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Molecules of interest

Does an apple a day keep the doctor away because a phytoestrogen a day keeps the virus at bay? A review of the anti-viral properties of phytoestrogens

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Abstract

From dengue to herpes and influenza to AIDS, the phytoestrogens that are present in many fruits and vegetables have been shown to exert anti-viral properties. Here we review the various different anti-viral mechanisms employed by phytoestrogens.

Keywords: Phytoestrogen; Diet; Apple; Virus; Anti-viral

1. Introduction: 'An apple a day'

While the author of the poem 'an apple a day keeps the doctor away' is unknown and the first publication date of the poem is untraceable, the saying is thought to be the modern version of an old English saying 'Ate an apfel avore gwain to bed makes the doctor beg his bread'. It is now thought that there is at least some truth in the old saying 'an apple a day keeps the doctor away' as apples are now known to contain various phytoestrogens e.g. quercetin, the consumption of which has been linked to a healthy diet (Boyer and Liu, 2004). We suggest in this review that one of the mechanisms by which the consumption of apples (and other fruits and vegetables) is beneficial to health is because of the anti-viral properties of the phytoestrogens that they contain. Although phytoestrogens have been reviewed from various perspectives (Bacciottini and Brandi, 2004; Dijsselbloem et al., 2004; Heber, 2004) to our knowledge no reviews to date have considered their role in virally induced diseases and there has been little consideration of their antiviral properties. The purpose of this paper is therefore to review the literature addressing the various anti-viral mechanisms employed by phytoestrogens.

2. Phytoestrogens

Plants contain a wide variety of naturally occurring chemicals. Many of these chemicals were named 'phytoestrogens' because ''phyto'' which means plant was combined

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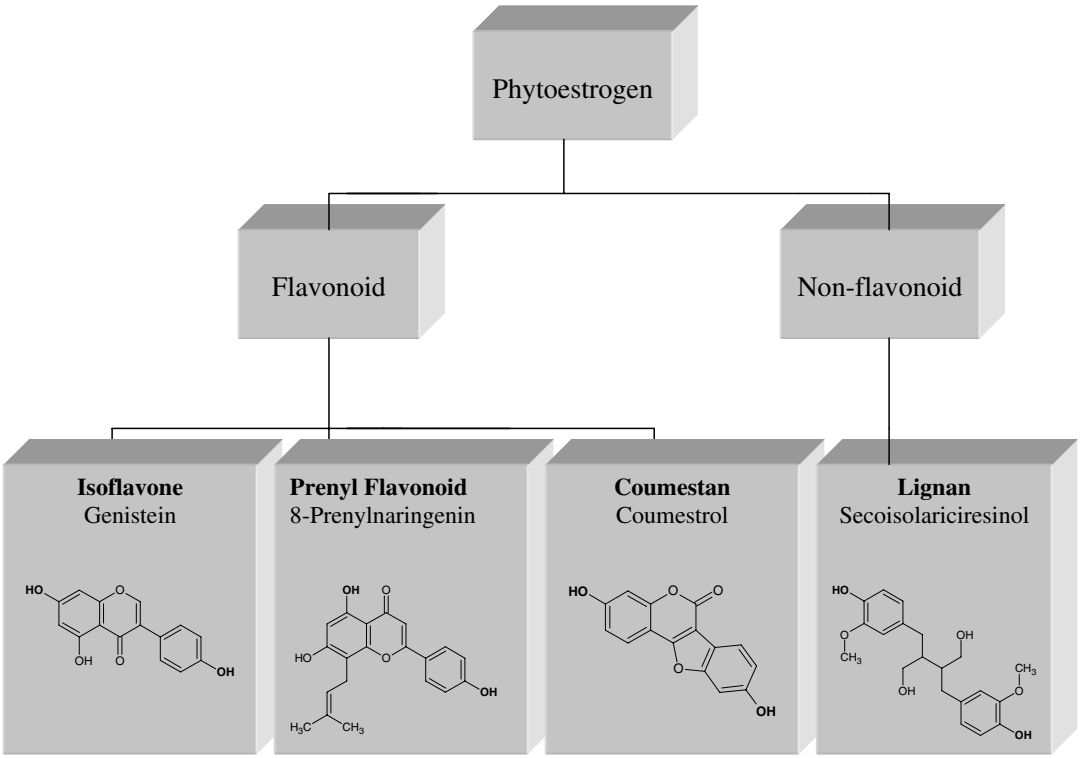

Fig. 1. The different classes of phytoestrogens.

with ''estrogen'' due to their estrogenic activity. They are non-steroidal naturally occurring phenolic compounds that can be divided into two groups: firstly, the flavonoids that are further subdivided into isoflavones, coumestans and prenyl flavonoids; and secondly the non-flavonoids, comprising the lignans (Fig. 1). All are polyphenols that have a structural similarity to estradiol (Fig. 2) and possess estrogenic activity due to having a similar 'A' ring to that of estradiol and possessing two hydroxyl groups (shown in bold in Fig. 1) at positions that afford the correct distance between them to facilitate binding to the estrogen receptor (Zand et al., 2000). The isoflavone phytoestrogens share a common structure (Fig. 2), with genistein having the important –OH groups at positions 7, and 4′. Biochanin A has a methoxy group at position 4′ and prunetin has a methoxy group at position 7 resulting in less estrogenic activity as the methoxy groups hinder binding to the estrogen receptor. In quercetin the 'B' ring is attached to position 2 and there is an –OH group at position 3.

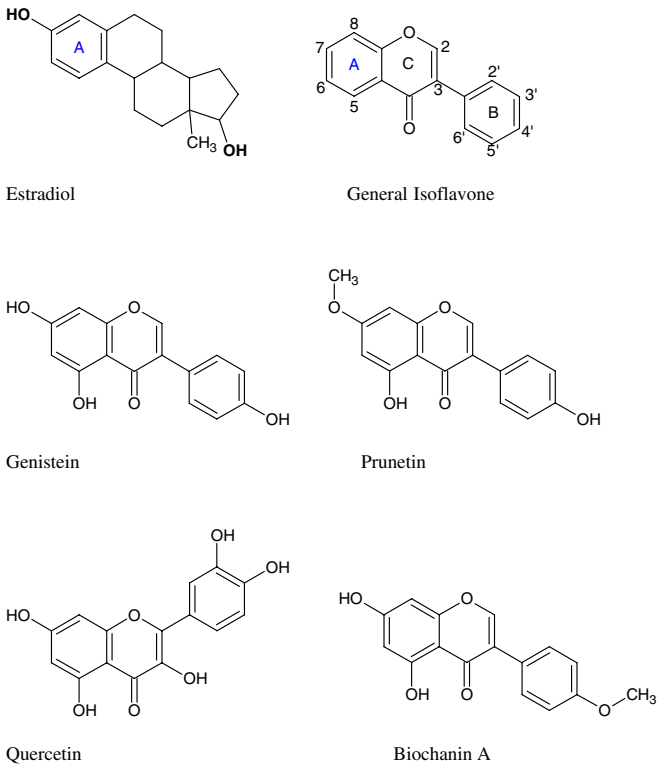

Fig. 2. The structure of estradiol and selected phytoestrogens.

Virtually all fruits and vegetables are known to be rich in phytoestrogens. Isoflavones are present in a wide variety of fruits and vegetables and Table 1 illustrates some of the richest known dietary sources of the specific phytoestrogens discussed in this review. Although genistein is found predominantly in soy-based foods such as soybeans, miso and tofu it is also present in some fruit and vegetables albeit at lower concentrations (Boker et al., 2002). Similarly, while the richest dietary source of lignans is flax seed they are also present in most fruit and vegetables. Although coumestans are much less commonly consumed they are found in some vegetables particularly clover sprouts, soy sprouts and alfalfa sprouts (Murphy et al., 1999).

Table 1
Dietary sources of selected phytoestrogens

Phytoestrogen	Dietary plant source	Mean concentration (mg/100 g)	References
Quercetin	Radish leaves	70	Sakakibara et al. (2003)
	Onions	13	Nuutila et al. (2002)
	Apples	4.7	Franke et al. (2004) and Tsao et al. (2003)
Biochanin A	Alfalfa sprouts	2946	Murphy et al. (1999)
	Red clover	833	Pettersson and Kiessling (1984)
	Chinese peas	9.3	Franke et al. (1995)
Genistein	Soybeans	80	Eldridge and Kwolek (1983) and Setchell and Walsh (1987))
	Miso	35	Coward et al. (1993)
	Tofu	28	Franke et al. (1998) and Murphy et al. (1999)

Source of data is The USDA-Iowa State University Database on the isoflavone content of foods (USDA, 2002) and the USDA Database for the flavonoid content of selected foods (USDA, 2006).

3. Entry of viruses into cells

In order to carry out replication within cells, viruses must first gain entry to those cells. The variety of different mechanisms for viral entry include the classical clathrin-mediated endocytosis, caveolae-mediated endocytosis, lipid-raft-mediated endocytosis and macropinocytosis which have all been reviewed in detail elsewhere (Pelkmans and Helenius, 2003; Smith and Helenius, 2004). Here, it will suffice to present a brief overview of both clathrin-mediated endocytosis and caveolae-mediated endocytosis, as these are the main viral entry mechanisms that have been shown to be influenced by phytoestrogens (see Table 2).

The first step in viral entry is cellular attachment, which is achieved by binding to a cell surface carbohydrate, lipid or protein (Smith and Helenius, 2004). For many viruses, the second step is facilitated by using one of the various types of endocytosis to enter the cell (Pelkmans and Helenius, 2003).

During clathrin-dependent endocytosis a virus such as influenza firstly binds to cell surface molecules such as sialic acid, which it employs as viral receptors. These are then sequestered into clathrin-coated pits at the plasma membrane (Fig. 3). This is followed by internalization of the clathrin-coated pit by receptor-mediated endocytosis into clathrin-coated vesicles which are then delivered to early endosomes. It has been shown that virus infection of cells then sets in motion virus-induced intracellular signals. These are significant early events in the infection process whereby the virus is able to manipulate the signaling pathways of the cell to transmit virus signals that facilitate viral entry.

Caveolae-mediated endocytosis (Anderson, 1998) is another mechanism used by viruses to enter cells (Fig. 3). Caveolae are invaginations of the plasma membrane that are lined with a protein coat of caveolin-1 (Anderson et al., 1996). Following internalization, caveolae travel to caveosomes where the virus is sorted and sent to either the endoplasmic reticulum or the Golgi (Pelkmans and Helenius, 2002). Some viruses, e.g. simian virus 40 (SV-40), have been shown to attach firstly to major histocompatibility complex class I molecules on the cell surface, and then to move laterally along the plasma membrane, eventually becoming trapped in a caveola (Anderson et al., 1998). This is followed by activation of tyrosine kinases that cause local phosphorylation (Kurzchalia and Parton, 1999). As several phytoestrogens are known to be tyrosine kinase inhibitors (Akiyama et al., 1987) there is much evidence to indicate that they can inhibit the phosphorylation required for these virus-induced events that are important for both viral entry and infection.

4. Anti-viral mechanisms used by phytoestrogens

4.1. Inhibition of virus entry

Simian virus 40 (SV-40) has been associated with pleural mesothelioma (Carbone et al., 1994) and osteosarcoma (Medoza et al., 1998). Treatment of kidney fibroblast cells with genistein has been shown to block SV-40 by inhibiting virus-induced signals that are required for its entry. When treated with genistein the SV-40 virions were shown to be delayed at the mouth of caveolae. This rendered the virions incapable of being internalized, with the SV-40 virus remaining at the cell surface (Chen and Norkin, 1999). Indeed, genistein may be able to block tyrosine phosphorylation of caveolin-1 resulting in inhibition of SV-40 entry into cells (Pelkmans et al., 2002). Furthermore, genistein has also been shown to block SV-40-induced upregulation of c-myc and c-jun and to cause a delay in the onset of SV-40 DNA synthesis (Dangoria et al., 1996).

4.2. Inhibition of signaling pathways

Having become internalized in the caveosome, the next step in the viral infection process involves transport from the caveosome to the endoplasmic reticulum. For the SV-40 virus, this step involves inducing a signal in the caveosome that facilitates transport of the virus from the

Table 2
Summary table

Phytoestrogen	Virus	Effect of phytoestrogen	References
Quercetin	Influenza virus	Reduces levels of superoxide radicals and lipid peroxidation products	Kumar et al. (1990)
	Human immuno-deficiency virus	Inhibits HIV integrase	Fesen et al. (1993)
	Human endogenous retrovirus	Upregulates HERV expression	Martin et al. (2005)
Biochanin A	Human herpesvirus 6	Blocks expression of early and late viral antigens	Cirone et al. (1996)
Genistein	Japanese encephalitis virus	Suppresses virus induced TNF and IL1 production	Raung et al. (2005)
	Simian virus 40	Delays virus at mouth of caveolae	Chen and Norkin (1999)
		Blocks tyrosine phosphorylation of caveolin-1	Pelkmans et al. (2002)
		Blocks virus induced upregulation of c-myc and c-jun	Dangoria et al. (1996)
	Bovine herpesvirus type 1	Inhibits phosphorylation of glycoprotein E	Akula et al. (2002)
	Human polyoma JC virus	Blocks virus induced phosphorylation of epidermal growth factor receptor pathway substrate clone 15	Querbes et al. (2004)
	Encephalomyocarditis virus	Inhibition of viral protein synthesis	Hirasawa et al. (2003)
	Mengo virus	Inhibition of viral replication	Hirasawa et al. (2003)
	Coxsackievirus B4	Inhibition of viral replication	Hirasawa et al. (2003)
	Moloney murine leukaemia virus	Reduced expression of the cellular CAT1 receptor Suppression of membrane fusion Abolition of virus-induced syncytium formation	Kubo et al. (2003)
	Dengue virus	Inhibition of phosphorylation of proteins involved in actin reorganisation	Talavera et al. (2004)
	Porcine reproductive and respiratory syndrome virus	Reduces replication of virus	Greiner et al. (2001)
	Bovine viral diarrhea virus	Inhibition of late steps of infection	Lecot et al. (2005)
Prunetin and genistein	Herpes simplex virus type 1	Inhibition of phosphorylation of viral proteins Reduced phosphorylation of cellular proteins Abolition of viral glycoprotein C expression	Yura et al. (1993)

caveosome to the endoplasmic reticulum (Pelkmans and Helenius, 2003). Genistein has been shown to block these downstream signals that are required for movement of the SV-40 virus from the caveosome to the endoplasmic reticulum (Dangoria et al., 1996). The human JC polyomavirus (JCV) which causes progressive multifocal leukoencephalopathy (Hou and Major, 2000) is a typical example of a virus that uses receptor-mediated clathrin-dependent endocytosis to enter human glial cells. It has been demonstrated that the virus triggers intracellular signals that involve phosphorylation of proteins from different signaling pathways of the cell. One of these is the epidermal growth factor receptor pathway substrate clone 15 (eps15) protein. Tyrosine phosphorylation of eps15 is required for viral entry into cells but treatment with genistein is able to significantly inhibit infectious entry of JCV (Querbes et al., 2004) possibly by inhibiting viral induced phosphorylation.

4.3. Inhibition of viral protein synthesis

Encephalomyocarditis virus (EMC), as the name suggests, can cause encephalitis and myocarditis (Yoon et al., 1982) and has also been shown to cause diabetes mellitus (Craighead and McLane, 1968). During its replication it induces activation of mitogen-activated protein kinases (MAPKs) from the cellular signaling pathways. It has been shown that, at 50μM, genistein is capable of suppressing replication of EMC virus in L929 cells to 0.5% of control level by a mechanism involving inhibition of viral protein synthesis (Hirasawa et al., 2003). In similar experiments, genistein also inhibited replication of both Mengo virus that causes non-pyrogenic meningitis, and Coxsackievirus B4 (CVB4) which causes nonpyrogenic meningitis, paralytic disease and pancreatitis (Vella et al., 1992).

4.4. Inhibition of the later stages of the viral cycle

Human herpesvirus 6 (HHV-6) is the causative agent of Roseola Infantum, an acute disease that occurs in young children and is characterized by a skin rash with an accompanying high fever (Caserta et al., 2001). HHV-6 is known to use a fusion/entry mechanism to enter cells and to cause

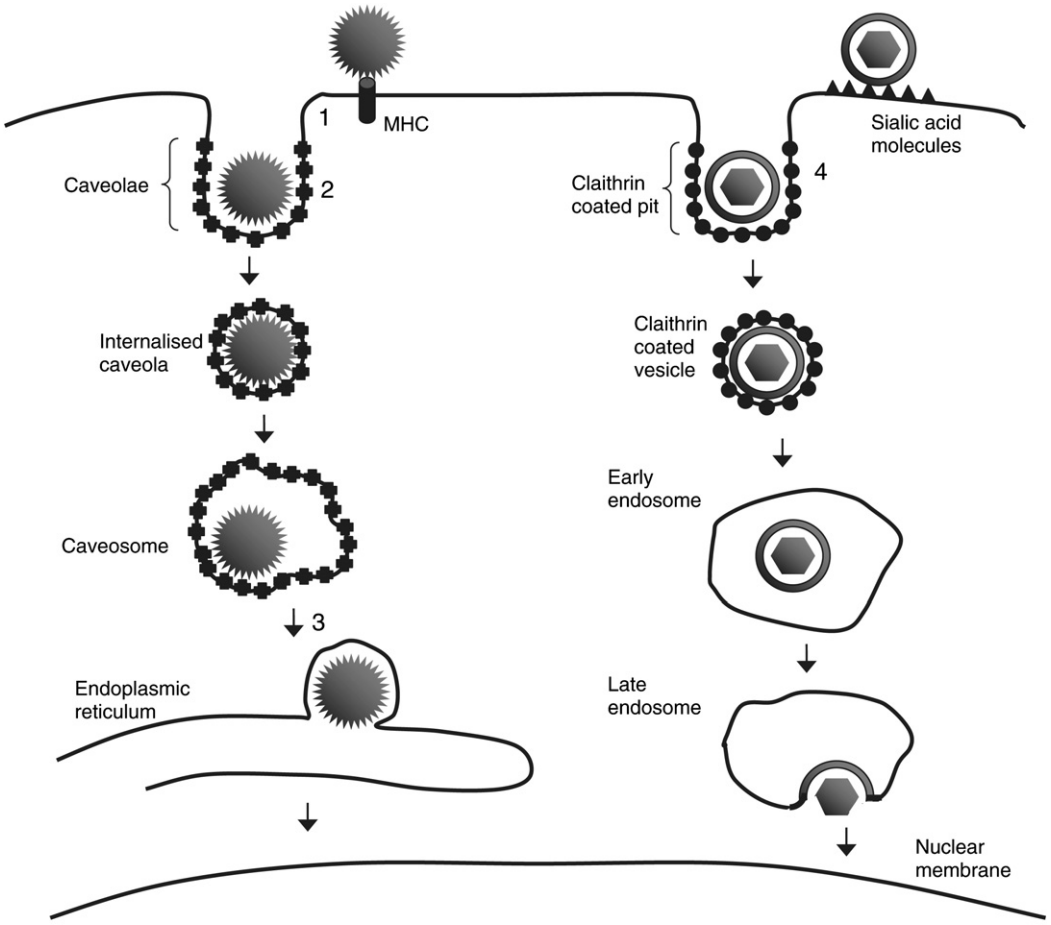

Fig. 3. Diagram of clathrin-dependent endocytosis and caveolae-mediated endocytosis.

subsequent phosphorylation of cellular proteins (Spear et al., 2000). In HSB-2 cells exposed to HHV-6, treatment with biochanin A blocks infection. Importantly, biochanin A causes significant inhibition at a late stage of the viral infectious cycle by blocking the expression of early and late viral antigens and causing inhibition of syncytium formation (Cirone et al., 1996).

Inhibition of syncytium formation is also caused by genistein in Moloney murine leukaemia virus (Mo-MLV) entry. The first step in Mo-MLV entry of XC cells is viral identification of the cellular cationic amino acid transporter 1 (CAT1) receptor (Wang et al., 1991) followed secondly by fusion of the viral envelope with the cell membrane (Jones and Rissser, 1993) and finally syncytium formation. However, when XC cells are treated with genistein it causes a reduction in expression of the CAT1 receptor by the cells, suppression of membrane fusion and abolition of virus-induced syncytium formation (Kubo et al., 2003).

When dengue virus, which is the causative agent for both dengue fever and dengue haemorrhagic fever (Rigau-Perez et al., 1998) infects cells, there is an increase in cellular release of Il-8 (Bosch et al., 2002) and other chemokines (Avirutnan et al., 1998) leading to alteration in cellular permeability with associated actin cytoskeleton rearrangements. There is also a corresponding increase in the thickness of stress fibres and focal adhesions. Treatment of human dermal microvascular endothelial cells with genistein has been shown to inhibit phosphorylation of proteins involved with actin reorganisation leading to a reduction in stress fibres and focal adhesions during dengue virus infection (Talavera et al., 2004).

Herpes simplex virus type 1 (HSV-1) infection of cells also induces phosphorylation of several polypeptides (Lemaster and Roizman, 1980) including basic fibroblast growth factor receptor (Baird et al., 1990). Genistein and prunetin have been shown to reduce HSV-1 plaque formation in Vero cells (Yura et al., 1993) due to inhibition of the phosphorylation of cellular and viral proteins during the late stage of HSV-1 infection and the abolition of viral glycoprotein C expression (Yura et al., 1993).

Another virus, bovine herpesvirus type 1 (BHV-1) infects cattle and is the aetiologic agent of infectious bovine rhinotracheitis (Trapp et al., 2003). One of the glycoproteins encoded for by BHV-1 is glycoprotein E (gE) which forms a complex with glycoprotein I to facilitate cell–cell spread of the virus (Dingwell and Johnson, 1998). Interestingly, genistein is able to significantly inhibit the replication of BHV-1 (Shaw et al., 2000) by interacting with gE and inhibiting its phosphorylation (Akula et al., 2002).

4.5. Protection of tissue damage

In addition to anti-viral mechanisms that employ direct inhibition of virus by blocking viral entry and internalization, phytoestrogens may also be able to exert anti-viral activity via other methods. Some phytoestrogens, particularly quercetin and rutin (Afanas'ev et al., 1989), have been shown to be free-radical scavengers (Cos et al., 1998) and are therefore able to reduce tissue damage caused by oxidative stress associated with viral infection. This has been demonstrated in influenza virus infection of the lung. Quercetin supplementation of the diet of mice infected with the influenza virus significantly reduced the levels of both superoxide radicals and lipid peroxidation products (Kumar et al., 2003), suggesting that quercetin may be useful as an anti-viral drug therapy to alleviate the cytopathological effects of virus infection.

Japanese encephalitis virus (JEV) infection initially causes fever, headache and vomiting and is then followed by the development of neuropathology (Kumar et al., 1990). When JEV infects cells there is a concomitant rise in tumour necrosis factor (TNF) and interleukin-1 (IL-1) levels, which contributes to JEV-induced neuropathology. Treatment of neuron/glia cultures with genistein has demonstrated a neuroprotective effect against JEV infection by suppression of virus-induced TNF and IL1 production (Raung et al., 2005).

4.6. Inhibition of other viral enzymes

While the major investigations of the anti-viral properties of phytoestrogens have concentrated on their ability to inhibit tyrosine-specific protein kinases, it has been recognized that flavonoids and isoflavonoids are also capable of inhibiting the activity of other ATP-utilising enzymes such as topoisomerase II (Constantinou et al., 1995). Some viruses are known to encode type I topoisomerases (Klemperer et al., 1995) that have been shown to be essential for viral replication (Shuman et al., 1989). Since phytoestrogens can act as inhibitors of topoisomerase, this mechanism may also be important.

During the HIV infection process, the integrase enzyme carries out integration of viral DNA into the host genome (Chiu and Davies, 2004). Quercetin has been shown to be a potent HIV integrase inhibitor (Fesen et al., 1993) and may provide yet another anti-viral molecular mechanism.

4.7. Human endogenous retroviruses

Human endogenous retroviruses (HERVs) constitute about 4.8% of the human genome and have structural similarity to exogenous retroviruses. They have been named 'fossil viruses' and are thought to represent past exogenous retroviral infection in which proviral DNA has been integrated and retained within the genome (Nelson et al., 2003; Nelson et al., 2004). There is evidence to suggest that HERVs could be beneficial as they regulate gene expression and cellular differentiation (Larsson and Andersson, 1998) or they could be harmful by alteration of genes important in cancer aetiology (Yi et al., 2004). Interestingly the long terminal repeats of HERVs may also modulate gene expression through hormone response elements. In this way phytoestrogens may exert effects through an entirely novel mechanism. Our current research suggests that, not only are phytoestrogens able to exert anti-viral properties towards exogenous viruses, but that phytoestrogens may also be able to influence HERV expression (Martin et al., 2005).

5. In vivo studies

While most of the studies have considered the anti-viral properties of phytoestrogens in vitro there have been a few animal studies that concur with the in vitro observations. It has been shown that pigs fed a soy genistein enriched diet exhibited reduced replication of porcine reproductive and respiratory syndrome (PRRS) virus when virally challenged (Greiner et al., 2001). Although the applicability of the in vitro and in vivo data to human dietary antiviral effects has yet to be established, human peak plasma phytoestrogen concentrations have been demonstrated which are comparable with the lower end of the in vitro concentration range required for antiviral activity. In the studies reviewed herein the in vitro concentration of phytoestrogen required to achieve antiviral activity ranged from 10 μM to 100 μM. Following a single dose of soybean milk young adult women have been shown to have a plasma concentration of total isoflavones of between 2 and 6 μM (Xu et al., 1994). Similarly after genistein ingestion peak plasma concentrations of 7.7 μM (Busby et al., 2002), 7 μM (Ullmann et al., 2005) and 2.44 μM have also been demonstrated (Watanabe et al., 1998).

6. Conclusion

We have presented evidence in this review that phytoestrogens do not act solely via their clearly established and well-accepted estrogen receptor mediated mechanisms but also via an alternative mechanism of expressing anti-viral activities. Although the data presented herein illustrates that genistein is the best-characterized phytoestrogen in terms of anti-viral activity, we have also shown that other phytoestrogens are capable of expressing this function and we predict that further phytoestrogens with anti-viral activity remain to be identified in the future. Although apples only contain genistein at very small quantities (Boker et al., 2002) they do contain several other phytoestrogens (Lee et al., 2003) and while it remains to be proven, the possibility does exist that phytoestrogens present within fruit may be capable of exerting greater anti-viral effects than that demonstrated during in vitro experiments. The use of a single purified substance fails to account for possible synergistic anti-viral effects with other components present within fruits and vegetables. We can conclude therefore that one of the ways in which the phytoestrogens that are present in the recommended 'an apple a day' may offer protection against a wide variety of human diseases is via their anti-viral properties. The ability of phytoestrogens to inhibit several different types of viruses is therefore worthy of further research not only to elucidate the mechanisms involved but also to design specific phytoestrogen based inhibitors to harness these valuable anti-viral properties.

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References

Afanas'ev, I.B., Dorozhko, A.I., Brodskii, A.V., Kostyuk, V.A., Potapovitch, A.I., 1989. Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. Biochem. Pharmacol. 38, 1763–1769.

Akiyama, T., Ishida, J., Nakagawa, S., Ogawars, H., Watanabe, S., Itoh, N., Shibuya, M., Fukami, Y., 1987. Genistein, a specific inhibitor of tyrosine specific protein kinases. J. Biol. Chem. 262, 5592–5595.

Akula, S.M., Hurley, D.J., Wixon, R.L., Wang, C., Chase, C.C., 2002. Effect of genistein on replication of bovine herpesvirus type 1. Am. J. Vet. Res. 63, 1124–1128.

Anderson, R.G.W., 1998. The caveolae membrane system. Annu. Rev. Biochem. 67, 199–225.

Anderson, H.A., Chen, Y., Norkin, L.C., 1996. Bound simian virus 40 translocates to caveolin-enriched membrane domains, and its entry is inhibited by drugs that selectively disrupt caveolae. Mol. Biol. Cell. 7, 1825–1834.

Anderson, H.A., Chen, Y., Norkin, L.C., 1998. MHC class I molecules are enriched in caveolae but do not enter with simian virus 40. J. Gen. Virol. 79, 1469–1477.

Avirutnan, P., Malasit, P., Seliger, B., Bhakdi, S., Husmann, M., 1998. Dengue virus infection of human endothelial cells leads to chemokine production, complement activation and apoptosis. J. Immunol. 161, 6338–6346.

Bacciottini, L., Brandi, M.L., 2004. Foods and new foods: the role of nutrition in skeletal health. J. Clin. Gastroenterol. 38, 115–117.

Baird, A., Florkiewicz, R.Z., Maher, P.A., Kaner, R.J., Hajjar, D.P., 1990. Mediation of virion penetration into vascular cells by association of basic fibroblast growth factor with herpes simplex virus type 1. Science 348, 344–346.

Boker, L.K., Van der Schouw, Y.T., De Kleijn, M.J., Jacques, P.F., Grobbee, D.E., Peeters, P.H., 2002. Intake of dietary phytoestrogens by Dutch women. J. Nutr. 132, 1319–1328.

Bosch, I., Xhaja, K., Estevez, L., Raines, G., Melichar, H., Warke, R.V., Fournier, M.V., Ennis, F.A., Rothman, A.L., 2002. Increased production of interleukinn-8 in primary human monocytes and in human epithelial and endothelial cell lines after dengue virus challenge. J. Virol. 76, 5588–5597.

Boyer, J., Liu, R.H., 2004. Apple phytochemicals and their health benefits. Nutr. J. 3, 5.

Busby, M.G., Jeffcoat, A.R., Bloeden, L.T., Koch, M.A., Black, T., Dix, K.J., Heizer, W.D., Thomas, B.F., Hill, J.M., Crowell, J.A., Zeisel, h S.H., 2002. Clinical characteristics and pharmacokinetics of purified soy isoflavones: single-dose administration to healthy men. Am. J. Clin. Nutr. 75, 126–136.

Carbone, M., Pass, H.I., Rizzo, P., Marinetti, M., DiMuzio, M., Mew, D.J., Levine, A.S., Procopio, A., 1994. Simian virus 40-like DNA sequences in human pleural mesothelioma. Oncogene 9, 1781–1790.

Caserta, M.T., Mock, D.J., Dewhurst, S., 2001. Human herpesvirus 6. Clin. Infect. Dis. 33, 829–833.

Chen, Y., Norkin, L.C., 1999. Extracellular simian virus 40 transmits a signal that promotes virus enclosure within caveolae. Exp. Cell. Res. 246, 83–90.

Chiu, T.K., Davies, D.R., 2004. Structure and function of HIV-1 integrase. Curr. Top. Med. Chem. 4, 965–977.

Cirone, M., Zompetta, C., Tarasi, D., Frati, L., Faggioni, A., 1996. Infection of human T lymphoid cells by human herpesvirus 6 blocked by two unrelated protein tyrosine kinase inhibitors, biochanin A and herbimycin. AIDS Res. Hum. Retroviruses 12, 1629–1634.

Constantinou, A., Mehta, R., Runyan, C., Rao, K., Vaughan, A., Moon, R., 1995. Flavonoids as DNA topoisomerase antagonists and poisons: structure–activity relationships. J. Nat. Prod. 58, 217–225.

Cos, P., Ying, L., Calomme, M., Hu, J.P., Cimanga, K., Van Poel, B., Pieters, L., Vlietinck, A.J., Vanden Berge, D., 1998. Structure–activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. J. Nat. Prod. 61, 71–76.

Coward, L., Barnes, N., Setchell, K.D.R., Barnes, S., 1993. Genistein, Daidzein and their glycoside conjugates: antitumour isoflavones in soybean foods from American and Asian diets. J. Agric. Food. Chem. 41, 1961–1967.

Craighead, J.E., McLane, M.F., 1968. Diabetes mellitus: induction in mice by encephalomyocarditis virus. Science 162, 913–914.

Dangoria, N.S., Breau, W.C., Anderson, H.A., Cishek, D.M., Norkin, L.C., 1996. Extracellular simian virus 40 induces an ERK/MAP kinase-independent signaling pathway that activates primary response genes and promotes virus entry. J. Gen. Virol. 77, 2173–2182.

Dijsselbloem, N., Vanden Berghe, W., De Naeyer, A., Haegeman, G., 2004. Soy isoflavone phyto-pharmaceuticals in interleukin-6 affections. Multi-purpose nutraceuticals at the crossroad of hormone replacement, anti-cancer and anti-inflammatory therapy. Biochem. Pharmacol. 68, 1171–1185.

Dingwell, K.S., Johnson, D.C., 1998. The herpes simplex virus gE–gI complex facilitates cell-to-cell spread and binds to components of cell junctions. J. Virol. 72, 8933–8942.

Eldridge, A.C., Kwolek, W.F., 1983. Soybean isoflavones: effect of environment and variety on composition. J. Agric. Food Chem. 31, 394–396.

Fesen, M.R., Kohn, K.W., Leteurtre, F., Pommier, Y., 1993. Inhibitors of human immunodeficiency virus integrase. Proc. Natl. Acad. Sci. USA 90, 2399–2403.

Franke, A.A., Custer, L.J., Cerna, C.M., Narala, K., 1995. Rapid HPLC analysis of dietary phytoestrogens from legumes and from human urine. Proc. Soc. Exp. Biol. Med. 208, 18–26.

Franke, A.A., Custer, L.J., Wang, W., Shi, C.Y., 1998. HPLC analysis of isoflavonoids and other phenolic agents from foods and from human fluids. Proc. Soc. Exp. Biol. Med. 217, 263–273.

Franke, A.A., Custer, L.J., Arakaki, C., Murphy, S.P., 2004. Vitamin C and flavonoid levels of fruits and vegetables consumed in Hawaii. J. Food Comp. Anal. 17, 1–35.

Greiner, L.L., Stahly, T.S., Stabel, T.J., 2001. The effect of dietary soy genistein on pig growth and viral replication during a viral challenge. J. Anim. Sci. 79, 1272–1279.

Heber, D., 2004. Vegetables, fruits and phytoestrogens in the prevention of diseases. J. Postgrad. Med. 50, 145–149.

Hirasawa, K., Kim, A., Han, H.S., Han, J., Jun, H.S., Yoon, J.W., 2003. Effect of p38 mitogen-activated protein kinase on the replication of encephalomyocarditis virus. J. Virol. 77, 5649–5656.

Hou, J., Major, E.O., 2000. Progressive multifocal leukoencephalopathy: JC virus induced demyelination in the immune compromised host. J. Neurovirol. 6, S98–S101.

Jones, J.S., Rissser, R., 1993. Cell fusion induced by the murine leukemia virus envelope glycoprotein. J. Virol. 67, 67–74.

Klemperer, N.D., Lyttle, J., Tauzin, D., Traktman, P., Robinson, A.J., 1995. Identification and characterization of the orf virus type I topoisomerase. Virology 206, 203–215.

Kubo, Y., Ishimoto, A., Amanuma, H., 2003. Genistein, a protein tyrosine kinase inhibitor, suppresses the fusogenicity of Moloney murine leukemia virus envelope protein in XC cells. Arch. Virol. 148, 1899–1914.

Kumar, R., Mathur, A., Kumar, A., Sethi, G.D., Sharma, S., Chaturvedi, U.C., 1990. Virological investigations of acute encephalopathy in India. Arch. Dis. Child. 65, 1227–1230.

Kumar, P., Sharma, S., Khanna, M., Raj, H.G., 2003. Effect of Quercetin on lipid peroxidation and changes in lung morphology in experimental influenza virus infection. Int. J. Exp. Pathol. 84, 127–133.

Kurzchalia, T.V., Parton, R.G., 1999. Membrane microdomains and caveolae. Curr. Opin. Cell. Biol. 11, 424–431.

Larsson, E., Andersson, G., 1998. Beneficial role of human endogenous retroviruses: facts and hypotheses. Scand. J. Immunol. 48, 329–338.

Lecot, S., Belouzard, S., Dubuisson, J., Rouille, Y., 2005. Bovine viral diarrhea virus entry is dependent on clathrin-mediated endocytosis. J. Virol. 79, 10826–10829.

Lee, K.W., Kim, Y.J., Kim, D.O., Lee, H.J., Lee, C.Y., 2003. Major phenolics in apple and their contribution to the total antioxidant capacity. J. Agric. Food Chem. 51, 6516–6520.

Lemaster, S., Roizman, B., 1980. Herpes simplex virus phosphoproteins. II. Characterisation of the virion protein kinase and of the polypeptides phosphorylated in the virion. J. Virol. 35, 798–811.

Martin, J.H.J., Crotty, S., Warren, P., Nelson, P. 2005. The effect of phytoestrogens on nitric oxide production and HERV-K10 expression of human breast cancer cell lines. National Cancer Research Institute Cancer Conference, Birmingham UK, Abstract number P66.

Medoza, S.M., Konishi, T., Miller, C.W., 1998. Integration of SV40 in human osteosarcoma Oncogene 17, 2457–2462.

Murphy, P.A., Song, T., Buseman, G., Barua, K., Beecher, G.R., Trainer, D., Holden, J., 1999. Isoflavones in retail and institutional soy foods. J. Agric. Food Chem. 47, 2697–2704.

Nelson, P.N., Davari Ejtehadi, H., Carnegie, P., Roden, D., Martin, J., Rowland-Jones, S., Hooley, P., Astley, J., Murray, P.G., 2003. Demystified... human endogenous retroviruses. J. Clin. Path.; Mol. Path. 56, 11–18.

Nelson, P.N., Hooley, P., Roden, D., Davari Ejtehadi, H., Rylance, P., Warren, P., Martin, J., Murray, P.G., 2004. Human endogenous retroviruses: transposable elements with potential? Clin. Exp. Immunol. 138, 1–9.

Nuutila, A.M., Kammiovirta, K., Oksman-Caldentey, K.-M., 2002. Comparison of methods for the hydroysis of flavonoids and phenolic acids from onion and spinach for HPLC analysis. Food Chem. 76, 519–525.

Pelkmans, L., Helenius, A., 2002. Endocytosis via caveolae. Traffic 3, 311–320.

Pelkmans, L., Helenius, A., 2003. Insider information: what viruses tell us about endocytosis. Curr. Opin. Cell. Biol. 15, 414–422.

Pelkmans, L., Puntener, D., Helenius, A., 2002. Local actin polymerization and dynamin recruitment in SV40-induced internalization of caveolae. Science 296, 535–539.

Pettersson, H., Kiessling, K.-H., 1984. Liquid chromatographic determination of the plant estrogens coumestrol and isoflavones in animal feed. J. Assoc. Off. Anal. Chem. 67, 503–506.

Querbes, W., Benmerah, A., Tosoni, D., Di Fiore, P.P., Atwood, W.J., 2004. A JC virus-induced signal is required for infection of glial cells by a clathrin- and eps15-dependent pathway. J. Virol. 78, 250–256.

Raung, S.L., Chen, S.Y., Liao, S.L., Chen, J.H., Chen, C.J., 2005. Tyrosine kinase inhibitors attenuate Japanese encephalitis virus-induced neurotoxicity. Biochem. Biophys. Res. Commun. 327, 399–406.

Rigau-Perez, J.G., Clark, G.G., Gubler, D.J., Reiter, D.J., Sanders, E.J., Vorndam, A.V., 1998. Dengue and dengue haemorrhagic fever. Lancet 352, 971–977.

Sakakibara, H., Honda, Y., Nakagawa, S., Ashida, H., Kanazawa, K., 2003. Simultaneous determination of all polyphenols in vegetables, fruits and teas. J. Agric. Food Chem. 51, 571–581.

Setchell, K.D.R., Walsh, M.B., 1987. High-performance liquid chromatographic analysis of phytoestrogens in soy protein preparations with ultraviolet, electrochemical and thermospray mass spectrometric detection. J. Chromatogr. 386, 315–323.

Shaw, A.M., Braun, L., Frew, T., Hurley, D.J., Rowland, R.R., Chase, C.C., 2000. A role for bovine herpesvirus 1 (BHV-1) glycoprotein E (gE) tyrosine phosphorylation in replication of BHV-1 wild-type virus but not BHV-1 gE deletion mutant virus. Virology 268, 159–166.

Shuman, S., Golder, M., Moss, B., 1989. Insertional mutagenesis of the vaccinia virus gene encoding a type I DNA topoisomerase: evidence that the gene is essential for virus growth. Virology 170, 302–306.

Smith, A.E., Helenius, A., 2004. How viruses enter cells. Science 304, 237–242.

Spear, P.G., Eisenberg, R.J., Cohen, G.H., 2000. Three classes of cell surface receptors for alphaherpesvirus entry. Virology 275, 1–8.

Talavera, D., Castillo, A.M., Dominguez, M.C., Gutierrez, A.E., Meza, I., 2004. IL8 release, tight junction and cytoskeleton dynamic reorganisation conducive to permeability increase are induced in dengue virus infection of microvascular endothelial monolayers. J. Gen. Virol. 85, 1801–1813.

Trapp, S., Beer, M., Mettenleiter, T.C., 2003. Biology of bovine herpesviruses. Berl Munch Tierarztl Wochenschr 116, 171–178.

Tsao, R., Yang, R., Young, J.C., Zhu, H., 2003. Polyphenolic profiles in eight apple cultivars using high-performance liquid chromatography (HPLC). J. Agric. Food Chem. 51, 6347–6353.

Ullmann, U., Metzner, J., Franke, T., Cohn, W., Riegger, C., 2005. Safety, tolerability, and pharmacokinetics of single ascending doses of synthetic genistein (Bonistein) in healthy volunteers. Adv. Ther. 22, 65–78.

US Department of Agriculture, Agricultural Research Service. 2002. USDA-Iowa State University Database on the Isoflavone Content of Foods, Release 1.3 – 2002. Nutrient Data Laboratory Web site: http://www.nal.usda.gov/fnic/foodcomp/Data/isoflav/isoflav.html.

US Department of Agriculture, Agricultural Research Service. 2006 USDA-Database for the Flavonoid Content of Selected Foods, Release 2 – 2006. Nutrient Data Laboratory Web site: http://www.nal.usda.gov/fnic/foodcomp/Data/Flav/Flav02.pdf.

Vella, C., Brown, C.L., McCarthy, D.A., 1992. Coxsackievirus B4 infection of the mouse pancreas: acute and persistent infection. J. Gen. Virol. 73, 1387–1394.

Wang, H., Kavanaugh, M.P., North, R.A., Kabat, D., 1991. Cell-surface receptor for ecotropic murine retroviruses is a basic amino-acid transporter. Nature 352, 729–731.

Watanabe, S., Yamaguchi, M., Sobue, T., Takahashi, T., Miura, T., Arai, Y., Mazur, W., Wahala, K., Adlercreutz, H., 1998. Pharma-

cokinetics of soybean isoflavones in plasma, urine and faeces of men after ingestion of 60 g baked soybean powder (kinako). J. Nutr. 128, 1710–1715.

Xu, X., Wang, H.J., Murphy, P.A., Cook, L., Hendrich, S., 1994. Daidzein is a more bioavailable soymilk isoflavone than is genistein in adult women. J. Nutr. 124, 825–832.

Yi, J.M., Kim, H.M., Kim, H.S., 2004. Expression of the human endogenous retrovirus HERV-W family in various human tissues and cancer cells. J. Gen. Virol. 85, 1203–1210.

Yoon, J.W., Rodrigues, M.M., Currier, C., Notkins, A.L., 1982. Long-term complications of virus-induced diabetes mellitus in mice. Nature 296, 566–569.

Yura, Y., Yoshida, H., Sato, M., 1993. Inhibition of herpes simplex virus replication by genistein, an inhibitor of protein-tyrosine kinase. Arch. Virol. 132, 451–461.

Zand, R.S., Jenkins, D.J., Diamandis, E.P., 2000. Steroid hormone activity of flavonoids and related compounds. Breast Cancer Res. Treat. 62, 35–49.