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DAIDZEIN: A REVIEW OF PHARMACOLOGICAL EFFECTS

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Abstract

Background: Daidzein is an isoflavone with extensive nutritious value and is mainly extracted from soy plants. It is also called phytoestrogen due to its structural similarity to the human hormone estrogen. However, daidzein is distinct from estrogen due to the specificity of the estrogen receptor (ER) complex. In recent years, the pharmacological properties of daidzein have been extensively investigated and considerable progress has been made. The present review aims to evaluate the pharmacological effects and mechanisms of daidzein as reported in scientific literature.

Materials and Methods: Studies were identified as reported in PubMed, Elsevier, Scholar, and Springer over the last ten years and this resulted in the identification of 112 papers.

Results: Daidzein is reported to play a significant role in the prevention and treatment of a variety of diseases such as cancer, cardiovascular disease, diabetes, osteoporosis, skin disease, and neurodegenerative disease. This pharmacological activity is attributed to various metabolites including equol and trihydroxy isoflavone.

Conclusion: Daidzein appears to play a significant role in the prevention of a variety of diseases and has the potential of being used in a clinical setting. However, further research is needed to understand its molecular mechanisms and safety for use in humans.

Key words: Plant, natural product, phytoestrogen, pharmacology

Introduction

Daidzein (4′, 7-dihydroxyisoflavone) whose chemical structure is shown in Figure 1 is a naturally occurring isoflavonic phytoestrogen belonging to the non-steroidal estrogens (Cassidy, 2003) and is mainly derived from the leguminous plants such as soybean and mung bean. It is also the major bioactive ingredient in traditional Chinese medicine *Gegen* (Wang et al., 2003) which is used frequently in the treatment of fever, acute dysentery, diarrhea, diabetes, cardiac dysfunctions, liver injury etc. (Wong et al., 2011). The chemical structure of daidzein is similar to mammalian estrogens and it exerts a dual-directional function by replacing/interfering with estrogen and the estrogen-receptor (ER) complex. Therefore, daidzein exerts protective effects against some diseases which are linked to the regulation of estrogen such as breast cancer, osteoporosis, diabetes, cardiovascular diseases (Vitale et al., 2013). It also has a number of other biological activities independent of the ER such as anti-inflammation, anticancer, inhibition of oxidative damage, protection of skin and the nerves. These beneficial effects are mainly due to regulation of the immune response (Masilamani et al., 2012), scavenging of oxygen free radicals, inhibition of proliferation and so on. However, when daidzein is presented in the bound form "daidzin", it becomes inactive and some metabolites of daidzein also display a similar pattern.

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The safety of phytoestrogens is rather controversial (Humfrey, 1998) as these may exert some negative effects on human health. In addition, the general absorption of daidzein is poor and many studies have been conducted to improve the bioavailability of daidzein. For example, self-micro emulsifying drug delivery system (SMEDDS) was used to formulate and enhance oral absorption of daidzein (Shen et al., 2010).

This review comprehensively evaluates the pharmacological properties of daidzein based on the summary of previously reported studies.

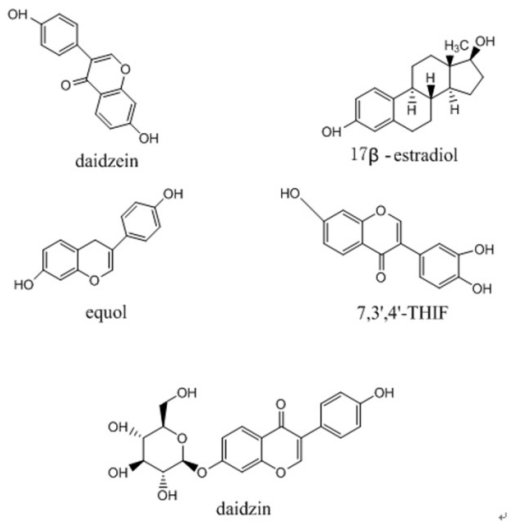

Figure 1: Molecular structure of daidzein and its related isoflavones

Pharmacological Effects

Anticancer and anti-Breast Cancer activities

Breast cancer is one of the most common malignant tumors in women which seriously threaten public health. Epidemiological studies have shown that the incidence of breast cancer in Asian women is lower than Western women due to the higher consumption of phytoestrogens (Adlercreutz, 2002). Thereby, the use of phytoestrogens may be a valid strategy in the prevention and treatment of breast cancer, via mechanisms including ER modulation and anti-angiogenesis (Liu et al., 2012a). Due to phytoestrogens being a significant constituent of daidzein its anticancer activity in breast cancer has attracted wide public attention.

Tumor necrosis factor-α (TNF-α), a type of endogenous cytokine, is able to affect tumorigenesis and dysregulation of TNF-α production contributes to cancer risk (Locksley et al., 2001; Paul A et al., 2013). Daidzein plays a vital role in the regulation of mammary tumor cell invasion induced by TNF-α. There are two distinct signaling pathways reported to elucidate the molecular basis of this, with one being the nuclear factor-kappa B (NF-κB) signaling pathway. In breast cancer cells MDA-MB-231, daidzein treatment suppressed TNF-α induced NF-κB and AP-1, followed by a reduction in the secretion of uPA from breast cancer cells, thus inhibiting the migration of breast cancer (Valachovicova et al., 2004). The other pathway is the Hedgehog (Hh) signaling pathway. Daidzein antagonized these effects via suppressing Gli1 activation and expression, thereby inhibiting migration and invasion of ER negative MCF10DCIS.com human breast cancer cells. The metabolites of daidzein *in vivo* exerted stronger activity at the same concentration. It was found that matrix metalloproteinase (MMP)-2 and MMP-9 also participated in breast cancer invasion. Daidzein inhibited the activity and expression of MMP-9 induced by TNF-α via Hh/Gli1 signaling pathway (Bao et al., 2014). Another study on MDA-MB-231 determined its anti-invasive effects partially by reducing expression of MMP-2 (Magee et al., 2014).

Moreover, daidzein displays anti-proliferative effects in breast cancer via cell cycle arrest in the G1 and G2/M phases and via the induction of apoptosis (Choi and Kim, 2008). The mechanism of apoptosis induced by daidzein is mitochondrial

caspase-dependent pathway. Daidzein increased intracellular reactive oxygen species (ROS) generation which changed mitochondrial transmembrane potential, leading to the release of cytochrome C. The reduced expression of anti-apoptotic proteins Bcl-2 and the increased expression of pro-apoptotic proteins Bax enhanced the release of cytochrome C. These factors further activated caspase-9 and caspase-7, resulting in eventual cell death (Jin et al., 2010).

Interestingly, the effect of daidzein in attenuating breast cancer progression is more effective than tamoxifen (TAM), which is a clinical drug currently used for the treatment of breast cancer (Liu et al., 2012b). However, some studies have raised concern that daidzein may not be safe as it may stimulate proliferation of tumor cells (Choi and Kim, 2013), boosting existing breast tumors and suppressing the pharmaceutical effects of TAM. Therefore, females with breast cancer should be aware of the risks of potential tumor progression when taking soy products (de Lemos, 2001), as co-administration of TAM with daidzein is reported to produce tumors of greater size than observed with TAM alone. These findings suggest that simultaneous consumption of isoflavone with TAM may not be safe (Tonetti et al., 2007) due to its estrogen-like effects; meanwhile, possible detrimental effects of daidzein in breast cancer patients have also been raised in other studies (Messina and Loprinzi, 2001). In fact, as an estrogen responsive marker, daidzein had a slight but significant stimulatory effect on MCF-7 tumor progression at the lower concentration (Ju et al., 2006) but when used at high concentrations, it exhibited anticancer capacity and could play a cooperative role in the treatment of TAM. Although daidzein has anticancer activity in breast tumor, its application should be applied with caution (Gaete et al., 2012).

Anti-Prostate Cancer

Epidemiological studies on risk factors of prostate cancer indicate the importance of consumption of soy (Adaramoye et al., 2015). As the main phytoestrogen of soy, daidzein displayed anti-proliferative properties in three prostate cancer cell lines (LNCaP, DU 145, PC-3) by the induction of cell cycle arrest at G0/G1 phase and the inhibition of angiogenesis via altering the expression of cyclin-dependent kinase-related pathway genes. Some of these genes are involved in DNA damage-signaling pathway, and also in the expression of angiogenesis genes, this can lead to the attenuation of growth factor EGF and IGF thus resulting in tumor growth inhibition (Rabiau et al., 2010). In androgen-dependent prostate cancer cells and LNCaP cells, the growth of prostate cancer is androgen dependent. Prostate androgen-regulated transcript-1 gene (PART-1) is a new gene that is responsive to androgens and could be potentially used as a biomarker of prostate cancer. Daidzein inhibited dihydrotestosterone (DHT)-induced expression of the PART-1 dose-dependently, implying that daidzein may have anti-androgen activity. Further *in vivo* studies have focused on the connection between prostate tumor growth and the inhibition of expression of PART-1 (Yu et al., 2003). Daidzein could induce apoptosis selectively in tumor cells by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) mediated apoptotic death. TRAIL is an endogenous anticancer agent, which induces disruption of mitochondrial membrane potential in the LNCaP cells thus promoting apoptosis (Szliszka and Krol, 2011). Studies in vitro and in vivo have suggested that daidzein can also be used as a radio sensitizer without inducing metastasis in lymph nodes as with genistein. The mechanism was AR-independent which enhance radiotherapy and inhibits tumor growth by down-regulating the expression of APE1/Ref-1 alter the activity of NF-κB and HIF-1α (Singh-Gupta et al., 2011). Overall, daidzein appears to have a role in prevention and treatment of prostate cancer.

Inhibition of Other Cancers

Daidzein was also reported to be beneficial in the treatment of colon cancer in that it produced biphasic effect on human colon cancer cells at different concentrations. Tumor suppressive effect on LoVo cells was by cell cycle arrest at G0/G1 phase and caspase-3 dependent apoptosis, which was irrelevant of differentiation (Guo et al., 2004). Using hepatocarcinoma SK-HEP-1 cells as the cell model, daidzein was reported to inhibit the growth of hepatocarcinoma cells, while having no effect on normal human hepatocytes. The apoptosis induced by daidzein was related to the regulation of Bcl-2 family via mitochondrial pathway (Park et al., 2013a). Daidzein still exhibited antitumor effect in a number of murine and human neuroblastoma cell lines by the inhibition of cell proliferation, cell cycle arrest at G2/M phase, and promotion of cell apoptosis (Lo et al., 2007). Some derivatives of daidzein such as 7, 3′, 4′-THIF a form of daidzein also have anticancer effect which has been demonstrated to play a chemo-preventive role in UVB

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induced non-melanoma skin cancer both *in vitro* and *in vivo*. The metabolite binds to Cot and MKK4 directly to inhibit the activity of Cot and MKK4, which further markedly suppresses the expression of UVB-induced cyclooxygenase 2 (COX-2) ultimately, inhibiting the elongation and the number and volume of tumors. Although daidzein does not have any influence on COX-2 expression, it could be used as a potential chemo-preventive agent in skin cancer due to its biotransformation (Lee et al., 2011a). 7-(O)-carboxymethyl daidzein conjugated to N-t-Boc-hexylenediamine (cD-tboc) elicits antithyroid cancer and anti-epithelial ovarian cancer properties by augmenting cell apoptosis (Somjen et al., 2012; Green et al., 2009).

Anti-Cardiovascular Diseases

Cardiovascular diseases(CVD), such as coronary heart disease, atherosclerosis, and hypertension, can be classified as a kind of estrogen-related disorder (Dubey et al., 2004) since it is prevalent in post-menopause women. Conventional hormone replacement therapy (HRT) may not be safe since the outcome of clinical trials has reported adverse cardiovascular effects in experimental studies investigating vascular benefits (Ross et al., 2008). Daidzein is a potential candidate in the treatment of cardiovascular diseases and it exerts its mechanism by mainly regulating of blood lipid metabolism, endothelial dysfunction attenuation, decreasing in blood pressure and increasing antioxidant activity.

In a study involving hypercholesteremic subjects, daidzein treatment for 6 months significantly decreased triglyceride (TG) concentration which is associated with ESR-β RsaI genotype while glucose and other lipids were not affected. In addition, uric acid, an independent risk factor of CVD, was also down-regulated by daidzein (Qin et al., 2014). In male middle-aged rats which included two groups, orchidectomized (Orx) and intact (IA), subcutaneous injection of high doses of genistein and daidzein decreased serum cholesterol levels (Sosić-Jurjević et al., 2007).

Ovariectomy, which produces endothelium dysfunction including attenuation in endothelium-dependent vasorelaxation and nitric formation increase in oxidative stress and damage to endothelium integrity, can be alleviated by daidzein. It exhibited estrogen-like effect on endothelium-dependent vasorelaxation and inhibited caveolin-1 leading to an increase in nitric oxide bioavailability and as a result, daidzein improved endothelium dysfunction (Sharma et al., 2012). Another study in streptozotocin-induced diabetic rats also demonstrated that chronic supplement with daidzein ameliorated endothelium dysfunction. It significantly improved the vascular contractile and relaxation response activity endothelium-dependently by NO and prostaglandin-dependent pathways and it also inhibited lipid peroxidation (Roghani et al., 2013).

Daidzein and its metabolite equol were also reported to play a significant impact on hypertension by controlling vascular smooth muscle tone via regulating a balance between vasodilator and vasoconstrictor, modulation of humoral systems and renal function, and this in turn lowers blood pressure. However, the anti-hypertension effect of daidzein has only been demonstrated in animal models currently, and it still needs further validation in human clinical trials (Martin et al., 2008). The effect of daidzein on catecholamine synthesis and secretion also contributed to a reduction in the risks of CVD (Yanagihara et al., 2014; Liu et al., 2007).

Pretreatment with daidzein in a rat ischemia/reperfusion model markedly reduced myocardial injury induced by ischemia reperfusion, such as improved myocardial contractile dysfunction, inhibition of myocardial apoptosis, and decreased myocardial infarct size. These beneficial effects could be largely due to the activation of NF-κB, which regulated expression of inflammatory cytokine by its antioxidant activity (Kimet al., 2009). However, the protective effect of daidzein on CVD is different because the ability is different for people to produce equol from daidzein which plays an important role in decreasing arterial stiffness and anti-atherosclerotic effects (Gil-Izquierdo et al., 2012).

In general, daidzein exhibits protective action on CVD risk factors although the mechanisms are not clearly defined. It has the potential to be an alternative agent for the therapy of CVD, especially in post-menopause CVD women (Gencel et al., 2012). However, the absorption and bioavailability of daidzein is poor when given in oral administration, but daidzein-loaded solid lipid nanoparticles (SLNs), a new type of daidzein, exerts a better effect on cardiovascular system and it has the potential to be used in the treatment of cardio-cerebrovascular diseases in the future(Gao et al., 2008).

Anti-Osteoporosis

Osteoporosis is a disease characterized by increased bone loss and fracture risks (Body, 2011). It is also common in menopausal women due to estrogen deficiency. Pretreatment with estrogen may prevent osteoporosis by affecting osteoclast formation and activation through promoting TGF-mediated apoptosis, which is produced by osteoblasts (Hughes et al., 1996). Estrogen could also directly induce osteoclast apoptosis by binding to ERα in osteoclast and then altering the expression of FasL (Novack, 2007). Although HRT is effective in the treatment of osteoporosis, its potential negative effects such as breast cancer cannot be ignored (Lewis, 2009).

As a phytoestrogen, daidzein displays estrogen-like effects. Moreover, equol, a metabolite of daidzein, showed stronger estrogenic activity than other isoflavones. Therefore, daidzein is unique compared to other isoflavones and has the potential to treat osteoporosis (Setchell et al., 2002). Daidzein can also inhibit bone reabsorption. A study on cultured osteoblasts from long bones of young female piglets showed that the low concentration of daidzein (1 nM) promoted differentiation of osteoblast via ERβ pathway, increased ALP activity and mineralization. Furthermore ERβ was evidenced by increase in the secretion of osteoprotegerin (OPG) and RANK ligand (RANK-L) which are involved in osteoclastogenesis and runx2/Cbfa1, thus daidzein plays a vital role in osteoblast differentiation and function (De Wilde et al., 2004). Moreover, daidzein inhibited differentiation and activation of osteoclast largely by activating caspase 3 to induce osteoclast progenitor apoptosis. The expression of osteoclastogenesis inhibitory factor (OCIF) in osteoblast-like cells downregulated secretion of some factors such as OPG, also associated with the inhibition of osteoclast differentiation. The mechanism was by ERS, mainly by ERβ pathway in porcine bone marrow cells (Rassi et al., 2002). In addition, daidzein exhibited bone protection and bone resorption inhibition indirectly by stimulating secretion of calcitonine, which is a kind of hormone produced by Thyroid C cells, inhibiting the activity of osteoclasts and inducing osteoblast-line cell proliferation. This study was conducted in ovariectomized (Ovx) male rats and it provided a feasible way in the treatment of male osteoporosis (Filipović et al., 2010). In vivo model using parietal bone defects from New Zealand white rabbits has demonstrated the effect of daidzein on bone formation in collagen matrix. There were 602% more new bone formation in collagen matrix with daidzein when compared to the control, suggesting that daidzein stimulated new bone formation and it could be applied to bone grafting (Wong and Rabie, 2009).

Mathey et al. (2007) pointed out that daidzein treatement together with equol improved not only total femoral BMD but also bone strength in Ovx rat. Thus, the addition of fructooligosaccharides (FOS) or live microbial to promote intestinal bacterial metabolism of daidzein could markedly enhance the protection action of daidzein on bone. Combination of daidzein with high dose of Ca also augmented the protective effect on bone mass and biomechanical strength, although their mechanisms were different (Fonseca and Ward, 2004). When daidzein was used in combination with kiwifruit, the effect on reducing bone loss caused by estrogen deficiency was little while exerting no effect on the production of equol (Tousen et al., 2014).

However, some side effects of daidzein such as low bioavailability, unfavorable metabolism and uterine estrogenicity have limited its clinical application. Recently, a number of daidzein analogs were found to exert protective impact on osteoporosis by promoting of differentiation in bone marrow-derived mesenchymal stem cells (BMSCs) and adipose-derived stromal stem cells (ASC) ER-independently. For example, isoformonetin, a methoxy daidzein, prevented osteoblasts from apoptosis and controlled bone loss (Strong et al., 2014; Srivastava et al., 2013).

Anti-Diabetic Activity

In recent years, due to an increase in living standards, diabetes has become a worldwide epidemic disease. International Diabetes Federation (IDF) report a persistent growth in diabetes incidence and it is projected that by the year 2030, there will be 439 million diabetics in the world. Moreover, about 90% of diabetic patients diagnosed had type II diabetes (Getek et al., 2014). Undoubtedly, finding an effective method to treat diabetes is urgent.

Soy phytoestrogen plays an important role in Type 2 diabetes. Daidzein is one of the most bioactive components exerting anti-diabetic activity in soy phytoestrogen (Jayagopal et al., 2002). Both *in vitro* and *in vivo* experiments have proved anti-hyperglycemic effect of daidzein. In Type 2 diabetic cell model, L6 myotubes, daidzein stimulated glucose uptake through

promoting AMPK phosphorylation to increase glucose transporter 4 translocation to PM of muscle cells, which in turn led to glucose homeostasis insulin-independently. *In vivo* studies used KK-Ay mice and db/db mice as Type II diabetic animal model. It has been observed that daidzein controlled increased blood glucose levels to exhibit its anti-hyperglycemia effect (Cheong et al., 2014). Another study in C57BL/KsJ-db/db mice found that the anti-diabetic effect of daidzein in type 2 diabetes was also associated with liver glucose and lipid metabolism by modulating related enzyme activities. Genistein and daidzein treatment regulated blood metabolism by significantly lowering the ratio of glucose-6-phosphatase (G6Pase)/GK and phosphoenolpyruvate carboxykinase (PEPCK) in the liver of db/db mice while had no effect on the levels of plasma insulin and C-peptide, but increased the ratio of insulin/glucagon. Insulin resistance is related to hepatic lipid accumulation, genistein and daidzein dramatically lowered the plasma FFA level to decrease β-oxidation via carnitine palmitoyltransferase (CPT-1) in type 2 diabetic mice. As a result, it improved the metabolism of liver lipid, and then controlled blood glucose concentration (Ae Park et al., 2006). To conclude, daidzein prevented against Type II diabetes and has the potential to be developed as a potent anti-diabetic phytochemical medical agent.

Daidzein could also play a beneficial role in regulation of fasting blood glucose level in type I diabetes which is also known as insulin dependent diabetes (IDDM). Insulin deficiency is the main pathogenetic mechanism responsible for type I diabetes. Daidzein treatment induced survival of pancreatic β-cells and insulin secretion while having no effect on glucagon in non-obese diabetic (NOD) mice, an animal model of human type 1diabetes. The regulation of hepatic glucose and lipid metabolism by altering a series of related enzyme activity has also been demonstrated, the mechanism of which is similar to the type II diabetes, such as reducing activities of G6Pase, PEPCK, fatty acid beta-oxidation and CPT and increasing activities of malic enzyme and G6PD (Choi et al., 2008). The molecular basis of daidzein regulating glucose and lipid metabolism is activation of peroxisome proliferator-activated receptors(PPAR) and further regulation of PPAR-α-mediated and PPAR-γ-mediated gene expression involved in glucose and lipid metabolism (Mezei et al., 2003). Daidzein also suppressed up-regulation of postprandial blood glucose levels by inhibiting the activity of carbohydrate digestive enzymes, α-glucosidase and α-amylase (Park et al., 2013b).

Taking daidzein and hemin together might decrease the expression of caveolin, inhibiting RAAS system and enhancing the level of renal nitric oxide in the wistar rat mode (Katyal et al., 2013). Comprehensive factors have to be taken into consideration as combined therapy in diabetes may protect kidney and related systems such as renin-angiotensin system (RAS).

Anti-Aging Activity

Daidzein has a role in the cosmetic industry due to its ability to prevent skin aging and photo-damaging. Skin aging is primarily associated with collagen reduction in the dermis, type I and type III collagens are the main component of extracellular matrix (ECM) which is vitally important in maintenance of the dermis structure.

Transforming growth factor (TGF-β) mediated by smad is involved in the regulation of ECM (Choi et al., 2007). It has been demonstrated *in vitro* and *in vivo* that daidzein promoted collagen deposition by stimulating collagen synthesis via up-regulating the expression of type I pro-collagen and inhibiting collagen degradation via down-regulating the levels of MMP1 (matrix metalloproteinase1), and MMP2. This collagen metabolic regulation was mediated by TGF-β/smad signal pathway, phosphorylated-smad2, smad3 and TGF-β was significantly higher in the daidzein-treated cells when compared to the control (Zhao et al., 2015). Exposure to solar UV radiation for a long time, in particular UVB radiation, accelerates skin aging due to induced production of oxygen free radicals in a study of pig skin model which is similar to the human skin. The photo-protection effect of daidzein was demonstrated by evaluating colorimeter-measured erythema and photo-damaged cell numbers after solar-simulated ultraviolet (ssuv) irradiation (Lin et al., 2008). It was widely believed that daidzein exhibited photo-protection due to its antioxidant activity by clearing free radical of keratinocytes induced by UV radiation (Huang et al., 2008a). However, another study found the primary mechanism related to ERβ. S-equol is a gut metabolite of daidzein which prevents skin from natural or photo-aging via activating ERβ directly. The selective activation of ERβ increased levels of the antioxidant enzymes which protected skin from harmful oxygen-free radical injury and reduced levels of snail which modulated proliferation and migration of keratinocyte, resulting in an increase in the expression of type I and type III collagens (Jackson et al., 2011). Retinoid a vitamin A derivative has been used to treat skin-aging and it enhances collagen accumulation in the dermis, however retinoid-induced skin irritation can lead to epidermal hyperplasia. Co-treatment with daidzein inhibited these side effects of retinoid (Varanl et al., 2004). Daidzein could bind

to RAR and RARγ directly to increase the activity of RAR and RARγ, further exerting photo-protective effects. Moreover, it inhibited the activity of matrix metalloproteinase-9 (Oh et al., 2013). Hyaluronic acid (HA) was another major ingredient present in the epidermis and dermis, maintaining hydration and inhibiting elasticity loss, which can prevent skin from aging. HA exhibits age-dependent loss similar to that observed with collagen. Both *in vitro* and *in vivo* studies demonstrated that daidzein stimulated the production of cutaneous HA (Miyazaki et al., 2002). Bifidobasterium-fermented (BE) soy milk extract mainly containing genistein and daidzein enhanced HA production and improved the elasticity and viscoelasticity of mouse skin, while non-fermented (SME) soy milk extract did not exert the stimulative effect of daidzein on HA production (Miyazaki et al., 2003).

Based on the ability of daidzein to protect the skin, the ability of percutaneous absorption of daidzein to achieve stable treatment concentration was studied. The results indicate that daidzein showed higher skin deposition in a non-ionized form than in an ionized form. Although aglycone mixture and PEG400 can improve skin permeation, the ability of daidzein absorption by the transdermal route was very weak (Minghetti et al., 2006), however, repeated transdermal application of daidzein could improve its concentration in plasma (Vänttinen and Moravcova, 2001).

Antioxidant Activity

Daidzein is a natural antioxidant and there are two mechanisms mediating its antioxidant activity. First, in liposomal membranes, daidzein inhibited lipid oxidation by clearing radical directly and impeded the migration of radicals by changing the fluidity of membrane via binding to it (Liang et al., 2008). Secondly, daidzein exerted antioxidant effect indirectly via improving the activity of anti-oxidative enzymes (AOE) which including catalase, glutathione peroxidase (GPx) and superoxide dismutase (SOD) (CuZn-and Mn-SOD) (Kampkötter et al., 2008a). The effect of daidzein on modulating the expression level of AOE in a study of rat hepatoma H4IIE cells reached the maximum at the concentration of 300 micromol/L. Transfection experiments suggested that daidzein can up-regulate the expression of catalase mRNA via activating catalase promoter region directly. However, the oxidative stress induced by H2O2 was not affected by daidzein through this mechanism and daidzein itself exhibited a weak antioxidant capacity (Röhrdanz et al., 2002). Furthermore, it was demonstrated that daidzein significantly enhanced the activity of catalase and the expression of catalase gene by acting on the proximal part of the catalase promoter (Kampkötter et al., 2008b). In general, changes in AOE system are more important than daidzein itself in exerting antioxidant activity.

Daidzein was reported to be beneficial to animal health due to its antioxidant activity. In streptozotocin-induced diabetic rats, daidzein down-regulated the increased concentration of MDA, a product of lipid peroxidation and stimulated the inhibited activity of SOD to attenuate the oxidative stress including the prevention of vascular dysfunction (Roghani et al., 2013). The administration of flutamide resulted in androgen (AE) deficiency which lowered the levels of endogenous AOE in Wistar rats. Daidzein exerted protective effect by restoring the levels of AOE and AE to normal in a dose dependent manner (Lateef et al., 2012). It was observed *in vitro* that some metabolites of daidzein such as O-DMA and equol generally exhibited stronger antioxidant potential by increasing the activity and expression of catalase and SOD compared to daidzein alone (Choi and Kim, 2014). 3′-OH-daidzein and 6-OH-daidzein, another two metabolites of daidzein were also more effective than daidzein (Liang et al., 2008). Thereby, these antioxidant metabolites might contribute to the antioxidant properties of dietary isoflavonone. In addition, gamma irradiation on soybean will significantly improve concentration of genistin and daidzein and the antioxidant activity at dose up to 10 kGy (Popović et al., 2013).

Anti-Inflammatory Activity

Failure to clear apoptotic cells in time can lead to the initiation of inflammatory diseases. Efferocytosis is defined as clearance of apoptotic cells and daidzein augmented efferocytosis capability of macrophage cell RAW264.7 by up-regulating the expressionofTG2 which is needed for effective engulfment during efferocytosis. The increased TG2 stimulated phosphorylation of Erk to activate Rac1 and the down-regulation of mitochondrial membrane potential eventually enhances efferocytosis (Yen and Yang, 2014).

Daidzein could also inhibit activation of NF-κB which is a type of transcription factor, closely related to inflammation by

regulating the transcriptional activation of array of target genes including pro-inflammatory mediators, such as iNOS, COX-2, various cytokines, chemokines, and adhesion molecules. It can be activated by many stimuli such as TNF-α (Pahl, 1999). TNF-α-treated murine lung MLE-12 epithelial cells were the cell models to elucidate the underlying anti-inflammatory mechanism of daidzein. Daidzein markedly decreased the level of TNF-α-induced protein poly-adenosine diphosphate-ribosylation (PARylation) by binding to PARP-1 directly, resulting in the suppression of the transcription of pro-inflammatory genes such as NF-κB, which further inhibited the expression of chemokine Cxcl2 (Li and Pan, 2014). Daidzein was effective in the treatment of periodontal inflammation which was induced by lipopolysaccharide (LPS) from Prevotella intermedia, a pathogen. In P. intermedia LPS-treated RAW264.7 cells, daidzein significantly inhibited the production of NO and IL-6 through NF-κB signal pathway via suppressing degradation of IκB-α and iNOS activation to alter the function of NF-κB, and STAT1 plays a cooperative role with NF-κB in this process via suppressing STAT1 phosphorylation. These reduced secretion of inflammatory factors from macrophage contributed to anti-inflammatory effect of daidzein in the periodontium (Choi et al., 2012).

As for obesity-related adipose inflammation, daidzein was reported to activate PPARγ directly to promote differentiation of adipocytes and regulate expression of adipokine. Mainly, it up-regulated the expression of adiponectin and further decreased the expression of pro-inflammatory factor TNF-α and MCP-1 which plays an important role in suppressing macrophage infiltration in adipose tissue. Moreover, daidzein inhibited hypertrophy in adipocyte size and it is apparent that daidzein can improve obesity-related inflammation which is related to insulin resistance (Sakamoto et al., 2014). In addition, daidzein is still used in the treatment of inflammatory damage of the skin caused by UVB or 12-O-tetradecanoylphorbol-13-acetate (TPA). Keratinocytes and fibroblasts were used to investigate UVB induced cutaneous inflammation, and the result indicated that daidzein suppressed macrophage infiltration to the dermis and epidermis induced by UVB, further decreasing the production of ROS, the expression of pro-inflammatory mediators such as iNOS and COX-2 and the pro-inflammatory factors such as TNF-α via inhibiting the mitogen-activated protein kinase (MAPK) signaling pathway (Lee et al., 2014).Besides, daidzein suppressed TPA-induced skin inflammation by reducing the activation of NF-κB, and the expression of IL-6,TNF-αand COX-2 (Khan et al., 2012). Consequently, daidzein has the potential to improve therapy in inflammatory diseases in the future.

Neuroprotective Activity

Daidzein has also been evaluated for its protective effect against neurodegenerative diseases. Stroke morbidity rate is high in the world, and currently there is no suitable drug for its treatment. It is well known that stroke is associated with brain injury which can result in lasting damage to the body. Rats were used to demonstrate the neuroprotective effect of daidzein after stroke. The results indicated that when treated with daidzein, rats expressed fewer deep slips in the skilled ladder rung walking task compared to rats treated with no daidzein, suggesting that daidzein was effective in neuroprotection and function recovery after stroke, although the mechanism is not clearly defined (Stout et al., 2013). There were three possible hypotheses reported to explain this neuroprotective effect of daidzein. Firstly, daidzein binds to ERβ and G-protein-coupled receptor 30 (GPR30) directly to inhibit neuron cell apoptosis by mitochondrial caspase-dependent pathway (Kajta et al., 2013). Secondly, daidzein induces the transcription of arginase 1 (Arg1), further stimulating the survival and regeneration of neuron in central nervous system (CNS) by inhibiting MAG cAMP independently (Ma et al., 2010). Thirdly, daidzein activates PPARγ by regulating nuclear translocation from cytoplasm to inhibit neural cells death and promote axon cells maturation. Moreover, the activation of PPARγ is not related to ligand binding of daizein (Hurtado et al., 2012). In addition, Yang et al. (2012) showed that daidzein may induce phosphorylation of Src kinase, further activating Src-protein kinase C delta (PKCδ)-ERK signal pathway to promote axon growth of rat dorsal root ganglion (DRG) neurons. In cerebellar granule cells, daidzein could reduce oxidative damage of mitochondria by down-regulation of the ROS levels, and thus subsequent inhibition of apoptosis (Atlante et al., 2010).

It is well known that hippocampus is mainly responsible for learning and memory. Scopolamine induced memory damage in male rats can be ameliorated by daidzein through ER and some behavioral tests have demonstrated this (Kim et al., 2010). Axon formation and extension was stimulated with daidzein treatment in hippocampus neuron. Daidzein activated ERβ in the membrane, further promoting phosphorylation of PKCα in growth-associated protein GAP-43. Eventually, the growth of axon in hippocampus neuron led further to the modulation of learning and memory ability (Wang et al., 2008). When exposed to a very high-fat diet,

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animals would suffer from high fat diet-induced energy metabolism imbalance which resulted in apoptosis and gliosis in the adult hippocampus. Pretreatment with daidzein enhanced cell proliferation, while inhibited apoptosis and gliosis by down regulating the expression of ERα, caspase3, GFAP and IBA1, in the hippocampus (Rivera et al., 2013).

In vitro, daidzein inhibited aggregation of Aβ and in the used pheochromocytoma PC12 neuronal cellular model, treatment with daidzein, Aβ-induced cytotoxicity was also inhibited. Moreover, the co-treatment in the cultures with baicalein further enhanced this effect. Therefore, daidzein may play a significant role in the treatment of Alzheimer's disease in the future (Choi et al., 2013).

Other Activities

In addition to the described pharmaceutical activities, daidzein exhibits other beneficial effects. Menopausal women are at higher risk of multiple problems like heart disease and bone loss which was mentioned earlier due to estrogen deficiency. The incidence of hot flashes is also high in that about 75% of postmenopausal women experience this leading to a reduction in their quality of life. Ricciotti HA found isoflavone supplement which is rich in daidzein significantly ameliorated hot flashes in a study of twenty-four postmenopausal women (Ricciotti et al., 2005). Daidzein was also effective in pulmonary fibrosis induced by bleomycin in rats, inflammation and alveolar epithelial cell apoptosis was mainly responsible for pulmonary fibrosis. Daidzein reversed this effect by decreasing the level of proteinase activated receptor 2 and TGF-β (Soumyakrishnan et al., 2014). In addition, daidzein can inhibit the over-secretion of mucin from airway epithelial cells which would cause respiratory diseases (Lee et al., 2011b). In OVX rats, daidzein was reported to stimulate cadmium excretion to avoid the damage of heavy metal accumulation on renal function (Om & Shim, 2007). It was reported that daidzein treatment in male Balb/cJ mice alleviated anxiety, increased locomotor activity and decreased their social behavior including aggression and sexual behavior, the mechanism may be related to ER (Zeng et al., 2010). Daidzein supplementation to the mother also affected social behavior of female offspring. Daidzein reduced the expression of ERα in the brain, resulting in behavioral masculinization in adult female mice while it had no effect on anxiety (Yu et al., 2010).

Conclusion

Daidzein as a plant extract has been extensively investigated recently. In this paper, pharmacological effects of daidzein have been reported and these include anticancer, anti-cardiovascular diseases, anti-osteoporosis, anti-diabetes, anti-inflammation, antioxidant, anti-aging activity, neuroprotective activity and some other activities as presented in Figure 2. Over the years, conventional HRT has been used to ameliorate menopause symptoms clinically but unfortunately various complications such as breast cancer have limited its clinical usage. Daidzein was discovered to have a structure similar to estrogen and to be selective to the ER. Currently it is being widely used in the treatment of some diseases and there is hope for further development in its clinical application. However, the mechanisms of action remain uncertain and its poor bioavailability limits its application, and some possible side effects of daidzein have been reported. Besides promotion of tumor growth mentioned earlier, daidzein can cause erectile dysfunction due to down-regulation of androgen and alternation of penile cavernosal structures (Pan et al., 2007; Huang et al., 2008a). Thus, the details of the mechanisms involved need to be further clarified to reduce the side effects.

Some bio-transformations in the body or synthetic analogues of daidzein maybe more effective on health or/and less toxic, such as isoformonetin and 7, 3′, 4′-THIF. Although some bioactive analogues of daidzein have been identified for their benefits on human health, many other potential metabolites remain unknown. Therefore, it is necessary to find novel effective substances based on the structure of daidzein. In addition, it is also crucial to enhance the absorption and bioavailability of daidzein, which may be realized via the appropriate way of administration or drug modification according to its physicochemical properties and pharmacokinetic parameters. Further studies are needed before daidzein can be widely promoted for use in clinical settings.

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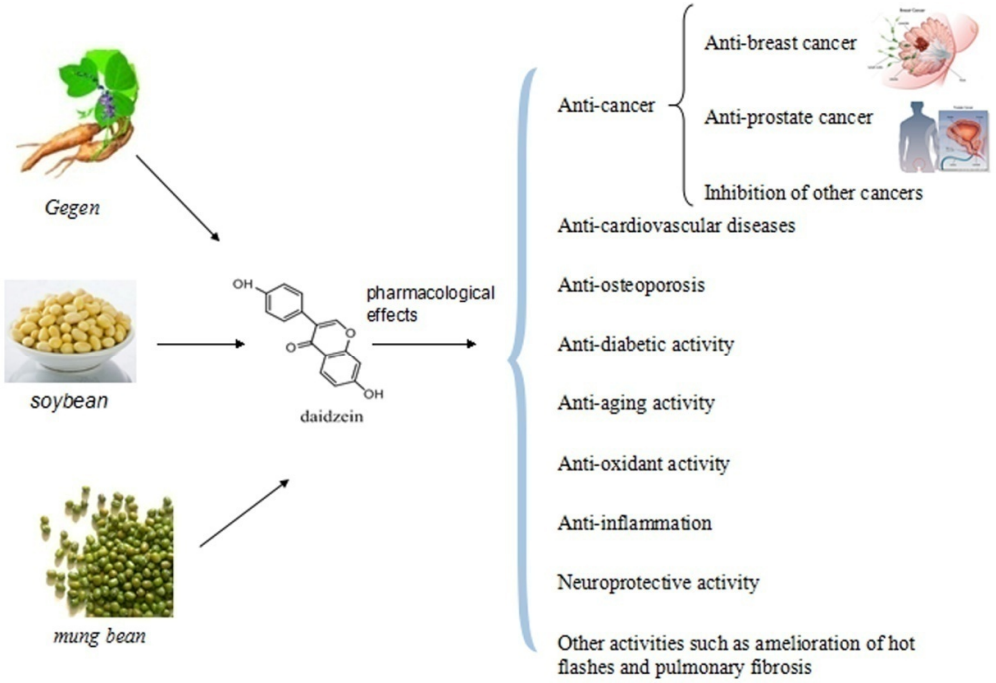

Figure 2: The pharmacological effects of daidzein

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