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Some natural flavonoids are competitive inhibitors of Caspase-1, −3 and −7 despite their cellular toxicity

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Abstract

A common feature of both apoptosis and inflammation is the activation of caspases. Caspases are aspartate-directed cysteine proteases that have numerous cellular targets. It has been discovered that several flavonoids are inhibitors of caspases. Flavonoids are members of a family of polyphenolic compounds from plants that have many biological properties, one of which is the ability to induce cell death. Some flavonoids are selective inhibitors of particular caspases. Since some of the inhibitory flavonoids are nevertheless cytotoxic, these results suggest that flavonoid-induced cell death may be occurring through a non-classical apoptosis pathway that is not dependent on caspase activity.

Keywords

Apoptosis; Caspase; Flavonoid; Breast Cancer; Enzyme kinetics; Small molecule inhibitor

1. Introduction

Flavonoids are phytochemicals found widely in the human diet, especially in yellow onion, kale, leek, parsley, soy, tea, cocoa, and blueberry.(Manach, Scalbert, Morand, Remesy, & Jimenez, 2004; Spencer, Vauzour, Vafeiadou, Rodriguez-Mateos, & Rendeiro, 2008) Flavonoids possess antioxidant properties and have been widely reported to be protective against conditions in which inflammation may play a role, including cancer and cardiac and neurodegenerative diseases.(Ramos, 2007; Schroeter, Spencer, Rice-Evans, & Williams, 2001) The mechanism of the protective effect against cancer is not fully understood, but may be related to flavonoids' ability to inhibit the NF-κB signalling pathway. NF-κB activity is thought to suppress apoptosis and promote cancer cell growth and metastasis. (Prasad, Phromnoi, Yadav, Chaturvedi, & Aggarwal, 2010) Several studies have suggested that flavonoids induce apoptosis in cultured cancer cells but not normal cells.(reviewed in (Ramos, 2007))

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Caspases are cysteine proteases that cleave peptide and protein substrates at sites next to Asp residues.(Reviewed in (Earnshaw, Martins, & Kaufmann, 1999)) There are a total of 14 mammalian caspases thus far identified, 12 of which exist in humans (caspases 1–10, 12 and 14).(Pop & Salvesen, 2009) The caspases can be divided into two groups, depending on their main function. One group consists of those caspases which mediate apoptosis, and includes caspases 2, 3 and 6–10. Caspase-3 and −7 are the final effector caspases in the apoptosis pathway. The second group is involved in maturation of cytokines, and includes caspase-1, −4, −5 and −12.

Activated caspases cleave a variety of substrates (approximately 400 targets have been identified to date).(Luthi & Martin, 2007) These include proteins involved in signal transduction (apoptosis regulators, cytokines, serine/threonine kinases), structural proteins (cytoskeletal and nuclear) and proteins involved in regulation of transcription, translation and RNA editing. The function of caspase-14 remains undefined; however, a role in keratinocyte differentiation has been suggested.(Eckhart, Declercq, Ban, Rendl, Lengauer, Mayer, et al., 2000)

Modulators of caspase activation may offer a novel therapeutic approach to disease processes such as neurodegeneration and chronic inflammation since both depend on activation of caspases.(Dinarello, 2004; Fischer & Schulze-Osthoff, 2005; Howley & Fearnhead, 2008) Recent studies indicate that inhibition of caspase activity is possible through the use of peptide analogs that bind the active site of caspases.(Caserta, Smith, Gultice, Reedy, & Brown, 2003; Mittl, Di Marco, Krebs, Bai, Karanewsky, Priestle, et al., 1997; Rano, Timkey, Peterson, Rotonda, Nicholson, Becker, et al., 1997) While these inhibitors have been very valuable in helping to understand the function of caspases, peptide inhibitors often make poor therapeutic agents. Therefore, identification of small molecules that modulate caspase is needed to advance the development of treatments for apoptosis-related and inflammatory diseases. (Lee, Long, Adams, Chan, Vaidya, Francis, et al., 2000)

Here, some naturally occurring flavonoids, are reported to be able to inhibit caspase activity in a dose-dependent manner *in vivo* and *in vitro*. These results are surprising because of prior work showing flavonoid-induced caspase activation as determined by cleavage of the pro-caspase to the active form.(Arafa, Zhu, Barakat, Wani, Zhao, El-Mahdy, et al., 2009; Chien, Wu, Chung, Yang, Lu, Tsou, et al., 2009; Das, Banik, & Ray, 2010; Sakao, Fujii, & Hou, 2009; Shen, Chen, Hsu, & Lee, 2003; Wang, Lin-Shiau, & Lin, 1999; Way, Kao, & Lin, 2005) However, Shimmyo et al. recently showed that myricetin is able to inhibit caspase-3 activity in rat neuronal cell cultures and deduced through molecular docking studies that myricetin can bind to the active site of caspase-3.(Shimmyo, Kihara, Akaike, Niidome, & Sugimoto, 2008) These studies have been extended and show that a variety of flavonoids can inhibit caspase-1, −3, and −7 and that inhibition by some flavonoids can be specific for certain caspases. These results suggest that flavonoids may provide an effective starting point for development of specific small molecule caspase inhibitors as therapeutic agents.

2. Methods and materials

2.1. Materials

Flavonoids and iso-flavonoids were purchased from Sigma, Alexis Biochemicals, or Indofine Chemicals and stocks were prepared at 50 mM in dimethyl sulfoxide (DMSO). Recombinant caspase-1, −3, and −7, Ac-DEVD-AMC, and Ac-LEHD-AMC were purchased from Biomol. AMC was purchased from Invitrogen.

2.2. Cell Culture

MDA-MB-231 cells were purchased from American Type Culture Collection (ATCC) and maintained in DMEM with high-glucose, L-glutamine, and sodium pyruvate (Hyclone), supplemented with 10% Bovine Growth Serum (BGS) and 1X antibiotic/antimycotic (Hyclone) in a 37 °C humidified atmosphere containing 5% CO_2. Cells were passaged based on dilutions and confluency as recommended by ATCC.

The creation of immortalized caspase $3^{-/-}$/caspase $7^{-/-}$ mouse embryonic fibroblast (MEF) and wild-type control cell lines was previously described and a kind gift of Richard Flavell. (Lakhani, Masud, Kuida, Porter, Booth, Mehal, et al., 2006). MEFs were grown in DMEM with high glucose, L-glutamine, and sodium pyruvate (Hyclone), supplemented with 10% Fetal Bovine Serum and 1X antibiotic/antimycotic (Hyclone) in a 37 °C humidified atmosphere containing 5% CO_2. Cells were used in all experiments at five passages or less.

2.3. RNA isolation, first strand cDNA synthesis and PCR

RNA was isolated from immortalized caspase $3^{-/-}$/caspase $7^{-/-}$ mouse embryonic fibroblast and wild-type control cell lines using Trizol (Invitrogen) following manufacturer's recommended protocol. First strand cDNA was generated from one microgramme of total RNA using SuperScript III (Invitrogen) and a PolyA primer. Reverse-Transcribed Polymerase Chain Reaction (RT-PCR) was used to genotype the MEF cell lines. Caspase-3 primers were Forward: 5′-TGAGGAGATGGCTTGCCAGA-3′, Reverse: 5′-TCCGTTGCCACCTTCCTGTT. Caspase-7 primers were Forward: 5′-AGTTGACGCCAAGCCAGACC-3, Reverse: 5′-CGGCATGCCTGAATGAAGAA-3′. Beta-Actin primers were Forward: 5′-CATGTACGTTGCTATCCAGGC-3′, Reverse: 5′-CTCCTTAATGTCACGCACGAT-3. RT-PCR was carried out using 2X GoTaq mastermix (25μl) (Promega) following manufacturer's recommended protocol. The cycling conditions comprised 95 °C for 3 min, 40 cycles at 95 °C for 30 sec, 60 °C for 30 sec and 72 °C, and a final extension 72 °C for 3 min. Bands were visualized on a 1.5% TBE-Agarose gel.

2.4. Caspase assays

MDA-MB-231 cells were seeded in 96-well plates (0.5×10^4 cells/well). After 24 h, cells were treated with flavonoid (200 μM) or the known apoptosis inducer staurosporine (1 μM) as a positive control. After 24 h of treatment, induction of apoptosis was determined by measurement of caspase-3 and −7 activity using the luminometric Caspase-Glo 3/7 assay (Promega) according to the manufacturer's protocol using a Veritas Luminometer (Turner Biosystems).

2.5. In vitro Caspase activity assays

Caspase assays were run in 100-μl volumes following the manufacturer's recommended protocol (Biomol). Briefly, recombinant caspases were diluted into the appropriate assay buffer to approximately 10 units/assay (1 unit = 1 pmol of AMC product formed per minute) and were added to the incubation mixtures. Reactions were initiated by the addition of substrate: 10 μM Ac-DEVD-AMC for caspase-3 or −7, and 10 μM Ac-LEHD-AMC for caspase-1. For screening studies, inhibitors were added to the final assay mixture prior to the addition of substrate. Inhibitors were used at 200 μM, 100 μM, and 50 μM. In all assays performed, the final DMSO concentration was 2% which had no effect on caspase activity (data not shown). Accumulation of AMC was measured at 30 °C with Modulus II plate reader (Turner Biosystems) at an excitation wavelength of 365 nm and an emission wavelength of 410–460 nm. Measurements were recorded at 2 min intervals for 0.5 s/well for a total of 30 min. AMC accumulation was plotted versus time and only data that were

linear were used in analysis. Linearity generally occurred over the first 15–20 min of the run.

For Michaelis-Menton studies, the procedure was the same as described above. The amount of substrate used in the assays was 150 μM, 90 μM, 60 μM, 30 μM, 15 μM, 6 μM, and 3 μM and the amount of inhibitor used was 100 μM, 50 μM, and 25 μM.

2.6. Correcting for quenching in Caspase assays

In performing our assays, we discovered that the flavonoids were capable of quenching the fluorescence of the AMC product (Figure 4). This quenching effect, if not corrected, results in an underestimate of the amount of product being formed and therefore would impede the analysis. To determine accurately the amount of product being formed, we corrected for quenching by generating AMC standard curves in the presence of the concentrations of flavonoids used in our assays using 30 μM, 15 μM, 7.5 μM, and 3.75 μM of purified AMC prepared in Assay Buffer.

2.7. Determination of Ki's for Caspases

For every flavonoid and caspase tested, Lineweaver-Burke plots intersected at the $1/V_o$ axis suggesting competitive inhibition. The K_i values for each flavonoid with each caspase tested were determined from the slopes and intercepts of the best lines through the data at each flavonoid concentration in the Lineweaver-Burke plots using the relationships shown below:

$$1/V_0=\alpha K_m/V_{max}(1/[\text{Substrate}])+1/V_{max}, \text{where } \alpha=1+[\text{flavonoid}]/K_i$$

2.8. Statistical data analysis

Dose-response data were analyzed using sigmoidal curve fits in Prism (GraphPad Software, Inc) with variable slope. We set the top part of the curve to 100% response (0% viability) and the bottom part of the curve to 0% (100% viability). IC50s are reported with 95% Confidence Intervals.

For comparisons between two groups, the data were analyzed using the two-tailed Student *t* test with 95% confidence interval. A *p* value <0.05 was regarded as statistically significant.

3. Results

3.1. Flavonoids cause a reduction in cellular viability

Flavonoids have been reported to be cytotoxic to cancer cells (reviewed in (Ramos, 2007)). Here, we investigated the cellular toxicity of ten structurally related flavonoids: apigenin, catechin, chrysin, daidzein, genistein, kaempferol, luteolin, myricetin, naringenin, and quercetin. The structures of these flavonoids are illustrated in Figure 1. Flavonoid-induced cellular cytotoxicity in the human breast cancer cell line, MDA-MB-231 has also been tested. After 72 hours of treatment, the percentage of living cells remaining was determined and is shown in Figure 2 and Supplemental Figure S3. From these data, we calculated IC_{50}'s (Table 1). Catechin, myricetin, and naringenin were the least effective at killing cells (IC_{50} >200 μM). Apigenin and luteolin were the most cytotoxic (IC_{50}<20 μM). Chrysin, daidzein, genistein, kaempferol, and quercetin varied in their toxicity with IC_{50}s ranging from 40 μM to 179 μM.

3.2. Flavonoids inhibit caspase-3/7 activity in a dose dependent manner

It was ideal to determine if cell death was occurring through apoptosis. Since activation of cellular caspases is an early hallmark of apoptosis, caspase activity during flavonoid-

induced cell death was monitored using a luminescence-based assay system with a peptide substrate for caspase-3 and −7. We could not detect appreciable caspase-3 or-7 activity above the control upon flavonoid treatment for any flavonoid tested (Figure 3A). Quercetin appeared to increase caspase-3 or −7 activity only slightly, however this was not statistically significant. Interestingly, a decrease in caspase-3 or −7 activity in flavonoid treated cells for apigenin, luteolin, and kaempferol that was statistically significant ($p<0.001$) was observed. Staurosporine was used as a positive control to induce caspase-3 and −7 activation in the MDA-MB-231 cells to verify that the assay was working properly. The staurosporine treatment did induce a very strong caspase-3 or −7 activation in these cells that was significantly higher than the DMSO treatment ($p<0.005$). To determine if the incubation time with the flavonoids was important for detecting caspase activation, these studies were repeated with incubation for 6, 12, 24, and 48 hours and flavonoid-induced activation of caspase-3 or −7 (data not shown) was not observed. These results suggested to us that (1) flavonoids did not activate caspase-3 or −7, and/or (2) the flavonoids were inhibiting the activity of the enzymes.

Caspase activation requires protease-induced zymogen cleavage.(Salvesen & Dixit, 1999) To determine whether the flavonoids were inhibiting zymogen cleavage, caspase activity, both, or neither, the effect of flavonoids on staurosporine-induced activity was examined. Figure 3B shows that staurosporine induced caspase-3 or −7 activation very strongly compared to control treated cells, but that incubation of cells with a combination of staurosporine and various flavonoids led to less caspase activity than without flavonoids. The flavonoid-induced reduction in activity was dose dependent for apigenin, luteolin, and kaempferol treated cells (* $p<0.05$, ** $p<0.005$). Myricetin and quercetin were able to reduce the staurosporine-induced activity (* $p<0.05$, ** $p<0.005$) but was not dose-dependent.

Reduction of caspase-3 or −7 activities could be occurring either through direct inhibition of caspase-3 or −7 activities or indirectly by inhibiting an upstream effector caspase (such as caspase-8 or −9) thereby preventing zymogen cleavage to produce active caspase-3 or −7. To determine which was correct, we treated cells with staurosporine for 24 hours and then added various flavonoids directly to the media immediately before the caspase-3/7 assay was initiated (Figure 3C). The flavonoids were able to inhibit staurosporine induced caspase-3 or −7 activity even when added immediately before assay (** $p<0.005$).

3.3. Purified recombinant caspases are inhibited by flavonoids

Flavonoids were tested to see if they could directly inhibit purified recombinant caspases. A preliminary survey of the inhibition of purified recombinant activated caspase -1, −3, and 7 by a variety of flavonoids at three concentrations (200 μM, 100 μM, and 50 μM) was performed and the results are shown in Figure 4. From these data, it was evident that some flavonoids (apigenin, catechin, chrysin, daidzein and naringenin) did not exhibit strong dose-dependent inhibition of purified caspase -1, −3, or −7 activity, while other flavonoids (kaempferol, luteolin, myricetin, and quercetin) showed significant, dose-dependent inhibition of purified caspase -1, −3, or −7 activity. Accordingly, the caspase inhibition was characterized by the latter group in more detail.

Kinetic analyses were performed to determine the type of caspase inhibition that was occurring with the flavonoids. Lineweaver-Burk plots were generated from the data (Supplemental Figure S1), and Ki's were calculated as described in the Methods for each flavonoid and caspase. Results are shown in Table 2. Shimmyo et al. (2008) previously reported that myricetin directly inhibits caspase-3 activity by binding to the active site. Our kinetic data have confirmed these findings and expanded the list of known flavonoids that inhibit caspases. Here we show through Michaelis-Menten kinetic analysis that myricetin as

well other flavonoids are able to inhibit caspase-1, −3, or −7 activity *in vitro* and that the inhibition is competitive.

Assessing caspase inhibition by flavonoids was complicated by the fact that the flavonoids quench the fluorescence of the AMC product (Supplemental Figure S2). Therefore, if uncorrected, this makes the flavonoids appear to be better inhibitors of caspase activity than they actually are. Accordingly, standard curves were ran of fluorescence intensity vs. concentration of AMC in the presence or absence of the tested flavonoids at the various concentrations used in our studies (25 μM, 50 μM, 100 uM and 200 μM). Using the equations of each line (Supplemental Table S1) in the standard curve, the actual amount of AMC produced in the caspase assays was calculated, effectively correcting for the quenching of AMC fluorescence by the flavonoid. Corrected results were then used for calculations of Ki's in Table 2. It should also be noted Shimmyo et al. reported that myricetin negligibly quenches AMC fluorescence, in contrast to our findings, potentially explaining why their IC_{50} value (10.2 μM) is lower than our IC_{50} (25μM) which was corrected for quenching by myricetin.

3.4. Caspase-3 and Caspase-7 are not required for cell death

Numerous studies have indicated the importance of caspase-3 and caspase-7 activation in flavonoid-induce cell death. Our results suggest that caspase-3 and caspase-7 may not be required for cell death. Therefore, to further explore this issue, MEF cells were obtained that do not contain caspase-3 or caspase-7.(Lakhani, et al., 2006) The ability of selected flavonoids to induce death in these cells compared to heterozygote control (known to be equivalent in response to wild-type MEFs) was tested. We confirmed, using RT-PCR, that the cell lines genotypes are correct (Figure 5A). There was no significant difference in the cytotoxicity of the flavonoids tested towards the caspase-containing MEFs compared with the caspase-deficient MEFs (Figure 5B) with the exception of myricetin which was weakly cytotoxic to the caspase-deficient MEFs ($p<0.05$).

4. Discussion

The concentration of flavonoid that inhibits caspases is similar to the concentration of flavonoids that also kills MDA-MB-231 cells. These concentrations are also similar to concentrations that other groups have shown to kill various other cancer cell lines.(reviewed in (Ramos, 2007)) Many groups have made strong arguments that flavonoids induce cell death through caspase activation and activity (Arafa, et al., 2009; Chien, et al., 2009; Das, Banik, & Ray, 2010; Sakao, Fujii, & Hou, 2009; Shen, Chen, Hsu, & Lee, 2003; Wang, Lin-Shiau, & Lin, 1999; Way, Kao, & Lin, 2005). However, it should be noted that in these papers, the authors only showed through western blots that caspase zymogens could be cleaved. Although cleavage of caspase zymogens should produce active caspases, the authors failed to test this directly. Here it is shown that flavonoids are able to inhibit caspase activity directly, even after staurosporine induced activation of caspase-3 and/or −7.

Suprisingly, myricetin was one of the more potent inhibitors of caspase-1, caspase-3, and caspase-7 in an *in vitro* assay (Figure 4) yet was dramatically weaker in the *in vivo* assay for caspase-3 and −7 (Figure 3). There are two plausible explanations to account for these differences. The first is that myricetin was being degraded either in the cell culture medium or within the cytoplasm of the cell and that therefore there was not enough present to fully inhibit the activity. The second explanation is that myricetin was not able to effectively cross the cell membrane and enter the cell. Current studies are underway to distinguish these possibilities. Preliminary data in our lab indicate that some flavonoids exhibit one or the other quality (i.e. degradation in cell culture medium or inability to cross the cell membrane).

In the context of their ability to inhibit caspases, how do flavonoids kill cells? On the one hand, the flavonoids are able to inhibit staurosporine-induced caspase activity at concentrations at which they are cytotoxic and we have been unable to detect phosphatidylserine flipping by annexin-V staining, considered to be an indicator of apoptosis (data not shown). These results suggest a non-apopototic form of cell death may be active. On the other hand, it has been shown that the flavonoids are able to induce DNA fragmentation and PARP-cleavage (data not shown). Interestingly, it was found that flavonoids can induce equivalent cell death in mouse MEF cells lacking caspase-3 and −7 (Figure 5B) whereas these cells exhibit improved viability after staurosporine treatment. (Lakhani, et al., 2006) At this stage, the mechanism of flavonoid cytotoxicity is unclear and may involve a caspase-independent form of cell-death.(Kroemer & Martin, 2005)

Finally, the results suggest that the hydroxyl groups on the C-ring of the flavonoid (Fig. 1) may be important in binding to the activity site of caspases. The C-ring must be at the C-2 position of the flavone nucleus as isoflavonoids (genistein and daidzein) were unable to inhibit caspase activity. Hydrogen bonding is probably occurring through the hydroxyl groups although this remains to be tested.

5. Conclusions

In conclusion, our results show that some natural flavonoids are able to inhibit caspase-1, −3, and −7 activity. Quercetin shows specificity for caspase-3 whereas myricetin shows specificity for caspase-1, suggesting that these flavonoids may be useful leads for the rational design of non-peptidic, caspase-specific inhibitors for therapeutic use.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A

Supplementary data

Refer to Web version on PubMed Central for supplementary material.

Highlights

- flavonoids induce cytotoxicity and apoptosis in cancer.
- flavonoids inhibit caspase activity *in vitro* and *in vivo*.
- flavonoids are selective inhibitors of specific caspases.
- flavonoids may be used to development specific small molecule caspase inhibitors.
- caspase inhibitors can be used as novel therapeutic strategies.

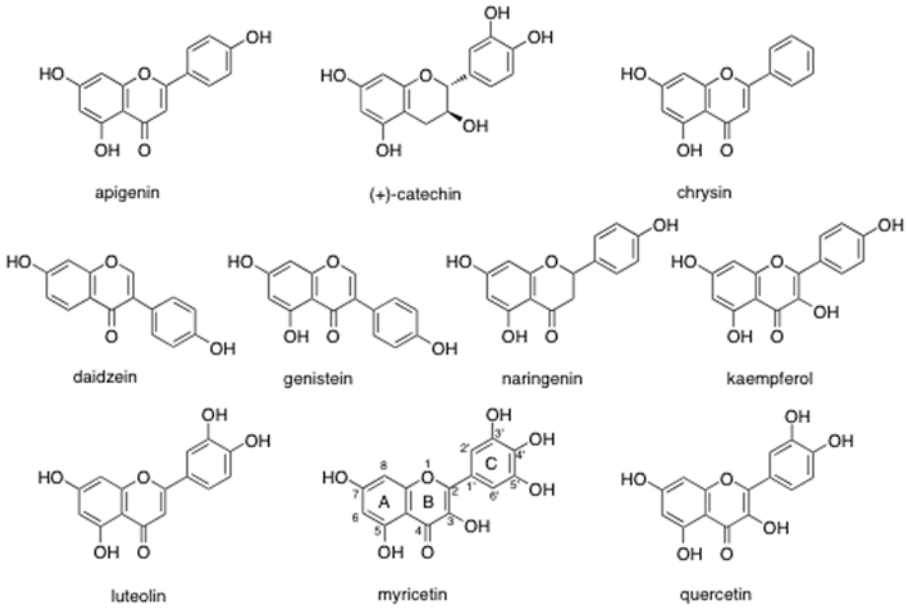

Figure 1. Natural flavonoids used in this study

Flavonoids are polyhydroxylated flavone (2-phenyl-1,4-benzopyrone) derivatives of plant origin. Isoflavonoids are similar in structure, but the phenyl substituent is at the 3-position of the 1,4-benzopyrone. The flavonoids may be hydroxylated at positions 3, 5, 7, 3′, 4′, and/or 5′ as shown for the fully hydroxylated myricetin, and the 2,3 bond may be a single bond rather than a double bond, as in naringenin or catechin. Numbering is indicated on myricetin.

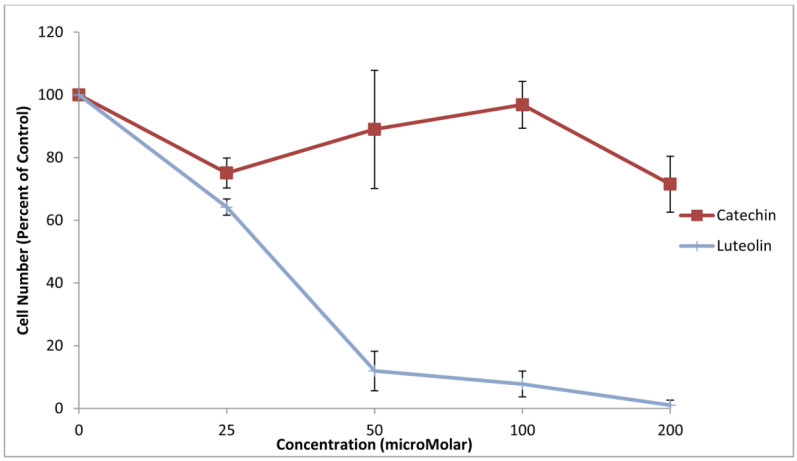

Figure 2. Example of dose-dependent flavonoid cytotoxicity for a strong killer (luteolin) and a weak killer (catechin) on MDA-MB-231 cells

The cell number, as a percent of the DMSO-treated cell number, was calculated by counting cells using Trypan Blue Exclusion. Data points represent means of 4 to 6 independent experiments; Error bars represent standard deviation. Data for other flavonoids can be found in supplemental Figure S3.

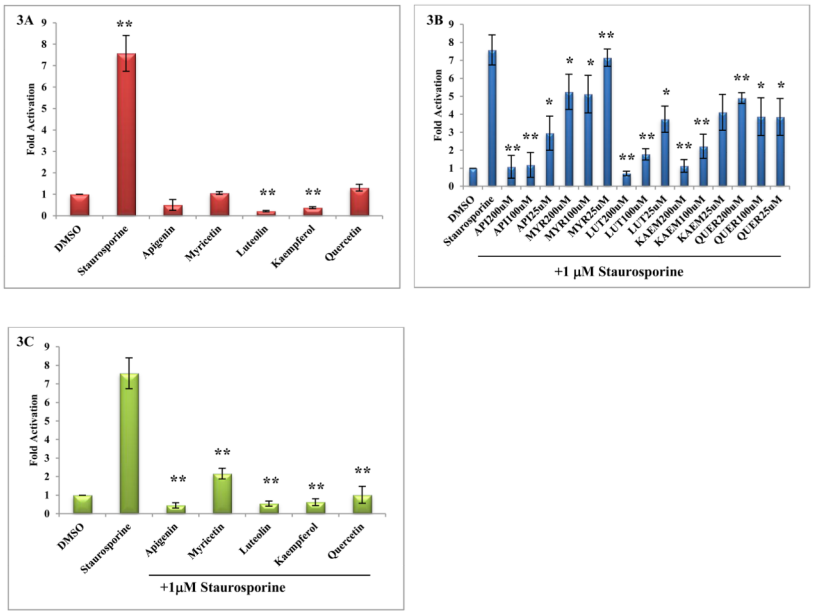

Figure 3. Flavonoids inhibit Caspase-3/−7 activity in MDA-MB-231 cells

A. Cells were treated with 200 μM flavonoids or 1 μM staurosporine for 24 hours and assayed. Only staurosporine induced caspase-3/−7 activity. B. Dose dependent inactivation of caspase-3/−7 by flavonoids. Cells were treated with 1 μM staurosporine alone or 1 μM staurosporine and apigenin (API), myricetin (MYR), luteolin (LUT), kaempferol (KAEM), and quercetin (QUER) (200 μM, 100 μM, and 25 μM respectively) for 24 h and then assayed for caspase-3/−7 activity. C. Flavonoids directly inhibit staurosporine induced caspase 3/7 activity. Cells were treated with 1 μM staurosporine for 24 h. Immediately before cells were to be assayed for caspase-3/−7 activation, media were replaced as indicated with 200 μM flavonoid and 1 μM staurosporine in media and then assayed. Results are reported as fold activation compared to DMSO treated cells. Error bars represent standard error of margin (n=3). * $p<0.05$, ** $p<0.005$, t-test.

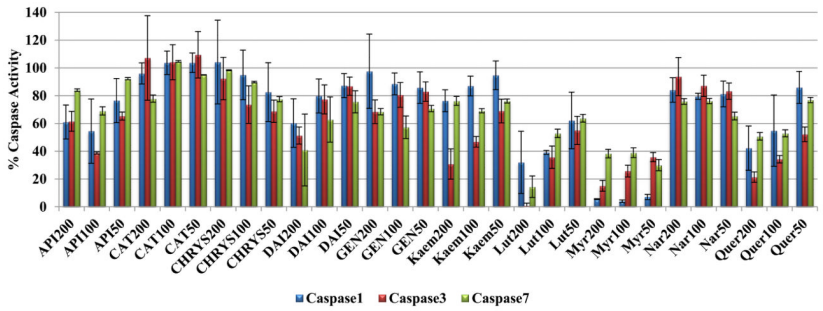

Figure 4. Flavonoids directly inhibit purified recombinant caspases

Various flavonoids were tested to inhibit purified, activated, recombinant caspase-1, −3, or −7 in the presence of their respective substrate. Results were normalized to caspase in the absence of inhibitor. Assays were performed on two separate occasions and the results are the average with error bars inidicating standard deviation. Abbreviations used for flavonoids are: API (apigenin), CAT (+-catechin), CHRYS (chrysin), DAI (daidzein), GEN (genistein), Kaem (kaempferol), Lut (luteolin), Myr (myricetin), Nar (narigenin), and Quer (quercetin).

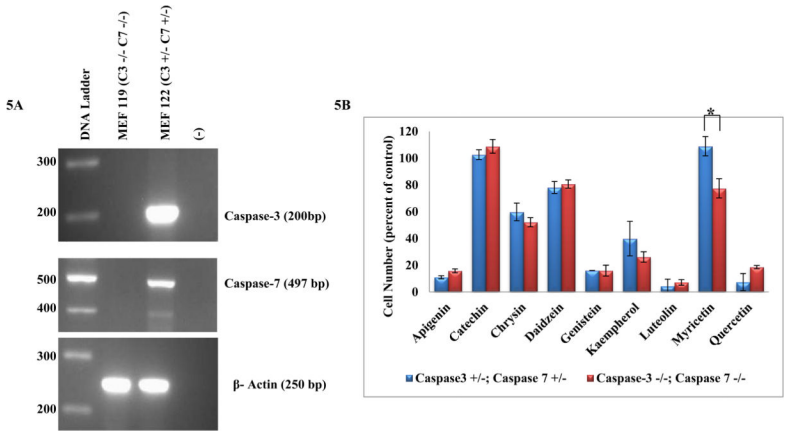

Figure 5. Flavonoid induced cell death does not require caspases

(A) RT-PCR for expression of Caspase-3, Caspase-7, and β-Actin from MEF 119 (Caspase 3 −/−/Caspase7 −/−) and MEF 122 (Caspase 3+/−/Caspase 7+/−, wild type) cells. (B) Cellular number as a percent of DMSO-treated cells after 72 hours of treatment with flavonoids as indicated. Trypan blue exclusion assays were performed as described. Error bars represent standard deviation from three independent experiments. *p<0.05, t-test.

Table 1

IC_{50} values for flavonoid-induced cell death at 72 hours. Values in parenthesis are the range for 95% confidence interval (CI).

Flavonoid	IC_{50} (μM, 95% CI)
Apigenin	14 (11–19)
Catechin	>200
Chrysin	40 (35–45)
Daidzein	179 (144–222)
Genistein	51 (43–61)
Kaempferol	38 (31–47)
Luteolin	16 (14–20)
Myricetin	>200
Narigenin	>200
Quercetin	101 (82–124)

Table 2

Ki's for flavonoid inhibition of caspase-1, −3, and −7.

	Caspase-1			Caspase-3			Caspase-7		
Compound	**K_i (uM)**	**SD**	**n**	**K_i (uM)**	**SD**	**n**	**K_i (uM)**	**SD**	**n**
none (Km)	125.2	147.2	4.0	23.6	7.3	5.0	51.6	21.5	4.0
luteolin	118.6	46.0	5.0	181.0	82.8	5.0	129.8	65.7	5.0
myricetin	14.3	3.9	6.0	68.7	30.8	3.0	65.9	22.7	6.0
quercetin	>300		3.0	96.4	41.6	4.0	>300		3.0
kaempferol	>300		1.0	194.3	216.6	4.0	>300		1.0