Correspondence

Apigenin: Is It a Pro- or Anti-Inflammatory Agent?

To the Editor-in Chief:

We read with special interest the elegant article by Sawatzky and colleagues¹ regarding the involvement of apoptosis-modulating proteins in the resolution of acute inflammation *in vivo*. One of the findings described by the authors was that in contrast to specific inhibitors of ERK-1/2 proteins, the flavonoid apigenin, which is a nonspecific inhibitor of ERK and Cox-2, actually exacerbated inflammation. Apigenin is a phytopolyphenol widely distributed in the human diet. Like many other flavonoids, apigenin has been reported to exert anti-inflammatory effects such as lowering oxidative stress and preventing the expression of several inflammatory factors, as confirmed by Sawatzky and collaborators.¹

We would like to draw attention to the fact that flavonoids are potent anti-inflammatory factors, even when administered after inflammation is settled. Our group has investigated the effect of xanthohumol, a prenylated flavonoid, in nude mice inoculated with human breast cancer MCF7 cells (Soares et al²). Administration of xanthohumol in the mouse's beverage for 2 months starting 24 hours after inoculation, resulted in dramatic decreases in inflammatory cells, both polymorphonuclear cells and lymphocytes, as compared to vehicle-treated mice. These findings imply that flavonoids prevent inflammation even when administered after the onset of disease.

Corroborating our findings, studies using apigenin and other flavonoids after inflammation has been initiated^{3,4} have reported anti-inflammatory effects of these flavonoids. Hendriks and colleagues⁴ examined the effect of another flavonoid compound, luteolin, in lesions of the central nervous system; they observed reduced inflammation and axonal damage when luteolin was administered either before or after disease onset.

Sawatzky and colleagues¹ argued that the increase in inflammation they observed after administration of apigenin could be due to inhibition of Cox-2, since Cox-2 inhibitors given at the peak of inflammation resulted in a prolonged inflammatory response. We think that another hypothesis may be applied: flavonoids are potent phytoestrogens and are capable of inducing estrogen-dependent gene transcription, resulting in activation of several tyrosine kinases.⁵ Estrogens are also able to activate several tyrosine kinase receptors, such as epidermal growth factor receptor and transforming growth factor- β .^{6,7} Therefore, it is quite possible that flavonoids interact with these signaling pathways as well.

In conclusion, the complexity of the inflammatory process and the variety of flavonoid effects may establish some unpredictability in their interactions. The potential relevance of flavonoids in health justifies the pursuit of this path of investigation.

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Authors' Reply:

We thank Drs. Soares and Azevedo for their special interest in our study and for highlighting the diverse biological effects and possible mechanisms of action of the dietary plant flavonoid apigenin. In our recent study¹ we described that by modulating signaling pathways by specific pharmacological inhibitors, we can influence the resolution of established inflammation in an acute model of pleurisy in the rat. In addition to using specific inhibitors of the mitogen-activated protein kinase (MAPK) pathway and the pro-apoptotic Bcl-2 family member Bax, we used another pharmacological reagent, the phytopolyphenol apigenin, which is a flavonoid that has been reported to have wide-ranging anti-inflammatory and anti-

cancer properties.^{2,3} The precise mechanisms by which apigenin and other flavonoids exert their anti-inflammatory properties, however, remain to be elucidated. Apigenin has been shown to affect a number of key signaling pathways, including MAPK, protein kinase C, phosphatidylinositol-3 kinase, nuclear factor- κ B and cyclooxygenase (COX),^{2,3} that likely lead to its diverse and complex biological profile in a wide variety of cell types and tissues. Thus, these effects are likely to influence, in a complex manner, all features of the inflammatory response, including the processes of initiation, progression, and resolution of inflammation.⁴

In our study¹ we found that apigenin, perhaps surprisingly given its better known anti-inflammatory properties, administered intrapleurally at the peak of inflammation in the rat model of carrageenan-induced pleurisy augmented inflammation, presumably by slowing the resolution phase of the inflammatory response. We suggested that a possible mechanism for its effects might be that the flavonoid could influence COX-2 expression and/or activity, possibly by inhibiting important signaling pathways. Our rationale for this explanation is that selective COX-2 inhibition by NS-398 and traditional nonsteroidal anti-inflammatory drugs, when administered at the peak of carrageenan pleurisy, cause a prolonged inflammatory response.⁵ This is likely attributable to inhibition of the anti-inflammatory cyclopentenone prostaglandins, 15-deoxy $\Delta^{12,14}$ PGJ₂ and PGD₂ synthesis, prostanoids that directly promote apoptosis of neutrophils and macrophages,^{6,7} apoptosis being a fundamental process for the successful resolution of inflammation.^{4,8} We have preliminary unpublished evidence that apigenin inhibits COX-2 expression in pleural inflammatory cells when administered 24 hours after established carrageenan-induced inflammation; however, given the recent interest in flavonoids, this finding merits further investigation.

Drs. Soares and Azevedo correctly highlight another interesting possible explanation. They have brought to our attention their recent preliminary study that another flavonoid, xanthohumol, given orally for 2 months starting 24 hours after nude mice had been inoculated with human breast cancer MCF cells reduced inflammatory cell recruitment.⁹ An interesting hypothesis that has been postulated is that flavonoids, being potent phytoestrogens,³ may be capable of inducing estrogen-dependent gene transcription thereby influencing certain tyrosine kinase-linked receptors.^{10,11} Although there is good evidence that estrogens can modulate carrageenan-induced pleurisy in the rat,^{12–14} whether flavonoids such as apigenin influence the resolution of inflammation in the acute pleurisy model described in our study by modulating estrogen-dependent pathways requires direct investigation. Given the many reported biological effects of

flavonoids, the structural diversity of this family (more than 5000 flavonoids have been described) and the relatively unknown pharmacological and pharmacokinetic properties of these compounds, further research in our opinion is warranted.

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