REVİEW
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ISOFLAVONES IN SOYBEAN AS A DAILY NUTRIENT: THE MECHANISM OF ACTION AND HOW THEY ALTER THE PHARMACOKINETICS OF DRUGS

GÜNLÜK BESLENME OLARAK SOYA'DA ISOFLAVONLAR: EYLEM MEKANİZMASI VE İLAÇLARIN FARMAKOKİNETİĞİNİ NASIL DEĞİŞTİRİR

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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 and, because of its pharmacological activity, soy isoflavone intake over a long period of time can result in interactions with drugs. This review summarises soy isoflavone-drug interactions based on pharmacokinetic parameters. Soy isoflavones have been reported to have pharmacokinetic interactions with celecoxib, theophylline, paclitaxel, midazolam, imatinib, carbamazepine, valproic acid, repaglinide, omeprazole and danofloxacin. This is due to changes in the AUC, C_{max}, t_{max}, Cl and $t_{1/2}$ of drugs when delivered together with soy isoflavones. This mechanism of pharmacokinetic interaction occurs through the inhibition/induction of drug metabolizing enzymes such as CYP3A4, CYP2A1 and CYP2C9, or through the inhibition of drug transporters such as P-gp and BCRP. Thus, the consumption of soybean, soy isoflavones or soy products with drugs needs to be reconsidered.
Keyword: Soybean, Isoflavones, Pharmacokinetic Interaction, Drug Metabolizing Enzyme, Drug Transporter
Öz
Soya fasulyesi (Glycine max (L.) iyi bir izoflavon kaynağıdır.Soyanın ana izoflavon bileşenleri daidzein, genistein ve glisitindir.Dünya soya fasulyesi üretimi çok yüksektir ve farmakolojik aktivitesi nedeniyle uzun süre soya izoflavon alımı zaman ilaçlarla etkileşimlere neden olabilir. Bu derleme soya izoflavon-ilaç etkileşimlerini farmakokinetik parametrelere göre özetlemektedir. Soya izoflavonlarının selekoksib, teofilin, paklitaksel, midazolam,

imatinib, karbamazepin, valproik asit, repaglinid, omeprazol ile farmakokinetik etkileşimleri olduğu bildirilmiştir. Danofloksasin Bu, soya izoflavonları ile birlikte verildiğinde ilaçların EAA, Cmax, tmax, Cl ve t1 / 2 değerlerinde meydana gelen değişikliklerden kaynaklanmaktadır. Bu farmakokinetik etkileşim mekanizması, CYP3A4, CYP2A1 ve CYP2C9 veya P-gp ve BCRP gibi ilaç taşıyıcılarının inhibisyonu yoluyla. Böylece soya fasulyesi, soya izoflavonları veya Uyuşturuculu soya ürünlerinin yeniden değerlendirilmesi gerekiyor.
Anahtar Kelime: Soya fasulyesi, İzoflavonlar, Farmakokinetik Etkileşim, İlaç Metabolize Edici Enzim, İlaç Taşıyıcı

Background

Soybeans (*Glycine max* (L.) are a source of isoflavones in daily meals. In 2016, global soybean production amounted to 334,894,085 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, 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 can have a similar effect to that of oestrogen.[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 that 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 cancer cell proliferation.[14]

Based on this pharmacological activity, soy isoflavones can be used as dietary nutrition over a long period of time. Soybean consumption continued to increase in 2011.[15] Fonsesca in 2014[16] showed that the amount of isoflavones taken in by 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 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 fulfil 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 show different results where the consumption of isoflavones per day can 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 can occur with soy isoflavones. Soy extracts, soy products, and soy isoflavones have interactions with 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 or drug transporters.[25]

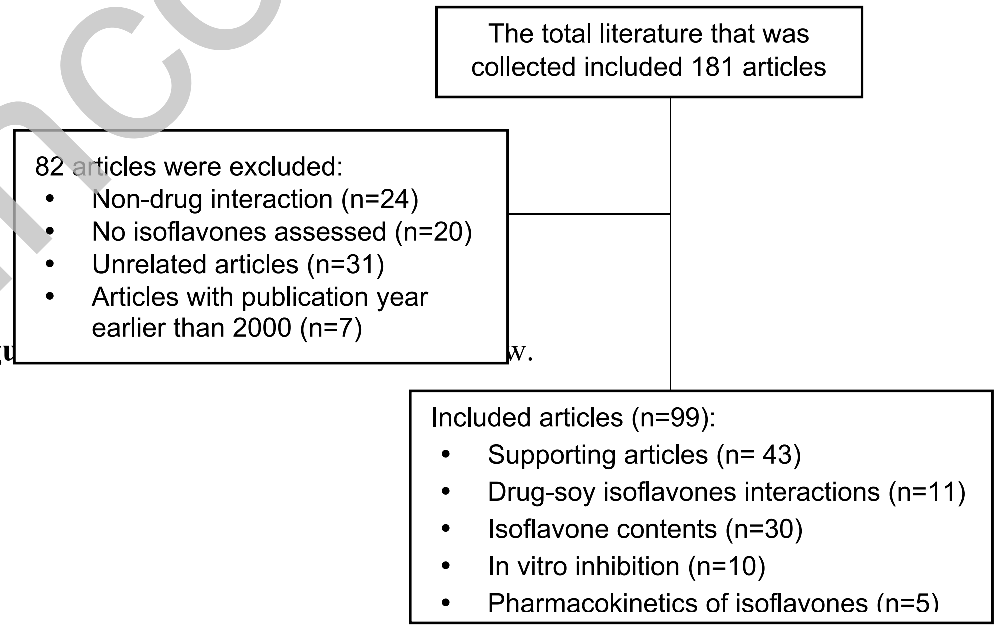
Almost all drug biotransformation reactions need a metabolic enzyme, and the enzymes most often used to process drugs are the liver microsomal cytochrome P450 (CYP) enzymes. The CYP enzymes involved in drug metabolism are CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5.[26] Drugs or bioactive compounds such 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 can also be involved in drug interactions, because drug transporters mediate the absorption, distribution, and excretion of 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 can be induced by various factors, including clinical drugs, environmental xenobiotics, and dietary compounds;[31] it is known to be involved in 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 can be inhibited by genistein at some doses[34] In addition to P-gp, interactions can occur through other drug transporters. So, drug metabolizing enzymes and drug transporters play important roles in the absorption, distribution, metabolism, and excretion (ADME) of drugs and are involved in interactions that will affect the pharmacokinetics and pharmacodynamics of drugs.

These pharmacokinetic interactions can be seen by assessing pharmacokinetic parameters including the area under the curve (AUC), C_{max}, volume of distribution (Vd), $t_{1/2}$, and clearance. Nagashima[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 can 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 drugs.

Methods

This review is based on 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 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.



Figu w.

Soy Isoflavones

Isoflavones are bioactive metabolites and include a group of phytoestrogens. Isoflavones have structures similar to those of mammalian oestrogens. 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 (genistin, 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 summarise the content of genistein, daidzein, and glycitein in soybeans, as seen in Table 1. 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 variety**,** location of 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]

Table 1. Summary of isoflavone contents in soybean

Sample	Genistein	Daidzein	Glycitein	Genistin	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

In each type of soybean product containing different soy isoflavones, we summarise the isoflavone content focusing only on the aglycone form, i.e. genistein, daidzein, and glycitein in various soy products from several studies, presented in Table 2. 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 the 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 several factors such as time and temperature.[53,54]

Table 2. Isoflavone contents of soybean products

Sample	Genistein	Daidzein	Glycitein	Reference

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

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 age, the hydrolysis process of glycosides by 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[63] found that urinary recovery of equol from tempeh is higher than from soymilk, although the solid food matrix and fermentation may increase the production of equol. Equol is a metabolite of daidzein produced by intestinal bacteria; the level of equol production has been linked to consumption and the content of the isoflavone daidzein. The solid food matrix of tempeh may protect isoflavones from degradation so they can reach the large intestine and be metabolised into equol by gut bacteria. This indicates that tempeh contains more daidzein than soymilk. Information about pharmacokinetics is very important to evaluate safety and understand efficacy. For example, from the $t_{1/2}$, we can predict how long isoflavones are still present in the body so that its consumption time can be regulated by medication.

It turns out that not only are isoflavone tablets high in isoflavones, but daily food processed from soy also contains quite high levels of isoflavones, and also can 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.

Table 3. Pharmacokinetics of isoflavones after oral administration in humans

Sample	Isoflavones	C_{max}	t_{max}	$t_{1/2}$	AUC	Vd/F	Cl/F	Reference
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]

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 drugs. This simultaneous use can lead to drug-isoflavone interactions. This is supported by Laurenzana,[64] who found that the content of natural materials such as flavones, isoflavones, and tangeretin affect the activity of human CYP enzymes when given orally together with drugs. These changes in ADME will certainly affect the pharmacokinetic parameters of drugs, because of interactions with drug metabolizing enzymes and interaction with drug transporters.

In this article, the pharmacokinetic interactions between isoflavones and some drugs and their mechanisms of interaction have been summarised, focusing on 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 changes in the AUC, C_{max}, and clearance. These changes can be either an increase or a decrease in pharmacokinetic parameters, depending on the mechanism. The mechanisms that will be discussed here involve enzymes and drug transporters.

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

Sample	Pharmacokinetic parameters					Significant effect	Mechanism	Method	Route of administration	Reference
	Cmax	tmax	$t_{1/2}$	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					

Effects of Soy Isoflavones on Drug Metabolizing Enzymes

Soybeans influence the metabolism of drugs and affect ADME through interactions with phase I or phase II drug metabolizing enzymes (DMEs). The enzymes involved in phase I metabolism are the cytochrome P450 (CYP) families, while the enzymes involved in phase II metabolism are sulfotransferases (SULTs), uridine diphosphate glucuronosyltransferases (UDPGT/UGTs), N-acetyl transferases (UDPGT/UGTs), glutathione-S-transferases (GSTs), and methyltransferases.[25]

Phase I Metabolism Enzymes

Cytochromes P450 are the main group of enzymes that catalyse the oxidative biotransformation of drugs and other lipophilic xenobiotics.[27] The enzymes involved in 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 summarised the inhibitory effect of soy isoflavones on CYP enzymes in Table 5.

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

Sample	Method	CYP	Reference
Standardised 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

CYP2C9

Pharmacokinetic interactions can occur through the inhibition or induction of drug metabolizing enzymes. Around 15% of all drug biotransformation is metabolised by 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 Zapletalova[77] based on in vitro studies (Table 5) showing that genistein can inhibit CYP2C9 at doses of 35.96 mmol/L and 100 µM.[65] The flavone structure of genistein (4,5,5,7-trihydroxyisoflavone) can suppress CYP2C9 by interacting with the active site of CYP2C9[79].

CYP1A2

The same effect was also seen by Peng[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 also C_{max}. Theophylline is mainly excreted through the hepatic metabolism pathway, and CYP1A2 catalyses all of these pathways; thus, the inhibition of CYP1A2 will inhibit the metabolism of this drug. This is also related to the results of Anderson[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 drug levels, as shown by Li et al.[33] In vivo, genistein can 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 Zapletalova's studies[77] from 2016 showing that genistein can inhibit CYP3A4 at a concentration of 23.25 mM. Another trial using different cells, namely V79 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 can alter the pharmacokinetics of drugs by the induction of enzymes. This can decrease AUC and C_{max}, and increase clearance. Studies by Xiao showed there is a change in the value of midazolam pharmacokinetic parameters after patients were given genistein tablets (1000 mg) for 14 days;[67] the same thing was also found for imatinib[80] and carbamazepine.[69] Midazolam and imatinib are primarily metabolised by CYP3A4 after oral administration.[67] Imatinib is metabolised into N-desmethyl imatinib by CYP3A4[81,80] 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 can 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 (CAR), glucocorticoid receptors (GR), and other receptors.[83] PXR is the main regulator of xenobiotic-induced CYP3A gene expression. Previous research has found that genistein can significantly activate human PXR and induce 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., 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 can be caused by 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 can be seen in Table 3. For example, fried 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 various studies, it appears that soybean extract can 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 can 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 Zapletalova.[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 can be said to be safe, but again further research is needed to obtain more accurate results.

Phase II Metabolism Enzymes

Uridine Diphosphate Glucuronosyltransferases (UDPGT/UGTs)

Soybean increases the Phase II metabolism of drugs to increase the detoxification and clearance of potentially carcinogenic intermediaries. The results of Marahatta[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 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 valproic acid in urine and is metabolised by UGT1A3, UGT1A4, UGT1A6, UGT1A8, UGT1A9, UGT1A10, UGT2B7, and UGT2B15. The metabolism and elimination of valproic acid 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 can 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 (TDM) and food-drug interactions depend on the therapeutic index of each drug.[72]

Effects of Soy Isoflavones 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 extracell, for example the ATP-binding cassette (ABC) group and solute carrier (SLC) transporters. The ABC family includes transporters for the elimination of drugs likes P-glycoprotein (P-gp) (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)

P-gp is a product of the multi drug resistance protein 1 (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 can influence the administration of 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, characterised by an increase in the repaglinide AUC of 53% and C_{max} by 36%[70]. Genistein affects P-gp by increasing intestinal absorption. Li et al.[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, Jin 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 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]

Breast Cancer Resistance Protein (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 can have effects on in vivo absorption, distribution, and excretion, 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 can see that the pharmacokinetic interaction of soy isoflavones with drugs occurs through several mechanisms, i.e. through drug metabolizing enzymes or drug transporters. These interactions will affect the bioavailability of drugs in the blood. The mechanisms are summarised and illustrated in Figure 2.

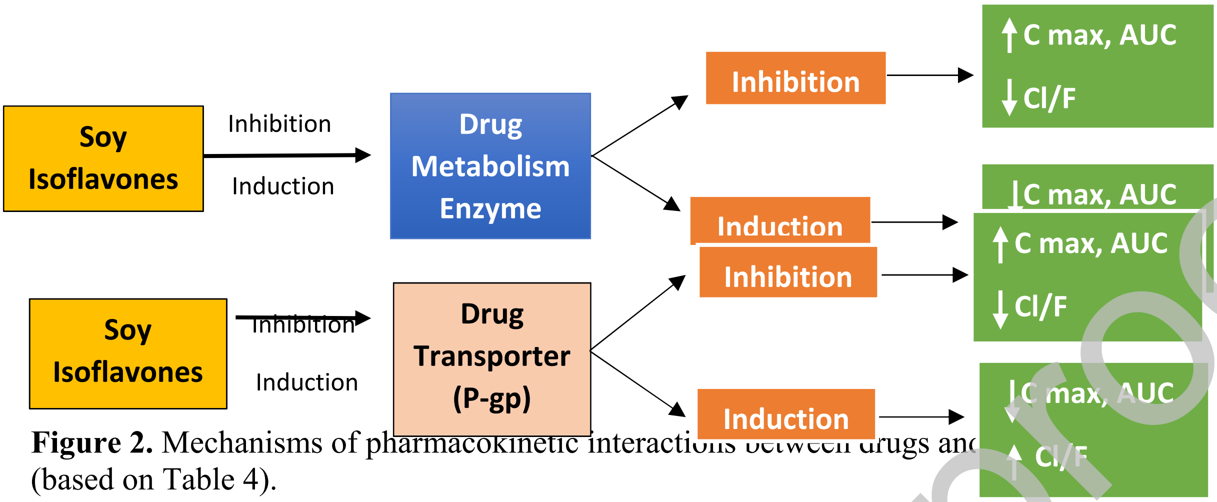


Figure 2. Mechanisms of pharmacokinetic interactions between drugs and
(based on Table 4).

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 drugs can cause pharmacokinetic interactions. These interactions can cause changes in the AUC, C_{max}, t_{max}, and $t_{1/2}$ of drugs. These interactions occur through mechanisms related to the inhibition/induction of drug metabolizing enzymes, 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 drugs needs to be considered because this diet can affect the efficacy of drugs. Furthermore, the timing and consumption of soy isoflavones with drugs should be monitored.

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