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Is it possible to change milk secretion of drugs with soy enriched diets in lactating ruminants?

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ABSTRACT

Soy is the most commonly used protein supplement in beef and dairy diets. Soy, which is also used as a common protein source in animal feed, is palatable and has a good amino acid balance and high bioavailability. In vivo and in vitro interaction of flavonoids, including isoflavones such as genistein and daidzein, with several ABC transporters, including breast cancer resistance protein (BCRP/ABCG2), has been demonstrated. BCRP presence in ruminants could affect the efflux of hydrophobic toxins and drugs, including their active secretion to milk and a reduction in the withdrawal time of the drug milk residues. As a result of inhibition of efflux transporters such as BCRP, changes in drug pharmacokinetics and drug transfer into milk have been observed. In this respect, the use of forage supplemented with BCRP inhibitors may be beneficial to control drug accumulation in milk and prevent undesirable contamination of milk. It is aimed to reduce the drug withdrawal periods for dairy animals with the procedure in question. In this review, it is aimed to give information about the importance of soy-enriched diets in the nutrition of ruminants during the lactation period and the effect of transport proteins on the transfer of drugs into milk.

Keywords: BCRP/ABCG2, pharmacokinetics, soy, withdrawal time, ruminant

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Introduction

Milk secretion of drugs and xenotoxins

The main mechanisms that ensure the milk secretion of drugs are; passive diffusion, active transport by ABC (ATP-binding cassette) transport proteins, and ion trapping. Other examples of mechanisms include facilitated diffusion, pinocytosis and exocytosis (Ito and Alcorn, 2003).

Drug excretion into milk varies depending on many factors such as lipophilicity, molecular weight, plasma protein binding rate, and ionization (Agatonovic-Kustrin et al., 2002; Ito and Lee, 2003).

Drug residues that are released due to the accumulation of drugs used for the treatment of diseases or to increase animal production or their metabolites in products such as animal tissues or milk pose risks to food safety and public health (Behnke et al., 2008). Since residue levels in milk are harmful to health, it is necessary to pay attention to drug withdrawal times (Alvarez et al., 2006). Maximum residue limit (MRL) values have been determined for many drugs by the European Union in order to eliminate possible public health problems that residues may cause (EMA, 1999).

Breast cancer resistance protein (BCRP/ABCG2), which is in the family of ABC transport proteins, is one of the main factors in the transfer of drugs into milk, which leads to the presence of undesirable products in milk; causes exposure of suckling pups and consumers

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to xenotoxins (Jonker et al., 2005; van Herwaarden and Schinkel, 2006; Schrickx and Fink-Gremmels, 2008).

BCRP affects the bioavailability of many compounds and causes drug-drug interactions by enabling the excretion of many toxic elements from the cells in the intestine, liver and other organs (Szakacs et al., 2006). BCRP/BCRP1 is located in the apical membrane of epithelial cells of the small and large intestines, the bile canalicular membrane of hepatocytes, the apical membrane of renal proximal tubular epithelial cells, the luminal membrane of endothelial cells of capillaries in the brain, the syncytiophoblasts of the placenta, and the apical of mammary alveolar epithelial cells, which is the main area of milk production (van Herwaarden and Schinkel, 2006).

BCRP is highly expressed in the mammary tissues of cows and sheep and the apical membrane of the alveolar epithelial cells that form the blood-milk barrier during lactation (Pulido et al., 2006). The blood -milk barrier consists of mammary alveolar epithelial cells, with tight junctions with the apical side facing the milk and the basolateral side facing the mammary gland capillaries (Zhao and Lacasse, 2008). BCRP/ ABCG2 provides active transport of various drugs and toxins to milk by crossing the blood-milk barrier (Jonker et al., 2005). BCRP plays an important role in the active transport of some drugs and carcinogenic xenotoxins (cimetidine, nitrofurantoin, topotecan, acyclovir, PhIP) into milk (Jonker et al., 2005; Merino et al., 2005). Lactation period affects BCRP expression, and BCRP in the mammary gland of lactating animals is higher than in non-lactating animals (Jonker et al., 2005). Although BCRP has been shown to have an increased expression in lactating mice, cows and humans, and play an important role in the transfer of substrate drugs into milk, ABC transport proteins similar in structure to BCRP, such as MRP1, MRP2 and P-gp, are not significantly expressed in lactating mice (Jonker et al., 2005). These results support the view that BCRP substrate drugs have a higher rate of excretion into milk (Jonker et al., 2005). In another similar study, immunoblot and Western blot experiments showed high BCRP expression in the mammary glands of sheep and cows. The results obtained by the researchers on the vanadate-sensitive ATPase activity of BCRP-specific substrates and inhibitors in cow mammary gland homogenates confirmed the functional expression of BCRP in cows and sheep (Pulido et al., 2006).

The role of BCRP in the excretion of drugs used in veterinary medicine

BCRP is critical to drug pharmacokinetics and safety in veterinary field. BCRP has been shown to mediate the excretion of some substrate drugs, such as enrofloxacin and nitrofurantoin, into milk in sheep. In addition to numerous studies on potential drug interactions mediated by P-glycoprotein (P-gp) in ruminants, BCRP-mediated interactions in the mammary gland are also gaining importance. In these studies, antiparasitic drugs such as monepantel, triclabendazole, ivermectin, moxidectin and antibiotics such as danofloxacin, nitrofurantoin, and enrofloxacin secreted into milk by BCRP-mediated active transport (Virkel et al., 2018).

The secretion of exogenous compounds into milk by BCRP can lead to many beneficial or undesirable results. A useful example of BCRP-mediated drug transfer into milk is to provide effective treatment with parenteral antibiotic administration in the treatment of mastitis. However, the excretion of parenterally administered fluoroquinolones into milk may cause some adverse drug reactions, such as fluoroquinolone-induced cartilage disorders in suckling puppies. Especially in dairy animals, the transfer of drugs into milk by BCRP may cause undesirable contamination in milk for human consumption (Mealey, 2012). In other studies in humans, mice, rats, cows, and sheep, bile acids, uric acid, enterolactone, enterodiol, ciprofloxacin, danofloxacin, moxidectin, flunixin, 5-hydroxyflunixin, pantoprazole, nifedipine, aflatoxin B1 and heterocyclic amines are transported into milk by BCRP (Garcia-Lino et al., 2019).

Drug excretion characteristics based on some studies in ruminants

Benzimidazoles, avermectins and milbemycins are excreted in ruminant milk, but the mechanisms underlying the excretion process have not been fully elucidated (De Liguoro et al., 1996; Barker and Kappel, 1997; Moreno et al., 2005).

Although most drugs secreted from maternal plasma to milk by passive diffusion, the milk/plasma (M/P) ratio, which is used to determine the equilibrium concentration between breast milk and blood, is affected by the composition of the milk and the physicochemical properties of the drug (Agatonovic-Kustrin et al., 2002; Ito and Lee, 2003).

Drugs with steady-state concentrations such as cimetidine, ranitidine, and nitrofurantoin are excreted in human and rat milk, possibly by active transport mechanism. High M/P ratio has been reported for some drugs such as moxidectin and ivermectin in

domestic animals and these compounds can be actively transported into milk (Imperiale et al., 2004).
In a study conducted in goats and cows, benzylpenicillin can cross the blood-milk barrier by active transport and this transport can be inhibited by probenecid (Schadewinkel-Scherkl et al., 1993). Furthermore, some transporters such as MDR1 and OCTN2, which have a potential role in drug disposition in the mammary gland, increase significantly in lactating cell (Alcorn et al., 2002).

Use of inhibitors to reduce the excretion of substrates of ABC transport proteins into milk

The importance given to the use of inhibitors to increase the oral bioavailability and penetration of drugs into tissues for effective treatment or to reduce the transfer of substrates of ABC transport proteins into milk has been increasing (Merino et al., 2010; Shukla et al., 2011). Many in vivo and in vitro studies have shown that inhibition of ABC transport proteins, such as BCRP, with various drugs may also leads to drug interactions. The bioavailability and therapeutic effect of the substrate drugs increase as a result of the use of BCRP substrate drugs co-administered with BCRP inhibitors. In contrast, the bioavailability and therapeutic effects of the substrate drugs are weakened as a result of the use of BCRP substrates co-administered with BCRP inducers (Kunta and Sinko, 2004).

BCRP inhibitors

BCRP inhibitors have two clinically significant pharmacological effects. The first and the reason for the intensification of research on these drugs is that they can overcome BCRP-mediated multi-drug resistance in tumor cells (Henrich et al., 2007). Second, when BCRP inhibitors are administered with drugs that are BCRP substrates, the BCRP substrate could change the disposition (absorption, distribution, and excretion) of the drug in the body (Breedveld et al., 2004; Kuppens et al., 2007).

Flavonoids are herbal components that have recently attracted attention as BCRP inhibitors and have been extensively researched. There are thought to be two possible mechanisms of BCRP inhibition by flavonoids (Morris and Zhang, 2006). The first is that flavonoids are inhibited by interactions with the nucleotide-binding domain of BCRP. The second mechanism appears to result from interaction with BCRP substrate binding sites; because many flavonoid BCRP substrates can stimulate BCRP ATPase activity as seen in mitoxantrone (Cooray et al., 2004, Pulido et al., 2006).

Soy

There are many studies showing the interaction and inhibition of BCRP with polyphenols (tannins, gossypol, fagopyrin) that are largely found in ruminant feed (Broderick 1995; Cooray et al., 2004; Zhang et al., 2004b). Soybean, which is used as a protein source in ruminant feed, is rich in isoflavonoids containing herbal active ingredients (100-200 mg of isoflavonoids are found in 100 g of soy) (Broderick 1995 and 2003). BCRP is inhibited as a result of the interaction of isoflavonoids, which are secondary metabolites of soy and are found in high amounts in polyphenols, and BCRP in the mammary gland (Pulido et al., 2006; Perez et al., 2009). Studies conducted in line with this information have shown that adding BCRP inhibitors such as isoflavonoids to forage prevents the secretion of toxins into milk and is beneficial in controlling drug residues. It is also expected that this procedure may make it possible to reduce the drug withdrawal time in lactating sheep (Pulido et al., 2006).

Soy Active Ingredients

Flavonoids are polyphenols commonly found in fruits and plants. Flavonoids, which give these products their flavor and color, are also the main component of many herbal products (Zhang et al., 2004a). Flavonoids contain two or more aromatic rings, each carrying at least one aromatic hydroxyl and connected by a carbon bridge.

Flavonoids are divided into different subclasses such as chalcones, flavonols, flavones, procyanidins, flavan-3 ol (catechins), flavanones and isoflavones according to the change of C ring and oxidation state (Morris and Zhang, 2006).

Isoflavonoids are secondary metabolites naturally found in plants of the Leguminosae family, such as soy, red clover, peanuts, chickpeas, and alfalfa (USDA, 2008). Isoflavonoids attract bacteria to plant roots to aid in nodulation and nitrogen fixation (Rolfe, 1988). The most abundant source of isoflavones is soybean containing genistein (approximately 2.3 mg/kg), daidzein and glycitine (USDA, 2008). In addition, they contain trace amounts of formononetin and biochanin A (Burdette and Marcus 2013). Isoflavones exist in plants in two forms: glycoside (genistin or genistein-7-O-β-D-glycoside) and aglycone (genistein) (Barnes, 2010).

Glycosides are found in higher concentrations than aglycones in soybean and other plants (Song et al., 1998). After oral ingestion, isoflavone glycosides are converted to aglycon forms with high biological activity by epithelial and microbial β-glucosidases in the oral cavity and small intestine (Akiyama et al., 1987; Day et al., 1998; Morito et al., 2001; Walle et al., 2005).

Isoflavones such as genistein, daidzein and biochanin A are found in soybeans, and their

precursors together with lignans found in a wide variety of plants (Yao et al., 2004).

BCRP-Inhibitory Effects of Some Soy Active Ingredients

Recent studies have begun to investigate the ability of isoflavones to alter the pharmacokinetics or pharmacodynamics of compounds transported by BCRP. To date, a variety of in vitro and in vivo experimental models have been used to detect isoflavones that inhibit BCRP function and to better understand the potential effects of concomitantly consumed isoflavones (e.g. soy-rich diet) with drugs transported by BCRP (Bircsak and Aleksunes, 2015).

Isoflavones in animal feeds and diets can alter the in vivo pharmacokinetics and pharmacodynamics of many drugs. For this reason, it is necessary to pay attention to the administration of BCRP substrate drugs, especially to animals in the lactation period. On the other hand, there are also therapeutic uses, such as inhibiting the secretion of xenobiotics into milk with this function, leading to the protection of the infants from drug-induced toxic effects (Bircsak and Aleksunes, 2015).

When BCRP inhibitors are administered together with drugs that are BCRP substrates, a change occurs in the in vivo disposition of drugs (absorption, distribution, excretion, and excretion into milk) (Ballent et al., 2012; Mealey, 2012). The bioavailability of drugs used in sheep increased with the inhibition of ABC transport proteins (Dupuy et al., 2003; Merino et al., 2003). Polyphenols (tannins, gossypol, fagopyrin and isoflavones) are present in moderate amounts in ruminant feeds. The main isoflavones genistein and daidzein in soy found in ruminant diets have been shown to act as BCRP inhibitors both in vitro and in vivo (Imai et al., 2004; Merino et al., 2010; Zhang et al., 2004b). Although genistein and daidzein are metabolized to glucuronide and sulfate forms in vivo, they interact with and inhibit BCRP (Alvarez et al., 2011). In a study conducted in ewes, daidzein monoglucurunoids and equol are the main isoflavone conjugates found in plasma and tissues (Urpi Sarda et al., 2008). Various studies have shown that flavonoids such as genistein, daidzein, apigenin and luteolin, which are also found in soybean and cow milk in ruminant feeds, reverse BCRP-mediated drug resistance (Antignac et al., 2003; Zhang and Morris, 2003; Cooray et al., 2004; Imai et al., 2004; Zhang et al., 2004b).

In a study conducted in sheep, the flavonoid quercetin increases the plasma moxidectin bioavailability, which is the result of decreasing the secretion of moxidectin into bile and intestine due to P -glycoprotein inhibition of quercetin (Dupuy et al., 2003). Studies in sheep have revealed that the drug penetration into milk decreases as a result of the use of BCRP substrates such as enrofloxacin, danofloxacin and nitrofurantoin together with BCRP inhibitors such as genistein and daidzein (Pulido et al., 2006; Perez et al. 2009 and 2013).

In a study to understand the effect of genistein on the concentrations of enrofloxacin in milk, enrofloxacin was administered to lactating sheep with genistein and without genistein (Pulido et al., 2006). There was no change in enrofloxacin peak plasma concentration (Cmax) value between sheep administered genistein and sheep not administered (control group). On the other hand, a 1.5-fold decrease was found in the area under the concentration versus time curve (AUC) of enrofloxacin in the milk of animals administered genistein, and genistein reduced the concentrations of enrofloxacin in milk. Researchers have suggested that the drug withdrawal period in milk may be shortened, as genistein reduces enrofloxacin concentrations in milk.

In another similar studies, the effect of isoflavone combinations (genistein + daidzein) on the secretion of BCRP substrates (danofloxacin and nitrofurantoin) into milk in lactating sheep was investigated (Perez et al. 2009 and 2013).

In the nitrofurantoin experiment, there was a significant decrease in milk AUC of nitrofurantoin in sheep given exogenous isoflavones (10 mg/kg genistein + 10 mg/kg daidzein) by oral gavage compared to the standard diet (Perez et al., 2009). In addition, higher plasma nitrofurantoin AUC and Cmax values were found in animals in the control group given only nitrofurantoin compared to sheep exposed to isoflavones (Perez et al., 2009). Since this is not compatible with the BCRP inhibition mechanism, the researchers suggested that the plasma nitrofurantoin concentration may be decreased in sheep given isoflavones due to decreased absorption or increased excretion of nitrofurantoin (Bircsak and Aleksunes, 2015).

In the danofloxacin experiment, the AUC and Cmax of danofloxacin in milk were significantly reduced in the group fed with soy-enriched diet for 15 days compared to lactating sheep fed with feed that do not contain isoflavones (Perez et al., 2013). A similar decrease in milk concentrations of danofloxacin was not observed in sheep given orally the soy active ingredients 10 mg/kg genistein and 10 mg/kg daidzein. Researchers have explained that this is because the form in which the compounds are administered (solid and liquid, aglycone and glycone) or the compounds in the diet can change the effect of other chemicals on the pharmacokinetics. Although the components in

the soy diet were specified in these studies, the amount of genistein, daidzein or other isoflavones in the rations was not reported.

Conclusion

Milk is one of the main sources of nutrients and bioactive components in all mammals and is essential in the early stages of neonatal development. Milk production is a very complex process that develops depending on transport mechanisms. Many studies in recent years have focused on transporters in the mammary gland to reveal this mechanism (Garcia-Lino et al., 2019).

One of the most important transporter superfamilies that ensure the secretion of compounds into milk is the ABC transporter protein family. The expression of these transport proteins vary according to the lactation period of the mammary gland. There is a relationship between BCRP, one of the ABC transporter proteins in the mammary gland of ruminants, and xenobiotic residues in milk, and this is important in shortening the drug withdrawal period as a result of interaction with ABC transport proteins (Garcia-Lino et al., 2019).

As a result of the interactions between BCRP drug substrate and inhibitors, many implications can be made for the pharmacotherapy of dairy animals. It is aimed to contribute to a better understanding of the potential role of BCRP inhibitors in the transfer of drug residues into milk, thereby protecting dairy consumers against possible drug residues and other xenobiotics.

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