Xanthohumol Stimulates the Secretion of Catecholamines and Induces M2 Polarization in RAW 264.7 Macrophages

Janaiya Samuels, Stephen Alvarez, Huy Dinh, Rangaiah Shashidharamurthy, Ph.D, Srujana Rayalam, Ph.D

Department of Pharmaceutical Sciences, School of Pharmacy, Philadelphia College of Osteopathic Medicine - GA Campus, Suwanee, GA

Abstract

Anti-inflammatory, anti-oxidant, and anti-cancer effects of xanthohumol (XN), a prenylated chalcone extracted from common hop plants, are gaining attention and research has been expanding on the beneficial effects of this compound. In this study, we have investigated the anti-inflammatory effects of XN using a mouse monocytic cell line, RAW 264.7. We hypothesized that the anti-inflammatory effects of XN are due to M2 polarization of macrophages which, in turn, is mediated partly through the adenosine monophosphate-activated protein kinase (AMPK) signaling pathway. RAW 264.7 cells were treated with either 0.1% DMSO or XN at varying concentrations for 24hrs. Cell culture supernatant was collected for ELISA and whole cell lysates were collected for Western blotting experiments. Our results suggest that XN upregulated the secretion of interleukin 10 (IL10), a signature cytokine for M2 polarization, in RAW 264.7 cells in a dose-dependent manner after 24hr. We further demonstrated that XN increased arginase expression, a marker for M2 polarization, and failed to increase inducible nitric oxide synthase (iNOS) expression, a marker for M1 polarization. XN decreased interferon-γ (IFNγ) induced elevation of nitrite release, indicating the inhibitory effects of XN against M1 polarization. Additionally, XN at 25μM increased the secretion of catecholamines from macrophages comparable to interleukin 4 (IL4), an inducer of the M2 phenotype. Finally, XN upregulated the expression of phospho-AMPK in RAW 264.7 cells, indicating the role of AMPK signaling pathway in XN-induced effects. These results provide evidence for the anti-inflammatory properties of XN – mediated induction of M2 polarization. The M2 macrophage mediated anti-inflammatory effects, coupled with catecholamine secretion, and previously anti-adipogenic effects, makes XN an attractive molecule to study its beneficial effects on metabolic disease, like obesity and diabetes, that are associated with underlying chronic, low-grade inflammation.

Macrophages and Obesity

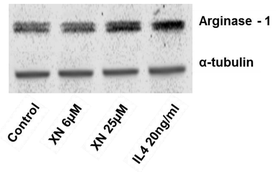
- Obesity is associated with chronic low grade inflammation.
- Macrophages that reside in the adipose tissue contribute not only to the obesity-induced inflammatory status but also altered metabolism.
- They adapt to their environment by altering their phenotype from anti-inflammatory to pro-inflammatory and the ratio of M1/M2 changes with the onset of obesity.
- During the formation of obesity, 'classically' activated, M1 macrophages infiltrate adipose tissue, form crown-like structures, and begin to secrete pro-inflammatory cytokines.
- In the lean state, adipose tissue macrophages are 'alternatively' activated, or M2 macrophages, and are characterized by their ability to secrete anti-inflammatory cytokines and catecholamines.
- Thus, the versatile phenotype of adipose tissue macrophages could help maintain adipose tissue homeostasis in the setting of obesity.

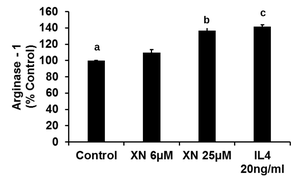
Research Goals

- Determine if the anti-inflammatory phytochemical, xanthohumol, can induce macrophages towards the M2 phenotype in RAW 264.7 cells.
- Demonstrate XN-induced upregulation of catecholamine secretion from RAW 264.7 cells.
- Investigate the possible role of AMPK signaling pathway in XN-induced M2 polarization.

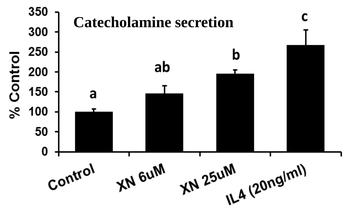
XN Promotes M2 Polarization in RAW 264.7 Macrophages

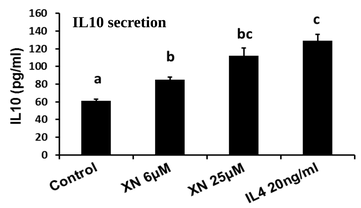
XN upregulates the expression of arginase – 1, a marker for M2 polarization





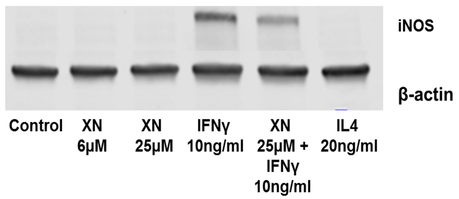
XN stimulates catecholamine and IL-10 secretion in RAW 264.7 cells



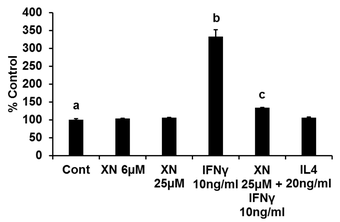


Inhibitory effects of XN against M1 polarization

XN downregulates IFNγ-induced iNOS expression



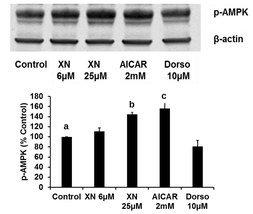
XN suppresses nitrite release in IFNγ-stimulated RAW 264.7 macrophages



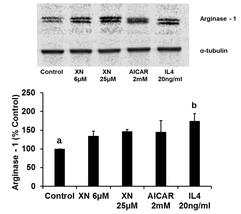
Unless specified, the time point for all the experiments was 24hr.
Means that are not denoted with a common letter are different: $P < 0.05$

XN upregulates p-AMPK expression in macrophages

XN and AICAR, an AMPK activator, increase the expression of p-AMPK in RAW 264.7 cells

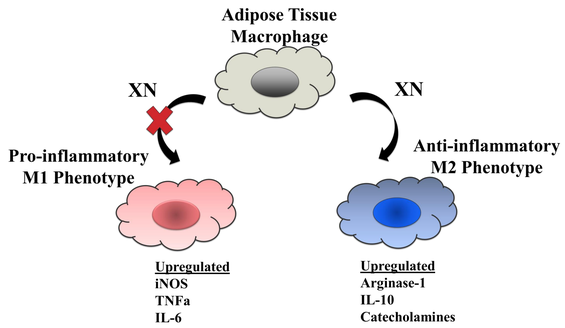


XN and AICAR increase the expression of arginase – 1 in RAW 264.7 cells



Both XN 25μM and AICAR significantly increase p-AMPK expression in 1hr

Working Model



Conclusions

- XN promotes macrophage polarization towards the anti-inflammatory phenotype as evidenced by the attenuation of M1 markers and an increase in anti-inflammatory cytokines, catecholamine secretion, and M2 markers.
- XN upregulated p-AMPK and arginase - 1 expression indicating that XN-mediated macrophage polarization might be partly mediated via the AMPK signaling pathway.

Financial Support

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