

Apigenin Overview

Apigenin is described as a nonmutagenic bioflavonoid which is presented in leafy plants and vegetables (e.g., parsley, artichoke, basil, celery) and has significant chemopreventive activity against UV-radiation. Current research trials indicate that it may reduce DNA oxidative damage; inhibit the growth of human leukemia cells and induced these cells to differentiate; inhibit cancer cell signal transduction and induce apoptosis; act as an anti-inflammatory; and as an anti-spasmodic or spasmolytic.

(Source: <http://www.nabio.net/products1.htm>)

Research Overview

1. TNF-induced transactivation was inhibited by apigenin.2. (BASIC RESEARCH)
2. The antiproliferative flavonoid apigenin led to an inhibition of protein kinase CK2 activity in prostate cell lines. (BASIC RESEARCH)
3. Apigenin is cytotoxic to a particular abnormal cell line in vitro. (BASIC RESEARCH)
4. Brined olives contain higher concentrations of phenolic antioxidants, including apigenin, than olive oil and may be more important modulators of cancer chemopreventive activity.
5. Apigenin may augments prostate cancer therapy. (BASIC RESEARCH)
6. Apigenin has strong potential for development as an agent for prevention against prostate cancer. (BASIC RESEARCH)
7. Apigenin is a pleiotropic effector affecting protease-dependent invasiveness and associated processes and proliferation of tumor cells. (BASIC RESEARCH)
8. Apigenin is a promising anti-breast cancer agent and its growth inhibitory effects. (BASIC RESEARCH)
9. Apigenin inhibits UV-induced skin tumorigenesis in mice when topically applied. (ANIMAL)
10. Apigenin markedly inhibited the proliferation, and, to a lesser degree, the migration of endothelial cells, and capillary formation in vitro. (BASIC RESEARCH)
11. Suppression of nuclear receptor levels is presented as a novel mechanism whereby flavonoids exert their pleiotropic effects. (BASIC RESEARCH)
12. Apigenin may provide a new approach for the treatment of human anaplastic thyroid carcinoma for which no effective therapy is presently available. (BASIC RESEARCH)
13. Modulation of COX-2 and iNOS by apigenin may be important in the prevention of carcinogenesis and inflammation. (BASIC RESEARCH)
14. Over 50% of tested flavonoids significantly inhibited aromatase activity, with greatest activity being demonstrated with apigenin. (HUMAN)
15. Intervention with parsley, containing high levels of apigenin, seemed, partly, to overcome the decrease in antioxidant SOD. (HUMAN)
16. The flavones apigenin and luteolin strongly inhibited the growth of HL60 cells and induced morphological differentiation into granulocytes. (BASIC RESEARCH)
17. The wide distribution of isoflavonoids and flavonoids (including apigenin) in the plant kingdom, together with their anti-angiogenic and anti-mitotic properties, suggest that these phytoestrogens may contribute to the preventive effect of a plant-based diet on chronic diseases, including solid tumours. (BASIC RESEARCH)
18. Luteolin and apigenin inhibited E2-induced DNA synthesis. (BASIC RESEARCH)
19. In skin tumors, apigenin may block several points in the process of tumor promotion, including inhibiting kinases, reducing transcription factors and regulating cell cycle. (BASIC RESEARCH)
20. The effects of 21 synthetic and naturally occurring flavonoids on the in vitro growth of cells of the human breast carcinoma showed that apigenin was the most potent. (BASIC RESEARCH)
21. Flavonoids, including apigenin, may have a role in ameliorating atherosclerosis.
22. Flavonoids, including apigenin, prevent in vitro LDL oxidation and probably would be important to prevent atherosclerosis.
23. Flavonoids containing phenol B rings, included apigenin, formed prooxidant metabolites that oxidized NADH upon oxidation by peroxidase/H₂O₂.

Apigenin Abstracts (35)

1_ Am J Physiol Gastrointest Liver Physiol. 2003 Jul 3 [Epub ahead of print]. 5,6dichlororibifuranosylbenzimidazole (DRB) and apigenin induced sensitization of colon cancer cells to TNF α mediated apoptosis. Farah M, Parhar K, Moussavi M, Eivemark S, Salh B. The Jack Bell Research Centre, Vancouver, BC, Canada.

Tumor necrosis factor alpha (TNF α) is a multifunctional cytokine involved in the expression of many genes integral to the inflammatory response. Additionally it activates both apoptotic and survival pathways, the latter being mediated through the activation of the transcription factor NF κ B. Protein kinase CK2, a serinethreonine kinase that is universally upregulated in human malignancies, may be involved at multiple levels in this process. However its role in mediating a survival response within colon cancer cells remains incompletely understood. Here we report that inhibition of CK2 in HCT116 and HT29 cells using two specific CK2 inhibitors, 5,6dichlororibifuranosylbenzimidazole (DRB) and apigenin, effected a synergistic reduction in cell survival when used in conjunction with TNF α . Furthermore, there was a demonstrable synergistic reduction in colony formation in soft agar using the same combinations. Western analysis showed that PARP and procaspase 3 cleavage complemented the FACS analysis findings of significantly increased subdiploid DNAcontaining cell populations using these conditions. Remarkably, these events occurred in the absence of any reduction in the expression of the Bcl2 family members Bcl2, Mcl1 and BclXL, or any change in the proapoptotic molecules Bad or Bax. Onehybrid NF κ B promoter assays utilizing a Gal4p65 transactivation domain construct revealed that the TNF α induced transactivation was inhibited by both DRB and apigenin. This was associated with a concomitant reduction in the expression of a recognized antiapoptotic NF κ B target, manganese superoxide dismutase (MnSOD) demonstrated by QPCR. Our findings indicate a potentially novel strategy for the treatment of colon cancer, one that targets CK2 simultaneous with TNF α administration.

2_ Int J Oncol. 2003 Jun;22(6):126370. Inhibition of CK2 activity provokes different responses in hormonesensitive and hormonerefractory prostate cancer cells. Hessenauer A, Montenarh M, Gotz C. Medizinische Biochemie und Molekularbiologie, Universitat des Saarlandes, D66424 Homburg, Germany.

Protein kinase CK2 seems to play an essential role in cellular growth regulation as well as in apoptosis. By using a pair of prostate carcinoma cell lines which are either hormonesensitive (LNCaP cells) or hormonerefractory (PC3 cells) we analysed the contribution of protein kinase CK2 to their different growth behaviour as well as to apoptosis. We found the same amount of CK2 subunits in both cell lines although the enzymatic activity of CK2 was much higher in the hormonerefractory cells. These results for the first time show a correlation between the specific activity of protein kinase CK2 and specific growth properties of prostate cancer cells. The antiproliferative flavonoid apigenin led to an inhibition of the CK2 activity in both types of cells but only the hormonesensitive LNCaP cells responded with apoptosis. Thus, these results demonstrate that a high CK2 activity is dispensable for growth and not necessary for a protection against apoptosis in hormonerefractory prostate cancer cells.

3_ Ai Zheng. 2003 Apr;22(4):35862. [Effect of emodin and apigenin on invasion of human ovarian carcinoma HO 8910PM cells in vitro] [Article in Chinese] Zhu F, Liu XG, Liang NC. Institute of Biochemistry and Molecular Biology, Guangdong Medical College, Zhanjiang, Guangdong, PR China.

BACKGROUND & OBJECTIVE: Emodin inhibited the activity of TPK and CK2 and the degradation of IkappaB. Apigenin inhibited the activity of MAPK and PI(3)K. In this study the authors observed the effect of emodin and apigenin on the invasion of HO8910PM cells in vitro. **METHODS:** Trypan blue dye exclusion assay was used to examine the cytotoxicity of emodin and apigenin. Reconstituted basement membrane invasion assay was utilized to evaluate the invasive activity. Type IV collagenase production was analyzed by PAGE substrate zymography. **RESULTS:** Emodin had weaker cytotoxicity on HO8910PM cells than apigenin, their IC(50) after treatment with the chemicals for 48 hours were (35.30+/-3.50) micromol/L, and (28.92+/-2.60)micromol/L, while emodin significantly inhibited membrane invasion and adhesion and migration of HO8910PM cells. Their inhibition rates after treated with the chemical of 40 micromol/L were (45.31+/-3.10)%,(25.42+/-1.70)%, and (41.59+/-1.90)%. Emodin inhibited the production but not activity of MMP9. Apigenin inhibited migration and adhesion of HO8910PM cells, their inhibition rates after treated with the chemical of 40 micromol/L were (29.04+/-1.70)% and (30.80+/-3.00)%, while weakly inhibited membrane invasion (the inhibition rate only was 12.1%) and inhibited neither production nor activity of MMP9. **CONCLUSION:** Both emodin and apigenin had cytotoxicity on HO8910PM cells. Emodin was a potential agent inhibiting tumor invasion and metastasis.

4_ Food Chem Toxicol. 2003 May;41(5):70317. Isolation, structure elucidation and antioxidant potential of the major phenolic and flavonoid compounds in brined olive drupes. Owen RW, Haubner R, Mier W, Giacosa A, Hull WE, Spiegelhalder B, Bartsch H. Division of Toxicology and Cancer Risk Factors, German Cancer Research Center, Im Neuenheimer Feld 280, D69120 Heidelberg, Germany. r.owen@dkfz heidelberg.de

Because olives represent an important component of the Mediterranean diet, it is necessary to establish unequivocal identification and quantitation of the major potential antioxidant phenolic compounds they contain. The major phenolic antioxidants in two types of brined olives were isolated and purified by semipreparative high performance liquid chromatography. Structural analysis was conducted using UV spectrophotometry, mass spectrometry and nuclear magnetic resonance spectroscopy. In particular, completely assigned ¹H and ¹³C NMR data are presented and errors in literature data are corrected. The data show that tyrosol,

hydroxytyrosol, 3(3, 4-dihydroxyphenyl) propanoic acid (dihydrocaffeic acid), dihydrocoumaric acid (phloreolic acid), the phenylpropanoid glucosides acteoside (verbascoside) and isoacteoside, along with the flavonoids luteolin and apigenin are major components of the phenolic fraction of brined black olives. Brined green olives contain only hydroxytyrosol and traces of other minor phenolics. Brined olives contain even higher concentrations of phenolic antioxidants than olive oil and may, therefore, be more important modulators of cancer chemopreventive activity.

5_ Zhonghua Yi Xue Za Zhi. 2002 Nov 10;82(21):14847. [Bystander effect mediated by herpes simplex virusthymidine kinase/ganciclovir approach on prostatic cancer cells and its regulation] [Article in Chinese] Xing Y, Lu G, Xiao Y, Zeng F, Zhang Q, Xiong P, Feng W. Department of Urology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China.

OBJECTIVE: To estimate the bystander effect mediated by herpes simplex virusthymidine kinase/ganciclovir (HSVTK/GCV) suicide gene therapy approach on PC3m, a prostate cancer cell line, to explore the role of connexin (Cx) mediated gap junctional intercellular communication (GJIC) in the procedure of bystander effect of HSVTK/GCV system and to investigate the modulation of apigenin, a Cx expression upregulator on the connexin43 (Cx43) expression and GJIC of PC3m cells. **METHODS:** PC3m cells were cultured and PC3m cells transfected with EBVbased expression vector containing HSVTK gene (TK(+) PC 3m cells) and TK() PC3m cells were mixed at the ratio of 1:9. GCV was added into the mixture. The bystander effect was evaluated by MTT assay. GJIC and HSV TK/GCV induced bystander effect in several typical cell lines, such as NIH3T3, Cos7, and L02 cells, were determined by crape loading dye tracing (SLDT) and MTT assay respectively. Cx43 mRNA expression and inherent GJIC capacity of PC3m cells were examined by RTPCR and SLDT. TK(+) PC3m cells and TK() cells were mixed and divided into 4 groups and added with GCV, apigenin, apigenin + GCV, and apigenin + GCV + 18alphaglycyrrhetic acid (AGA) respectively. Then the killing rate on PC3m cells was examined by MTT. **RESULTS:** After 72 h treatment of 100 micro mol/L GCV on the mixture of wildtype PC3m cells and HSVTK gene modified PC3m cells, only 23.5% +/- 3.2% cells were killed. The magnitude of HSVTK/GCV bystander effect were more powerful in NIH3T3, Cos7, and L02 cells which manifested excellent GJIC than in ACHN and HeLa cells (P < 0.001). Expression of Cx43 mRNA was shown by RTPCR, however, it is weaker than that in ACHN cells and normal prostate tissue. With the administration of apigenin, the expression of Cx43 mRNA and the GJIC function of PC3m cells were increased by 2.2 times (P < 0.01) The enhancing effect of apigenin on GJIC function of PC3m cells lasted 48 hours and could be inhibited by addition of AGA. Apigenin of the concentration of 10 micro mol/L could obviously improve the bystander effect of TK system on PC3m cells (P < 0.001). The killing rate of GCV on the mixed PC3m cells was 59.86% +/- 2.44%, and was only 25.34% +/- 2.89% with the addition of AGA. **CONCLUSION:** There is a positive correlation between the magnitude of bystander effect mediated by HSVTK/GCV approach and the potency of internal GJIC in the target cells. Downregulated Cx43 expression and disrupted inherent GJIC potential of PC3m cells result in the poor magnitude of HSVTK/GCV bystander effect. Chemical agent like apigenin up modulates Cx43 expression and invokes GJIC capacity of PC3m cells, thus enhancing the bystander effect and augmenting the efficacy of TK suicide therapy.

6_ Oncogene. 2002 May 23;21(23):372738. Involvement of nuclear factorkappa B, Bax and Bcl2 in induction of cell cycle arrest and apoptosis by apigenin in human prostate carcinoma cells. Gupta S, Afaq F, Mukhtar H. Department of Dermatology, Case Western Reserve University & The Research Institute of University Hospitals of Cleveland, 11100 Euclid Avenue, Cleveland, Ohio 44106, USA. gxs44@po.cwru.edu

Apigenin, a common dietary flavonoid abundantly present in fruits and vegetables, may have the potential for prevention and therapy for prostate cancer. Here, we report for the first time that apigenin inhibits the growth of androgenresponsive human prostate carcinoma LNCaP cells and provide molecular understanding of this effect. The cell growth inhibition achieved by apigenin treatment resulted in a significant decrease in AR protein expression along with a decrease in intracellular and secreted forms of PSA. These effects were also observed in DHTstimulated cells. Further, apigenin treatment of LNCaP cells resulted in G1 arrest in cell cycle progression which was associated with a marked decrease in the protein expression of cyclin D1, D2 and E and their activating partner cdk2, 4 and 6 with concomitant induction of WAF1/p21 and KIP1/p27. The induction of WAF1/p21 appears to be transcriptionally upregulated and is p53 dependent. In addition, apigenin inhibited the hyperphosphorylation of the pRb protein in these cells. Apigenin treatment also resulted in induction of apoptosis as determined by DNA fragmentation, PARP cleavage, fluorescence microscopy and flow cytometry. These effects were found to correlate with a shift in Bax/Bcl2 ratio more towards apoptosis. Apigenin treatment also resulted in downmodulation of the constitutive expression of NFkappaB/p65. Taken together, these findings suggest that apigenin has strong potential for development as an agent for prevention against prostate cancer.

7_ Cancer Lett. 2002 Feb 8;176(1):1723. Effect of flavonoids on cell cycle progression in prostate cancer cells. Kobayashi T, Nakata T, Kuzumaki T. Department of Biochemistry, Yamagata University School of Medicine, Yamagata 9909585, Japan.

The effect of some flavonoids, which are components of fruits, vegetables, and peas, on the cell cycle progression of human LNCaP prostate cancer cells has been investigated in this study. Genistein arrested the cell cycle at the G2/M phases, which is attributed to the suppression of cyclin B expression. In addition, genistein induced the cyclindependent kinase inhibitor p21, which does not depend on p53 activation. Apigenin and luteolin also increased p21 levels, but quercetin did not. Apigenin induced p21 production through a p53dependent pathway, but luteolin did so in a p53independent manner. These results suggest that flavonoids are potent regulators of cyclin B and p21 for cell cycle progression, which may play some roles in prevention of carcinogenesis.

8_ Nutr Cancer. 2001;39(1):13947. Apigenin acts on the tumor cell invasion process and regulates protease production. Lindenmeyer F, Li H, Menashi S, Soria C, Lu H. Institut National de la Sante et de la Recherche Medicale, U553, Bat. INSERM, Institut d'Hematologie, Hopital SaintLouis, Universite Paris 7, 75475 Paris, France.

Apigenin is a widely distributed plant flavonoid and was proposed as an antitumor agent. In this study, we investigated the apigenin effects on the proteasemediated invasiveness in an estrogeninsensitive breast tumor cell line MDAMB231. The results show that apigenin at 22.845.5 microM (2.510 micrograms/ml) strongly inhibited, in a dosedependent manner, tumor cell invasion through Matrigel, cell migration, and cell proliferation. We show that apigenin treatment from 22.8 microM (2.5 micrograms/ml) led to a partial decrease in urokinaseplasminogen activator expression and to a total inhibition of phorbol 12myristate 13acetateinduced matrix metalloproteinase9 secretion. We also demonstrate in the apigenintreated cells a defective adhesion to Matrigel and a G2M cell cycle arrest. Taken together, our results demonstrate that apigenin is a pleiotropic effector affecting proteasependent invasiveness and associated processes and proliferation of tumor cells.

9_ Biochem Biophys Res Commun. 2001 Oct 5;287(4):91420. Selective growthinhibitory, cellcycle deregulatory and apoptotic response of apigenin in normal versus human prostate carcinoma cells. Gupta S, Afaq F, Mukhtar H. Department of Dermatology, Case Western Reserve University, Research Institute of University Hospitals of Cleveland, 11100 Euclid Avenue, Cleveland, OH 44106, USA. gxs44@po.cwru.edu

Agents that are capable of inducing selective apoptosis of cancer cells are receiving considerable attention in developing novel cancerpreventive approaches. In the present study, employing normal human prostate epithelial cells (NHPE), virally transformed normal human prostate epithelial cells (PZHPV7), and human prostate adenocarcinoma (CAHPV10) cells, we evaluated the growthinhibitory effects of apigenin, a flavonoid abundantly present in fruits and vegetables. Apigenin treatment to NHPE and PZHPV7 resulted in almost similar growth inhibitory responses of low magnitude. In sharp contrast, apigenin treatment resulted in a significant decrease in cell viability of CAHPV10 cells. Similar selective growth inhibitory effects were also observed for human epidermoid carcinoma A431 cells compared to normal human epidermal keratinocytes. Apigenin treatment resulted in significant apoptosis of CAHPV10 cells as evident from (i) DNA ladder assay, (ii) fluorescence microscopy, and (iii) TUNEL assay, whereas the NHPE and PZHPV7 cells did not undergo apoptosis but showed exclusive necrotic staining only at a high dose of 40 microM. Apigenin (110 microM) also resulted in a dosedependent G2M phase cell cycle arrest of CAHPV10 cells but not of PZHPV7 cells. The growthinhibitory and apoptotic potential of apigenin was also observed in a variety of prostate carcinoma cells representing different stage and androgen responsiveness. Apigenin may be developed as a promising chemopreventive and/or chemotherapeutic agent against prostate cancer. Copyright 2001 Academic Press.

10_ Anticancer Res. 2001 JanFeb;21(1A):41320. Apigenin inhibits growth and induces G2/M arrest by modulating cyclinCDK regulators and ERK MAP kinase activation in breast carcinoma cells. Yin F, Giuliano AE, Law RE, Van Herle AJ. Division of Endocrinology, UCLA School of Medicine, Los Angeles, California 90024, USA.

We have previously reported that apigenin inhibits the growth of thyroid cancer cells by attenuating epidermal growth factor receptor (EGFR) tyrosine phosphorylation and phosphorylation of ERK mitogenactivated protein (MAP) kinase. In this study, we assessed the growth inhibitory effect of apigenin on MCF7 breast carcinoma cells that express two key cell cycle regulators, wildtype p53 and the retinoblastoma tumor suppressor protein (Rb), and MDAMB468 breast carcinoma cells that are mutant for p53 and Rb negative. We found that apigenin potently inhibited growth of both MCF7 and MDAMB468 breast carcinoma cells. The approximate IC50 values determined after 3 days incubation, were 7.8 micrograms/ml for MCF7 cells, and 8.9 micrograms/ml for MDAMB468 cells, respectively. Because the cell cycle studies using FACS showed that both MCF7 and MDAMB468 cells were arrested in G2/M phase after apigenin treatment, we studied the effects of apigenin on cell cycle regulatory molecules. We observed that G2/M arrest by apigenin involved a significant decrease in cyclin B1 and CDK1 protein levels, resulting in a marked inhibition of CDK1 kinase activity. Apigenin reduced the protein levels of CDK4, cyclins D1 and A, but did not affect cyclin E, CDK2 and CDK6 protein expression. In MCF7 cells, apigenin markedly reduced Rb phosphorylation after 12 h. We also found that apigenin treatment resulted in a dose and timedependent inhibition of ERK MAP kinase phosphorylation and activation in MDAMB468 cells. These results suggest that apigenin is a promising antibreast cancer agent and its growth inhibitory effects are mediated by targeting different signal transduction pathways in MCF7 and MDAMB468 breast carcinoma cells.

11_ Carcinogenesis 2000 Apr;21(4):6339 Increase in wildtype p53 stability and transactivational activity by the chemopreventive agent apigenin in keratinocytes. McVean M, Xiao H, Isobe K, Pelling JC Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS 66160, USA.

Apigenin, a naturally occurring, nonmutagenic flavonoid, has been shown to inhibit UVinduced skin tumorigenesis in mice when topically applied. In this report we have used the mouse keratinocyte 308 cell line, which contains a wildtype p53 gene, to study the effect of apigenin treatment on p53 protein levels and the expression of its downstream partner, p21/waf1. Cells were treated with 70 microM apigenin for various times and levels of p53 and p21/waf1 protein were assessed by western blot analysis. The level of p53 protein was induced 27fold after 4 h of apigenin treatment and levels remained elevated through 10 h of exposure. After 24 h of exposure to 70 microM apigenin, p53 protein levels returned to control levels. p21/waf1 protein levels increased approximately 1.52fold after 4 h and remained elevated at 24 h. To investigate the mechanism of p53 protein accumulation, we compared the halflife

of p53 protein in vehicle and apigenin treated cells. Cells were incubated for 4 h in the presence of apigenin, then cycloheximide was added to inhibit further protein synthesis and p53 protein levels were measured by western blot. The half-life of p53 protein was found to be increased an average of 8fold in apigenin treated cells compared with vehicle treated cells ($t(1/2) = 131$ min versus 16 min in apigenin versus vehicle treated cells, respectively). The mechanism of p53 protein stabilization is currently being investigated. To determine whether p53 was transcriptionally active, we also performed gel mobility shift assays and transient transfection studies using a luciferase plasmid under the control of the p21/waf1 promoter. Both p53 DNA binding activity and transcriptional activation peaked after 24 h of exposure to apigenin. These studies suggest that apigenin may exert antitumorigenic activity by stimulating the p53/p21/waf1 response pathway.

12_ *Int J Cancer* 2000 Mar 1;85(5):6916 Apigenin inhibits endothelial cell proliferation in G(2)/M phase whereas it stimulates smooth muscle cells by inhibiting P21 and P27 expression. Trochon V, Blot E, Cymbalista F, Engelmann C, Tang RP, Thomaidis A, Vasse M, Soria J, Lu H, Soria C INSERM U353, Institut d'Hematologie, Hopital SaintLouis, Universite Paris 7, Paris, France.

Apigenin is a plant flavonoid that is thought to play a role in the prevention of carcinogenesis. However, its mechanism of action has not yet been elucidated. Because of the importance of angiogenesis in tumor growth, we investigated the effect of apigenin on endothelial and smooth muscle cells in an in vitro model. Apigenin markedly inhibited the proliferation, and, to a lesser degree, the migration of endothelial cells, and capillary formation in vitro, independently of its inhibition of hyaluronidase activity. In contrast, it strongly stimulated vascular smooth muscle cell proliferation. The molecular mechanisms of apigenin activity were analyzed in these 2 types of cells. Our results show that apigenin inhibits endothelial cell proliferation by blocking the cells in the G(2)/M phase as a result of the accumulation of the hyperphosphorylated form of the retinoblastoma protein. Apigenin stimulation of smooth muscle cells was attributed to the reduced expression of 2 cyclin dependent kinase inhibitors, p21 and p27, which negatively regulate the G(1) phase cyclin dependent kinase. Copyright 2000 WileyLiss, Inc.

13_ *Biochem Biophys Res Commun* 2000 Feb 5;268(1):23741 The flavonoid apigenin suppresses vitamin D receptor expression and vitamin D responsiveness in normal human keratinocytes. Segaert S, Courtois S, Garmyn M, Degreef H, Bouillon R Laboratory for Experimental Medicine, Department of Dermatology, Katholieke Universiteit Leuven, Campus Gasthuisberg, Onderwijs en Navorsing, Herestraat 49, Leuven, B3000, Belgium.

Apigenin, a flavonoid with chemopreventive properties, induces cellular growth arrest, with concomitant inhibition of intracellular signaling cascades and decreased protooncogene expression. We report that apigenin potently inhibited vitamin D receptor (VDR) mRNA and protein expression in human keratinocytes without changes in VDR mRNA half-life. Concurrently, downregulation of retinoid X receptor alpha, a dramatic loss of cmyc mRNA, and upregulation of p21(WAF1) took place. Furthermore, a nearly complete suppression of vitamin D responsiveness was observed as estimated by induction of 24-hydroxylase mRNA. The apigenin effect on VDR expression was shared by some other (quercetin and fisetin) but not all tested flavonoids. Interestingly, the apigenin mediated VDR suppression was counteracted by the NF-kappaB inhibitors sodium salicylate and caffeic acid phenethyl ester. The presented results propose suppression of nuclear receptor levels as a novel mechanism whereby flavonoids exert their pleiotropic effects. This study may also contribute to the understanding of the regulation of VDR expression in epidermal keratinocytes. Copyright 2000 Academic Press.

14_ *Anticancer Res.* 1999 MarApr;19(2A):12619. Effect of citrus flavonoids on HL60 cell differentiation. Kawaii S, Tomono Y, Katase E, Ogawa K, Yano M. National Institute of Fruit Tree Science, Shizuoka, Japan.

Twentyseven Citrus flavonoids were examined for their activity of induction of terminal differentiation of human promyelocytic leukemia cells (HL60) by nitro blue tetrazolium (NBT) reducing, nonspecific esterase, specific esterase, and phagocytic activities. 10 flavonoids were judged to be active (percentage of NBT reducing cells was more than 40% at a concentration of 40 microM), and the rank order of potency was natsudaidain, luteolin, tangeretin, quercetin, apigenin, 3, 3', 4', 5', 6', 7', 8 heptamethoxyflavone, nobiletin, acacetin, eriodictyol, and taxifolin. These flavonoids exerted their activity in a dose dependent manner. HL60 cells treated with these flavonoids differentiated into mature monocyte/macrophage. The structure activity relationship established from comparison between flavones and flavanones revealed that orthocatechol moiety in ring B and C2C3 double bond had an important role for induction of differentiation of HL60. In polymethoxylated flavones, hydroxyl group at C3 and methoxyl group at C8 enhanced the differentiation inducing activity.

15_ *Eur J Cancer* 1999 Oct;35(10):151725 Induction of apoptosis by apigenin and related flavonoids through cytochrome c release and activation of caspase9 and caspase3 in leukaemia HL60 cells. Wang IK, Lin Shiau SY, Lin JK. Institute of Biochemistry, College of Medicine, National Taiwan University, Taipei, R.O.C.

The aim of this study was to investigate the mechanism of flavonoid induced apoptosis in HL60 leukaemic cells. Thus, the effect of structurally related flavonoids on cell viability, DNA fragmentation and caspase activity was assessed. Loss of membrane potential and reactive oxygen species generation were also monitored by flow cytometry. The structurally related flavonoids, such as apigenin, quercetin, myricetin, and kaempferol were able to induce apoptosis in human leukaemia HL60 cells. Treatment with flavonoids (60 microM) caused a rapid induction of caspase3 activity and stimulated proteolytic cleavage of poly(ADP-ribose) polymerase (PARP). Furthermore, these flavonoids induced loss of mitochondrial transmembrane potential, elevation of reactive

oxygen species (ROS) production, release of mitochondrial cytochrome c into the cytosol, and subsequent induction of procaspase9 processing. The potency of these flavonoids on these features of apoptosis were in the order of: apigenin > quercetin > myricetin > kaempferol in HL60 cells treated with 60 microM flavonoids. These results suggest that flavonoid-induced apoptosis is stimulated by the release of cytochrome c to the cytosol, by procaspase9 processing, and through a caspase3-dependent mechanism. The induction of apoptosis by flavonoids may be attributed to their cancer chemopreventive activity. Furthermore, the potency of flavonoids for inducing apoptosis may be dependent on the numbers of hydroxyl groups in the 2-phenyl group and on the absence of the 3-hydroxyl group. This provides new information on the structure-activity relationship of flavonoids.

16_ *Anticancer Res* 1999 Sep/Oct;19(5B):4297303 Signal pathways involved in apigenin inhibition of growth and induction of apoptosis of human anaplastic thyroid cancer cells (ARO). Yin F, Giuliano AE, Van Herle AJ Division of Endocrinology, UCLA School of Medicine 90024, USA. fyin@ucla.edu

Recently we demonstrated that several flavonoids can inhibit the proliferation of certain human thyroid cancer cell lines. Among the flavonoids tested, apigenin and luteolin are the most effective inhibitors of these tumor cell lines. In the present study, we investigated the signal transduction mechanism associated with the growth inhibitory effect of apigenin, using a human anaplastic thyroid carcinoma cell line, ARO (UCLA RO81A1). Using Western blot method, it was shown that the inhibitory effect of apigenin on ARO cell proliferation is associated with an inhibition of both EGFR tyrosine autophosphorylation and phosphorylation of its downstream effector mitogen activated protein (MAP) kinase. Protein levels of these signaling molecules were not affected. The inhibitor of phosphorylation by apigenin occurred within 30 min and continued for 4 h. A dose-dependent inhibition was demonstrable ranging from 12.5 microM to 50 microM. The level of phosphorylated cMyc, a nuclear substrate for MAPK, was depressed from 1648 h after apigenin treatment, finally leading to a programmed cell death involving DNA fragmentation. Furthermore, treatment with apigenin resulted in the inhibition of both anchorage-dependent and anchorage-independent thyroid cancer cell growth. In summary, apigenin is a promising inhibitor of signal transduction pathways that regulate the growth (anchorage-dependent and independent) and survival of human anaplastic thyroid cancer cells. Apigenin may provide a new approach for the treatment of human anaplastic thyroid carcinoma for which no effective therapy is presently available.

17_ *Carcinogenesis* 1999 Oct;20(10):194552 Suppression of inducible cyclooxygenase and inducible nitric oxide synthase by apigenin and related flavonoids in mouse macrophages. Liang YC, Huang YT, Tsai SH, Lin Shiau SY, Chen CF, Lin JK Institute of Biochemistry, College of Medicine, National Taiwan University, No. 1, Section 1, Taipei, Taiwan.

Prostaglandins biosynthesis and nitric oxide production have been implicated in the process of carcinogenesis and inflammation. In this study, we investigated the effect of various flavonoids and (epigallocatechin-3-gallate on the activities of inducible cyclooxygenase (COX2) and inducible nitric oxide synthase (iNOS) in lipopolysaccharide (LPS)-activated RAW 264.7 macrophages. Apigenin, genistein and kaempferol were markedly active inhibitors of transcriptional activation of COX2, with IC(50) < 15 microM. In addition, apigenin and kaempferol were also markedly active inhibitors of transcriptional activation of iNOS, with IC(50) < 15 microM. Of those compounds tested, apigenin was the most potent inhibitor of transcriptional activation of both COX2 and iNOS. Western and northern blot analyses demonstrated that apigenin significantly blocked protein and mRNA expression of COX2 and iNOS in LPS-activated macrophages. Transient transfection experiments showed that LPS caused an approximately 4-fold increase in both COX2 and iNOS promoter activities, these increments were suppressed by apigenin. Moreover, electrophoretic mobility shift assay (EMSA) experiments indicated that apigenin blocked the LPS-induced activation of nuclear factor-kappaB (NF-kappaB). The inhibition of NF-kappaB activation occurs through the prevention of inhibitor kappaB (I-kappaB) degradation. Transient transfection experiments also showed that apigenin inhibited NF-kappaB-dependent transcriptional activity. Finally, we showed that apigenin could inhibit the I-kappaB kinase activity induced by LPS or interferon-gamma. The results of further studies suggest that suppression of transcriptional activation of COX2 and iNOS by apigenin might mainly be mediated through inhibition of I-kappaB kinase activity. This study suggests that modulation of COX2 and iNOS by apigenin and related flavonoids may be important in the prevention of carcinogenesis and inflammation.

18_ *Arch Pharm Res* 1999 Jun;22(3):30912 Inhibition of aromatase activity by flavonoids. Jeong HJ, Shin YG, Kim IH, Pezzuto JM Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 60612, USA. hyehjean@nmdhst.cc.nih.gov

In searching for potent cancer chemopreventive agents from synthetic or natural products, 28 randomly selected flavonoids were screened for inhibitory effects against partially purified aromatase prepared from human placenta. Over 50% of the flavonoids significantly inhibited aromatase activity, with greatest activity being demonstrated with apigenin (IC50: 0.9 microg/mL), chrysin (IC50: 1.1 microg/mL), and hesperetin (IC50: 1.0 microg/mL).

Int J Cancer 1999 Jul 19;82(2):26873 Mitogen-activated protein kinase (MAPK) regulates the expression of progelatinase B (MMP9) in breast epithelial cells. Reddy KB, Krueger JS, Kondapaka SB, Diglio CA Department of Pathology, Wayne State University, Detroit, Michigan 48201, USA. kreddy@med.wayne.edu

Mitogen-activated protein kinases (MAPKs) play a major role in the mitogenic signal transduction pathway and are essential components of both growth and differentiation. Constitutive activation of the MAPK cascade is associated with the carcinogenesis

and metastasis of human breast and renal cell carcinomas. The gelatinases B (MMP9) and A (MMP2) are 2 members of the matrix metalloproteinase (MMPs) family which are expressed in human cancers and thought to play a critical role in tumor cell invasion and metastasis. In a previous study, we have shown that EGF and amphiregulin upregulate MMP9 in metastatic SKBR3 cells but have no effect on MMP2 secretion. We now investigated specific step(s) in EGF-induced signalling associated with regulation of cell proliferation and MMP9 induction. EGF-induced signalling in SKBR3 cells was blocked by relatively specific inhibitors either on ras (FPT inhibitor1) or P13 kinase (Wortmannin) or by reduction in EGF-induced tyrosine kinase activity (RG 13022). Blocking these signalling pathways significantly inhibited of EGF-induced cell proliferation but only partially reduced in EGF-induced MMP9 secretion. In contrast, when SKBR3 cells were exposed to MEK inhibitor (PD 98059) or MAPK inhibitors (Apigenin or MAPK antisense phosphorothioate oligodeoxynucleotides), EGF-induced cell proliferation, MMP9 induction and invasion through reconstituted basement membrane were significantly reduced. Our results suggest that interfering with MAPK activity may provide a novel means of controlling growth and invasiveness of tumors in which the signalling cascade is activated.

19_ Thyroid 1999 Apr;9(4):36976 Growth inhibitory effects of flavonoids in human thyroid cancer cell lines. Yin F, Giuliano AE, Van Herle AJ Division of Endocrinology, UCLA School of Medicine, Los Angeles, California 90024, USA.

Previous studies have indicated that flavonoids exhibit antiproliferative properties on some hormonedependent cancer cell lines, such as breast and prostate cancer. In the present study, the effects of some selected flavonoids, genistein, apigenin, luteolin, chrysin, kaempferol, and biochanin A on human thyroid carcinoma cell lines, UCLA NPA871 (NPA) (papillary carcinoma), UCLA RO82W1 (WRO) (follicular carcinoma), and UCLA RO81A1 (ARO) (anaplastic carcinoma) have been examined. Among the flavonoids tested, apigenin and luteolin are the most potent inhibitors of these cell lines with IC50 (concentration at which cell proliferation was inhibited by 50%) values ranging from 21.7 microM to 32.1 microM. The cells were viable at these concentrations. Using NPA cells known to be estrogen receptor positive (ER+), it was shown that no significant [3H]E2 displacement occurred with these flavonoids at the IC50 concentration. In WRO cells that are known to have an antiestrogen binding site (AEBS), biochanin A caused a stronger inhibitory growth effect (IC50 = 64.1 microM) than in NPA and ARO cells. In addition, it was observed that biochanin A has an appreciable binding affinity for the AEBS as indicated by the displacement of [3H]tamoxifen from the WRO cells. In summary, flavonoids have potent antiproliferative activity in vitro against various human thyroid cancer cell lines. The inhibitory activity of certain flavonoid compounds may be mediated via the AEBS and/or type II EBS. The observation that ARO cells that lack both the AEBS and the ER are effectively inhibited by apigenin and luteolin suggest that other mechanisms of action are operative as well. The present study suggests that flavonoids may represent a new class of therapeutic agents in the management of thyroid cancer.

20_ Br J Nutr 1999 Jun;81(6):44755 Effect of parsley (*Petroselinum crispum*) intake on urinary apigenin excretion, blood antioxidant enzymes and biomarkers for oxidative stress in human subjects. Nielsen SE, Young JF, Daneshvar B, Lauridsen ST, Knuthsen P, Sandstrom B, Dragsted LO Institute of Food Safety and Toxicology, Danish Veterinary and Food Administration, Copenhagen, Denmark.

Seven men and seven women participated in a randomized crossover trial to study the effect of intake of parsley (*Petroselinum crispum*), containing high levels of the flavone apigenin, on the urinary excretion of flavones and on biomarkers for oxidative stress. The subjects received a strictly controlled diet low in flavones and other naturally occurring antioxidants during the 2 weeks of intervention. This basic diet was supplemented with parsley providing 3.734.49 mg apigenin/MJ in one of the intervention weeks. Urinary excretion of apigenin was 1.59409.09 micrograms/MJ per 24 h during intervention with parsley and 0.112.27 micrograms/MJ per 24 h on the basic diet ($P < 0.05$). The fraction of apigenin intake excreted in the urine was 0.58 (SE 0.16)% during parsley intervention. Erythrocyte glutathione reductase (EC 1.6.4.1; GR) and superoxide dismutase (EC 1.15.1.1; SOD) activities increased during intervention with parsley ($P < 0.005$) as compared with the levels on the basic diet, whereas erythrocyte catalase (EC 1.11.1.6) and glutathione peroxidase (EC 1.11.1.9) activities did not change. No significant changes were observed in plasma protein 2adipic semialdehyde residues, a biomarker of plasma protein oxidation. In this shortterm investigation, an overall decreasing trend in the activity of antioxidant enzymes was observed during the 2week study. The decreased activity of SOD was strongly correlated at the individual level with an increased oxidative damage to plasma proteins. However, the intervention with parsley seemed, partly, to overcome this decrease and resulted in increased levels of GR and SOD.

21_ Biosci Biotechnol Biochem 1998 Nov;62(11):2199204 Structureactivity relationships of flavonoids and the induction of granulocytic or monocytic differentiation in HL60 human myeloid leukemia cells. Takahashi T, Kobori M, Shinmoto H, Tsushida T Iwate Industrial Research Institute, Japan.

The flavones apigenin and luteolin strongly inhibited the growth of HL60 cells and induced morphological differentiation into granulocytes. The flavonol quercetin inhibited the cell growth and induced a differentiation marker, i.e., NBT reducing ability. However quercetin-treated cells were not morphologically differentiated into granulocytes. The chalcone phloretin weakly induced NBT reducing ability and a marker of monocytic differentiation alphanaphthyl butyrate esterase activity in the cells. Quercetin and phloretin appeared to induce the differentiation of HL60 cells into monocytes. The proportion of alphanaphthyl butyrate esterase-positive cells induced by genistein was less than that of the NBT-positive cells. Some of the nuclei in genistein-treated HL60 cells morphologically changed. Genistein must have induced both granulocytic and monocytic differentiation of HL60 cells. The flavonols galangin and kaempferol, which had fewer hydroxyl group(s) in the B-ring than quercetin, and the flavanone naringenin inhibited the growth but did not induce the differentiation of HL60 cells.

22_ Baillieres Clin Endocrinol Metab 1998 Dec;12(4):64966 Phytoestrogens and inhibition of angiogenesis. Fotsis T, Pepper MS, Montesano R, Aktas E, Breit S, Schweigerer L, Rasku S, Wahala K, Adlercreutz H Laboratory of Biological Chemistry, Medical School, University of Ioannina, Greece.

The consumption of a plantbased diet can prevent the development and progression of chronic diseases associated with extensive neovascularization, including the progression and growth of solid malignant tumours. We have previously shown that the plantderived isoflavonoid genistein is a potent inhibitor of cell proliferation and in vitro angiogenesis. Moreover, the concentration of genistein in the urine of subjects consuming a plantbased diet is 30fold higher than that in subjects consuming a traditional Western diet. We have also reported that certain structurally related flavonoids are more potent inhibitors than genistein. Indeed, 3hydroxyflavone, 3',4'dihydroxyflavone, 2',3'dihydroxyflavone, fisetin, apigenin and luteolin inhibit the proliferation of normal and tumour cells as well as in vitro angiogenesis at halfmaximal concentrations in the lower micromolar range. The wide distribution of isoflavonoids and flavonoids in the plant kingdom, together with their antiangiogenic and antimitotic properties, suggest that these phytoestrogens may contribute to the preventive effect of a plantbased diet on chronic diseases, including solid tumours.

23_ Nutr Cancer 1998;31(2):90100 Effects of phytoestrogens on DNA synthesis in MCF7 cells in the presence of estradiol or growth factors. Wang C, Kurzer MS Department of Food Science and Nutrition, University of Minnesota, St. Paul 55108, USA.

Phytoestrogen effects on estrogen action and tyrosine kinase activity have been proposed to contribute to cancer prevention. To study these mechanisms, a number of phytoestrogens and related compounds were evaluated for their effects on DNA synthesis (estimated by thymidine incorporation analysis) in estrogendependent MCF7 cells in the presence of estradiol (E2), tamoxifen, insulin, or epidermal growth factor. We observed that 1) at 0.0110 microM, genistein and coumestrol enhanced E2induced DNA synthesis, as did 10 microM enterolactone. Chrysin at 1.010 microM and 10 microM luteolin or apigenin inhibited E2induced DNA synthesis, as did all compounds at > 10 microM, 2) tamoxifen enhanced genisteininduced DNA synthesis but inhibited DNA synthesis induced by all other compounds, and 3) genistein enhanced insulin and epidermal growth factorinduced DNA synthesis at 0.11.0 and 0.110 microM, respectively. At higher concentrations, inhibition was observed. Similar effects were seen with coumestrol. In conclusion, the effects of phytoestrogens in the presence of E2 or growth factors are concentration dependent and variable. At low concentrations, genistein and coumestrol significantly enhanced E2induced and tyrosine kinasemediated DNA synthesis; at high concentrations, inhibition was observed. Differing effects were observed with the other compounds. The variable effects of phytoestrogens on DNA synthesis must be considered when their roles in cancer prevention or treatment are evaluated.

24_ Am J Clin Nutr 1998 Jun;67(6):12108 Effects of flavonoids and vitamin C on oxidative DNA damage to human lymphocytes. Noroozi M, Angerson WJ, Lean ME Department of Human Nutrition, Glasgow University, Royal Infirmary, United Kingdom.

This study assessed the antioxidant potencies of several widespread dietary flavonoids across a range of concentrations and compared with vitamin C as a positive control. The antioxidant effects of pretreatment with flavonoids and vitamin C, at standardized concentrations (7.6, 23.2, 93, and 279.4 micromol/L), on oxygen radicalgenerated DNA damage from hydrogen peroxide (100 micromol/L) in human lymphocytes were examined by using the singlecell gel electrophoresis assay (comet assay). Pretreatment with all flavonoids and vitamin C produced dosedependent reductions in oxidative DNA damage. At a concentration of 279 micromol/L, they were ranked in decreasing order of potency as follows: luteolin (9% of damage from unopposed hydrogen peroxide), myricetin (10%), quercetin (22%), kaempferol (32%), quercitrin (quercetin3Lrhamnoside) (45%), apigenin (59%), quercetin3glucoside (62%), rutin (quercetin3betaDrutinoside) (82%), and vitamin C (78%). The protective effect of vitamin C against DNA damage at this concentration was significantly less than that of all the flavonoids except apigenin, quercetin3glucoside, and rutin. The ranking was similar with estimated ED50 (concentration to produce 50% protection) values. The protective effect of quercetin and vitamin C at a concentration of 23.2 micromol/L was found to be additive (quercetin: 71% of maximal DNA damage from unopposed hydrogen peroxide; vitamin C: 83%; both in combination: 62%). These data suggest that the free flavonoids are more protective than the conjugated flavonoids (eg, quercetin compared with its conjugate quercetin3glucoside, $P < 0.001$). Data are also consistent with the hypothesis that antioxidant activity of free flavonoids is related to the number and position of hydroxyl groups.

25_ Anticancer Res 1998 MarApr;18(2A):111721 Bioflavonoids commonly and potently induce tyrosine dephosphorylation/inactivation of oncogenic prolinedirected protein kinase FA in human prostate carcinoma cells. Lee SC, Kuan CY, Yang CC, Yang SD Department of Life Science, National Tsing Hua University, Hsinchu, Taiwan, R.O.C.

In this study, we investigate the effect of bioflavonoids on the activity and phosphotyrosine content of oncogenic prolinedirected protein kinase FA (PDPK FA) in human prostate carcinoma cells. Chronic treatment of human prostate carcinoma cells with low concentrations of quercetin, apigenin, and kaempferol commonly and potently induced tyrosine dephosphorylation and concurrent inactivated oncogenic PDPK FA in a concentrationdependent manner. This is demonstrated by a specific assay of this kinase's activity in the immunoprecipitates from the cell extracts followed by immunoblotting and phosphotyrosine analysis. The results indicate that bioflavonoids may function as common tyrosine kinase inhibitors to inhibit PDPK FAspecific tyrosine kinase and thereby to induce tyrosine dephosphorylation/inactivation of this oncogenic kinase in human carcinoma cells. Under this condition, quercetin, apigenin, and kaempferol can also inhibit cell growth in a similar concentrationdependent manner. The results further

indicate that inhibition of tyrosine phosphorylation/activation of this oncogenic PDPK represents a new mode of action mechanism for bioflavonoids during the antiproliferation process in human carcinoma cells.

26_ Suppression of protein kinase C and nuclear oncogene expression as possible molecular mechanisms of cancer chemoprevention by apigenin and curcumin. Lin JK, Chen YC, Huang YT, LinShiau SY. College of Medicine, National Taiwan University, Taipei, Taiwan. *J Cell Biochem Suppl* 1997;2829:3948 Apigenin, a less toxic and nonmutagenic flavonoid, suppressed 12O-tetradecanoyl phorbol-13-acetate (TPA) mediated tumor promotion of mouse skin. TPA had the ability to activate protein kinase C (PKC) and induced nuclear protooncogene expression. Our study indicates that apigenin inhibited PKC by competing with adenosine triphosphate (ATP). Apigenin also reduced the level of TPA-stimulated phosphorylation of cellular proteins and inhibited TPA-induced c-jun and c-fos expression. Curcumin, a dietary pigment phytopolyphenol, is also a potent inhibitor of tumor promotion induced by TPA in mouse skin. When mouse fibroblast cells were treated with TPA alone, PKC translocated from the cytosolic fraction to the particulate fraction. Treatment with 15 or 20 microM curcumin for 15 min inhibited TPA-induced PKC activity in the particulate fraction by 2660%. Curcumin also inhibited PKC activity in vitro by competing with phosphatidylserine. Curcumin (10 microM) suppressed the expression of c-jun in TPA-treated cells. Fifteen flavonoids were examined for their effects on morphological changes in soft agar and cellular growth in v-Hras transformed NIH3T3 cells. The results demonstrated that only apigenin, kaempferol, and genistein exhibited the reverting effect on the transformed morphology of these cells. Based on these findings, it is suggested that the suppression of PKC activity and nuclear oncogene expression might contribute to the molecular mechanisms of inhibition of TPA-induced tumor promotion by apigenin and curcumin.

27_ *Nutr Cancer* 1997;28(3):23647 Phytoestrogen concentration determines effects on DNA synthesis in human breast cancer cells. Wang C, Kurzer MS Department of Food Science and Nutrition, University of Minnesota, St. Paul 55108, USA.

Thirteen isoflavonoids, flavonoids, and lignans, including some known phytoestrogens, were evaluated for their effects on DNA synthesis in estrogen-dependent (MCF7) and independent (MDAMB231) human breast cancer cells. Treatment for 24 hours with most of the compounds at 2080 microM sharply inhibited DNA synthesis in MDAMB231 cells. In MCF7 cells, on the other hand, biphasic effects were seen. At 0.110 microM, coumestrol, genistein, biochanin A, apigenin, luteolin, kaempferol, and enterolactone induced DNA synthesis 150-235% and, at 2090 microM, inhibited DNA synthesis by 50%. Treatment of MCF7 cells for 10 days with genistein or coumestrol showed continuous stimulation of DNA synthesis at low concentrations. Time-course experiments with genistein in MCF7 cells showed effects to be reversed by 48-hour withdrawal of genistein at most concentrations. Induction of DNA synthesis in MCF7 cells, but not in MDAMB231 cells, is consistent with an estrogenic effect of these compounds. Inhibition of estrogen-dependent and independent breast cancer cells at high concentrations suggests additional mechanisms independent of the estrogen receptor. The current focus on the role of phytoestrogens in cancer prevention must take into account the biphasic effects observed in this study, showing inhibition of DNA synthesis at high concentrations but induction at concentrations close to probable levels in humans.

28_ *Prog Clin Biol Res* 1996;395:22334 Diet intervention for modifying cancer risk. Birt DF, Pelling JC, Nair S, Lepley DE Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha 681986805, USA.

Considerable evidence suggests that dietary differences between populations account for a significant proportion of the variation in cancer occurrence in different parts of the world. A major problem has been identifying the particular dietary components which predispose or protect individuals against cancer. For example, the high rates of breast and colon cancer in the United States have been associated with numerous dietary patterns including high fat, high dietary energy, and low fruit and vegetable intakes. Our laboratories have attempted to identify mechanisms whereby diet may modify cancer and it is anticipated that future studies will determine which of these potential mechanisms may be relevant in humans. A promising lead in understanding the mechanism of high dietary fat/high dietary energy promotion of cancer was the impact of these diets on cellular protein kinase C (PKC). PKC is important in cellular signaling events which are critical to tumor promotion. Our studies demonstrated increased PKC activity and/or protein expression observed in epidermis and pancreatic epithelial cells of rodents fed high fat/energy diets. The inverse association between cancer at a number of sites and fruit and vegetable intake may be due to both micronutrient and nonnutrient components of fruits and vegetables. We have studied the prevention of skin tumor promotion by apigenin, a plant flavonoid. Apigenin may block several points in the process of tumor promotion, including inhibiting kinases, reducing transcription factors and regulating cell cycle. The complexity of our diets and the multitude of potential dietary effects which may be important in cancer development make this a fertile area for future study.

29_ *Res Commun Chem Pathol Pharmacol* 1989 Apr;64(1):6978 Antiproliferative effects of synthetic and naturally occurring flavonoids on tumor cells of the human breast carcinoma cell line, ZR751. Hirano T, Oka K, Akiba M Division of Clinical Pharmacology, Tokyo College of Pharmacy, Japan.

An examination was made of the effects of 21 synthetic and naturally occurring flavonoids on the in vitro growth of cells of the human breast carcinoma, ZR751. In all cases, antiproliferative effects were noted, with an IC₅₀ ranging from 2.7 to 33.5 micrograms/ml, except for the isoflavonoid, daidzin (IC₅₀ greater than 50 micrograms/ml). No significant structure-activity relationship among the compounds could be found. Flavone, 6-hydroxyflavone and 4',5,7-trihydroxyflavone (apigenin) were the most potent with IC₅₀ of 2.7, 3.4, and 3.5 micrograms/ml, respectively. The flavonoid effects observed here were not due to cytostatic action alone, since cell death was found to increase dose-dependently, according to the results of a dye exclusion test.

30_ Prostaglandins Leukot Essent Fatty Acids. 2003 Jul;69(1):737. Effects of flavonoids on the susceptibility of lowdensity lipoprotein to oxidative modification. Safari MR, Sheikh N. Department of Biochemistry and Nutrition, School of Medicine, Hamadan University of Medical Sciences and Health Services, Hamadan, Iran. safari@umsha.ac.ir

Dietary flavonoid intake has been reported to be inversely associated with the incidence of coronary artery disease. To clarify the possible role of flavonoids in the prevention of atherosclerosis, we investigated the effects of some of these compounds on the susceptibility of lowdensity lipoprotein (LDL) to oxidative modification. In this study, six flavonoids, "apigenin, genistein, morin, naringin, pelargonidin and quercetin", were added to plasma and incubated for 3h at 37 degrees C. Then, the LDL fraction was separated by ultracentrifugation. The oxidizability of LDL was estimated by measuring conjugated diene (CD), lipid peroxides and thiobarbituric acidreactive substances (TBARS) after cupric sulfate solution was added. We showed that among flavonoids used, quercetin and morin significantly ($P < 0.01$ by ANOVA) and dosedependently prolonged the lag time before initiation of oxidation reaction. Also, these two flavonoids suppressed the formation of lipid peroxides and TBARS more markedly than others. Their ability to prolong lag time and suppression of lipid peroxides and TBARS formation resulted to be in the following order: quercetin>morin>pelargonidin>genistein>naringin>apigenin. LDL exposed to flavonoids in vitro reduced oxidizability. These findings show that flavonoids may have a role in ameliorating atherosclerosis.

31_ Mol Cell Biochem. 2003 Apr;246(12):1936. Antioxidant effect of flavonoids on the susceptibility of LDL oxidation. Naderi GA, Asgary S, SarrafZadegan N, Shirvany H. Department of Biochemistry, Isfahan Cardiovascular Research Center, Amin Hospital, Isfahan University of Medical Sciences, Isfahan, Iran. isfcarvasrc@hotmail.com

In vitro studies have demonstrated increased atherogenicity of oxidized lowdensity lipoprotein (oxLDL) compared to native LDL. Oxidative modification of LDL alters its structure allowing LDL to be taken up by scavenger receptors on macrophage, endothelial, and smooth muscle cells, leading to the formation of lipidladen foam cells, the hallmark of early atherosclerotic lesions. The susceptibility of LDL to in vitro oxidation was assessed essentially by the technique described by Esterbauer et al. LDL oxidation were monitored by change in 234absorbance in the presence and absence of pure flavonoids. Morin, genistein, apigenin and biochanin A, naringin and quercetin were used at different concentration. These flavonoids significantly inhibit in vitro LDL oxidation, genistein, morin and naringin have stronger inhibitory activity against LDL oxidation than biochanin A or apigenin. This study show that flavonoids prevent in vitro LDL oxidation and probably would be important to prevent atherosclerosis.

32_ J Nutr Sci Vitaminol (Tokyo). 2001 Oct;47(5):35762. Antioxidant ability of various flavonoids against DPPH radicals and LDL oxidation. Hirano R, Sasamoto W, Matsumoto A, Itakura H, Igarashi O, Kondo K. Internal Medicine I, National Defense Medical College, Tokorozawa, Saitama, Japan. rhirano@me.ndmc.ac.jp

Flavonoids, a group of polyphenolic compounds, exist naturally and serve as antioxidants in vegetables, fruits, and so on. The inhibition of low density lipoprotein (LDL) oxidation may be an effective way to prevent or delay the progression of atherosclerosis. In the present study, we analyzed the radical scavenging capacity of 10 flavonoids (catechin, epicatechin [EC], epigallocatechin [EGC], epicatechin gallate [ECg], epigallocatechin gallate [EGCg], myricetin, quercetin, apigenin, kaempferol, and luteolin) toward 1,1diphenylpicrylhydrazyl [DPPH]. After 20 min of incubation, EGCg was the most effective DPPH radical scavenger, luteolin being the least active of this flavonoid group. The mutual antioxidant effect of flavonoids with alphanatocopherol (alphanatoc) on LDL oxidizability was investigated by using the lipophilic azo radical initiator 2,2'azobis(4methoxy2,4dimethylvaleronitrile) [AMVNCH3O]. An inhibitory effect of flavonoids on LDL oxidation was observed in the order of luteolin>ECg>EC>quercetin>catechin>EGCg>EGC> myricetin>kaempferol> apigenin. The shortened lag time induced by higher doses of alphanatoc (6 mg/100 mL) was restored by flavonoids. These results suggest that 1) radical trapping effects of flavonoids differ according to their structure, and 2) flavonoids act as hydrogen donors to alphanatoc radical; furthermore, by interaction with alphanatoc, they have a greater potential to delay the oxidation of LDL.

33_ Chem Biol Interact. 1999 Aug 30;122(1):1525. Oxygen activation during peroxidase catalysed metabolism of flavones or flavanones. Chan T, Galati G, O'Brien PJ. Department of Pharmacology and Faculty of Pharmacy, University of Toronto, Ont, Canada.

Flavonoids containing phenol B rings, e.g. naringenin, naringin, hesperetin and apigenin, formed prooxidant metabolites that oxidised NADH upon oxidation by peroxidase/H₂O₂. Extensive oxygen uptake occurred which was proportional to the NADH oxidised and was increased up to twofold by superoxide dismutase. Only catalytic amounts of flavonoids and H₂O₂ were required indicating a redox cycling mechanism that activates oxygen and generates H₂O₂. NADH also prevented the oxidative destruction of flavonoids by peroxidase/H₂O₂ until the NADH was depleted. These results suggest that prooxidant phenoxyl radicals formed by these flavonoids cooxidise NADH to form NAD radicals which then activated oxygen. Similar oxygen activation mechanisms by other phenoxyl radicals have been implicated in the initiation of atherosclerosis and carcinogenesis by xenobiotic phenolic metabolites. This is the first time that a group of flavonoids have been identified as prooxidants independent of transition metal catalysed autoxidation reactions.

34_ J Nutr. 1998 Dec;128(12):230712. Grape juice but not orange or grapefruit juice inhibits platelet activity in dogs and monkeys.

Platelet aggregation (PA) contributes to both the development of atherosclerosis and acute platelet thrombus formation (APTF) followed by embolization producing cyclic flow reductions (CFR) in stenosed and damaged dog and human coronary arteries. In seven anesthetized dogs with coronary stenosis and medial damage, CFR occurred at $7 \pm 3/30$ min and were abolished 127 ± 18 min after gastric administration of 10 mL of purple grape juice/kg. Collageninduced ex vivo whole blood PA decreased by $49 \pm 9\%$ after the abolishment of CFR with grape juice. Ten mL of orange juice/kg ($n = 5$) and 10 mL of grapefruit juice/kg ($n = 5$) had no significant effect on the frequency of the CFR or on ex vivo PA. In vitro studies have suggested that flavonoids bind to platelet cell membranes and thus may have an accumulative or tissue-loading effect over time. To test this we fed 5 mL of grape juice/kg to 5 cynomolgous monkeys for 7 d. Collageninduced ex vivo PA decreased by $41 \pm 17\%$ compared to control (preretreatment) after 7 d of feeding. In the same 5 monkeys, neither 5 mL of orange juice/kg nor 5 mL of grapefruit juice/kg given orally for 7 d produced any significant change in PA. Grape juice contains the flavonoids quercetin, kaempferol and myricetin, which are known inhibitors of PA in vitro. Orange juice and grapefruit juice, while containing less quercetin than grape juice, primarily contain the flavonoids naringin, luteolin and apigenin glucoside. The flavonoids in grapes were shown in vitro to be good inhibitors of PA, whereas the flavonoids in oranges and grapefruit to be poor inhibitors of PA. The consumption of grape juice, containing these inhibitors of PA, may have some of the protection offered by red wine against the development of coronary artery disease (CAD) and acute occlusive thrombosis, whereas orange juice or grapefruit juice may be ineffective. Thus, grape juice may be a useful alternative dietary supplement to red wine without the concomitant alcohol intake.

35_ Phytomedicine. 2002 Sep;9(6):48995. Apigenin strong cytostatic and antiangiogenic action in vitro contrasted by lack of efficacy in vivo. Engelmann C, Blot E, Panis Y, Bauer S, Trochon V, Nagy HJ, Lu H, Soria C. Humboldt University, Charite, Berlin, Germany. carsten.engelmann@charite.de

The cancer chemopreventive agent apigenin also has strong cytostatic and antiangiogenic effects in vitro. We now investigated its efficacy against experimental Lewis lung carcinomas (LLC), C6 gliomas and DHDK 12 colonic cancers in vivo. Tumour bearing mice received 50 mg/kg/day apigenin in three different galenical formulations during 12 days in 8hourly intervals. Only weak effects of apigenin on the size and the number of new tumour blood vessels of both established and newly transplanted tumours were recorded although the intratumoural necrosis was elevated ($45 \pm 15\%$ vs. $20 \pm 7\%$ (control), $p < 0.05\%$). These results contrast sharply with the high in vitro sensitivity of LLC, C6, DHDK 12 and endothelial cells to apigenin where complete growth suppression occurs at concentrations beyond 30 g/ml. Possible causes are discussed.

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