

Biologically Active Components in Foods

by Elliot Middleton, M.D., Division of Allergy and Clinical Immunology, SUNY

The flavonoids comprise a large group of naturally occurring, low molecular weight compounds that are present in all vascular plants. They are present in fruits, vegetables, nuts, seeds, stems, flowers, bark, tea, and wine and the average Western diet contains approximately 100 to 1000 mgs per day. The remarkable properties exhibited by these compounds make it possible that they should be considered natural dietary biologic response modifiers, i.e. disease-preventing, health promoting substances. The flavonoids have been shown in both in vitro and in vivo experimental systems over several decades to possess antiallergic, antiinflammatory, antiviral, anticancer and anticarcinogenic activities. In addition, some of them are potent antioxidants.

With respect to antiallergic activity it is now well known that certain flavonoids, depending on structure, can inhibit the stimulated release of proinflammatory mast cell, basophil and eosinophil granular constituents that participate in the pathogenesis of diseases such as asthma, allergic rhinoconjunctivitis, urticaria and others. Quercetin is a particularly active inhibitor as described in greater detail below, while taxifolin (dihydroquercetin) lacked activity.

Antiinflammatory activity can be assessed by the effects of flavonoids on neutrophil function. Certain flavonoids including quercetin have the capacity to inhibit neutrophil activation with production of superoxide anion and activation of phospholipase A2 and NADPH oxidase.

Activation of the latter enzyme results in neutrophil generation of superoxide anion, a tissue-damaging oxygen radical. Quercetin inhibits the release of 3H-arachidonic acid from prelabelled cells following a non-immunologic stimulus (zymosan-activated serum) thus indicating that quercetin can inhibit neutrophil phospholipase A2.

Antiviral activity has been demonstrated with quite a few viruses although no clinically or commercially successful flavonoid has been discovered. Selected flavonoids can inhibit viruses such as polio virus type 1, parainfluenza virus type 3, respiratory syncytial virus and herpes virus type 1, amongst others. Four flavonoids were examined for their ability to affect viral infection of tissue culture monolayers or, once the monolayers were infected, the ability of the flavonoids to inhibit replication of the virus intracellularly. Quercetin (a flavonol) exhibited both antiinfective and antireplicative activity against each of the four viruses. Hesperetin (a flavanone) showed only antireplicative activity. Catechin (a flavan) had no antireplicative properties but showed antiinfective activity against respiratory syncytial virus and herpes simplex virus type 1. Naringin (a flavanone glycoside) exhibited neither anti-infective nor antireplicative activity against any of the four viruses. Clearly there are unique structure activity relationship to be considered with respect to antiviral activity of flavonoids. Of particular note with respect to antiviral activity is the fact that certain flavonoids have activity against three enzymes that are critically involved in the life cycle of the human immunodeficiency virus, namely, antireverse transcriptase activity, antiintegrase activity and antiprotease activity.

The antioxidant activity of certain flavonoids has been recognized for some time. Some of the flavonoids are as potent or more potent than familiar antioxidants such as vitamin E and beta carotene. Of current interest is the presence of potent antioxidant flavonoids in red wine. The explanation of the "French paradox" may lie in this observation. The French paradox indicates

that Frenchmen eat quite a lot of fatty food but do not have a very high incidence of coronary artery disease. A possible explanation is that the French consume a fair amount of red wine with their meals and that the antioxidant activity of the red wine may account for the antiatherogenic activity.

Chemically, the flavonoids are phenylbenzo-, γ -pyrones with two major groupings, i.e. flavones with the phenyl group at the 2 position of the chromone moiety and isoflavones with the phenyl group at the 3 position. There are a number of other chemical variations of the flavonoids that are characterized by the reduction of the C2-C3 bond and the degree of hydroxylation or methoxylation (or other substituents) in the A, B and C rings. There are now over 4,000 flavonoid compounds in nature that have been structurally characterized. Clearly these compounds must be of importance in plant physiology and biochemistry or they would not have survived evolution. The activity of various flavonoids in different mammalian cell test systems is profoundly affected by structure including changes in the degree of hydroxylation, methoxylation, the state of oxidation of the C2-C3 double bond and the presence of the C4 keto grouping. Certain flavonoids can undergo dimerization or oligomerization to provide very interesting and novel compounds. It is of considerable interest that the antiallergic drug cromolyn, which is used in the management of asthma and allergic rhinoconjunctivitis, is structurally very closely related to naturally occurring flavonoids. Cromolyn is a bischromone, that is, 2 chromone moieties (the benzopyrone ring system) attached to each other via a short carbon bridge.

Information accumulated over the decades indicates that a number of mammalian enzyme systems can be affected by selected flavonoids. Some of these enzyme systems can be listed: protein kinase C, protein tyrosine kinases, myosin light chain kinase, phospholipase C, phospholipase A2, lipoxygenase, cyclooxygenase, cyclic nucleotide phosphodiesterase, and ATPases, amongst others. A number of these enzymes such as protein kinase C, protein tyrosine kinase, phospholipase A2 and phospholipase C are intimately involved in cell activation and signal transduction processes that occur in all physiologically stimulated cells.

A number of mammalian cell systems have been studied with respect to the effects of flavonoids on their function. These include (a partial listing) B and T lymphocytes, monocytes/macrophages, mast cells, basophils, eosinophils, neutrophils, and platelets. The effects of flavonoids on some of these cell systems will be briefly described.

In the mid 1970's, work was published from England showing that rat peritoneal mast cell histamine release stimulated either with antigen (from sensitized animals), the mitogen concanavalin A or the calcium ionophore A23187 was significantly inhibited by certain flavonoids. We decided to examine the effects of one of the flavonoids, quercetin, on the release of histamine from a somewhat related granule-containing cell, the human peripheral blood basophil. In summary, we found that quercetin caused a concentration-dependent (IC₅₀ approximately 10 μ M) inhibition of histamine release; quercetin only affected antigen-activated cells (see below); its onset of action was instantaneous; the inhibitory activity of quercetin was partially reversed by increased buffer calcium concentration (a nonsignificant effect); quercetin was antagonistic to the histamine release-augmenting effect of heavy water (D₂O) suggesting an effect of the compound on microtubule assembly and function; and the action of quercetin was not potentiated by theophylline indicating that cyclic AMP-related mechanisms were probably not involved. Also, some interesting structure-activity relationships were discerned as will be described. A point of particular interest in these experiments was the observation that quercetin

only inhibited histamine release when the cells had been activated by antigen; that is, peripheral blood leukocyte suspensions containing basophils could be incubated for 30 minutes with the flavonoid in the absence of antigen and washed later and then found to respond perfectly normally to the antigen stimulus with appropriate histamine release. That is, the basophils did not appear to have a tight-binding receptor for quercetin in the unstimulated state. On the other hand, if antigen was added to a suspension of basophil-containing leukocytes thereby initiating the histamine release reaction, then, the addition of quercetin to the ongoing histamine release reaction (at 2 or 5 or 10 minutes) caused an abrupt cessation of further histamine release. This suggests that a flavonoid-sensitive substance is generated in the antigen-activated basophil with interaction of quercetin with which abolished further histamine release. This is a rather novel pharmacological mechanism of action and is of considerable interest with respect to drug design.

In subsequent experiments with basophil histamine release we found that compounds with a reduced C2-C3 double bond, such as taxifolin (dihydroquercetin), were inactive as inhibitors of histamine release. Likewise the rhamnonylglucoside of quercetin, rutin, also lacked activity as an inhibitor, perhaps by virtue of steric hindrance produced by the glycoside group. Certain other flavonoids also lacked activity if their chemical structure did not include certain basic features, namely, hydroxylation in the B-ring, double bond at C2-C3 and a keto group at C4.

Subsequent experiments were performed to find out whether selected flavonoids had an effect on histamine release from basophils stimulated by other secretagogues including anti-IgE, concanavalin A, the chemoattractant peptide f-met-leu-phe, tetradecanoyl phorbol acetate (TPA) (activates protein kinase C directly), and the calcium ionophore A23187. It was found that there were flavonoids that totally lacked activity against these various secretagogues but there were others that showed good activity as inhibitors of histamine release stimulated by these various secretagogues. The concentration-effect curves of flavonoids such as quercetin against each one of these secretagogues did differ, however. It is possible that these differences in concentration-effect relationships represent differences in the signal transduction mechanism employed by the basophil for each of the different secretagogues and shows differential sensitivity to the effects of the flavonoids.

The effect of TPA to cause basophil histamine release suggested that activation of protein kinase C was a necessary step in the secretory process. To study this further we prepared a partially purified protein kinase C preparation from rat brain and studied the effects of flavonoids on protein kinase C activation by TPA. Fisetin, quercetin and luteolin were all very active at (50 μ M) and a number of other compounds were less active and some totally lacked activity. Interestingly, the most active inhibitors of protein kinase C were also the most active inhibitors of TPA-induced basophil histamine release. Further kinetic studies with the rat brain preparation indicated that fisetin and quercetin caused inhibition of protein kinase C by virtue of blocking the ATP binding site in the catalytic portion of the enzyme.

The eosinophil participates in inflammatory reactions of various sorts including bronchial asthma, allergic rhinoconjunctivitis, inflammatory bowel disease, and certain dermatoses, to mention a few. The granules of eosinophils contain several very toxic proteins that contribute to the pathogenesis of these clinical disorders. The eosinophil, like the mast cell and basophil, is a secretory cell and, therefore, we were interested to find out if quercetin and taxifolin had any effect on the secretion of eosinophil cationic protein and Charcot-Leyden crystal protein from the granules of partially purified eosinophils. The results clearly indicated that quercetin (10-50 μ M) caused 70-90% inhibition of eosinophil cationic protein secretion while taxifolin had a negligible

effect. Essentially similar results were found with the effects of quercetin and taxifolin on the secretion of Charcot-Leyden crystal protein. The stimulus for the release of these toxic granular proteins was the calcium ionophore A23187. Thus the secretory function of eosinophils, like that of mast cells and basophils, can be inhibited by quercetin.

The development of an inflammatory process must begin by trapping leukocytes in the vascular system of the tissue that is becoming inflamed. That is, activation of endothelial cells within the capillaries and venules of the tissue, (for example, the lung in asthma), must take place in order for leukocytes to adhere to the endothelium and then to undergo diapedesis into the inflammatory focus within the tissue. The proteins involved in the business of making leukocytes stick to endothelium are called adhesion molecules. There are many adhesion molecules now recognized. Some are present on the surface of endothelial cells and others, called counter receptors, are present on the membranes of leukocytes.

We were interested to know whether or not quercetin would inhibit the expression of one particular endothelial cell adhesion molecule known as intracellular adhesion molecule-1 following stimulation of the endothelial cells with endotoxin. Interestingly, quercetin caused a concentration-dependent inhibition of the expression of ICAM-1 in endotoxin-stimulated endothelial cells. The cells which we studied were human umbilical vein endothelial cells (HUVEC) in tissue culture. The possible importance of this observation with respect to diet and inflammation must be obvious.

Other cells are also involved in immune function and effects of flavonoids on these cells will be briefly described. The macrophage/monocyte is a cell critical to the initiation of the immune response. These cells take up antigen and partially digest it and present it on the cell surface so that it can interact with the T cell receptor to initiate an immune response. Some investigations have indicated that the process of antigen presentation can be inhibited in these cells by quercetin. T and B lymphocytes are essential cells involved in immune function. Certain flavonoids can inhibit lymphocyte mitogenesis stimulated by phyto mitogens such as phytohemagglutinin and concanavalin A. Also, certain flavonoids, depending on structure, can inhibit the generation of cytotoxic lymphocytes in murine mixed spleen cell cultures. This observation clearly indicates that flavonoids can have an effect on cell-cell interactions and relates to the issue of adhesion molecules mentioned above. The B cell is the precursor of the plasma cell which is the principal antibody-producing cell of the immune system. Activation of the B cell antigen receptor causes B cell activation with accompanying phosphorylation of tyrosine residues in several proteins within the B cell. This process can be inhibited by the isoflavone genestein. Other experiments showed that B cell precursors stimulated with recombinant human IL-7 also resulted in phosphorylation of tyrosine residues in several proteins within the precursor B cell and this was accompanied by phosphatidylinositol turnover with increased production of inositol trisphosphate. It was also found that genestein inhibited these effects. Phosphatidylinositol turnover is a vital process in transmembrane signal transduction and a key enzyme involved in this process is phosphatidylinositol kinase (PI 4-kinase). This kinase turns out to be inhibited by the isoflavone orobol, and by quercetin and fisetin. The epidermal growth factor-induced phosphatidylinositol turnover in epidermoid carcinoma cells (A231 cells) is inhibited by the isoflavone piceatannin. The effects of flavonoids on antibody formation has also been studied. A unique flavonoid called plantagoside (a flavanone glucoside and an alpha mannosidase inhibitor) caused a concentration-dependent inhibition of mouse spleen cell antibody response to sheep red blood cells (antigen). Finally, cell mediated immunity

or delayed-type hypersensitivity reactions can also be affected by certain flavonoids. As noted, the related phenomenon of mitogenesis stimulated by PHA or conA is inhabitable by certain flavonoids.

Flavonoids also affect platelet function. The platelet is now appreciated as a participant in inflammatory processes and it is of interest, therefore, the platelet aggregation and release reaction can be inhibited by certain flavonoids as can calcium mobilization and the adhesion of platelets to collagen. The latter observation once again points to the effect of flavonoids on adhesion molecules-dependent interactions.

It is now widely recognized that diets rich in fruits and vegetables appear to be associated with a reduced frequency of cancer of various organ systems. Clearly there must be substances in fruits and vegetables which have anticancer effects. The flavonoids are known to possess a number anticancer activities which can be briefly described at this point. Actually there are eight different mechanisms by which flavonoids may affect cancer.

Certain flavonoids exert anticarcinogenic activity. This can take place by induction of enzymes affecting carcinogen metabolism. Also, certain flavonoids actually inhibit adduct formation between carcinogens and DNA and finally certain flavonoids inhibit *in vivo* experimental carcinogenesis. A good example of the latter is an experiment where female rats that developed mammary carcinoma in response to a particular carcinogen were found to have a 50% reduction in the number of tumors as compared to control when they consumed a diet containing 5% quercetin.

In addition, certain flavonoids possess antitumor-promotor activity in which case the flavonoids inhibit the various activities of tumor promoters that are involved in the process of carcinogenesis. Moreover, antitumor activity in flavonoids has been described with a number of different hormone-dependent tumors. Also, certain flavonoids turn out to be very active antiproliferative agents inhibiting cancer cell proliferation *in vitro*. Moreover, a rather extraordinary process stimulated by certain flavonoids is a prodifferentiation effect; that is, certain flavonoids can actually stimulate a malignant cell to develop into a mature phenotype. Also, inhibitory effects of certain flavonoids on the expression of the multi-drug resistance gene has been described. And, certain flavonoids affect topoisomerase activity which can be associated with reduced cancer growth. Finally, through effects on adhesion molecule expression and function certain flavonoids can have an antimetastatic activity and reduce the development of metastases.

In summary then, some biochemical properties of the flavonoids include alteration of enzyme activity, antioxidant activity, vitamin C sparing activity, chelation of metal cations, inhibition of lipid peroxidation, radical scavenging activity and effects on protein phosphorylation. Some basic life processes that are affected by flavonoids include immune mechanisms, inflammation, cellular differentiation, heat shock protein synthesis, cancer, atherosclerosis, metabolism and perhaps even aging.

It seems reasonable to consider seriously the possibility that dietary flavonoids may be very important disease-preventing and health-promoting substances. Further research is definitely warranted.

References

The interested reader will find an extensive bibliography regarding the contents of this article in: Middleton, E., Jr. and Kandaswami, C. (1993). The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer, Harborne, J.B., 619-652, Chapman & Hall, New York.