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REVIEWS: CURRENT TOPICS

Soy isoflavones and virus infections

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Abstract

Isoflavones and their related flavonoid compounds exert antiviral properties *in vitro* and *in vivo* against a wide range of viruses. Genistein is, by far, the most studied soy isoflavone in this regard, and it has been shown to inhibit the infectivity of enveloped or nonenveloped viruses, as well as single-stranded or double-stranded RNA or DNA viruses. At concentrations ranging from physiological to supraphysiological (3.7–370 μM), flavonoids, including genistein, have been shown to reduce the infectivity of a variety of viruses affecting humans and animals, including adenovirus, herpes simplex virus, human immunodeficiency virus, porcine reproductive and respiratory syndrome virus, and rotavirus. Although the biological properties of the flavonoids are well studied, the mechanisms of action underlying their antiviral properties have not been fully elucidated. Current results suggest a combination of effects on both the virus and the host cell. Isoflavones have been reported to affect virus binding, entry, replication, viral protein translation and formation of certain virus envelope glycoprotein complexes. Isoflavones also affect a variety of host cell signaling processes, including induction of gene transcription factors and secretion of cytokines. The efficacy of isoflavones and related flavonoids in virus infectivity in *in vitro* bioassays is dependent on the dose, frequency of administration and combination of isoflavones used. Despite promising *in vitro* results, there is lack of data confirming the *in vivo* efficacy of soy isoflavones. Thus, investigations using appropriate *in vivo* virus infectivity models to examine pharmacological and especially physiological doses of flavonoids are warranted.

Keywords: Isoflavones; Virus; Infections; Genistein

1. Introduction

There is great interest in the potential of soy and soy foods to prevent or treat chronic diseases, including cardiovascular disorders, osteoporosis and cancer. These potential benefits are mostly attributed to dietary isoflavones, a subclass of flavonoids that possess numerous biological properties and are most commonly found in legumes, with the highest amounts found in soybeans [1]. This review will focus on the potential therapeutic benefits of isoflavones present in soy and soy foods on *acute* viral infections, a topic that previously has received little attention. In order to understand the potential mechanisms of action of isoflavones against viral infections, the metabolism and pharmacokinetics of soy isoflavones, the differences in soy and isoflavones consumption and bioavailability in animal and human populations, and the *in vitro* and *in vivo* models used to examine the biological properties of isoflavones will be briefly described. After a review of the evidence for antiviral actions of isoflavones, the possible molecular mechanisms underlying the antiviral action of soy isoflavones, based both on direct experimental results and on inferences from the known biological properties of flavonoids and isoflavones, will be presented.

2. Metabolism and pharmacokinetics of soy isoflavones

The predominant isoflavones found in soybeans are the β-glycoside forms of genistein, daidzein and glycitein, which are not bioavailable [2–4]. Upon ingestion, small intestinal brushborder membrane enzymes and bacterial β-glycosidases remove the glycoside group, after which the isoflavones are readily absorbed and become bioactive.

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Some genistein (aglycone: 2.6%; glycoside: 2.9%) remains in the intestinal tissue, where it may influence cell proliferation and virus or bacterial infections [5–8]. Most of the absorbed isoflavones are glucuronidated or undergo sulfation at the gut level before entering the enterohepatic circulation [9–11]. In adult humans, aglycone forms attain maximal plasma concentrations at 4–8 h following a meal before being eliminated through the bile and urine within 16 h of ingestion [2]. If not absorbed along the small intestine, isoflavones are further metabolized by commensal bacteria in the colon into equol, *p*-ethyl phenol, *O*-desmethylangolensin or other metabolites through dehydroxylation, reduction, C-ring cleavage and demethylation [12,13].

3. *In vitro* and *in vivo* models of the isoflavone mechanisms of action

The evidence for the potential beneficial health effects of isoflavones is based on *in vitro* (a wide range of cell types), *in vivo* (rodents, swine and primates) and human epidemiological studies. The extrapolation of study results to humans is often challenging within the experimental model used due to important metabolic phenotype differences observed between species [14]. Recently, it has been shown that a woman's serum isoflavone profiles closely resemble those of a female swine, whereas a female monkey's profiles are more similar to those of laboratory rats after dietary intake of soy protein isolates. In addition, humans are non-equol or poor-equol producers, significantly differing from both monkeys and rats [14]. Interpretation of results is further complicated by differences in isoflavone consumption among different human populations.

4. Soy and isoflavone consumption in different populations

The mean isoflavone consumption by adults is higher in Asian countries (11–47 mg/day) than in Western countries (1–2 mg/day). However, infants fed soy formula (4 months' duration) in Western countries ingest much higher concentrations of isoflavones (22–45 mg/day) than adults [15]. These differences in isoflavone consumption are reflected by higher plasma concentrations in soy-formula-fed infants than in adults in Asian or Western countries. The mean plasma concentrations of isoflavones are 1640 nM for genistein and 1160 nM for daidzein for infants fed soy formula; 492.7 nM for genistein and 282.5 nM for daidzein in Japanese men; and 33.2 nM for genistein and 17.9 nM for daidzein in British men [15]. Although humans are able to absorb isoflavones from a range of soy-rich foods, the food matrix, as well as the form in which isoflavones are consumed (aglycone or glycoside), influences the pharmacokinetic profiles of isoflavones [2]. Overall, genistein appears to have a bioavailability higher than that of daidzein [13]. Thus, when attempting to extrapolate results from *in vitro* or animal models to possible effects in humans, it is critical to be aware of the differences in isoflavone consumption, absorption and effective serum and tissue concentrations among species, including different human populations.

5. General mechanisms of action of isoflavones

To better understand the potential mechanisms of the antiviral action of isoflavones, it is necessary to first understand their general biological functions. Isoflavones affect cellular functions through a number of mechanisms, including acting as estrogen receptor (ER) effectors, inhibition of protein tyrosine kinases (PTKs) and inhibition of topoisomerase II and others [16,17]. Isoflavones can exert both estrogenic and antiestrogenic properties depending on dosage, circulating endogenous estrogen concentration and target tissue [16]. In addition, isoflavones preferentially bind to ERβ, whereas classic estrogens exert their effects via both ERα and ERβ [18]. Accordingly, isoflavones may act as natural selective ER modulators but exhibit potency that is at least 1000-fold lower than that of estrogen [18,19]. Although this is a very important property of isoflavones, the estrogenic or antiestrogenic effects are unlikely to be involved in the modulation of viral infections at the intestinal or systemic level.

There is evidence that soy isoflavones induce antioxidant and phase II enzymes [20]. Quinine reductase, glutathione-*S*-transferase and UDP-glucuronosyltransferase activities were increased in various tissues of rats fed a high-isoflavone diet (0.81 mg/g) for 2 weeks [20]. In addition, genistein (0.1 mg/kg) injected subcutaneously in rats produced a mild anti-inflammatory effect in a model of chronic parasitic ileitis, as demonstrated by reduction in nitric oxide production, gametocyte infiltration and improved intestinal mucosal architecture [21]. The mechanism(s) by which genistein reduced inflammatory response may be linked to an inhibition of both transcription nuclear factor-κB (NF-κB) activation and chemokine-8 secretion [18,22,23] via a decrease in either caspase-3 or PTK activity [24,25]. This anti-inflammatory property of isoflavones may be critical in host responses to viral infection.

Genistein also acts as a PTK inhibitor through a variety of mechanisms, including competing with adenosine triphosphate (ATP) at the tyrosine kinase ATP-binding site of epidermal growth factor receptors [26,27], inhibiting c-src [28,29] (a PTK involved in mitogen-activated protein kinase (MAPK)] or activating p38 MAPK at the transforming growth factor-β receptor level [30]. Isoflavone-mediated inhibition of PTK has also been demonstrated in studies investigating tumor necrosis factor (TNF), Toll-like receptor and growth factor signaling cascades [18,31]. Isoflavone PTK-inhibitory activity has been identified as a mechanism of action in reducing the infectivity of a number of viruses. Isoflavones can also bind to nuclear receptors (such as, but not limited to, ER-related receptor, peroxisome proliferator-activated receptor and aryl hydrocarbon receptor), interfere

with Ca^{2+} transport and/or Na^{+}/K^{+} ATPases, alter lipid and lipoprotein profiles, block cell cycle in G_2/M transition and inhibit Akt kinase, topoisomerase II and cAMP-phosphodiesterase-4 enzymes. These effects have been reported to occur over a wide range of genistein concentrations ranging from less than 1 μM to greater than 0.5 mM [6,18,31,32].

6. Antiviral activity of isoflavones

Flavonoids and isoflavones possess antiviral properties against a wide range of viruses under both *in vitro and in vivo* conditions (Table 1). Flavonoids, including genistein, reduced the infectivity of nonenveloped viruses, including the following: single-stranded RNA poliovirus, coxsackie virus and echovirus from the Picornaviridae family [44,50,55–58]; double-stranded RNA viruses such as rotavirus [59] and double-stranded DNA viruses including adenovirus [33,34]; and John Cunningham (JC) virus [48] and simian virus 40 (SV40) [54] from the Adenoviridae and Polyomaviridae (JC and SV40) families (Table 1). Flavonoids also inhibited the infectivity of a variety of enveloped viruses such as single-stranded RNA viruses in the Reoviridae, Arenaviridae, Flaviviridae, Coronaviridae, Retroviridae [both human immunodeficiency virus (HIV) and Moloney murine leukemia virus (MoMLV)], Arteriviridae and Paramyxoviridae families, including arenavirus [35], bovine viral diarrhea virus [37], coronavirus [38], HIV [47], MoMLV [49], porcine reproductive and respiratory syndrome (PRRS) virus [51] and respiratory syncytial virus [52]; and double-stranded DNA viruses, including bovine herpes virus (BHV) [36], Epstein–Barr virus (EBV) [39], herpes simplex virus (HSV) 1 [34,40,41,44] and HSV-2 [41], herpes human virus (HHV) 8 [46] and human cytomegalovirus (CMV) [45,60] from the Herpesviridae family.

Although flavonoids and isoflavones possess antiviral activity against a variety of viruses, this property must be considered in the context of the flavonoid concentrations or doses used. The amounts of genistein necessary to exert antiviral activities span a range from physiological to supraphysiological doses (3.7–370 μM; Table 1). Although genistein is found at approximately 33 μM in soy-based infant formula, which provides 6–11 mg/kg/day genistein to infants [59,61], the concentrations in intracellular compartments and blood are estimated to be much lower. In a recent review by Klein and King [31], concerns about the definition of a 'physiological' dose for genistein were

Table 1
Viruses inhibited by isoflavones or its related compounds

	Inhibitory compound	Inhibitory dose	Model	References
Adenovirus	Genistein	5–20 μM	SW480 cells	[33]
	Quercetin	0.2 mM[a]	BCC-1/KMC cells	[34]
Arenaviruses	Genistein	100 μM	Vero cells	[35]
BHV-1	Genistein	25 μM	MDBK cells	[36]
Bovine viral diarrhea virus	Genistein	185–370 μM	MDBK cells	[37]
Coronavirus	Luteolin	11 μM[a]	Vero cells	[38]
	Quercetin	83 μM[a]	Vero cells	[38]
EBV	Genistein	30–50 μM	Ramos cells	[39]
HSV-1	Genistein	5–25 μM	Vero cells	[40,41]
	Torvanol A[b]	21 μM	Vero cells	[42]
	Kaempferol	0.4 mM	Vero cells	[43]
	5,6,7-Trimethoxyflavone[c]	10–30 μM[a]	Vero and MRC-5 cells	[44]
HSV-2	Genistein	50 μM	Vero cells	[41]
Human CMV	Genistein	50 μM	HEL 299 cells	[45]
	5,6,7-Trimethoxyflavone[c]	26 μM	Vero and MRC-5 cells	[44]
HHV-8	Genistein	100–200 μM	HFF cells	[46]
HIV	Genistein	3.7–37 μM	Primary macrophages	[47]
JC virus	Irisolidone	7.5 μM[a]	Primary astrocytes	[48]
MoMLV	Genistein	62–92 μM	XC cells	[49]
Poliovirus	5,6,7-Trimethoxyflavone[c]	0.1 mM	Vero and MRC-5 cells	[44]
	3-Methylkaempferol[d]	<10 μM	Vero cells	[50]
	3(2*H*)-Isoflavene	20 μM	HeLa R19 cells	[55]
PRRS virus	Isoflavones mix[e]	15–37 mg/kg/day	Pigs (*in vivo*)	[51]
Respiratory syncytial virus	Genistein	25–50 μM	Vero cells	[52]
Rotavirus	Genistein	33 μM	MA-104	[59]
SV40	Genistein	200 μM	CV-1 cells	[53,54]

[a] IC_{50}.
[b] From *Solanum torvum*.
[c] From *Callicarpa japonica*.
[d] From *Psiadia dentate*.
[e] Reported as genistein equivalent.

raised. According to their analysis, a low concentration of genistein should be defined as less than or equal to 5 μM, which corresponds to the serum or plasma levels of human adults following ingestion of soy foods or an isoflavone supplement (1–5 μM). Considering this definition, very few studies have examined the effects of physiological doses of isoflavones. Nevertheless, if one considers isoflavones and genistein as nutraceuticals or pharmaceuticals, then studies examining even the highest isoflavone doses are still relevant, assuming possible toxicity effects are also examined.

Flavonoids and isoflavones other than genistein have also been tested for their potential antiviral properties. These include flavanols (quercetin, kaempferol and 3-methylkaempferol), flavones (luteolin and 5,6,7-trimethoxyflavone), isoflavans [3(2*H*)-isoflavenes] and isoflavones (torvanol A and irisolidone). Such compounds are found in fruits, vegetables, plants and trees. Quercetin inhibited adenovirus and coronavirus infections at 0.2 mM and 83 μM, respectively [34,38]. Kaempferol and 3-methylkaempferol reduced HSV-1 and poliovirus at 0.4 mM and <10 μM, respectively [43,50]. Luteolin was effective against coronavirus at 11 μM, whereas 5,6,7-trimethoxyflavone inhibited HSV-1, HIV and poliovirus at doses ranging from 10 μM to 0.1 mM [38,44]. The isoflavones torvanol A and irisolidone were effective against HSV-1 and JC virus, respectively, at concentrations ranging between 7.5 and 11 μM [42,48]. In addition, seven flavonoids isolated from *Rhus succedanea* and *Garcinia multiflora* (from the Anacardiaceae and Clusiaceae families, respectively) inhibited influenza A and influenza B viruses, HSV-1, HSV-2 and measles *in vitro* [62]. Finally, 3(2*H*)-isoflavene inhibited poliovirus type 2 infection at 20 μM [55,56,63]. In a recent study, the order of potency of flavonoids in inhibiting the neuraminidase activity of influenza virus was found to be as follows: aurones>flavon(ol)es>isoflavones>flavanon(ol)es and flavan(ol)es. Optimal inhibitory activity required the presence of 4′-OH, 7-OH, C_4O and C_2C_3 functional groups, but was markedly reduced by the presence of a glycosyl moiety [64].

7. Possible mechanisms of the antiviral activity of isoflavone

The mechanism(s) whereby isoflavones and related flavonoids inhibit virus infectivity has yet to be fully elucidated. Given the variety of reported effects of isoflavones on numerous viruses and their target host cells, antiviral activity is likely due to a combination of several effects modulating both the viral particle and the host cell. A review of the current literature indicated that isoflavones affected virus binding to cell membranes, entry into the cell, replication and virus protein translation within the host cell, and formation of certain glycoprotein complexes of the virus envelope (Fig. 1). At the host cell level, isoflavones can affect the induction of certain transcription factors and secretion of cytokines; most of these effects have been attributed to a reduction in PTK activity [33,49,53,54]. Inhibition of PTK activity decreased adenovirus, HHV-8, MoMLV and SV40 viral entry in host cells. Proposed mechanisms included a reorganization of the cytoskeleton, which may include blocking of virus-induced actin changes and recruitment of dynamin II to membrane-bound virus particles [33,49,53,54]. Inhibition of PTK activity at later stages of virus infection resulted in decreased phosphorylation of HSV-1 polypeptides [40] and BHV-1 glycoprotein E [36], which in turn decreased overall virus replication. In the presence of 50 μM genistein,

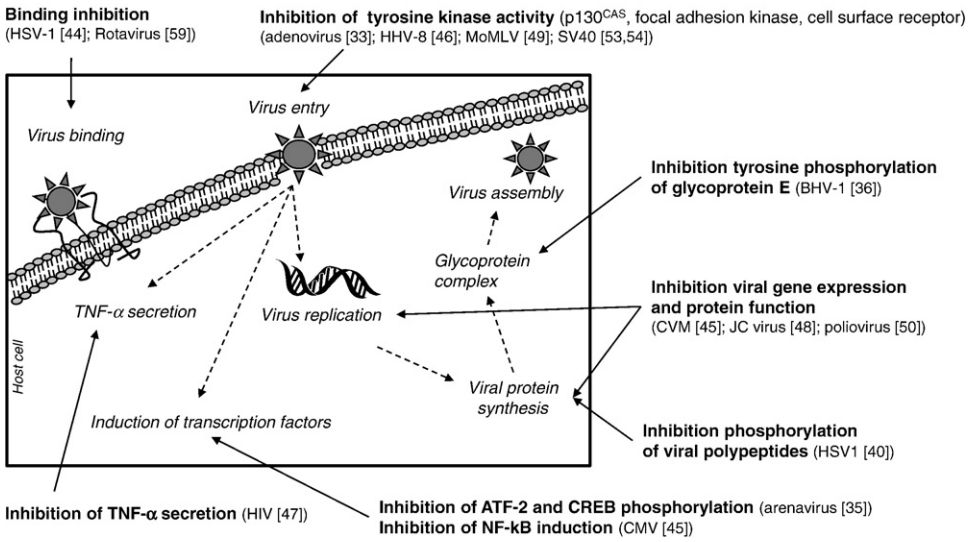

Fig. 1. Antiviral mechanisms of action of isoflavones and related compounds. This diagram summarizes the various steps in virus–host cell interactions (dotted lines and italicized script). The flavonoid-mediated inhibition of virus infectivity and propagation is represented in solid lines. Isoflavones and related compounds have been shown to affect virus binding, entry and replication, viral protein synthesis and assembly of the glycoprotein complex, as well as TNF-α secretion and induction of transcription factors. Each mechanism of action is followed by the name of the virus(es) and the corresponding reference(s).

the reduction in HSV-1 viral replication was not due to a reduction in viral adsorption into the cells, but rather due to a significant reduction in the phosphorylation of tyrosine residues in specific viral polypeptides ICP-6, ICP-19 and ICP-26 at a late stage of HSV-1 infection, without apparent cytotoxicity to Vero cells [40].

Genistein-mediated inhibition of protein phosphorylation also reduced the activation of the activator of Fe transcription 2 (ATF-2), cyclic AMP response element-binding protein (CREB) and NF-κB transcription factors in arenavirus and CMV-infected host cells, leading to a reduction in viral infectivity [35,45]. Using a model of arenavirus infection in Vero cells, pretreatment of cells with genistein at 100 μM for 1 h prior to infection decreased virus titers by 90% in a plaque assay and reduced virus nucleoprotein and glycoprotein expressions without altering cellular transferring receptor levels compared to untreated Vero cells [35]. When genistein (100 μM) was added at 0 h, 30 min and 8 h postinfection, it induced a 66.5%, 59.6% and 33.6% reduction in virus titers, respectively, via inhibition of transcription factor ATF-2 and CREB phosphorylation and via stimulation of pERK phosphorylation.

Isoflavones also inhibit virus infectivity by mechanisms independent of the reduction in PTK activity. Reduction in virus binding to host cells has been demonstrated in HSV-1 and rotavirus infections [44,59], whereas a reduction in virus gene expression has been shown in CMV, JC virus and poliovirus, through a possible inhibition of transcriptional factor binding to the viral promoter [45,48,50] or an alteration of virus protein function [45]. Although the binding of poliovirus type 2 to its cellular receptor was unaffected by 3(2*H*)-isoflavene, virus uncoating was efficiently blocked by the isoflavene ring interacting with the VP1 virus capsid protein [55,56]. In a model of human CMV infection, genistein blocked early and late viral gene expressions and protein synthesis [45]. Genistein also inhibited human CMV DNA replication by approximately 95% at 72 h postinfection [45]. Unlike its well-known property, genistein did not prevent viral entry by targeting PTK activity in this model [45]. In addition, viral cell cycle perturbation and induction of transcription factor NF-κB were absent in cells treated with genistein compared to untreated cells [45]. At the host cell level, isoflavones have also been shown to inhibit TNF-α secretion following infection [47,65].

Importantly, genistein concentration has been shown to decrease rapidly in a culture medium used in BHV-1 infectivity studies [45]. Genistein was metabolized faster by cells infected with BHV-1 than by noninfected cells. Accordingly, sequential doses of genistein (25 μM) were more effective than a single dose in decreasing the BHV-1 titer at 24 h postinfection [45]. Therefore, it is possible that isoflavones may be more effective as antiviral agents if they are ingested more frequently at low doses rather than administered as a single high dose. In a different study, kaempferol and its derivatives were more efficient inhibitors of HSV-1 infection when used in combination, suggesting a possible synergy in the antiviral properties of isoflavones and related flavonoids [43]. Thus, when investigating the antiviral properties of isoflavone or flavonoid mixtures, the dose, frequency and combination of the flavonoids used should be carefully considered.

To our knowledge, there was only one study that examined the antiviral properties of genistein *in vivo*. Dietary genistein (11–37.3 mg/kg body weight/day) provided for 19 days prior to inoculation with PRRS virus enhanced systemic serum virus elimination and body growth in postweaning pigs [51]. Virus concentrations in serum decreased, whereas interferon activity and body weight increased proportionally with dietary genistein concentration. It should be noted that pigs were exposed to higher doses of genistein (15.2–37.3 mg/kg body weight/day genistein equivalent) than were infants fed soy-based formula (6–11 mg/kg/day [61]). Using the same model, Greiner et al. [66] demonstrated that daidzein did not alter virus elimination from serum or affect the immune response of the host in virally challenged pigs. Thus, genistein, but not daidzein, was demonstrated as a potential antiviral compound that can modulate the inflammatory and immune responses of a challenged host. Since genistein, but not daidzein, inhibits PTK activity, the authors speculated that PTK inhibition was the primary mechanism of action of genistein against PRRS virus.

8. Conclusions

According to the current literature, isoflavones and related flavonoids exert antiviral activity against a wide range of viruses. However, caution needs to be taken when interpreting currently available data. The majority of these studies were conducted *in vitro* using a single host cell line and supraphysiological doses of isoflavones that may not be obtained from target cellular compartments *in vivo*. Differences in the reported *in vivo* and *in vitro* virus-inhibitory potencies of various isoflavones also add confusion regarding their efficacy as antiviral agents. Although several studies have reported on the use of a selective index (defined as the ratio of isoflavone concentration causing 50% cell toxicity to isoflavone concentration causing 50% inhibition of virus infectivity), the use of such a normalizing index has not been widely adopted. Finally, there are very few studies addressing the *in vivo* efficacy of isoflavones as antiviral agents. Clearly, comprehensive investigations using appropriate *in vivo* virus infectivity models and physiological doses of flavonoids are needed to validate the wide range of effects observed *in vitro*. To avoid future confusion when comparing the results of different studies, researchers should provide a detailed and unambiguous description of the flavonoids (e.g., glycoside vs. aglycone), concentrations and dietary formulations used in their studies, as well as a discussion of the physiological significance of their results relative to the range of dietary or pharmacological doses of

soy and isoflavones typically ingested and/or tissue concentrations achieved [67].

References

[1] Munro IC, Harwood M, Hlywka JJ, Stephen AM, Doull J, Flamm WG, et al. Soy isoflavones: a safety review. Nutr Rev 2003;61:1–33.

[2] Cassidy A, Brown JE, Hawdon A, Faughnan MS, King LJ, Millward J, et al. Factors affecting the bioavailability of soy isoflavones in humans after ingestion of physiologically relevant levels from different soy foods. J Nutr 2006;136:45–51.

[3] Dixon RA. Phytoestrogens. Annu Rev Plant Biol 2004;55:225–61.

[4] Cornwell T, Cohick W, Raskin I. Dietary phytoestrogens and health. Phytochemistry 2004;65:995–1016.

[5] Andlauer W, Kolb J, Stehle P, Furst P. Absorption and metabolism of genistein in isolated rat small intestine. J Nutr 2000;130:843–6.

[6] Chen AC, Berhow MA, Tappenden KA, Donovan SM. Genistein inhibits intestinal cell proliferation in piglets. Pediatr Res 2005;57: 192–200.

[7] Chen AC, Donovan SM. Genistein at a concentration present in soy infant formula inhibits Caco-2BBe cell proliferation by causing G_2/M cell cycle arrest. J Nutr 2004;134:1303–8.

[8] Tanaka H, Sato M, Oh-Uchi T, Yamaguchi R, Etoh H, Shimizu H, et al. Antibacterial properties of a new isoflavonoid from *Erythrina poeppigiana* against methicillin-resistant *Staphylococcus aureus*. Phytomedicine 2004;11:331–7.

[9] Sfakianos J, Coward L, Kirk M, Barnes S. Intestinal uptake and biliary excretion of the isoflavone genistein in rats. J Nutr 1997;127:1260–8.

[10] Zhang Y, Hendrich S, Murphy PA. Glucuronides are the main isoflavone metabolites in women. J Nutr 2003;133:399–404.

[11] Ronis MJ, Little JM, Barone GW, Chen G, Radominska-Pandya A, Badger TM. Sulfation of the isoflavones genistein and daidzein in human and rat liver and gastrointestinal tract. J Med Food 2006;9: 348–55.

[12] Setchell KD, Brown NM, Lydeking-Olsen E. The clinical importance of the metabolite equol — a clue to the effectiveness of soy and its isoflavones. J Nutr 2002;132:3577–84.

[13] Faughnan MS, Hawdon A, Ah-Singh E, Brown J, Millward DJ, Cassidy A. Urinary isoflavone kinetics: the effect of age, gender, food matrix and chemical composition. Br J Nutr 2004;91:567–74.

[14] Gu L, House SE, Prior RL, Fang N, Ronis MJ, Clarkson TB, et al. Metabolic phenotype of isoflavones differ among female rats, pigs, monkeys, and women. J Nutr 2006;136:1215–21.

[15] Xiao CW. Health effects of soy protein and isoflavones in humans. J Nutr 2008;138:1244S–9S.

[16] Barnes S. Soy isoflavones — phytoestrogens and what else? J Nutr 2004;134:1225S–8S.

[17] Ososki AL, Kennelly EJ. Phytoestrogens: a review of the present state of research. Phytother Res 2003;17:845–69.

[18] Dijsselbloem N, Vanden Berghe W, De Naeyer A, Haegeman G. 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 2004;68: 1171–85.

[19] Chen A, Rogan WJ. Isoflavones in soy infant formula: a review of evidence for endocrine and other activity in infants. Annu Rev Nutr 2004;24:33–54.

[20] Omoni AO, Aluko RE. Soybean foods and their benefits: potential mechanisms of action. Nutr Rev 2005;63:272–83.

[21] Sadowska-Krowicka H, Mannick EE, Oliver PD, Sandoval M, Zhang XJ, Eloby-Childess S, et al. Genistein and gut inflammation: role of nitric oxide. Proc Soc Exp Biol Med 1998;217:351–7.

[22] Choi C, Cho H, Park J, Cho C, Song Y. Suppressive effects of genistein on oxidative stress and NFkappaB activation in RAW 264.7 macrophages. Biosci Biotechnol Biochem 2003;67:1916–22.

[23] Tabary O, Escotte S, Couetil JP, Hubert D, Dusser D, Puchelle E, et al. Genistein inhibits constitutive and inducible NFkappaB activation and decreases IL-8 production by human cystic fibrosis bronchial gland cells. Am J Pathol 1999;155:473–81.

[24] Nozawa Y, Nishihara K, Peek RM, Nakano M, Uji T, Ajioka H, et al. Identification of a signaling cascade for interleukin-8 production by *Helicobacter pylori* in human gastric epithelial cells. Biochem Pharmacol 2002;64:21–30.

[25] Baxa DM, Yoshimura FK. Genistein reduces NF-kappa B in T lymphoma cells via a caspase-mediated cleavage of I kappa B alpha. Biochem Pharmacol 2003;66:1009–18.

[26] Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, et al. Genistein, a specific inhibitor of tyrosine-specific protein kinases. J Biol Chem 1987;262:5592–5.

[27] Lamartiniere CA. Protection against breast cancer with genistein: a component of soy. Am J Clin Nutr 2000;71:1705S–7S.

[28] Migliaccio A, Castoria G, Di Domenico M, de Falco A, Bilancio A, Lombardi M, et al. Steroid-induced androgen receptor-oestradiol receptor beta-Src complex triggers prostate cancer cell proliferation. EMBO J 2000;19:5406–17.

[29] Santen RJ, Song RX, McPherson R, Kumar R, Adam L, Jeng MH, et al. The role of mitogen-activated protein (MAP) kinase in breast cancer. J Steroid Biochem Mol Biol 2002;80:239–56.

[30] Han HJ, Lee YJ. Insulin stimulates Ca^{2+} uptake via PKC, cAMP, and p38 MAPK in mouse embryonic stem cells. Life Sci 2005;76: 2903–19.

[31] Klein CB, King AA. Genistein genotoxicity: critical considerations of *in vitro* exposure dose. Toxicol Appl Pharmacol 2007;224:1–11.

[32] Salti GI, Grewal S, Mehta RR, Das Gupta TK, Boddie Jr AW, Constantinou AI. Genistein induces apoptosis and topoisomerase II-mediated DNA breakage in colon cancer cells. Eur J Cancer 2000; 36:796–802.

[33] Li E, Stupack DG, Brown SL, Klemke R, Schlaepfer DD, Nemerow GR. Association of p130CAS with phosphatidylinositol-3-OH kinase mediates adenovirus cell entry. J Biol Chem 2000;275:14729–35.

[34] Chiang LC, Chiang W, Liu MC, Lin CC. *In vitro* antiviral activities of *Caesalpinia pulcherrima* and its related flavonoids. J Antimicrob Chemother 2003;52:194–8.

[35] Vela EM, Bowick GC, Herzog NK, Aronson JF. Genistein treatment of cells inhibits arenavirus infection. Antiviral Res 2008;77:153–6.

[36] Akula SM, Hurley DJ, Wixon RL, Wang C, Chase CC. Effect of genistein on replication of bovine herpesvirus type 1. Am J Vet Res 2002;63:1124–8.

[37] Lecot S, Belouzard S, Dubuisson J, Rouille Y. Bovine viral diarrhea virus entry is dependent on clathrin-mediated endocytosis. J Virol 2005;79:10826–9.

[38] Yi L, Li Z, Yuan K, Qu X, Chen J, Wang G, et al. Small molecules blocking the entry of severe acute respiratory syndrome coronavirus into host cells. J Virol 2004;78:11334–9.

[39] Fukuda M, Longnecker R. Epstein–Barr virus (EBV) latent membrane protein 2A regulates B-cell receptor-induced apoptosis and EBV reactivation through tyrosine phosphorylation. J Virol 2005;79: 8655–60.

[40] Yura Y, Yoshida H, Sato M. Inhibition of herpes simplex virus replication by genistein, an inhibitor of protein-tyrosine kinase. Arch Virol 1993;132:451–61.

[41] Lyu SY, Rhim JY, Park WB. Antiherpetic activities of flavonoids against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) *in vitro*. Arch Pharm Res 2005;28:1293–301.

[42] Arthan D, Svasti J, Kittakoop P, Pittayakhachonwut D, Tanticharoen M, Thebtaranonth Y. Antiviral isoflavonoid sulfate and steroidal glycosides from the fruits of *Solanum torvum*. Phytochemistry 2002; 59:459–63.

[43] Amoros M, Simoes CM, Girre L, Sauvager F, Cormier M. Synergistic effect of flavones and flavonols against herpes simplex virus type 1 in cell culture. Comparison with the antiviral activity of propolis. J Nat Prod 1992;55:1732–40.

[44] Hayashi K, Hayashi T, Otsuka H, Takeda Y. Antiviral activity of 5,6,7-trimethoxyflavone and its potentiation of the antiherpes activity of acyclovir. J Antimicrob Chemother 1997;39:821–4.
[45] Evers DL, Chao CF, Wang X, Zhang Z, Huong SM, Huang ES. Human cytomegalovirus-inhibitory flavonoids: studies on antiviral activity and mechanism of action. Antiviral Res 2005;68:124–34.
[46] Sharma-Walia N, Naranatt PP, Krishnan HH, Zeng L, Chandran B. Kaposi's sarcoma-associated herpesvirus/human herpesvirus 8 envelope glycoprotein gB induces the integrin-dependent focal adhesion kinase-Src-phosphatidylinositol 3-kinase-rho GTPase signal pathways and cytoskeletal rearrangements. J Virol 2004;78:4207–23.
[47] Stantchev TS, Markovic I, Telford WG, Clouse KA, Broder CC. The tyrosine kinase inhibitor genistein blocks HIV-1 infection in primary human macrophages. Virus Res 2007;123:178–89.
[48] Kim SY, Kim DH, Hyun JW, Henson JW, Kim HS. Irisolidone, an isoflavone metabolite, represses JC virus gene expression via inhibition of Sp1 binding in human glial cells. Biochem Biophys Res Commun 2006;344:3–8.
[49] Kubo Y, Ishimoto A, Amanuma H. Genistein, a protein tyrosine kinase inhibitor, suppresses the fusogenicity of Moloney murine leukemia virus envelope protein in XC cells. Arch Virol 2003;148:1899–914.
[50] Robin V, Irurzun A, Amoros M, Boustie J, Carrasco L. Antipoliovirus flavonoids from *Psiadia dentata*. Antiviral Chem Chemother 2001;12:283–91.
[51] Greiner LL, Stahly TS, Stabel TJ. The effect of dietary soy genistein on pig growth and viral replication during a viral challenge. J Anim Sci 2001;79:1272–9.
[52] Rixon HW, Brown G, Murray JT, Sugrue RJ. The respiratory syncytial virus small hydrophobic protein is phosphorylated via a mitogen-activated protein kinase p38-dependent tyrosine kinase activity during virus infection. J Gen Virol 2005;86:375–84.
[53] Dangoria NS, Breau WC, Anderson HA, Cishek DM, Norkin LC. Extracellular simian virus 40 induces an ERK/MAP kinase-independent signalling pathway that activates primary response genes and promotes virus entry. J Gen Virol 1996;77(Pt 9):2173–82.
[54] Pelkmans L, Puntener D, Helenius A. Local actin polymerization and dynamin recruitment in SV40-induced internalization of caveolae. Science 2002;296:535–9.
[55] Salvati AL, De Dominicis A, Tait S, Canitano A, Lahm A, Fiore L. Mechanism of action at the molecular level of the antiviral drug 3(2*H*)-isoflavene against type 2 poliovirus. Antimicrob Agents Chemother 2004;48:2233–43.
[56] Genovese D, Conti C, Tomao P, Desideri N, Stein ML, Catone S, et al. Effect of chloro-, cyano-, and amidino-substituted flavanoids on enterovirus infection *in vitro*. Antiviral Res 1995;27:123–36.
[57] Conti C, Genovese D, Santoro R, Stein ML, Orsi N, Fiore L. Activities and mechanisms of action of halogen-substituted flavanoids against poliovirus type 2 infection *in vitro*. Antimicrob Agents Chemother 1990;34:460–6.
[58] Tait S, Salvati AL, Desideri N, Fiore L. Antiviral activity of substituted homoisoflavonoids on enteroviruses. Antiviral Res 2006;72:252–5.
[59] Andres A, Donovan SM, Kuhlenschmidt TB, Kuhlenschmidt MS. Isoflavones at concentrations present in soy infant formula inhibit rotavirus infection *in vitro*. J Nutr 2007;137:2068–73.
[60] Mitrocotsa D, Mitaku S, Axarlis S, Harvala C, Malamas M. Evaluation of the antiviral activity of kaempferol and its glycosides against human cytomegalovirus. Planta Med 2000;66:377–9.
[61] Setchell KD, Zimmer-Nechemias L, Cai J, Heubi JE. Isoflavone content of infant formulas and the metabolic fate of these phytoestrogens in early life. Am J Clin Nutr 1998;68:1453S–61S.
[62] Lin YM, Flavin MT, Schure R, Chen FC, Sidwell R, Barnard DL, et al. Antiviral activities of biflavonoids. Planta Med 1999;65:120–5.
[63] Fisher D, Baird J, Payne L, Lucas P, Kleijnen J, Roberts H, et al. Are infant size and growth related to burden of disease in adulthood? A systematic review of literature. Int J Epidemiol 2006;35:1196–210.
[64] Liu AL, Wang HD, Lee SM, Wang YT, Du GH. Structure–activity relationship of flavonoids as influenza virus neuraminidase inhibitors and their *in vitro* anti-viral activities. Bioorg Med Chem 2008;16:7141–7.
[65] Formica JV, Regelson W. Review of the biology of quercetin and related bioflavonoids. Food Chem Toxicol 1995;33:1061–80.
[66] Greiner LL, Stahly TS, Stabel TJ. The effect of dietary soy daidzein on pig growth and viral replication during a viral challenge. J Anim Sci 2001;79:3113–9.
[67] Erdman Jr JW, Badger TM, Lampe JW, Setchell KD, Messina M. Not all soy products are created equal: caution needed in interpretation of research results. J Nutr 2004;134:1229S–33S.