Phytoestrogens found in milk inhibit COX enzyme activity in murine macrophages

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Phytoestrogens are a large group of naturally occurring non-steroidal plant-derived compounds with potential beneficial effects on health, as indicated in epidemiological studies and experimental data from animal studies. We have previously documented that bovine milk contains various phytoestrogens, especially the isoflavone equol, when cows are fed high amounts of especially clover. Because long-term ingestion of foods rich in phytoestrogens and long-term ingestion of non-steroidal anti-inflammatory drugs (NSAIDs), have been associated with reduced cancer incidence in humans, the anti-inflammatory activity of individual phytoestrogens found in bovine milk were examined. Phytoestrogens included equol, formononetin, daidzein, biochanin A and genistein. Cells of the murine macrophage cell line RAW 264.7 were treated with lipopolysaccharide (LPS; 50 ng/ml) or peptidoglycan (PGN; 30 μg/ml) and either phytoestrogen at 1, 10 and 40 μM or vehicle (DMSO or DMSO/ethanol) in duplicate. After incubation for 16 hours thromboxane B2 (TrxB2) and prostaglandin E2 (PGE2) concentrations were measured in the culture media by ELISA. In a separate assay cell viability was measured by a resazurin metabolism assay. All phytoestrogens inhibited the LPS and PGN stimulated response of TrxB2 and PGE2. Formononetin was the most potent inhibitor. Maximal inhibition of all phytoestrogens at 40 μM corresponded to 74-100% inhibition of the increase in TrxB2 and PGE2. The viability of RAW 264.7 cells was not affected by any of the five phytoestrogens. In conclusion, the results demonstrate that the phytoestrogens that can all be found in cow's milk, have the potential to contribute to a COX-inhibitory effect. Future studies will show if milk containing a full spectrum of different phytoestrogens can provide an even stronger anti-inflammatory effect than single phytoestrogens.