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REVIEW

Isoflavones in Soybean as a Daily Nutrient: The Mechanisms of Action and How They Alter the Pharmacokinetics of Drugs

Günlük Besin Olarak Soya Fasulyesindeki İzoflavonlar: Etki Mekanizmaları ve İlaçların Farmakokinetiğini Değiştirmeleri

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ABSTRACT

Soybeans [*Glycine max* (L.)] are a good source of isoflavones. The main isoflavone components of soybean are daidzein, genistein, and glycitein. World soybean production is very high. Because of its pharmacological activity, soy isoflavone intake over a long period of time may result in interactions with the drugs. This review summarizes soy isoflavone-drug interactions based on the pharmacokinetic parameters. Soy isoflavones have pharmacokinetic interactions with celecoxib, theophylline, paclitaxel, midazolam, imatinib, carbamazepine, valproic acid, repaglinide, omeprazole and danofloxacin. This is due to the changes in the area under the curve, maximum serum concentration, time that a drug is present at the maximum concentration in serum, clearance and half-life of the drugs when delivered together with soy isoflavones. The mechanisms of pharmacokinetic interactions occurs through the inhibition/induction of drug metabolizing cytochrome P450 (CYP450) enzymes such as CYP3A4, CYP2A1, and CYP2C9 or through the inhibition of drug transporters such as P-glycoprotein and breast cancer resistance protein. Thus, the consumption of soybean, soy isoflavones or soy products with drugs needs to be reconsidered.

Key words: Soybean, isoflavones, pharmacokinetic ınteraction, drug metabolizing enzyme, drug transporter

ÖZ

Soya fasulyesi [*Glycine max* (L.)] iyi bir izoflavon kaynağıdır. Soya fasulyesinin ana izoflavon bileşenleri daidzein, genistein ve glisitindir. Dünyada soya üretimi çok yüksektir. Farmakolojik aktivitesi nedeniyle, uzun süre soya izoflavon alımı, ilaçlarla etkileşime neden olabilir. Bu derleme, farmakokinetik parametrelere dayalı olarak soya izoflavon-ilaç etkileşimlerini özetlemektedir. Soya izoflavonları, selekoksib, teofilin, paklitaksel, midazolam, imatinib, karbamazepin, valproik asit, repaglinid, omeprazol ve danofloksasin ile farmakokinetik etkileşimlere sahiptir. Bunun nedeni, soya izoflavonları ile birlikte verildiğinde ilaçların eğrinin altındaki alan, maksimum serum konsantrasyonu, ilacın maksimum serum konsantrasyonunda bulunduğu süre, klirens ve yarılanma ömründe yaptığı değişikliklerdir. Farmakokinetik etkileşimlerin mekanizmaları ilaç metabolize eden sitokrom P450 (CYP450) enzimlerinin (örneğin; CYP3A4, CYP2A1 ve CYP2C9) inhibisyonu/indüklenmesi veya P-glikoprotein ve meme kanseri direnç proteini gibi ilaç taşıyıcılarının inhibisyonu yoluyla gerçekleşir. Bu nedenle soya fasulyesi, soya izoflavonları veya soya ürünlerinin ilaçlarla tüketimi yeniden gözden geçirilmelidir.

Anahtar kelimeler: Soya fasulyesi, izoflavonlar, farmakokinetik etkileşim, ilaç metabolize eden enzim, ilaç taşıyıcısı

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INTRODUCTION

Soybeans [*Glycine max* (L.)] are a source of isoflavones in the daily meals. In 2016, global soybean production amounted to be 34,894,085 tons; with 293,414,006 tons from the Americas, 28,808,950 tons from Asia, 10,488,759 tons from Europe, and 2,119,814 tons from Africa. In 2016, total of 89.05% of soybean production was from five countries: India (4.18%), China (3.57%), Argentina (17.56%), Brazil (28.75%), and the USA (34.99%).[1] Soybeans contain non-steroidal polyphenol compounds[2] with a chemical structure similar to that of oestradiol-17β, so these compounds may have a similar effect to that of the estrogen.[3,4] The main isoflavone content of soybean is in aglycone form, including genistein, daidzein, and glycitein; the glycosidic forms are genistin, daidzin, and glycitin, which are precursors of the metabolic process which forms daidzein and genistein aglycones.[5] The total glycitein and glycoside content in soybeans is only 5-10% of the total isoflavones, while the remaining is comprised of daidzein and genistein.[6] Isoflavones have effects on postmenopausal nutrition,[7] relief of postmenopausal vasomotor symptoms,[8] osteoporosis,[9] inflammation,[10] and cardiovascular disease.[11] The compounds also have antioxidant activity,[12] increase the efficacy of cancer therapy,[13] and inhibit the cancer cell proliferation.[14]

Based on this pharmacological activity, soy isoflavones could be used as a dietary nutrition over a long period of time. Soybean consumption continued to increase in 2011.[15] Fonseca et al.[16] showed that the amount of isoflavones taken in by the infants fed with soy-based formula is 0.8 mg/day/kg of body weight; this number is two-fold higher than the level of isoflavones consumed by the adults in Japan. The daily intake of isoflavones is related to how much soy is consumed and differs in each country, [i.e., it is much higher in east and south Asian countries (20-50 mg/day), than in Europe (0.49-1 mg/day)].[17,18] To fulfill the daily nutrient needs, the Chinese government has recommended that every citizen consumes 50 mg of soy food daily. Simple processed soy foods from Asia usually contain 3.5 mg of isoflavones in every gram. Large studies performed in the United States showed that each adult there consumes 2.5 mg of isoflavones per day, but other research data shows different results where the consumption of isoflavones per day may reach the range of 30-50 mg. In China, the average daily consumption of isoflavones is 40.8±28.7 mg/day.[19]

Isoflavone consumption patterns in this community therefore raise the possibility of drug interactions when used together, so their use must be monitored. Drug interactions occur when other substances affect the activity of a drug.[20] These interactions may occur with the soy isoflavones. Soy extracts, soy products, and soy isoflavones have interactions with the drugs such as: Warfarin,[21] tamoxifen,[22] levodopa,[23] and ciprofloxacin.[24] The mechanism of the drug-isoflavone interaction is by the inhibition or induction of drug metabolizing enzymes (DMEs) or drug transporters.[25]

Almost all the drug biotransformation reactions need a metabolic enzyme, and the enzymes most often used to process the drugs are the liver microsomal cytochrome P450 (CYP450) enzymes. The CYP enzymes involved in the drug metabolism are CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5.[26] Drugs or bioactive compounds such as isoflavonoids interact with these enzymes, and change the efficacy and action of the drug.[27] Soybean products (infusions) have an inhibitory effect on human CYP enzymes, including CYP2C9, CYP2C19, CYP3A4, and CYP2D6.[28] It has also been reported that soy isoflavones reduce the hepatic CYP2E1 and CYP3A activities related to acetaminophen metabolism.[29]

Furthermore, drug transporters could be involved in the drug interactions, because drug transporters mediate the absorption, distribution, and excretion of the drugs in the transport process across the plasma membrane.[30] There are two classifications of these drug transporters: The ATP-binding cassette (ABC) family and the solute carrier (SLC) family. P-glycoprotein (P-gp) is a member of the ABC family, and could be induced by various factors, including clinical drugs, environmental xenobiotics, and dietary compounds;[31] which is known to be involved in the drug interactions. There are reports that genistein from soy inhibits the efflux of the P-gp substrates cimetidine[32] and paclitaxel.[33] The efflux of vinblastine in KB-V1 cells highly expressing P-gp, and the P-gp substrate paclitaxel could be inhibited by genistein at some doses.[34] In addition to the P-gp, interactions may occur through other drug transporters. So, DMEs and drug transporters play important roles in the absorption, distribution, metabolism, and the excretion (ADME) of the drugs, and are involved in the interactions that will affect the pharmacokinetics and pharmacodynamics of the drugs.

These pharmacokinetic interactions could be seen by assessing the pharmacokinetic parameters including the area under the curve (AUC), maximum concentration (C_{max}), volume of distribution (Vd), half-life ($t_{1/2}$), and clearance. Nagashima et al.[23] found that soybean increases the AUC of levodopa. Soybean also reduces the AUC and C_{max} of losartan.[35] These differences in pharmacokinetic parameters depend on the mechanism. Until now, there has been no summary to explain how soy isoflavones could affect the pharmacokinetic profile of a drug and the mechanisms involved. This is needed as a reference regarding the safety of using soy isoflavones as daily nutrients with the co-administration of the drugs.

Methods

This review is based on the literature collected from the internet through Google Scholar, Elsevier, PubMed, and NCBI, using the keywords soybean, soy products, soy isoflavones, soy drug interaction, isoflavone content, daidzein, genistein, isoflavone interaction, pharmacokinetic parameter, and the pharmacokinetic interaction. In total, 181 articles were collected, but only 99 articles were included based on the inclusion criteria. The inclusion criteria were articles with a publication year before 2000, containing a description of pharmacokinetic parameter values, describing interactions with soybeans, containing isoflavone content data, or related to isoflavones, soybeans, and pharmacokinetic interactions. The flowchart of the search is illustrated in Figure 1.

Soy isoflavones

Isoflavones are bioactive metabolites and include a group of phytoestrogens. Isoflavones have structures like those of mammalian estrogens. The largest source of isoflavones is soybean. Soy isoflavones are present in 12 different isoforms, divided into four chemical forms: Acetylglucoside (acetylgenistin, acetylglycitin, acetyldaidzin), malonylglucoside (malonylgenistin, malonyldaidzin, malonylglycitin), glucoside (genistein, daidzin, and glycitin), and aglycone (genistein, daidzein, and glycitein).[36] After the metabolism process in the human gut, glucoside isoflavones become aglycones through the effect of gastrointestinal enzymes.[5] Genistein, daidzein, and glycitein comprise approximately 50%, 40%, and 10% of the isoflavones in soybean.[37] The isoflavone content is influenced by several factors; in this article, we summarize the content of genistein, daidzein, and glycitein in soybeans, as seen in Table 1.[37-47] The amount of isoflavones is in the order genistein › daidzein › glycitein, and the content of the glycoside form is lower than that of the aglycone form; differences arise based on the variety, location of the production, humidity etc.[38] Sources of isoflavones include soy products such as traditional soy foods (such as tofu and soy milk), isolated soy protein, soybean paste, soy flakes, soy flour, fermented soybean products (such as tempeh, miso, and natto), and soy sauce.[39]

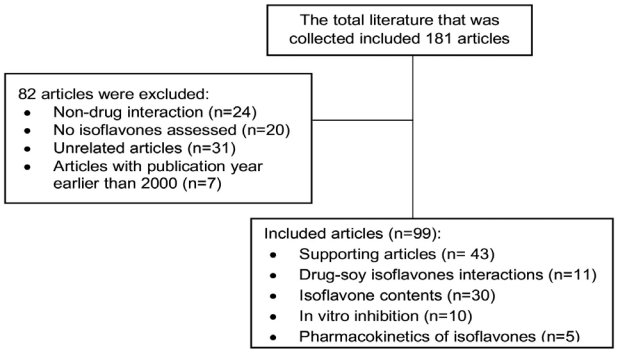

Figure 1. Flow chart of the literature review

In each type of soybean product containing different soy isoflavones, we summarize the isoflavone content focusing only on the aglycone form, (i.e., genistein, daidzein, and glycitein in various soy products from the several studies, presented in Table 2).[48-62] It appears that soy tablets commercially contain the highest levels of isoflavones, because soy tablets are usually used as additional nutrients so the soy isoflavone content is adjusted to nutritional requirements. Of the soy products shown Table 2, sufu has the highest content compared to others. Sufu is a traditional food from China, and it is made of fermented soybean curd.[48] Other fermented foods that also have high soy isoflavones content are natto, tempeh, and miso. The fermentation process influences the isoflavone content. Fermentation can increase aglycone isoflavones from black soybean pulp[49] in tempeh and tofu.[50,51] Another study reported a 75% increase in aglycone isoflavones in soybean flour after fermentation.[52] The fermentation process is also influenced by the several factors such as time and temperature.[53,54]

The differences in the isoflavone content of soybean products also leads to variations in the pharmacokinetic profile of isoflavones, as presented in Table 3. There are variations in the different levels, caused by many factors, [i.e., differences in the test subjects used (human, rats, or mice), variations in the age, the hydrolysis process of glycosides by the gut bacteria or gut wall enzymes, uptake, ethnicity, etc].[44] The content of daidzein and genistein in soy products depends on the raw material and the conditions while processing, Faughnan et al.[63] found that urinary recovery of equol from tempeh is higher than the soymilk, although the solid food matrix and fermentation may increase the production of equol. Equol is a metabolite of daidzein produced by the intestinal bacteria; the level of equol production has been linked to the consumption, and the content

Table 1. Summary of isoflavone contents in soybean

Sample	Genistein	Daidzein	Glycitein	Genistein	Daidzin	Glycitin	Reference
Soybean extract	36.55 µg/g	88.87 µg/g	34.42 µg/g	-	-	-	40
Soybean extract	1260 µg/g	849 µg/g	174 µg/g	-	-	-	41
Soybean	0.126 µg/g	0.71 µg/g	-	-	-	-	37
Soybean	330 µg/g	100 µg/g	50 µg/g	100 µg/g	69 µg/g	-	42
Soybean (culture origin)	3771 µg/g	3366 µg/g	-	-	-	-	43
Soybean (market origin)	2971 µg/g	2579 µg/g	-	-	-	-	43
Soy sprout	232 µg/g	177 µg/g	-				43
Soy flour	-	-	-	700 µg/g	620 µg/g		44
Soybean	42 µg/g	47.8 µg/g	2.7 µg/g	-	-	-	45
Isogen (refined soy isoflavones)	368 µg/g	782 µg/g		-	-	-	46
Soybean seed	-	-	-	465.78 µg/g	251.64 µg/g	108.25 µg/g	47

of the isoflavone daidzein. The solid food matrix of tempeh may protect isoflavones from degradation, so they could reach the large intestine and metabolized into equol by the gut bacteria. This indicates that tempeh contains more daidzein than soymilk. Information about pharmacokinetics is very important to evaluate the safety and understand the efficacy. For example, from the $t_{1/2}$, we could predict that how long isoflavones are still present in the body, so that its consumption time could be regulated by the medication.

It turns out that not only isoflavone tablets are high in isoflavones, but daily food processed from the soy also contains quite high levels of isoflavones, and may interact if taken together with certain drugs. Thus, there is a need for careful monitoring. An assessment of the pharmacokinetic profile of several other processed soybean products needs to be done, for example tofu, to obtain more information.

The mechanism of drug-isoflavone pharmacokinetic interactions

Drug interactions not only occur between drugs, but also occur between drugs and herbal or natural compounds, such as isoflavones. Isoflavones are a component of dietary foods or herbal supplements, so there is a possibility of long-term exposure together with the drugs. This simultaneous use may lead to the drug-isoflavone interactions. This is supported by Laurenzana et al.[64], who found that the content of natural materials such as flavones, isoflavones, and tangeretin affects the activity of human CYP enzymes when given orally together with the drugs. These changes in ADME will certainly affect the pharmacokinetic parameters of the drugs, because of the interactions with DMEs and interaction with the drug transporters.

In this article, the pharmacokinetic interactions between isoflavones and some drugs and their mechanisms of interaction have been summarized, focusing on the enzymes and drug transporters, as shown in Table 4. It has been reported that the co-administration of soy isoflavones (genistein or daidzein), soy tablets, or soybean extract with drugs results in changes in the pharmacokinetic parameters of the drug, which indicates an interaction. These effects include the changes in the AUC, C_{max}, and the clearance. These changes may be either an increase or

Table 2. Isoflavone contents of soybean products

Sample	Genistein	Daidzein	Glycitein	References
Isoflavin tablet	31.863 mg	12.803 mg	-	43
Novasoy tablet	19.9 mg	24.9 mg	3.4 mg	55
Soy nut	0.039 µg /mL	0.032 µg/mL	-	37
Soy milk	0.043 µg/mL	0.027 µg/mL	-	37
Soy milk	25.86 µg/mL	8.25 µg/mL	-	56
Soy milk	47.6 µg /mL	47.3 µg /mL	-	57
Soy milk	26.46 µg/mL	-	-	58
Soy milk	22.3 µg/g	19.6 µg/g	22 µg/g	59
Soy milk	71.1 µg/g	67.9 µg/g	11 µg/g	45
Soy milk	56 µg/mL	52 µg/mL	-	46
Tempeh	0.0196 µg/mL	0.0107 µg/mL	-	37
Tempeh	186.4 µg/g	137.1 µg/g	22.1 µg/g	59
Raw tempeh	280 µg/g	260 µg/g	-	60
Fried tempeh	310 µg/g	350 µg/g	-	60
Firm tofu	4.916 µg/g	7.306 µg/g	-	61
Tofu	14.5 mg	24.6 mg	-	57
Tofu	98.7 µg/g	104.9 µg/g	18.8 µg/g	45
Soybean meal	92.4 µg/g	109.2 µg/g	13.8 µg/g	45
Natto	224 µg/g	411 µg/g	-	57
Natto	147.4 µg/g	234.4 µg/g	8.8 µg/g	45
Fermented soybean miso	145 µg/g	166.8 µg/g	17 µg/g	45
Sufu	617.7 µg/g	536.9 µg/g	103.2 µg/g	45
Fermented tofu	321 µg/g	319 µg/g	-	62
Sufu	99.98 mg	65.48 mg	16.42 mg	48

a decrease in the pharmacokinetic parameters, depending on the mechanism. The mechanisms that will be discussed here involve enzymes and drug transporters.

Effects of soy isoflavones on drug metabolizing enzymes

Soybeans influence the metabolism of the drugs, and affect ADME through the interactions with phase-I or phase-II DMEs. The enzymes involved in phase-I metabolism are the CYP450 families, while the enzymes involved in phase-II metabolism are sulfotransferases, uridine diphosphate glucuronosyltransferases (UDPGT/UGTs), N-acetyl transferases (UDPGT/UGTs), glutathione-S-transferases, and methyltransferases.[25]

Phase-I metabolism enzymes

CYP450 are the main group of enzymes that catalyze the oxidative biotransformation of the drugs and other lipophilic xenobiotics.[27] The enzymes involved in the drugs metabolism are CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4.[26] The enzymes that are influenced by soy isoflavone are discussed below based on the pharmacokinetic interaction mechanism of some drugs (Table 4). To support the discussion, we have summarized the inhibitory effect of soy isoflavones on CYP enzymes in Table 5.

CYP2C9

Pharmacokinetic interactions may occur through the inhibition or induction of DMEs. Around 15% of all drug biotransformation is metabolized by the CYP2C9.[78] An interaction between celecoxib and genistein has been reported;[65] as shown in Table 4, there is an increase in C_{max} and AUC is almost 2.7 times higher than celecoxib alone, because of the inhibition of the CYP2C9 enzyme by genistein. Thus, the metabolism of celecoxib is reduced, clearance also decreases, and celecoxib accumulates in the body. This mechanism is also in line with the results of Kopecna-Zapletalova et al.[77] based on *in vitro* studies (Table 5) showing that genistein may inhibit CYP2C9 at doses of 35.96 mmol/L and 100 μM.[65] The flavone structure of genistein (4,5,5,7-trihydroxyisoflavone) may suppress CYP2C9 by interacting with the active site of CYP2C9.[79]

CYP1A2

The same effect was also seen by Peng et al.[66] when theophylline was given with soy isoflavones such as daidzein at a dose of 200 mg twice a day to healthy volunteers. There was an increase in the AUC and C_{max}. Theophylline is mainly excreted through the hepatic metabolism pathway, and CYP1A2 catalyzes all these pathways; thus, the inhibition of CYP1A2 will inhibit the metabolism of this drug. This is also related to the

Table 3. Pharmacokinetics of isoflavones after oral administration in humans

Sample	Isoflavones	C_{max}	t_{max}	$t_{1/2}$	AUC	Vd/F	Cl/F	References
Soy milk	Daidzein	2.19 μmol/L.mg dose	6.1 h	8 h	22.09 μmol.h/L mg dose	1.53 L/kg	8.47 L/h	59
Tempeh	Daidzein	2.33 μmol/L.mg dose	8.4 h	9.4 h	15.28 μmol.h/L mg dose	2.07 L/kg	9.86 L/h	59
Soy beverage	Daidzein	96.31 ng/mL	5.92 h	7.68 h	11.50 ng.h/mL	-	-	44
Soy extract capsule	Daidzein	96.02 ng/mL	6.25 h	6.67 h	1211.93 ng.h/mL	-	-	44
Soy isoflavones (isogen)	Daidzein	230 ng/mL	3.78 h	9.75 h	2629 ng.h/mL	211.4 L	12.2 L/h	46
Fermented soybean	Daidzein	214 ng/mL	2.88 h	9.54 h	2594 ng.h/mL	295.4 L	12.9 L/h	46
Soymilk	Daidzein	211.2 ng/mL	3.71 h	5.92 h	2101 ng.h/mL	131.4 L	19 L/h	46
Soymilk	Genistein	4.07 μmol/L.mg dose	5.6 h	9.9 h	50.01 μmol.h/L mg dose	0.72 L/kg	3.31 L/h	59
Soymilk	Genistein	231.1 ng/mL	4.86 h	5.64 h	2326 ng.h/mL	104 L	13.5 L/h	46
Tempeh	Genistein	2.35 μmol/L.mg dose	7.2 h	9.4 h	32.28 μmol.h/L mg dose	1.12 L/kg	6.58 L/h	59
Soy beverage	Genistein	116.37 ng/mL	5.75 h	7.61 h	1437.23 ng. h/mL	-	-	44
Soy extract capsule	Genistein	261.84 ng/mL	7 h	7.96 h	3259.54 ng. h/mL	-	-	44
Soy isoflavones (isogen)	Genistein	160 ng/mL	4.67 h	8.53 h	2356 ng.h/mL	226 L	15.1 L/h	46
Fermented soybean	Genistein	195.7 ng/mL	3.5 h	8.22 h	2279 ng.h/mL	347 L	17.4 L/h	46

C_{max}: Maximum concentration, t_{max}: Time-to-maximum, $t_{1/2}$: Half-life, AUC: Area under the curve, Vd: Volume of distribution

Table 4. Interaction of soy isoflavones with drugs based on pharmacokinetic parameters

Sample	Pharmacokinetic parameters					Significant effect	Mechanism	Method	Route of administration	Reference
	C_{max}	t_{max}	$t_{1/2}$	AUC	Clz/F					
Celecoxib	1380 µg/L	2.6 h	4.34 h	11455 µg/L.h	3.49 L/kg.h	Increased C_{max} and AUC, decreased Clz/F	Inhibitor of CYP2C9	Rats	Oral	65
Celecoxib + genistein 100 mg/kg	3756.71 µg/L	3.4 h	2.93 h	30835.89 µg/L. h	1.64 L/kg.h					
Theophylline	1.33 µg/ mL	2.77 h	9.88 h	18.52 µg h/mL	-	Increased C_{max}, AUC, $t_{1/2}$	Inhibitor of CYP1A2	Human	Oral	66
Theophylline + daidzein	1.63 µg/mL	2.65 h	12.01 h	24.41 µg h/mL	-					
Midazolam	48.86 ng/mL	0.83 h	2.01 h	209.18 ng.h/mL	1.68 L/h	Decreased C_{max}, AUC, increased Cl/F	Inducer of CYP3A4	Human	Oral	67
Midazolam + genistein tablet 1000 mg	36.25 ng /mL	1.13 h	1.67 h	180.59 ng.h/mL	3.98L/h					
Imatinib	14511 mg/L	2.6 h	2.89 h	109010 mg.h/L	300.125 L/kg	Decreased C_{max} and AUC	Inducer of CYP3A4	Rats	Oral	68
Imatinib + genistein 50 mg/kg	10810 mg/L	2.8 h	2.29 h	79070 mg.h/L	406.776 L/kg					
Carbamazepine	634 ng/mL	1.83 h	7.95 h	6087.77 ng/L.h	0.7791 L/h	Decreased C_{max}, AUC, t_{max}, increase Cl/F	Inducer of CYP3A4	Rats	Oral	69
Carbamazepine + soybean	320.16 ng/mL	1 h	6.69 h	1928 ng/L.h	0.9086 L/h					
Paclitaxel	36.8 ng/mL	1 h	14.7 h	702 ng.h/mL	712 mL/min.kg	Increased C_{max}, AUC, decreased Cl/F	Inhibitor of CYP3A4 and inhibitor of P-gp	Rats	Oral	33
Paclitaxel + genistein 10 mg/kg	70.6 ng/mL	0.5 h	16.2 h	1086 ng.h/mL	461 mL/min.kg					
Repaglinide	70.8 ng/mL	0.7 h	1.13 h	134.89 ng.h/mL	3.06 L/kg.h	Increased C_{max} and AUC	Inhibitor of P-gp	Rats	Oral	70
Repaglinide + genistein 10 mg/kg	124.71 ng/mL	0.75 h	1.39 h	245.71 ng.h/mL	2.23 L/kg.h					
Omeprazole	2007.33 ng/mL	0.5 h	1.31 h	1586.25 ng/L.h	0.156 L/h	Increased C_{max} and AUC, decreased Clz /F	Inhibitor of Pg-p	Rats	Oral	69
Omeprazole + soybean	3242.33 ng/ mL	0.5 h	2.21 h	7115.83 ng/L.h	0.134 L/h					
Danofloxacin	2.72 µg/mL	4.5 h	-	9.58 µg.h/mL	-	Decreased C_{max} and AUC	Inhibitor of BCRP	Sheep	Oral	71
Danofloxacin + soy diet	1.16 µg/mL	2.6 h	-	4.9 µg.h/mL	-					
Valproic acid	216.94 µg/mL	0.08 h	3.95 h	656.579 µg.h/mL	88.02 mL /h.kg	Decreased C_{max}, AUC, increased Cl/F, $t_{1/2}$	Inducer of UGT	Rats	Intravenous	72
Valproic acid + soy 500 mg	143.64 µg/mL	0.08 h	4.98 h	456.491 µg.h/mL	118.97 mL/h.kg					

C_{max}: Maximum concentration, t_{max}: Time-to-maximum, $t_{1/2}$: Half-life, AUC: Area under the curve, CYP: Cytochrome, P-gp: P-glycoprotein

results of Anderson et al.[76] who showed that soybean extract inhibits the CYP1A2 enzyme *in vitro*; one of the isoflavones contained in soy extract is daidzein.

CYP3A4

Inhibition of CYP3A4 will increase the drug levels, as shown by Li and Choi.[33] *In vivo*, genistein may increase the value of AUC and C_{max} of paclitaxel through the inhibition of CYP3A4; this is also supported by *in vitro* studies. It has been widely reported that genistein from soybean inhibits CYP3A4.[28,76,77] *In vitro* studies have reported that genistein inhibits CYP3A4 at a concentration of 0.5 mM/well, supported by Kopecna-Zapletalova et al.[77] from 2016 showing that genistein may inhibit CYP3A4 at a concentration of 23.25 mM. Another trial using different cells, namely V_{79} cells, showed the inhibitory activity of genistein on CYP3A4.[75] The inhibitory effect of isoflavones on CYP3A4 is classified as moderate inhibition and is non-competitive.[77]

In addition to the inhibitory effect described above, some studies show that the mechanism of isoflavones also may alter the pharmacokinetics of drugs by the induction of enzymes. This may decrease the AUC and C_{max} and increase clearance. Studies by Xiao et al.[67] showed there is a change in the value of midazolam pharmacokinetic parameters after the patients were given genistein tablets (1000 mg) for 14 days; the same thing was also found for imatinib[80] and carbamazepine.[69] Midazolam and imatinib are primarily metabolized by CYP3A4 after oral administration.[67] Imatinib is metabolized into N-desmethyl imatinib by CYP3A4[80,81] and is a prodrug. This means that there is a different mechanism for the prodrug. Prodrugs are activated by a CYP, so it is important to know if metabolism or the activation of enzymes may alter CYP activity.[82] Genistein increases the C_{max} and AUC of N-desmethyl imatinib by the induction of CYP3A4. In the future, to clarify the mechanism, it will be necessary to carry out a deeper investigation related to the effect of soy isoflavones on prodrugs.

Induction of xenobiotic-mediated *CYP3A* genes in humans is known to be regulated by pregnane X receptors (PXR), constitutive and immune receptors, glucocorticoid receptors, and other receptors.[83] PXR is the main regulator of xenobiotic-induced *CYP3A* gene expression. Previous research has found that genistein may significantly activate the human PXR, and induce the human CYP3A4 luciferase reporter activity.[84] According to this study, we consider that genistein acts as an inducer of CYP3A4 in humans. However, the $_{CYP3A4}$ induction mechanism is contrary to *in-vitro* studies (Table 4) because many studies report that soy, and its isoflavones have an inhibitory effect rather than induction, so there is no *in vivo*/*in vitro* correlation related to the effect of soybean on CYP3A4.

According to Cheng et al.[48] soybean contains 42 µg/g genistein and 4.78 µg/g daidzein, while some have reported extracts containing and 1260 µg/g genistein and 849 µg/g daidzein,[41] or 36.55 µg/g genistein and 88.87 µg/g daidzein.[40] These variations in the content may be caused by the differences in the soybean variety assessed, the location of growth, plant age, etc. Other than that, processed soybean foods such as tofu, tempeh, soy milk, natto, miso, and sufu also have variable contents, which could be seen in Table 3. For example, fried

Table 5. Summary of the inhibitory effects of soy isoflavones on CYP enzymes

Sample	Method	CYP450	References
Standardized soybean extract containing 37% isoflavones	*In vivo* (rat)	CYP3A1 (homologue to human CYP3A4)	73
Soybean 100 mg/kg	*In vivo* (rat)	CYP3A1 (homologue to human CYP3A4) CYP2D2 (homologue to human CYP2D6)	74
Soy 129 mg/day	*In vivo* (monkey)	CYP3A4	75
Soybean powder 375 µg/mL	*In vitro*	CYP3A4	28
Genistein 0.5 mM/well	*In vitro*	CYP3A4	28
Isoflavones	*In vitro* (V79 cells)	CYP3A4	75
Soy extract 12.2 µg/mL	*In vitro*	CYP3A4	76
Genistein 23.25 mmol/L	*In vitro*	CYP3A4	77
Genistein 35.95 mmol/L	*In vitro*	CYP2C9	77
Daidzein 60.56 mmol/L	*In vitro*	CYP2C9	77
Soy extract 2.6 µg/mL	*In vitro*	CYP2C9	76
Genistein 100 µM	*In vitro*	CYP2C9	65
Genistein 62.73 mmol/L	*In vitro*	CYP2C19	77
Genistein 20.97±1.27 mmol/L	*In vitro*	CYP2C8	77
Soy extract 23.6 µg/mL	*In vitro*	CYP1A2	76

CYP450: Cytochrome P450

tempeh contains 310 µg/g genistein and 350 µg/g daidzein.[60] In natto, the level of genistein is 224 µg/g and that of daidzein is 411 µg/g[57], but soymilk has a lower content of 56 µg/mL and 52 µg/mL, respectively.[46] When linked with experimental data *in vitro* from the various studies, it appears that soybean extract may inhibit the CYP3A4 enzyme at a concentration of 12.2 µg/mL, CYP2C9 at 2.6 µg/mL, and CYP1A2 at 23.6 µg/mL.[76] This means that consuming 1 gram of soybean extract may influence these enzymes. The same thing is also the case with soybeans and soybean products, because the content of genistein and daidzein (shown in Table 3) in each gram exceeds the inhibitory dose reported by Kopecna-Zapletalova et al.[77] However, further *in vivo* studies in human subjects need to be performed, as *in vivo* studies have only been conducted on mice with soybean doses that inhibit CYP3A1 (the homologue to CYP3A4 in humans), (i.e., 100 mg/kg).[74] If simplified, the dose is equivalent to 100 µg/g, so based on this the consumption of soymilk, tofu, soybeans could be said to be safe, but again further research is needed to obtain more accurate results.

Phase-II metabolism enzymes

Uridine diphosphate glucuronosyltransferases

Soybean increases the phase-II metabolism of drugs to increase the detoxification and clearance of potentially carcinogenic intermediaries. The results of Marahatta et al.[72] report that the administration of 500 mg for 5 days could affect valproic acid (VPA) in terms of its pharmacokinetic parameters. Specifically, the C_{max} decreased by 65%, but time-to-maximum (t_{max}) was not significantly different. AUC decreased by 69%. There were significant differences in C_{max}, $t_{1/2}$, AUC, and clearance between the treatment and control groups.[72] Soybean contributes to VPA excretion, which is very effective as it increases VPA glucuronidation. Valproate glucuronide is the main metabolite of VPA in urine and is metabolized by UGT1A3, UGT1A4, UGT1A6, UGT1A8, UGT1A9, UGT1A10, UGT2B7, and UGT2B15. The metabolism and elimination of VPA is affected by glucuronidation, especially by uridine 59-diphosphate-glucuronosyltransferase. Similarly, previous studies have shown that soy induces the UGT enzyme, an important component of glucuronidation.[85] Daidzein may stimulate glucuronidation.[86] Similarly, genistein has been reported to induce UGT activity.[87] The inhibition or induction of important enzymes for drugs that require therapeutic drug monitoring and food-drug interactions depend on the therapeutic index of each drug.[72]

Effects of soy ısoflavones on drug transporters

Drug transporters have an important role in the ADME of drugs and xenobiotics.[88] Drug transporters are also related to disposition of drug and drug interactions.[89] Drug transporters are classified as uptake and efflux transporters. Uptake transporters play a role in facilitating the translocation of drugs into cells such as organic anion transporting polypeptides (OATP; SLCO)[90], organic anion transporters (OAT; SLC22A)[91], and organic cation transporters (OCT; SLC22A)[92], while efflux transporters transfer or remove drugs from the intracell to the extra cell, for example the ABC group and SLC transporters. The ABC family includes transporters for the elimination of drugs likes P-gp [multidrug resistance protein 1 (MDR1); ABCB1], certain members of the multidrug resistance-associated protein (MRP; ABCC) family, and breast cancer resistance protein [(BCRP); ABCG]. These drug transporters are expressed in the intestine or liver, two main locations that affect how much drug will enter the body after the administration of an oral dose. Thus, the effect of isoflavones on drug transporters is important because it will affect the pharmacokinetic profile of a drug.[93] As shown in Table 4, the pharmacokinetic interaction mechanisms of some drugs occur only through efflux transporters.

Efflux drug transporters

P-glycoprotein

P-gp is a product of the *MDR1* gene, which is an efflux transporter that is widely studied and known for its ability to limit the entry of drugs into various organ compartments. P-gp functions as an efflux pump, such that it facilitates the transfer of intracellular drugs to the extracellular space.[26] Genistein may influence the administration of the drugs by modulating efflux proteins such as MDR1 and P-gp. P-gp is expressed mainly in the apical membrane of the intestine. MDR1 has been reported to increase the elimination of drugs in the intestinal lumen.[34] This mechanism is shown in Figure 2. Genistein inhibits P-gp and causes pharmacokinetic interactions with repaglinide at a genistein concentration of 10 mg/kg, characterized by an increase in the repaglinide AUC of 53% and C_{max} by 36%.[70] Genistein affects P-gp by increasing intestinal absorption. Li and Choi.[33] found an increase in the paclitaxel plasma concentration with a mechanism of P-gp inhibition, similar to what was also found with midazolam.[69] To confirm, Li et al.[54] tested Caco-2 cells and IEC-6 cells to investigate further repaglinide absorption in human cells and in mice, resulting in significantly increased intracellular repaglinide accumulation with genistein administration.[70] This means that P-gp transporters, which are supposed to carry drugs to the extracellular are blocked by genistein, resulting in the intracellular accumulation of repaglinide.

The mechanism by which genistein inhibits P-gp was revealed by molecular docking studies. The basic structure of P-gp includes four main core regions, with two nucleotide-binding

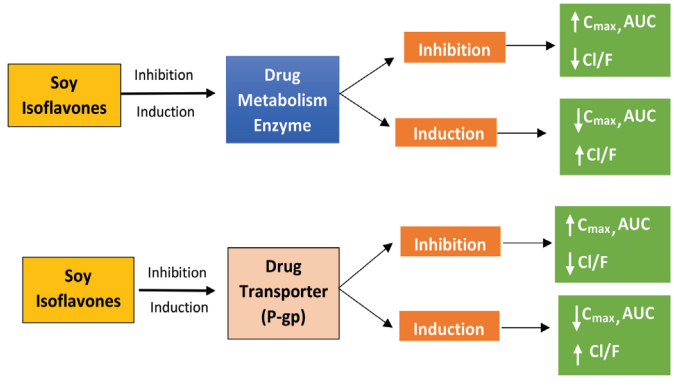

Figure 2. Mechanisms of pharmacokinetic interactions between drugs and soy isoflavones (based on Table 4)

C_{max}: Maximum concentration, AUC: Area under the curve, P-gp: P-glycoprotein, CL/F: Oral clearance

domains (NBD) located in the cytoplasm and two hydrophobic transmembrane domains (TMD).[94] The TMD serve as a channel to facilitate drug transport, whereas the NBD located in the cytoplasm have binding sites for ATP, used as the energy supply for drug transport.[95] 6C0V was chosen as a P-gp molecule with a three-dimensional structure combined with NBD simulation; it was found that genistein has a certain binding affinity for NBD and shares several binding sites with ATP in the corresponding functional area, which affects the energy supply when the drug is transported by P-gp. This is what causes the inhibition of the efflux function of P-gp.[70]

BCRP

Drug interactions that lead to the inhibition of efflux transporters can cause changes in the pharmacokinetics of the drug. For example, in the case of BCRP, several drugs are secreted into milk, such as danofloxacin as shown in a study performed in sheep given a soy diet to see its effect on drug levels in milk. A change was observed in the pharmacokinetic parameters of danofloxacin, namely a 50% decrease in C_{max} and AUC.[71] *BCRP* inhibitors administered with drugs that are substrates of the transporter could have effects on *in vivo* ADME, as well as the presence of drugs in milk.[96,97] A soy diet contains daidzein and genistein, which are BCRP inhibitors.[98,99]

From what has been discussed above, we could see that the pharmacokinetic interaction of soy isoflavones with drugs occurs through the several mechanisms, (i.e., through DMEs or drug transporters). These interactions will affect the bioavailability of drugs in the blood. The mechanisms are summarized and illustrated in Figure 2.

CONCLUSION

Soybeans are a good source of isoflavones. The isoflavone content of soybean is mainly in the aglycone form as daidzein, genistein, and glycitein. Soybean products also contain variable levels of isoflavones. Co-administration of soy isoflavones with the drugs may cause pharmacokinetic interactions. These interactions may cause changes in the AUC, C_{max}, t_{max}, and $t_{1/2}$ of the drugs. These interactions occur through mechanisms related to the inhibition/induction of DMEs, namely CYP3A4, CYP2C9, CYP1A2, and UGT or the inhibition/induction of drug transporters, such as P-gp and BCRP. Thus, the consumption of soy, soy isoflavones, or soy products together with the drugs needs to be considered because this diet may affect the efficacy of the drugs. Furthermore, the timing and consumption of soy isoflavones with the drugs should be monitored.

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