleaves type II collagen. Currently, there are no FDA-approved drug inhibitors of MMP-13 to treat the disease process of osteoarthritis. Curcumin, demethoxycurcumin, and bisdemethoxycurcumin are curcuminoids, molecules derived from the rhizome of the *Curcuma longa* plant. Growing evidence supports the use of curcuminoids to support joint health. The goal of this study is to determine whether curcumin, demethoxycurcumin, and bisdemethoxycurcumin are novel inhibitors of MMP-13 activity. Curcumin could be an inhibitor of MMP-13 by working as a chelating agent of Zn^{2+}, an ion which MMP-13 is dependent upon; demethoxycurcumin and bisdemethoxycurcumin could have similar Zn-chelating properties. Inhibition is measured by recording the rate of fluorescence increase as MMP-13 cleaves Mca-PLGL-Dpa-AR-NH_2, a type II collagen-mimicking fluorescent substrate. The rate of fluorescence increase in the presence of a curcuminoid is compared to the rate of fluorescence increase in the absence of any curcuminoids.

Introduction

- Osteoarthritis is a serious and growing problem as the worldwide population ages. There is no known cure
- Loss of articular cartilage is one of the key features of osteoarthritis and is accelerated by the overexpression of matrix metalloproteinase-13 (MMP-13) which cleaves Type II collagen[1]
- There is no FDA-approved MMP-13 inhibitor to slow the osteoarthritis disease process
- Curcuminoids support joint health and are inhibitors of some enzymes[2,3]
- The purpose of this study was to investigate whether curcuminoids (curcumin, demethoxycurcumin, bisdemethoxycurcumin) inhibit MMP-13

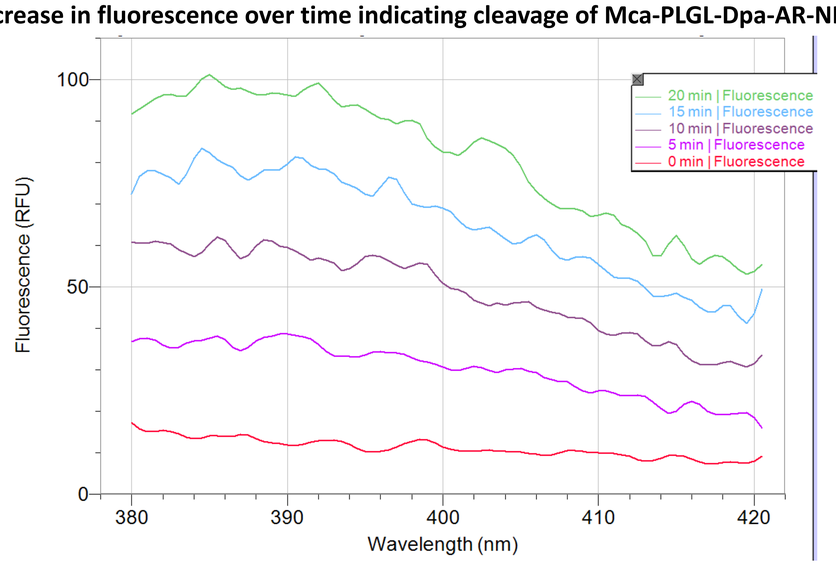
Methods

- The activity of MMP-13 was measured by a fluorogenic assay using the fluorescence resonance energy transfer substrate Mca-PLGL-Dpa-AR-NH_2 [4]
- The rate of fluorescence increase over time (RFU/min) was used as an indicator of the reaction rate (**Figure 1)**
- MMP-13 activities at different substrate concentrations with no curcuminoids added were used as control assays
- MMP-13 activity in the presence of 16.2µM bisdemethoxycurcumin, curcumin, or demethoxycurcumin was measured and compared to control assay measurements

Table 1: Summary of Reaction Rate Differences Between Control and Curcuminoid Assays

Curcuminoid (B = Bisdemethoxycurcumin, C = Curcumin, D = Demethoxycurcumin)	Assay Conditions	Slope of Control Assay, no curcuminoid (RFU/min)	Slope of Curcuminoid Assay, 16.2 µM curcuminoid (RFU/min)	Inhibition (Slope of Control Assay/Slope of Curcuminoid Assay)
B	2.5 µM substrate, 7.7 nM MMP-13	4.50	1.51	**2.97**
B	2.5 µM substrate, 9.0 nM MMP-13	2.55	1.56	**1.63**
B	5.0 µM substrate, 7.7 nM MMP-13	32.20	8.59	**3.75**
B	10.0 µM substrate, 7.7 nM MMP-13	14.73	11.76	**1.25**
C	2.5 µM substrate, 10.3 nM MMP-13	4.41	23.58	**0.19**
C	10.0 µM substrate; 9.0 nM MMP-13 for control assay, 10.3 nM MMP-13 for curcumin assay	9.45	5.54	**1.70**
D	2.5 µM substrate, 10.3 nM MMP-13	4.41	20.08	**0.22**
D	10.0 µM substrate; 9.0 nM MMP-13 for control assay, 10.3 nM MMP-13 for demethoxycurcumin assay	9.45	3.27	**2.89**

Figure 1: Increase in fluorescence over time indicating cleavage of Mca-PLGL-Dpa-AR-NH_2 by MMP-13



Discussion

- The results suggest that bisdemethoxycurcumin inhibits MMP-13 **(Table 1).** Increasing [substrate] did not decrease inhibition, so this suggests that bisdemethoxycurcumin does not act as a competitive inhibitor.
- Approximately 2-fold inhibition of MMP-13 occurred with 16.2 µM bisdemethoxycurcumin. This suggests that the IC_{50} of bisdemethoxycurcumin may be ~ 16.2 µM
- Curcumin and demethoxycurcumin seem to behave as MMP-13 activators at low [substrate] and as MMP-13 inhibitors at high [substrate] **(Table 1)**
- Bisdemethoxycurcumin may be a promising MMP-13 inhibitor for osteoarthritis treatment since it behaves as an inhibitor at both low and high [substrate]

Acknowledgements

Special thanks to the Saint Vincent College Chemistry Department for funding this project

References

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